The Genetics Society of America Conferences

19th International *C. elegans* Meeting

University of California Los Angeles, California

Program & Abstracts Volume

2013 *C. elegans* Meeting Organizing Committee

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Logo design courtesy of Ahna Skop

Genetics Society of America

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Please note: Abstracts published in the book should not be cited in bibliographies. Material contained herein should be treated as personal communication and should be cited as such only with the consent of the author.

GENERAL INFORMATION

Registration and Information Desk

The Conference registration desk will be open according to the following schedule:

Date	Time	Location
Wednesday, June 26	1:30 pm – 9:00 pm	Sunset Village Plaza
Thursday, June 27	8:00 am – 3:00 pm	Covel Commons
Friday, June 28	8:30 am – 12:30 pm	Covel Commons
Saturday, June 29	8:30 am – 12:30 pm	Covel Commons

Instructions for Speakers

Please arrive 60 minutes before the beginning of your session with your usb drive to load your presentation on the MAC meeting computer. If you plan to show Quicktime movies, please DO NOT attach them to your presentation. You should include them as separate files on the disk and be prepared to place them back into your presentation after loading on the meeting computer. All plenary speakers should go to the back of Royce Hall and enter through the door marked "Artist's Entrance".

Poster Sessions

All poster sessions will be located in Pauley Pavilion. There will be three poster sessions. There will be two posters on each 4' high x 8' wide poster board. Each author will have a net useable space of 3' 8" (111.8cm) high x 3' 8" (111.8cm) wide.

Presenters may mount abstracts beginning at 8:00 am on the day of their presentation. All abstracts will be up for one day. Authors will present according to the following schedule:

Date	Time	Presenters
Thursday, June 27	8:00 pm – 9:30 pm	Presenters of all even "A" posters
	9:30 pm – 11:00 pm	Presenters of all odd "A" posters
Friday, June 28	8:00 pm – 9:30 pm	Presenters of all even "B" posters
	9:30 pm – 11:00 pm	Presenters of all odd "B" posters
Saturday, June 29	3:30 pm – 5:00 pm	Presenters of all even "C" posters
	5:00 pm – 6:30 pm	Presenters of all odd "C" posters

All presenters should remove their abstracts at the end of their poster session. After that time, remaining posters will be removed and may be lost or thrown away. The meeting does not take responsibility for posters that are not removed on time.

Pauley Pavilion will be open at the following times for poster viewing:

Thursday, June 27	8:00 am – 11:00 pm
Friday, June 28	8:00 am – 11:00 pm
Saturday, June 29	8:00 am – 6:30 pm

All presenters **MUST** remove their abstracts by 6:30 pm on Saturday, June 29. The poster boards will be removed starting promptly at 6:30 pm on Saturday, June 29.

GENERAL INFORMATION

Photography

Absolutely no photography is allowed in the exhibit hall/poster session area.

Poster Competition

Poster prizes will be awarded to graduate students. Judges attempt to visit each poster at the time when the authors are presenting. The competition is open to posters from GSA graduate student members that are the first and presenting author on the abstract. Authors indicated at the time of their abstract submission that they wanted to be considered for the competition.

C. elegans Art Show

The *C. elegans* Art Show will be in Pauley Pavilion throughout the poster viewing time. The prizes will be awarded on Saturday, June 29 at 8:15 pm in Royce Hall, prior to the Worm Variety Show.

Social Events

Opening Mixer, Wednesday, June 26: Meet friends and colleagues at the Opening Mixer in Royce Quad immediately following Plenary Session 1 from 10:00 pm to 11:30 pm. Complimentary beer, wine and sodas will be available.

The GSA Faculty Mentoring Social will be held Thursday, June 27, 7:00 pm to 8:00 pm on the Grand Horizon Ballroom Terrace. This event is organized by senior *C. elegans* faculty for current and recently hired junior faculty members. New faculty members will be introduced followed by informal discussion. Heads of *C. elegans* laboratories are all encouraged to attend.

Evening Socials, Thursday, June 27 and Friday, June 28: There will be evening socials in the lobby of Pauley Pavilion from 10:00 pm – 12:00 midnight. Complimentary beer, wine and sodas will be available. Drinks must stay in the lobby. No drinks are permitted in the poster session area.

Saturday Evening Events, June 29: A barbecue is planned for 6:30 pm – 8:00 pm in Royce Quad. The *C. elegans* Art Show awards will be presented at 8:15 pm in Royce Hall followed by the Worm Variety Show, 8:30 pm – 9:00 pm. The conference party will be held 9:00 pm – 11:30 pm in the Ackerman Grand Ballroom. Beer, wine and soft drinks will be available.

Meal Times

	De Neve Plaza Commons	Covel Dining Hall
Breakfast	7:00 am – 9:00 am	7:00 am – 10:00 am
Lunch	11:00 am – 2:00 pm	11:30 am – 2:00 pm
Dinner	5:00 pm – 8:00 pm	5:00 pm – 9:00 pm

Join the Conversation
#WORM2013

GENERAL INFORMATION

Internet Access

UCLA provides a complimentary computer lab in De Neve Plaza and in the Covel Business Center (next to Sunset Village) for guests to check their e-mails. The hours are:

Monday – Thursday, June 24 – 27	7:30 am – 8:00 pm
Friday, June 28	7:30 am – 6:00 pm
Saturday, June 29	9:00 am – 6:00 pm
Sunday, June 30	12:00 noon – 5:00 pm

Attendees staying on campus who bring their own laptop computers can access the Internet directly via the Ethernet port in their sleeping rooms. In order to access the Internet, guest must have the Ethernet card on their computer software and an Ethernet cable. If you do not have your own Ethernet card or cable, you may purchase one through the Covel Business Center.

Recreational Facilities

Campus recreation facilities are available for residential meeting attendees. The meal/access card, provided with each room key at check-in, allows admission to all the recreation facilities. The John Wooden Center and Sunset Canyon Recreational Center include swimming pools, weight rooms, tennis courts, racquetball courts and handball courts. Attendees not staying on campus may use the facilities with payment.

Message Boards

Message boards are located in the Poster Session in Pauley Pavilion.

Meeting Announcements/Employment Opportunities/Seeking Employment Notices

Individuals and institutions offering or seeking employment may post notices and resumes on the "Employment Opportunities" bulletin boards in Pauley Pavilion. Organizers of meetings of interest to *C. elegans* researchers may post announcements on the boards provided.

Smoking

UCLA is a tobacco free campus. The use of tobacco is prohibited on all university property.

EDUCATION OPPORTUNITIES

Thursday, June 27

Genetics Society of America Careers Luncheon
12:15 pm – 1:15 pm, DeNeve Private Dining Room
The GSA Careers Luncheon is an excellent opportunity for undergraduates, graduate students, and postdoctoral fellows to have informal conversations with senior career scientists. After getting your lunch, you can proceed to the Private Dining Room to get a seat.

Undergraduate Mixer
6:30 pm – 8:00 pm, Northridge Room
Undergraduate researchers attending the conference will network on their own, forming a peer group that will help provide lasting support for the remainder of the conference.

Teaching Workshop I – What is Life Like at a Predominantly Undergraduate (i.e. Teaching) Institution and How Can I Get a Job at One?
8:00 pm – 9:00 pm, Northwest Auditorium
This workshop should be of interest to graduate students and postdocs who are considering a career at a predominately undergraduate institution (PUI). The challenges facing faculty members at PUIs are substantially different from those typically encountered at a research university and the job search process is also quite different. A panel of *C. elegans* researchers who are employed at different types of PUIs will discuss issues such as teaching loads, research expectations and opportunities, how to find PUI job openings, and hiring criteria at PUIs.

Friday, June 28

Genetics Society of America Careers Luncheon
12:15 pm – 1:15 pm, DeNeve Private Dining Room

See description of the lunch above on Thursday, June 27.

Publishers Discussion
7:00 pm – 8:00 pm, Grand Horizon Ballroom
This GSA-sponsored discussion is an excellent opportunity for graduate students, postdoctoral fellows and undergraduate students to have informal conversations with GENETICS and G3 journal editors about the peer-review and scientific publishing process in general, and more specifically, the GSA's two journals.

Education Special Interest Group Social
7:00 pm – 8:00 pm, Northwest Auditorium
Faculty with a passion for genetics education are encouraged to attend this mixer, where they can mix and mingle with other educators. Current members of the Education SIG can catch up on actions taken by GSA with regards to the education initiative, and those who are not yet SIG members can learn about the Education SIG.

Teaching Workshop II – Building a Successful and Sustainable Program of Scholarship at a PUI.
8:00 pm – 9:00 pm, Northwest Auditorium
This workshop will be of primary interest to those currently employed at predominantly undergraduate institutions (PUIs) at any stage in their career. A panel of PUI veterans will lead a discussion focused on the balance between teaching and research, best practices for research with undergraduate students, how to write successful grant proposals, and issues surrounding publications including the idea of the Least Publishable Unit (LPU), recommended journals to target, and personal experiences with various journals, their editorial boards, reviewers, etc.

EDUCATION OPPORTUNITIES

Saturday, June 29

Advocacy Luncheon
12:15 pm – 1:15 pm, DeNeve Private Dining Room
Adam Fagen, Executive Director of GSA, will discuss the latest policy and legislative news affecting funding for NIH, describe why it is important for scientists to speak out about issue that affect their grants and research and share tips for engaging in advocacy. Sign up online with registration.

GSA Plenary Session and Workshop for Undergraduate Researchers
1:30 pm – 3:00 pm, Southbay Room
Undergraduate conference attendees are invited to an "Undergraduate Plenary Session", with talks presented at a level appropriate for an undergraduate audience. Undergraduates will have a chance to talk to a panel of graduate students about graduate school, after which students will have a chance to break into small groups and participate in a discussion about a specific topic relevant to graduate school applications, admission, CV preparation and funding.

Teaching Workshop III - Educating Scientists for the 21st Century: Focus on High-Impact Learning Strategies
2:30 pm – 4:30 pm, Sunset Village Study Lounge
This workshop should be of interest to educators at all levels and types of institutions who have a dedicated commitment to teaching. The presenters at this workshop will share innovative teaching materials and techniques, with an emphasis on those that are used in, or can be adapted to, smaller class sizes. The presentations will include, but are not limited to, *C. elegans*-based approaches, interdisciplinary courses/programs related to developmental biology, active learning exercises, and high-tech applications that have been used effectively in the classroom.

EXHIBITS

The following companies have contributed to the support of this meeting. Registrants are encouraged to visit the exhibits in Pauley Pavilion during the Poster Sessions.

Andor Technology — Booth #109

425 Sullivan Avenue
South Windsor, CT 06074
Tel: 860-290-9211
Email: m.fish@andor.com
Website: www.andor.com

Andor Technology is a global leader in the pioneering and manufacturing high performance scientific imaging cameras, spectroscopy solutions and microscopy systems for research and OEM markets. Andor has been innovating the photonics industry for over 20 years and aims to continue to set the standard for high performance light measuring solutions.

Exo Labs — Booth #114

3131 Western Avenue
Suite M325
Seattle, WA 98121
Tel: 206-850-5642
Email: asalkin@exolabs.com
Website: www.exolabs.com

Worm pics and video! Exo Labs has launched the Focus Microscope Camera that connects your microscope to your iPad, where our app opens up the image to exploration. Take pictures and video. Make annotations and do point-to-point measurements. Project images. We want to ignite curiosity and inspire the next generation of scientists!

Firefly BioWorks, Inc. — Booth #107

One Kendall Square
Building 1400W
Cambridge, MA 02139
Tel: 888-240-7789
Email: tom_kelly@fireflybio.com
Website: www.fireflybio.com

Firefly BioWorks, Incorporated has developed FirePlex™, a customizable microRNA detection platform for rapid profiling of diverse biological samples. The FirePlex™ advantage lies in its streamlined, high-throughput protocol, affordable customization, and low capital cost. FirePlex™ readout is performed on flow cytometers, and is offered as a service for users with limited access to instrumentation.

Genesee Scientific — Booth #117

8430 Juniper Creek Lane
San Diego, CA 92126
Tel: 800-789-5550
Email: support@geneseesci.com
Website: www.wormstuff.com

Genesee Scientific, creators of www.WormStuff.com, is dedicated to supply the *C. elegans* research community with high quality products at competitive prices. In fact, we've made it our priority to innovate, source or manufacture the best *C. elegans* products on the market to help labs everywhere achieve the best results.

Genetics Society of America — Booth #101

9650 Rockville Pike
Bethesda, MD 20814
Tel: 301-634-7300
Email: society@genetics-gsa.org
Website: www.genetics-gsa.org

Come explore the resources and opportunities that GSA has to offer including education, career development and policy, meet members of the GSA staff and leadership; and find out about publishing in *GENETICS* and *G3: Genes|Genomes|Genetics.*

Kramer Scientific LLC — Booth #103

91 High Street
Amesbury, MA 01913
Tel: 978-388-7159
Email: abby@kramerscientific.com
Website: www.kramerscientific.com

Kramer Scientific fluorescence microscopes are made specifically with *C. elegans* in mind. Researchers across the world have used our systems to document discoveries for over 20 years. Kramer has solutions for full systems and cost-effective conversions of new and used microscopes. Check out our systems and "*C.*" what you've been missing.

EXHIBITS

MBF Bioscience **Booth #110**
185 Allen Brook Lane
Williston, VT 05495
Tel: 802-288-9290
Email: info@mbfscience.com
Website: www.mbfbioscience.com

MBF Bioscience develops software for tracking and analyzing the locomotion and behavior of *C. elegans*. Our WormLab software makes worm tracking simple. With four mouse clicks you can obtain fourteen different types of analyses about your worms. Track and analyze a single worm or multiple worms, even through entanglements.

Microscope World **Booth #102**
6122 Innovation Way
Carlsbad, CA 92009
Tel: 760-438-0528
Email: kbauer@microscopeworld.com
Website: www.microscopeworld.com

Microscope World announces the introduction of a new stereo fluorescence system for picking, sorting and manipulating GFP or RFP embryos. This reasonably priced system, while priced lower than our competitors, provides high intensity metal halide illumination through a filter direct to the field for brighter images and less light loss.

modENCODE Data Coordinating Center Booth 114
101 College Street
Toronto, Ontario, Canada M5G 0A3
Tel: 416-673-8593

Olympus **Booth #106**
3500 Corporate Parkway
Center Valley, PA 18034
Tel: 484-896-5844
Email: barbara.bach@olympus.com
Website: www.olympus-global.com

Olympus America Scientific Equipment Group provides innovative microscope imaging solutions for researchers, doctors, clinicians and educators. Olympus microscope systems offer unsurpassed optics, superior construction and system versatility to meet the ever-changing needs of microscopists, paving the way for future advances in life science.

PhylumTech Consortium **Booth #112**
Patricias Argentinas 435
Buenos Aires (C1405BWE)
Argentina
Tel: 54-5238-7500 ext 4200
Email: info@phylumtech.com
Website: www.phylumtech.com

PhylumTech Consortium brings solutions for high-throughput drug discovery using small animal models. We offer easy-to-use infrared devices for tracking *C.elegans* (WMicrotracker products), plus custom machines and compound testing services. We distribute worldwide since 2009 from Argentina and Italian offices, having reached more than 30 laboratories from all around the world.

Southland Instruments, Inc. **Booth #105**
PO Box 1517
Huntington Beach, CA 92647
Tel: 800-862-0447
Email: emascio@southlandinstruments.com
Website: www.southlandinstruments.com

Southland Instruments Inc. the authorized distributor of Motic, Labomed and Meiji Techno in the Western United States will be exhibiting stereomicroscopes, inverted microscopes and digital cameras and software. We are also an authorized service center for these manufacturers and other fine brands. Please stop by to see the “New Kid on the Block”.

Source BioScience **Booth #113**
1 Orchard Place
United Kingdom, NG8 6PX
Tel: 011 597 39012
Email: sales@sourcebioscience.com
Website: www.sourcebioscience.com

Source BioScience LifeSciences are European leaders in DNA sequencing, genomic services, and bioinformatics analyses and offers a comprehensive portfolio of genomic reagents and antibodies. Source BioScience LifeSciences offer a unique Sanger sequencing service; our local facilities in London, Cambridge, Oxford, Nottingham, Dublin and Berlin combined with our Overnight Service mean we have the flexibility to meet your entire DNA sequencing requirements. Our contract research services feature a broad range of microarray, genotyping and gene expression applications on Affymetrix, Agilent, and NimbleGen Fluidigm platforms.

EXHIBITS

Union Biometrica, Inc. **Booth #104**
84 October Hill Road
Holliston, MA 01746
Tel: 508-893-3115
E-mail: thofhuis@unionbio.com
Website: www.unionbio.com

Union Biometrica Large Particle Flow Cytometers automate the analysis, sorting & dispensing of objects too big/fragile for traditional cytometers, e.g., small model organisms like C.elegans, large cells / cell clusters, and beads. COPAS or BioSorter with interchangeable modules to cover the full 10-1500µm range are ideal for shared instrument grants.

WormAtlas **Booth #115**
Alert Einstein College of Medicine
Dept. of Neuroscience
1410 Pelham Parkway
Bronx, NY 10461
Tel: 718-430-2195
Email: david.hall@einstein.yu.edu
Website: www.wormatlas.org

We will demonstrate new features and offerings of the online databases WormAtlas and WormImage. WormAtlas now has a new handbook for dauer larva, updated pharynx atlas, updated neuron pages, more movies, and new anatomical methods. WormImage continues to grow, and SlidableWorm has a new interface and more slices available.

WormBase Consortium **Booth #116**
Caltech 156-29
1200 E. California Blvd.
Pasadena, CA 91125
Tel: 626-395-2686
Email: help@wormbase.org
Website: www.wormbase.org

WormGUIDES **Booth #111**
1275 York Avenue, Box 416
New York, NY 10065
Tel: 212-639-7744
Email: baoz@mskcc.org
Website: www.wormguides.org

WormGUIDES is an ongoing project for a 4D atlas of embryogenesis tracking cells and neuronal outgrowth over time and space. We will feature an early version as an app on PCs and mobile devices, as well as the StarryNite software underlying cell tracking.

PLENARY, PARALLEL AND WORKSHOP LISTINGS

Wednesday, June 26 7:00 PM–10:00 PM
Royce Hall

7:00 pm **Welcome and Opening Remarks**
Meeting Organizers:
Monica Colaiacovo, Harvard Medical School, USA and Ralf Sommer, Max-Planck Institute for Developmental Biology, Germany

7:05 pm **GSA Welcome**
Adam Fagen, Genetics Society of America

Plenary Session 1

Chair: Abby Dernburg, University of CA, Berkeley, USA

1- 7:10 – Invited Speaker
Feedback and Self-Organization in Meiosis. **Anne Villeneuve**, Stanford University.

2- 7:40
WormBase - still growing upwards and outwards. **Kevin Howe**, The WormBase Consortium.

3- 7:45
Tactile Toy, Teaching Tool, or Transformative Technology? WormGUIDES EmbryoAtlas 1.0. **William A. Mohler**, Zhirong Bao, Daniel Colon Ramos, Hari Shroff, Jim Schaff, Ion Moraru, wormguides.org.

4- 7:50
WormAtlas Update. L. A. Herndon, Z. F. Altun, C. A. Wolkow, K. Fisher, C. Crocker, **D. H. Hall**.

5- 7:55
WormBook News. **Jane E. Mendel**, Qinghua Wang, Todd Harris, Paul Sternberg, Oliver Hobert, Martin Chalfie.

6- 8:00
Caenorhabditis Genetics Center. Aric Daul, Theresa Stiernagle, Julie Knott, Brittany Werre, **Ann E. Rougvie**.

7- 8:05
The viral 2A peptide technology to express multiple functional proteins from a single ORF in Caenorhabditis elegans. **Arnaud Ahier**, Sophie Jarriault.

8- 8:10
A genome-scale resource for *in vivo* tag-based protein function exploration in *C. elegans.*. **Mihail S. Sarov**, John I. Murray, Susanne Ernst, Andrei Pozniakovski, Elisabeth Loester, Stephan Janosch, Wadim Kapulkin, Siegfried Schloissnig, Anthony A. Hyman, The ModENCODE C. elegans TF binding sites group.

9- 8:15
The Million Mutation Project and Beyond. Owen Thompson, **Mark Edgley**, Pnina Strasbourger, Stephane Flibotte, Brent Ewing, Ryan Adair, Vinci Au, Iasha Chaudhry, Lisa Fernando, Harald Hutter, Joanne Lau, Angela Miller, Greta Raymant, Bin Shen, Jay Shendure, Jon Taylor, Emily Turner, LaDeana Hillier, Donald G. Moerman, Robert H. Waterston.

8:27 - **Break**

10- 9:00 Advances in Targeted Genome Editing Across Species: Heritable Designer "Knock-In" and "Knock-Out" Modifications. **Te-Wen Lo**, Catherine Pickle, Mark Gurling, Caitlin Schartner, Erika Anderson, Ed Ralston, Barbara J. Meyer.

11- 9:12 Heritable genome editing in C. elegans via CRISPR-Cas systems. **Ari E. Friedland**, Yonatan B. Tzur, Kevin M. Esvelt, Monica P. Colaiacovo, George M. Church, John A. Calarco.

12- 9:24 CRE-LoxP mediated gene inactivation to study the coordination between proliferation and differentiation. **Suzan Ruijtenberg**, Sander van den Heuvel.

13- 9:36 Magnetotaxis in *C. elegans*. **Andrés G. Vidal-Gadea**, Kristi A. Ward, Jonathan T. Pierce-Shimomura.

14- 9:48 FLP-13 neuropeptides released from the ALA neuron signal through FRPR-4 to regulate behavioral quiescence. **Matthew Nelson**, Tom Janssen, Liliane Schoofs, David Raizen.

Join the Conversation
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Thursday, June 27 9:00 AM–12:00 NOON
Ackerman Grand Ballroom

Physiology I: Aging and Stress I

Chairs: Veena Prahlad, University of Iowa, USA and Jeremy Van Raamsdonk, Van Andel Research Institute, USA

15- 9:00 Counterbalance between BAG and URX neurons via guanylate cyclases controls lifespan homeostasis in *C. elegans*. **T. Liu**, D. Cai.

16- 9:12 PQM-1: the missing "DAE Factor" and key regulator of DAF-2-mediated development, longevity, and homeostasis. **Ronald G. Tepper**, J. Ashraf, R. Kaletsky, G. Kleemann, C. T. Murphy, H. J. Bussemaker.

17- 9:24 Analysis of mutation accumulation by large scale C. elegans whole genome mutation profiling. B. Meier, S. Cook, J. Weiss, A. Bailly, P. Campbell, **Anton Gartner**.

18- 9:36 TORC2 regulates SGK-1 in two opposing longevity pathways. **M. Mizunuma**, E. Neumann-Haefelin, N. Moroz, K. Blackwell.

19- 9:48 A CREB-Dependent Neuropeptide Signal from the Thermosensory AFD Neuron Regulates *C. elegans* Life Span at Warm Temperatures. **Yen-Chih Chen**, Wei-Chin Tseng, Chun-Liang Pan.

20- 10:00 FGT-1 is the sole glucose transporter in C. elegans and is central to aging pathways. Ying Feng, B. Williams, F. Koumanov, **A. J. Wolstenholme**, G. D. Holman.

10:12 - **Break**

21- 10:36 Sensory neuronal regulation of lifespan through modulating insulin-like peptides in *C. elegan*s. **Murat Artan**, Dae-Eun Jeong, Dongyeop Lee, Young-Il Kim, Joy Alcedo, Seung-Jae Lee.

22- 10:48 HLH-30/TFEB is a conserved regulator of autophagy and modulates longevity in *C. elegans*. **Louis R. Lapierre**, C. Daniel De Magalhaes Filho, Philip R. McQuary, C.-C. Chu, O. Visvikis, J. T. Chang, S. Gelino, B. Ong, Andrew Davis, J. E. Irazoqui, Andrew Dillin, M. Hansen.

23- 11:00 Regulation of SKN-1/Nrf by the germline longevity pathway. **Michael J. Steinbaugh**, Sri Devi Narasimhan, Stacey Robida-Stubbs, Prashant Raghavan, Theresa Operana, T. Keith Blackwell.

24- 11:12 Importance of Growth, Stress Defense, and NAD+-related Pathways for Dietary Restriction Longevity. **Natalie Moroz**, Juan J. Carmona, Edward Anderson, Anne Hart, David A. Sinclair, T. Keith Blackwell.

25- 11:24 Nonsense-mediated decay as a novel modulator of toxic CUG repeats in *C. elegans*. **Susana M. Garcia**, Yuval Tabach, Guinevere Lourenço.

26- 11:36 A high throughput chemical screen identifies a novel activator of dietary restriction. **Mark S. Lucanic**, Ravi Shah, Ivan Yu, Bob Hughes, Gordon Lithgow.

27- 11:48 Mating-induced somatic collapse reveals a novel soma-germline interaction. **Cheng Shi**, Coleen Murphy.

Thursday, June 27 9:00 AM–12:00 NOON
Bradley International Ballroom

Neurobiology I: Behavior

Chairs: Jagan Srinivasan, Worcester Polytechnic Institute, USA and Alexander Gottschalk, Goethe University, Germany

28- 9:00 Neuromodulation of *C. elegans* mechanosensation. **Xiaoyin Chen**, Martin Chalfie.

29- 9:12 Humidity sensation requires a conserved DEG/ENaC complex in multi-dendritic FLP neurons. **Josh Russell**, Jonathan Pierce-Shimomura.

30- 9:24 CEPsh glia modulate a sleep-related neuronal circuit in *C. elegans*. **Menachem Katz**, Francis Corson, Shachar Iwanir, Elena Dragomir, David Biron, Shai Shaham.

31- 9:36 A neuronal mechanism for navigation along a repulsive odor gradient. Akiko Yamazoe, Yuki Tanimoto, Kosuke Fujita, Yuya Kawazoe, Yosuke Miyanishi, Shuhei Yamazaki, Xianfeng Fei, Karl Emanuel Busch, Keiko Gengyo-Ando, Junichi Nakai, Yuichi Iino, Yuishi Iwasaki, Koichi Hashimoto, **Kotaro Kimura**.

32- 9:48 Multiple cholinergic pathways for excitation of the *Caenorhabditis elegans* pharynx. **Nicholas Trojanowski**, Olivia Padovan-Merhar, David Raizen, Christopher Fang-Yen.

33- 10:00 Pathogen-induced changes of neuronal TGF-β signaling promote avoidance behavior and survival. **Joshua D. Meisel**, Dennis H. Kim.

10:12 - **Break**

34- 10:36 Nematophagous fungi eavesdrop on nematode pheromones, and lure their prey with attractive volatile organic compounds. **Yen-Ping Hsueh**, E. Schwarz, W. Zeng, Z. Xian, P. Mahanti, M. Gronquist, F. Schroeder, A. Mortazavi, P. Sternberg.

35- 10:48 Sensory neurons override recurrent motor programs to induce ejaculation during mating. **Brigitte L. LeBoeuf**, L. Rene Garcia.

36- 11:00 *eol-1*, the homolog of mammalian *Dom3z*, is a novel genetic regulator of *C. elegans* olfactory learning. **Yu Shen**, Jiangwen Zhang, John Calarco, Yun Zhang.

37- 11:12 Membrane phospholipids that contain arachidonic acid regulate touch receptor neuron mechanics and touch sensation. **V. Vásquez**, M. Krieg, D. Lockhead, M. B. Goodman.

38- 11:24 Serotonin and PDF are opposing neuromodulators that control a bistable foraging behavior in C. elegans. **Steven W. Flavell**, N. Pokala, E. Z. Macosko, D. A. Albrecht, J. Larsch, C. I. Bargmann.

39- 11:36 Adenosine signaling in *C. elegans*: does skin rule the brain? **Hsiao-Fen Han**, Michael Ailion, Mary Beckerle, Erik Jorgensen.

40- 11:48 Chemosensing a predator: *Pristionchus pacificus* and *C. elegans*. **Kevin Curran**, A. Tong, M. Joens, J. Fitzpatrick, J. Srinivasan, S. Chalasani.

Thursday, June 27 9:00 AM–12:00 NOON
Grand Horizon Ballroom

Development and Evolution I: Germline Development, Meisosis and Sex Determination

Chairs: Sarit Smolikove, University of Iowa, USA and Swathi Arur, University of Texas MD Anderson Cancer Center, USA

41- 9:00 ZTF-8 interacts with the 9-1-1 complex and is required for DNA damage response and double-strand break repair in the C. elegans germline. **Hyun-Min Kim**, Monica Colaiacovo.

42- 9:12 Interplay between structure-specific endonucleases for crossover control during meiosis. **Takamune T. Saito**, Doris Y. Lui, Hyun-Min Kim, Katherine Meyer, Monica P. Colaiácovo.

43- 9:24 Sperm-derived TRP-3 channel specifies the onset of the fertilization Ca^{2+} wave in the oocyte of *C. elegans*. **J. Takayama**, S. Onami.

44- 9:36 Gap junctions between soma and germline regulate germ cell proliferation, meiotic maturation, and early embryogenesis. **Todd Starich**, David Hall, David Greenstein.

45- 9:48 Evidence for a meiotic crossover surveillance system. T. Machovina, O. McGovern, A. Woglar, D. Paouneskou, V. Jantsch, **J. Yanowitz**.

46- 10:00 Interchangeable α-kleisin subunits specify meiotic cohesin function in *C. elegans*. **Aaron F. Severson**, Barbara J. Meyer.

10:12 - **Break**

47- 10:36 A spatial and temporal transcriptomic survey of gene expression in the C. elegans embryo reveals organizing principles in cell fate specification. T. Hashimshony, M. Feder, D. Silver, A. Polsky, M. Levin, **I. Yanai**.

48- 10:48 LIN-41 and OMA-1/2 spatially control M-phase entry during oogenesis. **Caroline Spike**, Donna Coetzee, David Greenstein.

49- 11:00 Molecular Antagonism between X-Chromosome and Autosome Signals Determines Sex. **Behnom Farboud**, Paola Nix, Margaret Jow, John Gladden, Barbara Meyer.

50- 11:12 The role of an SLC6 family transporter in *C. elegans* sperm activation. **Kristin Fenker**, A. Hansen, C. Chong, M. Jud, G. Stanfield.

51- 11:24 Regulation of lineage-specific transcription factors by Wnt signaling in *C. elegans* embryogenesis: more than one way to regulate expression. **A. L. Zacharias**, T. Walton, J. T. Burdick, E. Preston, J. I. Murray.

52- 11:36 Two molecularly distinct 3'end-directed translational control mechanisms establish two identical protein gradients in germ cells. **Ryuji Minasaki**, N. Jourjine, A. Solovyeva, B. Kuechler, C. R. Eckmann.

53- 11:48 The hazards of love: Sterilization and lethality in interspecies crosses. JJ Ting, **GC Woodruff**, T. Maugel, N. Kanzaki, R Sommer, AD Cutter, ES Haag.

Thursday, June 27 9:00 AM–12:00 NOON
Northwest Auditorium

Cell Biology I: Morphogenesis, Migration, Cytoskeleton

Chairs: Shuichi Onami, RIKEN Quantitative Biology Center, Japan and Bryan Phillips, University of Iowa, USA

54- 9:00 A pathway for unicellular tube extension depending on the lymphatic vessel determinant Prox1 and on osmoregulation. **Irina Kolotueva**, Vincent Hyenne, Yannick Schwab, David Rodrigues, Michel Labouesse.

55- 9:12 NHR-67 mediates cell cycle arrest and promotes the differentiation of the invasive phenotype. **David Q. Matus**, Laura C. Kelley, Michalis Barkoulas, Adam J. Schindler, Qiuyi Chi, Marie-Anne Félix, David R. Sherwood.

56- 9:24 UNC-84 spans the nuclear envelope and connects the nucleoskeleton to KASH proteins at the outer nuclear membrane. Natalie Cain, Courtney Bone, Erin Tapley, Ben Lorton, Kent McDonald, **Daniel Starr**.

57- 9:36 Endocytosis controls EFF-1 mediated cell fusion. **K. Smurova**, B. Podbilewicz.

58- 9:48 A new model system for studying cell shape change: identifying the molecular mechanisms necessary for *C. elegans* uterine seam cell development. **Srimoyee Ghosh**, Paul Sternberg.

59- 10:00 Dynamic interaction between hemidesmosomes and actin cytoskeleton regulated by RNA alternative splicing in elongating *C.elegans* epidermis. **H. Zhang**, R. Fu, H. Zahreddine, M. Labouesse.

10:12 - **Break**

60- 10:36 Excretory canal development requires conserved kinases and *exc-6/INF2*, a formin implicated in kidney disease. **Daniel Shaye**, Iva Greenwald.

61- 10:48 A Rho-specific GAP functions in response to axonal guidance signals to regulate embryonic morphogenesis. **Andre Wallace**, Sanese Brown, Martha Soto.

62- 11:00 ZEN-4/MKLP1 and the establishment of epithelial polarity in the *C. elegans* foregut. **Stephen E. Von Stetina**, Susan E. Mango.

63- 11:12
A Pre-Stressed UNC-70 β-Spectrin Network Governs the Sense of Touch. **Michael Krieg**, Alexander R. Dunn, Miriam B. Goodman.

64- 11:24 Understanding the Role of MMPs In Basement Membrane Breaching *In Vivo*. **Laura C. Kelley**, David Q. Matus, Qiuyi Chi, David R. Sherwood.

65- 11:36 The PAF1 complex is essential for epidermal morphogenesis in *C. elegans* embryos. **Yukihiko Kubota**, Yusuke Takabayashi, Kenji Tsuyama, Nami Haruta, Rika Maruyama, Asako Sugimoto.

66- 11:48 Nuclear membrane proteins act in transport of the Netrin receptor, UNC-5 in cell migration in *C. elegans*. **Hon-Song Kim**, Kiyoji Nishiwaki.

WORKSHOP

Thursday, June 27 1:30 PM–2:30 PM

Strategies for Transgenesis

Room: Ackerman Grand Ballroom

Organizer: **Peter Boag**, Monash University, Melbourne, Australia

A key technology for understanding gene function is the generation of transgenic animals. The aim of this workshop is to highlight the recent technological advances that allow for rapid generation of expression constructs and transformation of C. elegans. The advantages and disadvantages of various approaches will be discussed.

Speakers:
Recombineering: Mihail Sarov, MPI-CBG

MosSCI: Christian Frøkjaer-Jensen, Jorgensen Lab, HHMI, Utah

Bombardment: Jenny Semple, Lehner lab, EMBL/CRG

Single/low-copy integration of transgenes: Shohei Mitani, Tokyo Women's Medical University School of Medicine

WORKSHOP

Thursday, June 27 1:30 PM–2:30 PM

WormBase tutorial: Genomes, Comparative Genomics, Genomic Variation and modENCODE

Room: Bradley International Ballroom

Organizer: **Kevin Howe**, WormBase, European Bioinformatics Institute

One of the core roles of WormBase is to act as a central repository for the genome sequences and genomic annotations that form the foundation of many nematode molecular biology studies. In this workshop, WormBase biologists will present an overview of the genomic data served by the resource and how to access it. The workshop will be presented in five parts: (a) an introduction to the new features of the WormBase web-site; (b) genomes, transcriptomes and proteomes in WormBase, including discourse on the curation and analysis pipelines employed, nomenclature, and how to access these core data types; (c) the tools and views provided for comparative genomics, with focus on gene orthology and paralogy, and genomic alignments; (d) genomic variation data - how it is collected, analysed, represented and displayed, including an overview of nomenclature used; and (e) an update from modENCODE, with focus on data sets produced by the project in the last two years.

Speakers:
Abigail Cabunoc, WormBase, Ontario Institute for Cancer Research (OICR), An introduction to WormBase 2.0
Gareth Williams, WormBase, European Bioinformatics Institute (EBI), An overview of genomic data in WormBase
Michael Paulini, WormBase, European Bioinformatics Institute (EBI), Comparative genomics in WormBase
Mary Ann Tuli, WormBase, European Bioinformatics Institute (EBI), Genomic variation data in WormBase
Marc Perry, Ontario Inst for Cancer Research, modENCODE - an update

WORKSHOP

Thursday, June 27 1:30 PM–2:30 PM

Mapping and Cloning Mutants from Whole Genome Sequence Data Using a Free, Cloud-based Pipeline

Room: Grand Horizon Ballroom

Organizer: **Gregory Minevich**, Columbia University

Whole genome sequencing (WGS) allows researchers to pinpoint genetic differences between individuals and significantly shortcuts the costly and time-consuming part of forward genetic analysis in model organism systems. Currently, the most effort-intensive part of WGS is the bioinformatic analysis of the relatively short reads generated by second generation sequencing platforms. We will present a free cloud-based pipeline, called CloudMap, which greatly simplifies the analysis of mutant genome sequences. Available on the Galaxy web platform, CloudMap requires no software installation when run on the cloud, but it can also be run locally or via Amazon's Elastic Compute Cloud (EC2) service. CloudMap uses a series of predefined workflows to pinpoint sequence variations in animal genomes, such as those of pre-mutagenized and mutagenized Caenorhabditis elegans strains. In combination with a variant-based mapping procedure, CloudMap allows users to sharply define genetic map intervals graphically and to retrieve very short lists of candidate variants with a few simple clicks. Automated workflows and extensive video user guides are available to detail the individual analysis steps performed (http://usegalaxy.org/cloudmap).

Topics covered:
Choosing the correct type of mapping cross for a given genetic screen.

Analyzing mapping cross data.Variant subtraction of previously sequenced strains.

In silico complementation analysis.

Analyzing putative deletions.

Using CloudMap for nematodes or organisms other than C.elegans.

Running CloudMap on the Cloud, locally, or on Amazon's servers.

Speakers:
Gregory Minevich, Columbia University

Richard Poole, University College London

WORKSHOP

Thursday, June 27 1:30 PM–2:30 PM

New Tools and Insights from Mining Genotype-phenotype Relations across Multiple Genotypes of Caenorhabditis spp.

Room: Northwest Auditorium

Organizers: **Jan Kammenga**, Wageningen University, and **Basten Snoek**, Wageningen University, and **Gino Poulin**, University of Manchester

Research in C. elegans has been instrumental for unravelling many genotype-phenotype relations. But almost all studies, including forward and reverse genetic screens, are dominated by investigations in the canonical single strain Bristol N2. In order to explore the full potential of the natural genetic variation and evolutionary context of the genotype-phenotype map, it is important to study these relations in other genotypes and related species. In this workshop we will highlight new tools and resources for investigating genotype-phenotype relations and illustrate the potential of exploring natural genetic variation in *Caenorhabditis spp*.

Speakers:
Basten Snoek, Wageningen University, The Netherlands, Introducing WormQTL, a public archive and analysis web portal for natural variation data in *Caenorhabditis spp*.

Ben Lehner, Centre for Genomic Regulation, Barcelona, Spain, Insights into the natural variation of gene expression dynamics.

Patrick Phillips, University of Oregon, US, Presenting natural genetic variation and resources in *C. remanei*.

Gino Poulin, University of Manchester, UK, Deciphering natural genetic variation effects on hyper activated LET-60(RAS) signalling using an RNAi-based fitness assay.

Reza Farhadifar, Harvard, Cambridge, US, Evolution and genetic architecture of the first mitotic spindle in *C. elegans*.

Benjamin Lang, MRC, Cambridge, UK, Natural genetic variation as the basis for gene network reconstruction in *C. elegans*.

WORKSHOP

Thursday, June 27 1:30 PM–2:30 PM

WormBase tutorial: Tools and Techniques for Data Mining

Room: West Coast

Organizer: **Todd Harris**, WormBase, Ontario Institute for Cancer Research (OICR)

During this workshop, WormBase developers will present tools available for querying WormBase and fetching data en masse. This will include topics ranging from the availability of precomputed analyses and where to find them; query languages; programmatic APIs; and cloud resources available for data analysis. We will introduce users to WormMine, our new data mining platform and the successor to WormMart. WormMine is WormBase's implementation of the InterMine data warehouse platform and will serve as an interface for complex data querying and the integration of new features to WormBase. An introduction to the software, querying for complex data, account management, template queries, extracting data, and current datasets available will be covered.

Speakers:
Todd Harris, WormBase, Ontario Institute for Cancer Research (OICR), An overview of data mining options at WormBase
JD Wong, WormBase, Ontario Institute for Cancer Research (OICR), Introducing WormMine, a powerful new data mining platform for WormBase

Thursday, June 27 3:00 PM–6:00 PM
Royce Hall

Plenary Session 2

Chair: Bruce Bowerman, University of Oregon, USA

67- 3:00 – Invited Speaker
Tony Hyman, Max Planck Institute.

68- 3:30
In vivo forced reprogramming and remodeling of differentiated somatic cells and organs by brief expression of a single transcription factor. Misty R. Riddle, Ken K. C. Q. Nguyen, David H. Hall, **Joel H. Rothman**.

69- 3:42
Evolution and genetic architecture of the first mitotic spindle in *C. elegans*. **R. Farhadifar**, C. Baer, E. Andersen, G. Fabig, T. Müller-Reichert, M. Delattre, D. Needleman.

70- 3:54
Meiotic chromosome structures constrain and respond to designation of crossover sites. **Diana E. Libuda**, Satoru Uzawa, Barbara J. Meyer, Anne M. Villeneuve.

71- 4:06
A sulfatase encodes a developmental switch for a feeding-structure dimorphism and controls micro- and macroevolutionary patterns in *Pristionchus*. **Erik J. Ragsdale**, Manuela R. Müller, Ralf J. Sommer.

72- 4:18
Axons degenerate in the absence of mitochondria. **Randi Rawson**, Lung Yam, Robby Weimer, Eric Bend, Erika Hartwieg, H. Robert Horvitz, Scott Clark, Erik Jorgensen.

4:30 - **Break**

73- 5:00
MATH-33, a conserved DUB required for DAF-16/FOXO stabilization and function. **Thomas Heimbucher**, Zheng Liu, Carine Bossard, Andrea Carrano, Richard McCloskey, Christian G. Riedel, Bryan R. Fonslow, Christian Klammt, Celine Riera, Kenneth Kemphues, Björn F. Lillemeier, John R. Yates III, Clodagh O'Shea, Tony Hunter, Andrew Dillin.

74- 5:12
DAF-16(FOXO) employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. **Christian G. Riedel**, Robert H. Dowen, Guinevere F. Lourenco, Natalia V. Kirienko, Thomas Heimbucher, Jason A. West, Sarah K. Bowman, Robert E. Kingston, Andrew Dillin, John M. Asara, Gary Ruvkun.

75- 5:24
Life in the hot seat: Comparing aging and stress resistance. **Nicholas Stroustrup**, Zachary Nash, Javier Apfeld, Walter Fontana.

76- 5:36
The functional and regulatory organization of the *C. elegans* insulin-like peptide network. **D. A. Fernandes de Abreu**, **A. Caballero**, P. Fardel, N. Stroustrup, Z. Chen, K. Lee, W. D. Keyes, Z. M. Nash, I. F. López Moyado, F. Vaggi, A. Cornils, M. Regenass, A. Neagu, I. Ostojic, C. Liu, D. Sifoglu, W. Fontana, A. Csikasz-Nagy, C. Murphy, A. Antebi, E. Blanc[+1], J. Apfeld[+4], Y. Zhang[+5], J. Alcedo[+2,8], Q. Ch'ng[+1] * Shared first author + Corresponding author.

77- 5:48
Succinylated octopamine ascarosides and a new pathway of biogenic amine metabolism in C. elegans. Alexander B. Artyukhin, Joshua J. Yim, Jagan Srinivasan, Yevgeniy Izrayelit, Neelanjan Bose, Stephan H. von Reuss, James M. Jordan, L. Ryan Baugh, Paul W. Sternberg, Leon Avery, **Frank C. Schroeder**.

WORKSHOP

Thursday, June 27 8:00 PM–8:30 PM

N-Genomes Discussion Group

Room: West Coast

Organizer: **Erich Schwarz**, Cornell University

The *Caenorhabditis* Genome Analysis Consortium has been working to decipher the genomes from six gonochoristic(male-female) relatives of *C. elegans (C. remanei, C. brenneri, C. japonica, C.* sp. 5 JU800, C. sp. 9 JU1422, and *C. angaria* PS1010) versus three hermaphroditicgenomes (the published *C. elegans* and *C. briggsae*, and the recently sequenced C. sp. 11 JU1373). This work has been delayed by misassembly, cryptic microbial contamination,unresolved alleles from heterozygosity in outbreeding species, and third-generation sequencing data which are both promising and difficult to incorporate. Recentadvances in genomic software have surmounted these difficulties, allowing a mature set of genomes to be assembled and analyzed. I will review the state of the "Ngenomes" (with N currently = 9) and lead a discussion on their analysis. Non-Consortium members with an interest in multigenome analysis of *C. elegans* are welcome to attend!

WORKSHOP

Thursday, June 27 8:00 PM–9:00 PM

Teaching Workshop 1- What is Life Like at a Predominantly Undergraduate (i.e. Teaching) Institution and How Can I Get a Job at One?

Room: Northwest Auditorium

Organizers: **Jennifer Miskowski**, Univ. of WI, La Crosse, and **Jonathan Karpel**, Southern Utah University

This workshop should be of interest to graduate students and postdocs who are considering a career at a predominately undergraduate institution (PUI). The challenges facing faculty members at PUIs are substantially different from those typically encountered at a research university and the job search process is also quite different. A panel of *C. elegans* researchers who are employed at different types of PUIs will discuss issues such as teaching loads, research expectations and opportunities, how to find PUI job openings, and hiring criteria at PUIs.

Friday, June 28 9:00 AM–12:00 NOON
Ackerman Grand Ballroom

Physiology II: Aging and Stress II

Chairs: Elke Neumann-Haefelin, Freiburg University, Germany and Arjumand Ghazi, University of Pittsburgh School of Medicine, USA

78- 9:00 Discovering Conserved Mechanisms of Protection against Ischemia-reperfusion Injury Using a Novel *C. elegans* Behavioral Model. **Dengke K. MA**, Bob Horvitz.

79- 9:12 Apoptotic hyperfunction causes gonadal atrophy in aging *C. elegans*. **Yila de la Guardia**, Ann Gilliat, Josephine Hellberg, David Gems.

80- 9:24 Molecular Outsourcing: Reproductive Signals Deploy NHR-49/PPARα to Reorganize Lipid Homeostasis and Alter Lifespan. **Ramesh Ratnappan**, Jordan Ward, Francis RG Amrit, Hasreet Gill, Kyle Holden, Keith Yamamoto, Arjumand Ghazi.

81- 9:36 Serotonergic signaling modulates the heat shock response in *C. elegans*. **Veena Prahlad**, Richard Morimoto.

82- 9:48 The conserved SKN-1/Nrf2 stress response pathway regulates synaptic function in *Caenorhabditis elegans*. **Trisha Staab**, Trevor Griffen, Connor Corcoran, Oleg Evgrafov, James Knowles, Derek Sieburth.

83- 10:00 Worms That Exercise Age Better. Daniel Burke, **Mary Anne Royal**, Leo Gefter, Christina Chang, Monica Driscoll.

10:12 - **Break**

84- 10:36 Perturbations of Glycolytic Flux Differentially Impact Healthspan via the Insulin Signaling and Dietary Restriction Pathways. **Brian Onken**, Monica Driscoll.

85- 10:48 Regulation of *C. elegans* Reproductive Aging by a Novel Gene-Environment Signaling Mechanism. **Jessica Sowa**, Meng Wang.

86- 11:00 Molecular determinants of longevity in *C. elegans* and the relationship between lifespan, “health span”, and the rate of aging. **Zachary Pincus**, Frank Slack.

87- 11:12 The conserved PBAF nucleosome remodeling complex mediates the response to stress in C. elegans. Aleksandra Kuzmanov, Evguenia Karina, Natalia Kirienko, **David Fay**.

88- 11:24 The tune of Insulin/IGF-1 signaling pathway set by lincRNAs. Pengpeng Liu, M. Liu, L. Zhang, Z. Yang, K. Xiong, W. Dong, W. Zhang, Z. Zhu, Q. Fan, **D. Liu**.

89- 11:36 Mitochondrial ROS promote longevity and innate immunity via a feedback loop involving HIF-1 and AMPK. **Ara B. Hwang**, Eun-A Ryu, Murat Artan, William Mair, Seung-Jae Lee.

90- 11:48 How to live without water: Molecular strategies of the dauer larva to survive extreme desiccation. **Cihan Erkut**, S. Penkov, S. Boland, A. Vasilj, H. Khesbak, Daniela Vorkel, B. Habermann, Jean-Marc Verbavatz, K. Fahmy, A. Shevchenko, T. V. Kurzchalia.

Friday, June 28 9:00 AM–12:00 NOON
Bradley International Ballroom

Gene Expression I: RNA Interference and Small RNAs

Chairs: John Murray, University of Pennsylvania, USA and Julie Claycomb, University of Toronto, Canada

91- 9:00 Heterochromatin organization through development: regulated anchorage by H3K9 methylation and a novel chromodomain protein. **Susan M. Gasser**, B. Towbin, A. Gonzalez, P. Zeller, V. Kalck.

92- 9:12 Identification of small RNA pathway genes using patterns of phylogenetic conservation and divergence. **Yuval Tabach**, A. Billi, G. Hayes, M. Newman, O. Zuk, H. Gabel, R. Kamath, B. Chapman, S. Garcia, M. Borowsky, J. Kim, G. Ruvkun.

93- 9:24 The CSR-1 22G-RNA pathway modulates histone H3 modifications associated with euchromatin. **Christopher J. Wedeles**, Julie M. Claycomb.

94- 9:36 Positive regulation of Pol II transcription by CSR-1 RNAi pathway in *C. elegans*. **Germano Cecere**, R. Sachidanandam, S. Hoersh, A. Grishok.

95- 9:48 Characterization of the AGO protein VSR-1 in small RNA-mediated gene silencing pathways in the worm. **Monica Z. Wu**, Julie M. Claycomb.

96- 10:00 Involvement of *C. elegans let-7-Family* developmental timing microRNAs in bacterial pathogen response. **Zhiji Ren**, Victor Ambros.

10:12 - **Break**

97- 10:36 *In vivo* quantitative analysis of the heterochronic pathway reveals extensive target specificity of individual *let-7* miRNA family members. **Matyas Ecsedi**, Helge Grosshans.

98- 10:48 MUT-14 and SMUT-1 are redundantly required for germline RNAi and endogenous siRNA production. **Carolyn M. Phillips**, Taiowa A. Montgomery, Peter C. Breen, Gary Ruvkun.

99- 11:00 The essential CHORD protein CHP-1 functions in small RNA pathways in *C. elegans*. **Wendy X. Cao**, Julie M. Claycomb.

100- 11:12 Periodic A/T rich DNA structures promote germline expression. **C. Frokjaer-Jensen**, M. W. Davis, E. M. Jorgensen.

101- 11:24 The period protein homolog LIN-42 negatively regulates microRNA biogenesis in *C. elegans*. **Priscilla M. Van Wynsberghe**, E. F. Finnegan, T. J. Stark, Evan P. Angelus, K. Homan, G. W. Yeo, Amy E. Pasquinelli.

102- 11:36 Arginine methylation is required for piRNA mediated gene silencing in *Caenorhabditis elegans*. **Alexandra Sapetschnig**, Peter Sarkies, Eric Miska.

103- 11:48 ALG-3/4 acts through the CSR-1 pathway to promote spermiogenic gene expression and to provide a paternally inherited memory of past gene expression. **Colin Conine**, James Moresco, John Yates, Craig Mello.

Friday, June 28 9:00 AM–12:00 NOON
Grand Horizon Ballroom

Development and Evolution II: Cell Death, Development and Evolution

Chairs: Sophie Jarriault, Strasbourg University, France and Andre Pires da Silva, University of Warwick, United Kingdom

104- 9:00 *let-70*, an E2 ubiquitin-conjugating enzyme, promotes linker cell death in *C. elegans*. **Jennifer A. Zuckerman**, Yun Lu, Shai Shaham.

105- 9:12 Translational Regulators GCN-1 and ABCF-3 Maternally Contribute to General Programmed Cell Death. **Takashi Hirose**, Bob Horvitz.

106- 9:24 The importance of multiple caspase downstream pathways to execution of cell death in *C. elegans*. **Akihisa Nakagawa**, Yu-Zen Chen, Ding Xue.

107- 9:36 A Redox Signaling Globin Regulates Germ Cell Apoptosis in *Caenorhabditis elegans*. **S. De Henau**, L. Tilleman, M. Pauwels, A. Pesce, M. Nardini, M. Bolognesi, K. De Wael, L. Moens, S. Dewilde, B. P. Braeckman.

108- 9:48 A non-canonical role for the Caenorhabditis elegans dosage compensation complex in growth and metabolic regulation downstream of TOR complex 2. **Christopher M. Webster**, Denzil Douglas, Alexander A. Soukas.

109- 10:00 WormGUIDES: an overview. Zhirong Bao, William Mohler, Javier Marquina, Hari Shroff, **Daniel A. Colon-Ramos**.

10:12 - **Break**

110- 10:36 Using natural variation to decipher the complex genetic causes of *C. elegans* drug sensitivities. **Erik C. Andersen**, Tyler Shimko.

111- 10:48 Evolution of sperm activation in *Caenorhaditis* hermaphrodites. **Qing Wei**, Ronald E. Ellis.

112- 11:00 Specificity of interaction between *Caenorhabditis* and their natural viruses. **Gautier Bresard**, Marie-Anne Felix.

113- 11:12 Influence of the Microbiome on *C. elegans* Growth in the Wild. **Buck S. Samuel**, Holli Rowedder, Christian Braendle, Marie-Anne Félix, Gary Ruvkun.

114- 11:24 Evolution of *Caenorhabditis* Dosage Compensation. **Te-Wen Lo**, Caitlin Schartner, Barbara J. Meyer.

115- 11:36 Sumoylated NHR-25/NR5A regulates cell fate during *C. elegans* vulval development. **Jordan D. Ward**, Nagagireesh Bojanala, Teresita Bernal, Kaveh Ashrafi, Masako Asahina, Keith Yamamoto.

116- 11:48 Vulva Precursor Cells dynamically regulate their sensitivity to the LIN-3/EGF morphogen gradient to control Notch ligand expression during vulva induction. **Jeroen S. van Zon**, Alexander van Oudenaarden.

Friday, June 28 9:00 AM–12:00 NOON
Northwest Auditorium

Neurobiology II: Neuronal Development

Chairs: Xiao Liu, Tsinghua University, China and Dan Chase, University of Massachusetts, Amherst, USA

117- 9:00 Synapse location during growth depends on glia location. **Z. Shao**, S. Watanabe, R. Christensen, E. Jorgensen, D. Colón-Ramos.

118- 9:12 Attenuation of insulin signaling contributes to FSN-1-mediated regulation of synapse development. **Wesley L. Hung**, Christine Hwang, ShangBang Gao, Edward H. Liao, Jyothsna Chitturi, Ying Wang, Hang Li, Christian Stigloher, Jean-Louis Bessereau, Mei Zhen.

119- 9:24 MicroRNA regulation of proteoglycan biosynthesis controls cell migration in *C. elegans*. Mikael Pedersen, Goda Snieckute, Konstantinos Kagias, Camilla Nehammer, Hinke Mulhaupt, John Couchman, **Roger Pocock**.

120- 9:36 Identification of a novel axon guidance regulator. **Nanna Torpe**, Roger Pocock.

121- 9:48 Reversible dendrite arborization in dauers is regulated by KPC-1/furin. **Nathan Schroeder**, Rebecca Androwski, Alina Rashid, Harksun Lee, Junho Lee, Maureen Barr.

122- 10:00 Axonal fusion in regenerating axons shares molecular components with the apoptotic cell recognition pathway. **Brent Neumann**, Sean Coakley, Hengwen Yang, Ding Xue, Massimo Hilliard.

10:12 - **Break**

123- 10:36 Inhibition of precocious DD motor neuron synapse formation by the single Ig domain protein, OIG-1. **Kelly L. Howell**, Oliver Hobert.

124- 10:48 GRDN-1/Girdin and SAX-7/L1CAM establish a glial guide for sensory dendrite extension. **Ian G. McLachlan**, Maxwell G. Heiman.

125- 11:00 Genes that function downstream of Notch define a novel mechanism for inhibiting axon regeneration. **Rachid El Bejjani**, Marc Hammarlund.

126- 11:12 The LAD-2/L1CAM functions in EFN-4/ephrin-mediated axon guidance. Binyun Dong, Melinda Moseley-Alldredge, **Lihsia Chen**.

127- 11:24
Brain-wide Ca^{2+}-imaging of neural activity in *Caenorhabditis elegans*. **T. Schroedel**, R. Prevedel, K. Aumayr, A. Vaziri, M. Zimmer.

128- 11:36
A database of C. elegans behavioral phenotypes. **Eviatar I. Yemini**, Laura J. Grundy, Tadas Jucikas, Andre E. X. Brown, William R. Schafer.

Friday, June 28 1:30 PM–4:30 PM
Royce Hall

Plenary Session 3

Chair: Michael Hengartner, University of Zurich, Switzerland

129- 1:30 - Invited Speaker
Cell-cell fusion, sculpting and mechanisms. **Benjamin Podbilewicz**, Technion - Israel Institute of Technology.

130- 2:00
The midbody ring, not the midbody microtubules, dictates abscission patterning *in vivo*. **Rebecca A. Green**, Jonathan Mayers, Lindsay Lewellyn, Arshad Desai, Anjon Audhya, Karen Oegema.

131- 2:12
In vivo visualization of chromosome synapsis in *C. elegans*. **Ofer Rog**, Abby F. Dernburg.

132- 2:24
The development of non-centrosomal MTOCs during epithelial polarization. **Jessica L. Feldman**, James R. Priess.

133- 2:36
C. elegans meets single-molecule detection technologies; The embryonic cell polarity system is driven by state transition of PAR-2 protein molecules. **Yukinobu Arata**, Tetsuya Kobayashi, Michio Hiroshima, Chan-gi Pack, Tatsuo Shibata, Yasushi Sako.

134- 2:48
Analyses of C. elegans enhancer and promoter architectures reveals CpG island-like sequences and promoter activity of HOT regions. **R. Chen**, T. Down, E. Zeiser, P. Stempor, Q. Chen, T. Egelhofer, L. Hillier, T. Jeffers, J. Ahringer.

3:00 - **Break**

135- 3:30
Neuropeptide Secreted from a Pacemaker Activates Neurons to Control a Rhythmic Behavior. **Han Wang**, Kelly Girskis, Tom Janssen, Jason P. Chan, Krishnakali Dasgupta, James A. Knowles, Liliane Schoofs, Derek Sieburth.

136- 3:42
Tasting Light: A *C. elegans* Pharyngeal Neuron Senses Hydrogen Peroxide Produced by Light. **Nikhil Bhatla**, Bob Horvitz.

137- 3:54
The connectome of the anterior nervous system of the *C. elegans* adult male. Travis A. Jarrell, Yi Wang, Adam E. Bloniarz, Steven J. Cook, Christopher A. Brittin, Kenneth Nguyen, Meng Xu, David H. Hall, **Scott W. Emmons**.

138- 4:06
The degenerin family ion channel UNC-8 promotes activity-dependent remodeling of GABAergic synapses in *C. elegans*. **Tyne W. Miller**, Sarah C. Petersen, Megan E. Gornet, Ying Wang, Han Lu, Cristina Matthewman, Shohei Mitani, Sayaka Hori, Laura Bianchi, Janet Richmond, David M. Miller.

139- 4:18
Epidermal Growth Factor signaling mediates heat-induced quiescence in *C. elegans*. **Andrew Hill**, Cheryl Van Buskirk.

Friday, June 28 5:00 PM–6:00 PM
Royce Hall

Plenary Session 4 - Keynote Address

Chair: Gary Ruvkun, Massachusetts General Hospital, USA

Victor Ambros, Univ. of Massachusetts Medical School. *Heterochronic Genes and Developmental Timing in C. elegans.*

WORKSHOP

Friday, June 28 8:00 PM–9:00 PM

Teaching Workshop II - Building a Successful and Sustainable Program of Scholarship at a PUI.

Room: Northwest Auditorium

Organizers: **Jennifer Miskowski**, Univ. of WI, La Crosse, and **Jonathan Karpel**, Southern Utah Univ.

This workshop will be of primary interest to those currently employed at predominantly undergraduate institutions (PUIs) at any stage in their career. A panel of PUI veterans will lead a discussion focused on the balance between teaching and research, best practices for research with undergraduate students, how to write successful grant proposals, and issues surrounding publications including the idea of the Least Publishable Unit (LPU), recommended journals to target, and personal experiences with various journals, their editorial boards, reviewers, etc.

Saturday, June 29 9:00 AM–12:00 NOON
Ackerman Grand Ballroom

Gene Expression II: Gene Expression, Genomics and Epigenetics

Chairs: Amy Walker, University of Massachusetts, USA and Sam Gu, Rutgers University, USA

141- 9:00 Feedback Control of Gene Expression Variability in the *Caenorhabditis elegans* Wnt pathway. **Ni Ji**, Teije Middelkoop, Remco Mentink, Hendrik Korswagen, Alexander van Oudenaarden.

142- 9:12 Integral nuclear pore components associate with Pol III-transcribed genes and are required for Pol III transcript processing in *C. elegans*. **Kohta Ikegami**, Jason Lieb.

143- 9:24 The regulation of global histone acetylation during meiotic prophase in C.elegans. **Jinmin Gao**, Hyun-Min Kim, Andrew E. Elia, Stephen J. Elledge, Monica P. Colaiácovo.

144- 9:36 Tissue integrity and laminopathic phenotypes correlate with subnuclear heterochromatin positioning. **A. Mattout**, SM. Gasser.

145- 9:48 A Comprehensive Expression Map of Lysine Methyltransferases Reveals Germline-specific Function of *set-17*. **Christoph G. Engert**, Alexander van Oudenaarden, Bob Horvitz.

146- 10:00 MRG-1 acts as an epigenome interpreter of Lys36 methylation on histone H3. **Teruaki Takasaki**, Thea Egelhofer, Andreas Rechtsteiner, Hiroshi Sakamoto, Susan Strome.

10:12 - **Break**

147- 10:36 Control of DNA accessibility by histone H2A variants revealed using *in vitro* analysis of *C. elegans* nucleosomes. **Ahmad N. Nabhan**, Francisco Guerrero, Geeta Narlikar, Diana Chu.

148- 10:48 Epigenetic reprograming during germ line development. B. Hargitai, I. Kalchhauser, **S. Gutnik**, R. Ciosk.

149- 11:00 Spatial Control of Gene Expression in the *C. elegans* Intestine. **Aidan Dineen**, Jim McGhee.

150- 11:12 Defining regulatory pathway coupling cell division timing and cell fate differentiation in *C. elegans* by automated lineaging. **Vincy Wing Sze Ho**, M.-K. Wong, X. An, J. Shao, K. He, D. Xie, J. Liao, L. Chen, X. Huang, L. Chan, K. Chow, H. Yan, Z. Zhao.

151- 11:24 Linking dosage compensation complex assembly to X chromosome gene regulation. **Bayly Wheeler**, C. Frøkjær-Jensen, E. Jorgensen, B. J. Meyer.

152- 11:36 Silencing of Germline-Expressed Genes by DNA Elimination in Somatic Cells and a Mechanism for Selective DNA Segregation. **Richard E. Davis**, J. Wang, M. Mitreva, M. Beriman, A. Thorne, V. Magrini, S. Kratzer, M. Balas, G. Koutsovoulos, S. Kumar, M. Blaxter.

153- 11:48 Exploring *C. elegans* heterochromatin through the HP1 homolog HPL-2. **Jacob M. Garrigues**, Susan Strome.

Saturday, June 29 9:00 AM–12:00 NOON
Northwest Auditorium

Cell Biology II: Cell Division, Cell Polarity and Fate

Chairs: Mike Boxem, Utrecht University, The Netherlands and Huimin Zhang, Suzhou University, China

154- 9:00 Aurora A is essential for the organization of the female meiotic spindle in late anaphase. **Eisuke Sumiyoshi**, Yuma Fukata, Asako Sugimoto.

155- 9:12 Formation of the nuclear envelope as a distinct subdomain of the endoplasmic reticulum requires spatial regulation of Lipin activation. **Shirin Bahmanyar**, Ronald Biggs, Jon Audhya, Arshad Desai, Jack Dixon, Thomas Mullert-Reichert, Karen Oegema.

156- 9:24 Homolog pairing and feedback control during meiosis are mediated through CHK-2 phosphorylation of pairing center proteins. **Y. Kim**, A. F. Dernburg.

157- 9:36 Intestinal pathogens hijack the host apical recycling pathway for fecal-oral transmission. **Suzy Szumowski**, Emily Troemel.

158- 9:48 Essential function for the exocyst complex in seamless tube formation. **Stephen Armenti**, Emily Chan, Jeremy Nance.

159- 10:00 A context-specific role for Syndecan/SDN-1 in Wnt-dependent spindle orientation. **Katsufumi Dejima**, Sukryool Kang, Andrew Chisholm.

10:12 - **Break**

160- 10:36 Cell signalling and membrane trafficking - an unbreakable relationship. **Zita Balklava**, Navin Rathnakumar.

161- 10:48 *ttm-1* encodes CDF transporters that excrete zinc from intestinal cells of *C. elegans* and act in a parallel negative feedback circuit that promotes homeostasis. Hyun Cheol Roh, Sara Collier, Krupa Deshmukh, James Guthrie, J. David Robertson, **Kerry Kornfeld**.

162- 11:00 The SYS-1/β-catenin regulatory machinery controls multiple functions of APR-1/APC during seam cell division. **Austin T. Baldwin**, Bryan T. Phillips.

163- 11:12 CEH-20/Pbx and UNC-62/Meis function upstream of *rnt-1*/Runx to regulate asymmetric divisions of the *C. elegans* stem-like seam cells. **Samantha L. Hughes**, Charles Brabin, Alison Woollard.

164- 11:24 Regulation of maternal Wnt mRNA translation in *C. elegans* embryos reveals mechanistic parallels between 3' UTRs and transcription enhancers. **Marieke Oldenbroek**, Scott Robertson, Tugba Guven-Ozkan, Caroline Spike, David Greenstein, Rueyling Lin.

165- 11:36 Life at the edge of robustness: Partial guts suggest that endoderm specification is not all-or-none. **Morris F. Maduro**, Francisco Carranza, Farhad Ghamsari, Gurjot Walia, Gina Broitman-Maduro.

166- 11:48 Identification of SEL-10/Fbw7 substrates regulated in cell fate patterning events via a conserved phosphodegron motif. **Claire de la Cova**, Iva Greenwald.

Saturday, June 29 9:00 AM–12:00 NOON
Grand Horizon Ballroom

Physiology III: Pathogenesis, Dauer Larvae and Metabolism

Chairs: William Mair, Harvard School of Public Health, USA and Sean Curran, University of Southern California, USA

167- 9:00 A Genome-Wide RNAi Screen of Caenorhabditis elegans Identifies Translational Machinery Genes Involved in Fat Regulation. **Elizabeth Pino**, Christopher Carr, Alexander Soukas.

168- 9:12 Identification of a novel *C. elegans* protein that detects bacteria. **Darym Alden**, Jonathan Dworkin.

169- 9:24 Nuclear Receptor NHR-8 Regulates Cholesterol, Bile Acid and Fat Metabolism, and Modulates Reproduction and Lifespan. **Daniel Magner**, Joshua Wollam, Yidong Shen, Caroline Hoppe, Dongling Li, Christian Latza, Veerle Rottiers, Harald Hutter, Adam Antebi.

170- 9:36 The Tumor Suppressor Rb Critically Regulates Starvation-induced Stress Response in *C. elegans*. **Mingxue Cui**, Huanhu Zhu, Max Cohen, Cindy Teng, Min Han.

171- 9:48 A novel ascaroside controls the parasitic life cycle of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Jaime H. Noguez, Joshawna K. Nunnery, Elizabeth S. Connor, Yue Zhou, Todd A. Ciche, Justin R. Ragains, **Rebecca A. Butcher**.

172- 10:00 A Conserved SREBP/Transketolase Regulatory Circuit Governing Lipid Homeostasis in Metazoans. **V. Rottiers**, P. Mulligan, A. K. Walker, J. L. Watts, A. C. Hart, A. M. Näär.

10:12 - **Break**

173- 10:36 C.elegans community behavior affects the dynamics of pathogen avoidance. **Andrzej Nowojewski**, Erel Levine.

174- 10:48 Triggering antifungal innate immunity. **Olivier Zugasti**, B. Squiban, J. Belougne, L. Kurz, N. Bose, F. Schroeder, N. Pujol, J. Ewbank.

175- 11:00 The *mir-58* family of microRNAs regulates the tissue-specific expression of PMK-2 p38 MAPK that functions in host defense. **Daniel J. Pagano**, Elena R. Kingston, Dennis H. Kim.

176- 11:12 Role of Manganese Homeostasis in Aging and Disease: Implications for Parkinson's Disease. **Suzanne Angeli**, Kathryn Page, David Killilea, Gordon Lithgow, Julie Andersen.

177- 11:24 HIF-1, DAF-16, and ZIP-2 coordinate the *C. elegans* defense against *P. aeruginosa* pathogenesis. **Natalia Kirienko**, Daniel Kirienko, Jonah Larkins-Ford, Gary Ruvkun, Fred Ausubel.

178- 11:36 A new role of DCR-1/DICER in C. elegans innate immunity against the highly virulent bacterium Bacillus thuringiensis DB27. **Igor Iatsenko**, Amit Sinha, Christian Rödelsperger, Ralf J. Sommer.

179- 11:48 A candidate host receptor exploited by microsporidia for intestinal infection in *C. elegans*. **R. J. Luallen**, M. Bakowski, E. Troemel.

Saturday, June 29 9:00 AM–12:00 NOON
De Neve Plaza Room

Neurobiology III: Synaptic Function

Chairs: Manuel Zimmer, IMP-Research of Molecular Pathology, Austria and Elissa Hallem, Univ. of California, Los Angeles, USA

180- 9:00 The EBAX-type Cullin-RING E3 ligase and Hsp90 guard the protein quality of the SAX-3/Robo receptor in developing neurons. **Zhiping Wang**, Yanli Hou, Xing Guo, Monique van der Voet, Jack Dixon, Mike Boxem, Yishi Jin.

181- 9:12 Feeding state, NPR-1 and circuit dynamics regulate chemoreceptor expression. **Matt Gruner**, Rebecca Hintz, Samuel Chung, Chris Gabel, Alexander van der Linden.

182- 9:24 Sensory responses to graded stimuli: a role in bidirectional chemotaxis? **Michael Hendricks**, Luo Linjiao, Aravi Samuel, Yun Zhang.

183- 9:36 Neuropeptide signaling remodels chemosensory circuit composition. **Sarah Leinwand**, Sreekanth Chalasani.

184- 9:48 Thermal memory and behavioral regulation revealed by calcium imaging of the cultured neurons and neural circuits. **Kyogo Kobayashi**, Ikue Mori.

185- 10:00 Functional memory loss: Msi-1 is an inhibitor of memory. Nils Hadziselimovic, Fabian Peter, Petra Hieber, Vanja Vukojevic, Philippe Demougin, Andreas Papassotiropoulos, **Attila Stetak**.

10:12 - **Break**

186- 10:36 Betaine acts on a ligand-gated ion channel in C. elegans. **Aude S. Peden**, Patrick Mac, You-Jun Fei, Cecilia Castro, Guoliang Jiang, Kenneth J. Murfitt, Eric A. Miska, Julian L. Griffin, Vadivel Ganapathy, Erik M. Jorgensen.

187- 10:48 Novel function of the polarity gene PAR-1 in control of activity at the NMJ. **Clara L. Essmann**, Emma Hiley, Zhitao Hu, Joshua Kaplan, Stephen Nurrish.

188- 11:00 Neuroligin organizes C. elegans GABAergic NMJs. **Géraldine Maro**, Shangbang Gao, Michael Liu, Mei Zhen, Kang Shen.

189- 11:12 Synaptic engineering: an ionic switch of behavior. **Jennifer K. Pirri**, Diego Rayes, Mark J. Alkema.

190- 11:24 Multiple independent calcium pools in a nociceptive neuron in *C. elegans*. **Jeffrey Zahratka**, Richard Komuniecki, Paul Williams, Bruce Bamber.

191- 11:36 Two Minds of a Worm: Comparison of the L4 and Adult Hermaphrodite Connectomes. **S. Cook**, C. Brittin, T. Jarrell, D. Hall, S. Emmons.

WORKSHOP

Saturday, June 29 1:30 PM–3:00 PM

Engineered nucleases for genome editing in nematodes

Room: Grand Horizon Ballroom

Organizer: **Hillel Schwartz**, , HHMI and Division of Biology, CalTech

Co-chairs:
John Calarco, FAS Center for Systems Biology, Harvard University

Ari Friedland, Dept. Genetics, Harvard Medical School

Te-Wen Lo, HHMI and Dept. Molecular and Cell Biology, UC Berkeley

Yonatan Tzur, Dept. Genetics, Harvard Medical School

Jordan Ward, Dept. Cellular and Molecular Pharmacology, UCSF

Speakers:
Te-Wen Lo, HHMI and Dept. Molecular and Cell Biology, UC Berkeley, Advances in Targeted Genome Editing Across Species: Heritable Designer "Knock-In" and "Knock-Out" Modifications

Jordan Ward, Dept. Cellular and Molecular Pharmacology, UCSF, Using CRISPRs to engineer the C. elegans genome

Vivian Chiu, HHMI and Division of Biology, CalTech, Transgene-free genome editing in nematodes using CRISPR-Cas
Ari Friedland, Dept. Genetics, Harvard Medical School, Heritable Genome Editing in C. elegans via a CRISPR-Cas System

Recent technological developments have made it possible to construct nucleases with targeted specificity: ZFNs (Zinc Finger Nucleases), TALENs (Transcription Activator-Like Effector Nucleases), and CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats). Using any of these methods, a researcher can induce double-strand breaks at the sequence of their choice. These breaks are repaired imperfectly by non-homologous end-joining (NHEJ) to leave small deletions or are repaired by homologous combination with an intact copy of the locus, which should permit the user to knock in precise changes at any site in the genome. Researchers will describe their experiences using these techniques in *C.elegans* and other nematodes and will discuss the potential for further developments.

WORKSHOP

Saturday, June 29 1:30 PM–3:00 PM

WormBase tutorial: Phenotypes, Interactions, Pathways, and Human Disease

Room: Bradley International Ballroom

Organizer: **Chris Grove**, California Institute of Technology

One core mission of WormBase is to capture information from the research literature about how all of the various biological entities of the nematode function together in the context of the organism. These entities include anatomy objects such as cells and tissues, sequence-based objects such as genes and proteins, as well as small molecules of endogenous or exogenous origin. This workshop will focus on explaining how users of WormBase can best access information about interactions between these various entities, as well as what phenotypes result from perturbation of their function. We will showcase some of our new visualization tools including our embedded Cytoscape interaction network viewer. We will also discuss how users may determine the relevance of various genes to human diseases and how C. elegans can be used as a model system for studying these pathologies. In addition we will introduce WormBase "Process Pages" and WikiPathways. Process Pages integrate genetic data with anatomical, developmental, and temporal information to focus on the larger biological picture of the nematode rather than on the discrete biological entity, such as the gene. To better visualize these intersecting details, we are using WikiPathways to diagram genetic and physical relationships. WikiPathways is a powerful community-driven pathways database with online and desktop editing tools. In this workshop we will introduce WikiPathways and demonstrate how to use the tools to build your own pathways and how to submit them to WormBase.

Speakers:
Abigail Cabunoc, WormBase, Ontario Institute for Cancer Research (OICR), An introduction to WormBase 2.0
Chris Grove, WormBase, California Institute of Technology, Accessing phenotypes, interactions, anatomy, and human disease data
Karen Yook, WormBase, California Institute of Technology, Introduction to WormBase process pages, WikiPathways
Alexander Pico, WikiPathways, The Gladstone Institutes, University of California San Francisco (UCSF), WikiPathways

WORKSHOP

Saturday, June 29 1:30 PM–3:00 PM

Novel Microscopy Techniques

Room: De Neve Plaza Room

Organizer: **Javier Apfeld**, Harvard Medical School

New advances in microscopy are opening new avenues for scientific inquiry. These innovations include new imaging devises (e.g. scanners, lensless optofluidic microscopes) and up and coming imaging techniques (e.g. optogenetics, novel genetically encoded sensors). The aim of this workshop is to highlight how these advances are being deployed in C. elegans, paying particular attention to the opportunities and challenges presented by these new methods.

Speakers:
Electron microscopy: Erik Jorgensen, University of Utah

Optogenetic tools: Alexander Gottschalk, Goethe University, Frankfurt, Germany

Microfluidic devices: Hang Lu, Georgia Institute of Technology

Non-linear microscopy and optical projection tomography: Nektarios Tavernarakis, University of Crete, Greece

Automated lifespan acquisition: Walter Fontana, Harvard Medical School

Multineuronal calcium imaging: Rex Kerr, Janelia Farm

WORKSHOP

Saturday, June 29 1:30 PM–3:00 PM

Dynamical Modeling From Data, aka "Worm Theory"

Room: Northridge

Organizers: **Saul Kato**, Center for Theoretical Neuroscience, Columbia University, and
Michael Hendricks, Center for Brain Science, Harvard University

To understand how real-time behavior emerges from a nervous system, we must quantitatively characterize the dynamics of the nervous system as well as of the behavior itself. More work has been done in the area of quantifying worm behavior, but as methods of imaging the activity of neurons and muscles are becoming more precise and comprehensive, a quantitative modeling approach of neural activity is needed and should ultimately dovetail with quantitative models of behavior. What kinds of models are right for *C elegans*? For inspiration, we can look to approaches that have worked well in other settings and organisms -- e.g. differential equations, integral/cascade systems models, deterministic, and/or stochastic -- but in the end, model selection will be driven by what the data tells us and what questions we are asking. The speakers in this workshop will each present a project involving dynamical modeling, and in particular will discuss the decisions they made in selecting their model framework. Time will be allocated for questions and interruptions are encouraged!

Speakers:
Shawn Lockery, Institute of Neuroscience, University of Oregon, Stochastic models of *C. elegans* neurons.
Greg Stephens, Vrije Universiteit Amsterdam and Okinawa Institute of Science and Technology, Dynamics of control in the behavior of *C. elegans.*
Quan Wen, Harvard University, How motor circuit and sensory feedback drive *C. elegans* locomotion.

WORKSHOP

Saturday, June 29 1:30 PM–3:00 PM

WormBase tutorial: Tools and Techniques for Data Mining

Room: West Coast

Organizer: **Todd Harris**, WormBase, Ontario Institute for Cancer Research (OICR)

During this workshop, WormBase developers will present tools available for querying WormBase and fetching data en masse. This will include topics ranging from the availability of precomputed analyses and where to find them; query languages; programmatic APIs; and cloud resources available for data analysis. We will introduce users to WormMine, our new data mining platform and the successor to WormMart. WormMine is WormBase's implementation of the InterMine data warehouse platform and will serve as an interface for complex data querying and the integration of new features to WormBase. An introduction to the software, querying for complex data, account management, template queries, extracting data, and current datasets available will be covered.The Saturday workshop will include InterMine developer Sergio Contrino on mining ModEncode data with InterMine.

Speakers:
Todd Harris, WormBase, Ontario Institute for Cancer Research (OICR), An overview of data mining options at WormBase
JD Wong, WormBase, Ontario Institute for Cancer Research (OICR), Introducing WormMine, a powerful new data mining platform for WormBase
Sergio Contrino, modENCODE, University of Cambridge, ModMine, a data mining platform of modENCODE data

WORKSHOP

Saturday, June 29 1:30 PM–3:00 PM

GSA Plenary Session and Workshop for Undergraduate Researchers

Room: Southbay

Organizer: **Elizabeth Ruedi**, Genetics Society of America

Undergraduate conference attendees are invited to an "Undergraduate Plenary Session", with talks presented at a level appropriate for an undergraduate audience. Undergraduates will have a chance to talk to a panel of graduate students about graduate school, after which students will have a chance to break into small groups and participate in a discussion about a specific topic relevant to graduate school applications, admission, CV preparation and funding.

Speakers:
Morris Maduro, Probing Gene Regulatory Networks in the Early *C. elegans* Embryo.
Diana Chu, Using Worm Sperm to Understand the Fundamentals of Fertility.

WORKSHOP

Saturday, June 29 1:30 PM–6:15 PM

Bridging the Divide between the C. elegans and Parasitic Nematode Communities

Room: Northwest Auditorium

Organizer: **Martin Chalfie**, Columbia University

Organizing Committee:
Barton Slatko, New England Biolabs
Susan Strome, University of California, Santa Cruz
William Sullivan, University of California, Santa Cruz

Each year infections of animals and plants by parasitic nematodes cause many billions of dollars of agricultural damage. Over a billion people worldwide, particularly in developing nations, are infected by nematodes and suffer from the resulting debilitating diseases. Currently, only a few investigators address problems of parasitic nematodes using *C. elegans*. We would like to encourage and facilitate more *C. elegans* researchers to do so. In this workshop, experts studying parasitic nematodes and their control will talk about the problems faced by their field and indicate areas where the *C. elegans* community may help. The workshop will provide information about parasitic nematodes and efforts to combat them as well asample opportunity for discussions.

Speakers:
Dr. Heidi Goodrich-Blair, Dept. of Bacteriology, Madison, WI, A tripartite association:Steinernema nematodes, Xenorhabdusbacteria, and insects

Dr. David Bird, Dept. of Plant Pathology, North Carolina State University, Omic-empowered genetics of the root-knot nematode, Meloidogyne hapla:Mendelian and QT loci defining parasitism traits
Dr. Peter Jay Hotez, National School of Tropical Medicine at Baylor College of Medicine, Urgent needs for R&D on human helminth infections

Dr. Frederic Landmann, Dept of MCD Biology, UC Santa Cruz, The cell biology of Wolbachia-Filarial nematode symbiosis

Dr. Conor Caffrey, Center for Discovery and Innovation in Parasitic Diseases, Dept. of Pathology University of California San Francisco, Schistosomics:developing a discovery tool kit for the global flatworm disease, schistosomiasis

Dr. Mark Blaxter, Institute of Evolutionary Biology, University of Edinburgh, More than just one "worm": nematode diversity revealed by the first fruits of the 959 nematode genomes initiative.

WORKSHOP

Saturday, June 29 2:30 PM–4:30 PM

Teaching Workshop III - Educating Scientists for the 21st Century: Focus on High-Impact Learning Strategies

Room: Sunset Village Study Lounge

Organizers: **Jennifer Miskowski**, Univ. of WI, La Crosse, and **Jonathan Karpel**, Southern Utah Univ.

This workshop should be of interest to educators at all levels and types of institutions who have a dedicated commitment to teaching. The presenters at this workshop will share innovative teaching materials and techniques, with an emphasis on those that are used in, or can be adapted to, smaller class sizes. The presentations will include, but are not limited to, *C. elegans*-based approaches, interdisciplinary courses/programs related to developmental biology, active learning exercises, and high-tech applications that have been used effectively in the classroom.

Sunday, June 30 9:00 AM–12:00 NOON
Royce Hall

Plenary Session 5

Chair: Ian Hope, University of Leeds, United Kingdom

192- 9:00 - Invited Speaker
Gene Regulatory Networks. **Marian Walhout**, Univ. of Massachusetts Medical School.

193- 9:30
A Transdifferentiating Cell Requires Dynamic Histone Modifying Activities. **Steven Zuryn**, Arnaud Ahier, Marie Charlotte Morin, Sophie Jarriault.

194- 9:42
Holocentromeres are dispersed point centromeres localized at transcription factor hotspots. **Florian A. Steiner**, Steven Henikoff.

195- 9:54
A gene-activating pathway mediated by small RNAs (RNAa) protects self-transcripts from epigenetic silencing in C. elegans. **Meetu Seth**, Masaki Shirayama, Weifeng Gu, Takao Ishidate, Darryl Conte Jr., Craig C. Mello.

196- 10:06
X-Chromosome Restructuring Imposed by the Dosage Compensation Complex and Its Relationship to Nuclear Pores. **Qian Bian**, Emily Crane, Satoru Uzawa, Barbara J. Meyer.

197- 10:18
A Developmental Time Course of Transcription and Subsequent Computational Bayesian Unification Reveals Multiple Global Waves of Gene Regulation. **Max E. Boeck**, Chau Huynh, Lou Gevirtzman, Daniel Mace, LaDeana Hillier, Owen Thompson, Pnina Strasbourger, Guilin Wang, Valerie Reinke, Robert Waterston.

10:30 - **Break**

198- 11:00 Epigenetic control of terminal neuronal differentiation in *Caenorhabditis elegans*. **Chaogu Zheng**, Siavash Karimzadegan, Martin Chalfie.

199- 11:12
MNR-1/menorin, a novel skin-derived cue, controls arborization of sensory dendrites in *C. elegans*. **Y. Salzberg**, C. A. Diaz-Balzac, N. Ramirez, M. Attreed, E. Tecle, Z. Kaprielian, H. Buelow.

200- 11:24
Reduced Insulin/IGF1 signaling restores germ cell immortality to *Caenorhabditis elegans prg-1* Piwi mutants. **Matt A. Simon**, Peter Sarkies, Kohta Ikegami, Leonard Goldstein, Aisa Sakaguchi, Eric Miska, Shawn Ahmed.

201- 11:36
A deletion polymorphism in the *C. elegans* RIG-I homolog disables antiviral siRNA formation and immunity. **Jeremie Le Pen**, Alyson Ashe, Peter Sarkies, Tony Bélicard, Amy Cording, Nicolas J. Lehrbach, Marie-Anne Félix, Eric A. Miska.

202- 11:48
Death by worm-star: a new way of killing *C. elegans*, with implications for ether lipid metabolism. **Jonathan Hodgkin**, Marie-Anne Félix, Laura C. Clark, Delia M. O'Rourke, Dave Stroud, Maria J. Gravato-Nobre.

Physiology: Aging and Stress

203A. The novel regulators of RNT-1 stabilization in stress response. **Soungyub Ahn**, Kiho Lee, Peter Swoboda, Junho Lee.

204B. Trade-offs and bet-hedging in reproductive performance during heat stress. **Erin Zucker Aprison**, Ilya Ruvinsky.

205C. Protective effect of *Paullinia cupana*, the Guarana, on *Caenorhabditis elegans* under oxidative stress. **Leticia P. Arantes**, Marina Machado, Daniele Zamberlan, Cintia Tassi, João da Rocha, Felix Soares.

206A. Evaluation of the activity of *Luehea divaricata* Mart. leaf extract against different pro-oxidants in *Caenorhabditis elegans* . **Leticia P. Arantes**, Dirleise Colle, Marina Machado, Cintia Tassi, Daniele Zamberlan, Ritiel da Cruz, João da Rocha, Melânia Manfron, Margareth Athayde, Felix Soares.

207B. Screening of antioxidant organochalcogen compounds in Caenorhabditis elegans: focus on modulation of DAF-16/FOXO pathway. **Daiana S. Avila**, Suzi Wollenhaupt, Ana Thalita Soares, Willian Salgueiro, Diego Avila.

208C. Role of amino-acid pool size in aging-related muscle atrophy. **Meenakshisundaram Balasubramaniam**, Ramani Alla, Robert J. Shmookler Reis, Srinivas Ayyadevara.

209A. Microfluidic devices for electrical measures of pharyngeal health. **Stephen A. Banse**, John. H. Willis, Kristin J. Robinson, Janis C. Weeks, Patrick C. Phillips, Shawn R. Lockery.

210B. Persistent effects of starvation on multiple life history traits. Moses Sandrof, Meghan Jobson, James Jordan, **Ryan Baugh**.

211C. RER-1 - Finding New Roles For An Old Protein. **Kunal Baxi**, Ata Ghavidel, Troy Harkness, Carlos Carvalho.

212A. 7-ketocholesterol acts through steroid receptor DAF-12 to regulate dauer formation and longevity. **Ben Becker**, Adam Antebi.

213B. Identifying the role of *apl-1* through an RNAi Screen and Mosaic Analysis. **Maisam T. Begum**, Pei Zhao, Chris Li.

214C. Modulation of HIF-1 activity and its effect on stress tolerance and longevity in C. elegans. **P. Bharill**, F. Fabretti, H. Gharbi, B. Schermer, T. Benzing, R. Mueller.

215A. The Mechanistic Basis of Neuroendocrine Control of Longevity. **Konstantinos Boulias**, Bob Horvitz.

216B. Proteomics approach to identify potential targets of tyrosol, an olive oil phenol that stimulates longevity and stress resistance in Caenorhabditis elegans. **Ana Cañuelo**, J. Peragón, P. Pacheco.

217C. Mutations in the Translation Initiation Factor Subunit *eIF-3.k* Suppress the Stress Sensitivity of *xbp-1* Mutants. **Douglas Cattie**, Kirthi Reddy, Claire Richardson, Dennis Kim.

218A. Biological behavior of carbon nanoparticles in *Caenorhabditis elegans*. **Yun Jeong Cha**, Shin Sik Choi.

219B. Novel Kinases that interact with the Insulin-IGF-1-like signaling pathway to control longevity. **Manish Chamoli**, Awadhesh Pandit, Mukund Sudharsan, Arnab Mukhopadhyay.

220C. Characterization of a gene whose expression correlates with the food type-dependent effects on lifespan. Wolfgang Maier, **Rashmi Chandra**, Joy Alcedo.

221A. *daf-16/FoxO* isoform-specific mutants reveal differential contributions to longevity in the contexts of reduced DAF-2 insulin-like signaling and germline ablation. **Albert Chen**, Chunfang Guo, Kathleen Dumas, Travis Williams, Sawako Yoshina, Shohei Mitani, Patrick Hu.

222B. Effects of *C. elegans sgk-1* mutations on life span, stress resistance, and DAF-16/FoxO regulation. **Albert Chen**, Chunfang Guo, Kathleen Dumas, Kaveh Ashrafi, Patrick Hu.

223C. Sestrin confers the regulations of muscle aging and lifespan in *Caenorhabditis elegans*. Ya-Luen Yang, Kah-Sin Loh, Bang-Yu Liou, I-Hua Chu, Cheng-Ju Kuo, Huan-Da Chen, **Chang-Shi Chen**.

224A. The Effect of Hydrolysable Tannins from Eucalyptus Leaves on C. elegans Lifespan. **Y. chen**, B. Onken, H. Chen, Q. Huang, S. Xiao, M. Driscoll, Y. Cao.

225B. Nutritional Control of Insulin-Like Peptide Expression during L1 Arrest and Recovery. **Yutao Chen**, Ryan Baugh.

226C. Transcriptional profiling reveals a principle role for *wdr-23* in regulating SKN-1 and potential interactions with molting and the cuticle. Lanlan Tang, Andrew Deonarine, Chi K. Leung, **Keith P. Choe**.

227A. Peroxide Stress Response and Ferritin Synthesis Regulation by the REF-1 Family Member HLH-29. **H. T. Chou**, T. K. Quach, C. M. Johnson.

228B. Down regulation of miR-124 in both Werner syndrome DNA helicase mutant mice and mutant *Caenorhabditis elegans wrn-1* reveals the importance of this microRNA in accelerated aging. **A. Dallaire**, C. Garand, E. R. Paquet, S. J. Mitchell, R. De Cabo, M. J. Simard, M. Lebel.

229C. Activation of the hexosamine pathway improves ER protein quality control and slows aging. **Martin S. Denzel**, Nadia J. Storm, Adam Antebi.

230A. Proteostasis in the aging model *Caenorhabditis elegans*. **I. Dhondt**, G. Depuydt, H. Cai, J. Staal, A. Borghi, L. Verstrepen, L. Baten, R. Beyaert, B. P. Braeckman.

231B. Pharmacological enhancers of physical activity and their impact on metabolic functions and longevity in C. elegans. **Sharon Epstein**, Matthew McGee, Pankaj Kapahi.

232C. An underlying dauer-independent DAF-2 longevity program implicates collagen homeostasis in longevity. **Collin Ewald**, Jess Landis, Jess Porter Abate, Coleen Murphy, T. Keith Blackwell.

POSTER SESSION LISTINGS

233A. Adaptation to hydrogen sulfide induces a reversible developmental plasticity in *C. elegans*. **Emily Fawcett**, Dana Miller.

234B. Examining neuromuscular deficits and oxidative damage after exposure to common anthropogenic chemicals in *Caenorhabditis elegans*. **Denise B. Flaherty**, Christopher W. Dukes, Seth V. Malhotra, Ashlin L. Niedzwiecki, Daniel C. Kovarik, Julia M. Billington.

235C. Lysosomal lipolysis promotes longevity through a lipid-responsive nuclear hormone receptor signaling pathway. **Andrew Folick**, Holly Doebbler, Yong Yu, Meng Wang.

236A. Genes that Affect Glucose-Fed *C. elegans* Exposed to Oxygen-Deprivation. **Anastacia Garcia**, Pamela Padilla.

237B. Role of Autophagy in Long-lived *C. elegans* Subjected to Dietary Restriction. **Sara Gelino**, Jessica Chang, Malene Hansen.

238C. Quasi-programmed yolk synthesis contributes to *C. elegans* aging. Yila de la Guardia, Eleanor Tyler, Alex Benedetto, **David Gems**.

239A. Characterizing Adult Reproductive Diapause Longevity. **B. Gerisch**, D. Magner, A. Antebi.

240B. The Dual Roles of TCER-1/TCERG1 in Balancing Reproductive Fitness and Longevity. Francis RG Amrit, Arshi Arora, Takis Benos, **Arjumand Ghazi**.

241C. Genetics of praziquantel resistance in *C. elegans*. **Rajarshi Ghosh**, Anya Levinson, Conrad Tenenbaum, Leonid Kruglyak.

242A. The Mediator subunit MDT-15 is required for the oxidative stress response. **Grace Y. S. Goh**, Kulveer S. Parhar, Ada W. L. Kwong, Marcus A. Wong, Stefan Taubert.

243B. Proteotoxicity Models of Transthyretin Amyloid Disease in *C. elegans*. **E. Greiner**, J. Paulsson, S. Choi, S. Wolff, D. Ong, A. Dillin, S. Encalada, J. Kelly.

244C. The neuroglobin GLB-5 regulates C. elegans responses to hypoxic exposure. **E. Gross**, Z. Soltesz, V. Zelmanovich, M. de-Bono.

245A. SIR-2.1, an HDAC, is required to maintain male mating ability during aging of C. elegans. **Xiaoyan Guo**, Luis Rene Garcia.

246B. Bacterial nitric oxide extends C. elegans lifespan. **Ivan Gusarov**, Laurent Gautier, Olga Smolentseva, Ilya Shamovsky, Svetlana Eremina, Alexander Mironov, Evgeny Nudler.

247C. Temperature-dependent effects of *C. elegans* N-acylethanolamine biosynthetic enzymes. **Neale Harrison**, Ifedayo Victor Ogungbe, Pedro Reis-Rodrigues, Thomas Gallagher, Young-Jai You, Matthew S. Gill.

248A. The E3 Ligase LIN-23/βTRCP Influences SKN-1/NRF2 Activity and Reduces Toxic Proteins in *daf-2* Mutants. **Kyle Holden**, Emmanuel Schrieber, Mani Balasubramani, Arjumand Ghazi.

249B. Profiling of genotoxic stress response of *C. elegans* and investigation of protective effects of selected natural compounds. **S. Honnen**, C. Henninger, C. Büchter, Y. Chovolou, P. Proksch, G. Fritz.

250C. A Novel Link between Ubiquitin-dependent Proteolysis and Mitochondrial Metabolism. **Thorsten Hoppe**, Alexandra Segref.

251A. The *Caenorhabditis elegans* homolog of co-chaperone p23/prostaglandin E synthase-3 regulates lifespan in response to temperature. **M. Horikawa**, A. Antebi.

252B. Translational Effect of Hydrogen Sulfide and a Novel Role for HIF-1. **Joe Horsman**, Dana Miller.

253C. SAMS-1: A protein that senses nutrient levels and mediates the longevity response to dietary restriction in the intestine. Tsui-Ting Ching, Alex Kramer, Alisha Paal, Linda Zhang, **Ao-Lin Hsu**.

254A. The role of *C. elegans* BRAP-2 in the SKN-1 mediated oxygen radical detoxification response. **Queenie Hu**, Lesley MacNeil, Marian Walhout, Terrance J. Kubiseski.

255B. Nanoparticle size, shape, coat, and charge alter the bioactivity of nanosilver in *C. elegans*. **Piper R. Hunt**, Steven J. Oldenburg, Nicholas Olejnik, Robert L. Sprando.

256C. Analysis of *miro-1*. **Takao Inoue**, Yanqing Shen, Natarie Pei Wen Low, Thilo Hagen, Li Fang Ng, Jan Gruber.

257A. AMPK regulates protein homeostasis in response to hypoxia and nutrient deprivation. **Nicole Iranon**, Dana Miller.

258B. Genes that influence longevity, dauer formation and pathogen responses downstream of sensory neurons in *C. elegans*. **Dae-Eun Jeong**, Marta M. Gaglia, Eun-A Ryu, Dongyeop Lee, Cynthia Kenyon, Seung-Jae Lee.

259C. *nhr-176* regulates *cyp-35d1* to control hydroxylation-dependent metabolism of thiabendazole in *C. elegans*. **Laura M. Jones**, Anthony Flemming, Peter E. Urwin.

260A. Modulation of the Ubiquitin-Proteasome System and C. elegans Longevity by Neuroendocrine and Growth Factor Signaling Pathways. **Kishore K. Joshi**, Tejash Shah, Christopher Rongo.

261B. Role of mir-35-41 family in embryonic development and hypoxia. **Konstantinos Kagias**, Roger Pocock.

262C. LEA genes in Antarctic nematode, *Panagrolaimus davidi*. **Hiroshi Kagoshima**, Yuji Kohara.

263A. 20-hydroxyecdysone prevents age-associated decline in *C. elegans* . **Shaunak Kamat**, Shrutika Yeola, Monica Driscoll.

264B. Quest for life-lengthening signals within gonadal longevity. **Oezlem Karalay**, Shuhei Nakamura, Adam Antebi.

265C. The HIF-1 Pathway and organismal senescence. **Jordan Kardos**, Sudhir Nayak.

266A. *C. elegans* EXO-3 contributes to progeny production by repairing AP site in gonad. **Yuichi Kato**, Takahito Moriwaki, Masafumi Funakoshi, Qiu-Mei Zhang-Akiyama.

267B. TAF-4 is Required for the Life Extension of *isp-1*, *clk-1* and *tpk-1* Mit Mutants. **Maruf H. Khan**, Lauren Temmer, Melissa Ligon, Bryce Hufnal, Robert Farber II, Andrew Dillow, Erynn Kahlig, Amanda Rodriguez, Shane L. Rea.

268C. Fat, reproduction and longevity phenotypes associated with altered protein synthesis capacity. **Akshat Khanna**, Sean P. Curran.

269A. Gene expression studies to evaluate the size related toxicity of ZnO nanoparticles on the soil nematode, *Caenorhabditis elegans*. **Priyanka Khare**, Madhavi Sonane, K. C. Gupta, Aruna Satish.

270B. Characterization of changes in metal composition in C. elegans with age. **Ida M. Klang**, David Killilea, Tracy Barhydt, Peter Swoboda, Daniel Edgar, Gordon Lithgow.

271C. Natural genetic polymorphisms in *set-15* and a casein kinase 1α homolog shape the mortality schedule of worms. **Gunnar Kleemann**, Hua-Jay Cherng, Alina Yang, Thomas Kuhlman, Joshua Bloom, Edward Cox, Leonid Kruglyak, Coleen Murphy.

272A. Molecular aging driven by Wnt signaling in *C. elegans*. **B. Koenders- van Sintanneland**, M. Lezzerini, Y. Budovskaya.

273B. Age-related degeneration of the egg-laying system promotes matricidal hatching in *Caenorhabditis elegans*. Christopher L. Pickett, **Kerry Kornfeld**.

274C. Regulation of the ER Stress Response by HPL-2 and other chromatin associated proteins in *Caenorhabditis elegans* . **Lucie Kozlowski**, Steve Garvis, Cécile Bedet, Francesca Palladino.

275A. Age-dependent neuronal changes are the result of multiple intrinsic and extrinsic factors. **Anagha Kulkarni**, Claire Bénard.

276B. An ACE inhibitor extends *Caenorhabditis elegans* life span. **Sandeep Kumar**, Nicholas Deitrich, Kerry Koenfeld.

277C. Integrin-linked kinase regulates longevity and thermo-tolerance via HSF-1 in *C. elegans*. **C. Kumsta**, A. Davis, T.-T. Ching, M. Nishimura, C.-C. Chu, S. Gelino, B. Ong, R. Bodmer, A.-L. Hsu, M. Hansen.

278A. The Impact of Age and Lipid Stores: An Analysis of Oxygen Deprivation Response in *C. elegans*. **M. L. Ladage**, J. M. Goy, V. Shulaev, P. A. Padilla.

279B. SIR-2.1 regulation by the NAD+ Salvage Pathway and Environmental Stress. **Stephanie E. Lange**, Wendy Hanna-Rose.

280C. DNA damage leads to replicative aging but extends lifespan of long lived mutant animals. Jessica M. Lindvall, Karen Thijssen, Andrea E. Karambelas, Daniel Cupac, Øyvind Fensgård, Gert Jansen, Jan H. J. Hoeijmakers, Hilde Nilsen, Wim Vermeulen, **Hannes Lans**.

281A. Dissecting Ageing-Related Pathways by Studying Protein Changes after Calorie Restriction. **Mark Larance**, Ehsan Pourkarimi, Anton Gartner, Angus Lamond.

282B. Mutations in ribosomal S6 kinase lengthen lifespan through increasing the activity of heat shock transcription factor 1. Keunhee Seo, Eunseok Choi, Dongyeop Lee, Dae-Eun Jeong, Sung Key Jang, **Seung-Jae Lee**.

283C. Investigating the role of the *wrn-1* helicase in the nematode worm, *C. elegans*. **Hayley Lees**, Alison Woollard, Lynne Cox.

284A. Development of small molecule inhibitors of SKN-1 dependent detoxification genes identified in a screen of ~364,000 compounds. **Chi K. Leung**, Satyamaheshwar Peddibhotlal, Patrick Maloney, Paul M. Hershberger, Michelle Bousquet, Hendrik Luesch, Siobhan Malany, Keith P. Choe.

285B. A dual role of the Wnt signaling pathway during aging in *Caenorhabditis elegans*. **M. Lezzerini**, Y. Budovskaya.

286C. Characterization of novel mutants with dysfunctional mitochondrial stress pathways. **Jacqueline Lo**, Sean P. Curran.

287A. New roles for *wdr-23* in organismal survival and stress adaptation. **Jacqueline Lo**, Akshat Khanna, Elaine Roh, Megan Bernstein, Sean P. Curran.

288B. Exploring the health benefits of phytoestrogens in C. elegans. Jessica M. Ochoa, Vanessa Parada, Gabriela Gutierrez, Breann De Santiago, Erika Perez, Peaches Ulrich, Emanuel Zamora, **Sylvia A. Lopez-Vetrone**.

289C. Investigating the Toxic Effect of Biosensor Nanoparticles Using the Caenorhabditis elegans Nematode. Michelle Callaway, Erika Perez, John D. Alocilja, Evangelyn Alocilja, **Sylvia A. Lopez-Vetrone**.

290A. Drift of ELT-2 as a cause of aging in C. elegans. **Frederick G. Mann**, Eric Van Nostrand, Ari Friedland, Stuart Kim.

291B. Modulation of Excitotoxic Neurodegeneration in *C. elegans* by the Cell Stress-Resistance Signaling Pathway. Nazila Tehrani, Moises Dominguez, **Itzhak Mano**.

292C. Vitamin D3 slows aging in *c. elegans*. **Karla Mark**, Dipa Bhaumik, Milena Price, Birgit Schilling, Bradford Gibson, Michael Holick, Gordon Lithgow.

293A. Insulin/IGF-1 Signaling Regulates Proteasome Activity through the Deubiquitinating Enzyme UBH-4. **Olli Matilainen**, Leena Arpalahti, Ville Rantanen, Sampsa Hautaniemi, Carina I. Holmberg.

294B. A redox sensor as a potential regulator of ROS signaling in C. elegans. **Katie McCallum**, Antonio Miranda-Vizuete, Danielle Garsin.

295C. Does iron dyshomeostasis drive ageing? **Gawain McColl**, B. R. Roberts, S. A. James, R. A. Cherny, A. I. Bush.

296A. Exploring the Flexibility of NAD+ Biosynthesis in *C. elegans*. **Melanie R. McReynolds**, Wendy Hanna-Rose.

297B. Sugar Stress Reduces Fertility in *C. elegans* via Multiple Mechanisms. Marjorie R. Liggett, Amanda K. Engstrom, Uyen Ho, Michael Mastroianni, **Michelle A. Mondoux**.

298C. A novel kinase regulates dietary restriction-mediated longevity in *C. elegans*. **Arnab Mukhopadhyay**, Manish Chamoli, Anupama Singh.

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299A. Regulation of self-renewal and differentiation capacities of germline stem cells during ageing. **Patrick Narbonne**, Jean-Claude Labbé, Paul S. Maddox.

300B. A role for the insulin signaling pathway in development of neuronal aging markers in a polyglutamine protein aggregation *C. elegans* model. **Courtney Rose Nichols**, Elena Vayndorf, J. Alex Parker, Christian Neri, Monica Driscoll, Barbara Taylor.

301C. Oligomeric proanthocyanidins extracts are putative anti-obesity targets in the nematode *Caenorhabditis elegans*. **Yu Nie**, Sukhi Bansal, Bob Hider, Peter Hylands, Stephen Stürzenbaum.

302A. HIF-independent processes in hypoxia in *C.elegans*. **Divya Padmanabha**, Young-Jai You, Keith Baker.

303B. The inner mitochondrial membrane translocase complex TIM23 modulates mitochondrial biogenesis and function during ageing in *C. elegans*. E. Lionaki, **K. Palikaras**, N. Tavernarakis.

304C. Coordination of mitophagy and the mitochondrial retrograde response during ageing in *C. elegans*. **Konstantinos Palikaras**, Nektarios Tavernarakis.

305A. Mitochondrial Dynamics And Behavioral Plasticity In Response To Oxygen Deprivation Are Linked Through HIF-1. **Eun Chan Park**, Piya Ghose, Alexandra Tabakin, Nathaly Salazar-Vasquez, Christopher Rongo.

306B. Independent genetic pathways for stress response and longevity revealed by experimental evolution in the nematode *Caenorhabditis remanei*. Rose Reynolds, Kristin Sikkink, Catherine Ituarte, Janna Fierst, John Willis, William Cresko, **Patrick Phillips**.

307C. Deciphering the microRNA responses to high temperature stress. C. Nehammer, **A. Podolska**, K. Kagias, S. Mackowiak, N. Rajewski, R. Pocock.

308A. Folliculin is an evolutionary conserved regulator of AMPK function. **E. Possik**, Z. Jalali, Y. Nouet, M. Yan, MC. Gingras, L. Chotard, F. Dupuy, C. Rocheleau, D. Hall, R. Jones, A. Pause.

309B. Epigenetic Mechanism of Longevity Regulation in *C. elegans*. **Mintie Pu**, Xiujuan Wang, Zhuoyu Ni, Haiyuan Yu, Siu Sylvia Lee.

310C. The mitochondrial stress machinery protects cells from inhibition of the mevalonate pathway. **Manish Rauthan**, Marc Pilon.

311A. Is mitochondrial fragmentation a bio marker of aging? **Saroj G. Regmi**, Barbara Conradt.

312B. Regulation of eIF4E compartmentalization by the heat shock response pathway during ageing in *C. elegans*. **M. Rieckher**, A. Princz, N. Tavernarakis.

313C. Systemic control of the cytosolic redox environment in *C. elegans*. **C. Romero**, W. Fontana, J. Apfeld.

314A. Developmental Exposure to Ultraviolet C Radiation Results in Altered Energy Production Later in Life in Caenorhabditis elegans. **John P. Rooney**, Rakesh Bodhicharla, Amanda Bess, Maxwell Leung, Ian Ryde, Alex Ji, Joel Meyer.

315B. Assaying nickel toxicity using nematodes. **David Rudel**, Ian Huffnagle, Chandler Douglas, John Atkinson.

316C. D-Aspartate oxidase is involved in the caloric restriction-induced lifespan extension in *C. elegans*. **Yasuaki Saitoh**, Mari Okutsu, Masumi Katane, Masae Sekine, Takemitsu Furuchi, Taro Sakamoto, Takao Inoue, Hiroyuki Arai, Hiroshi Homma.

317A. Genetic analyses of hypoxia response and the roles of HIF-1. **Jenifer Saldanha**, Dingxia Feng, Korinna Radke, Jo Anne Powell-Coffman.

318B. The Max/Mlx transcriptional network influences aging in *C. elegans*. David W. Johnson, Jesse Llop, Sara Farrell, **Andrew V. Samuelson**.

319C. ULP-4 SUMO protease controls HMGS-1 activity in cytosolic and mitochondrial metabolic networks during development, aging, and stress. **Amir Sapir**, Assaf Tsur, Thijs Koorman, Mike Boxem, Paul Sternberg, Limor Broday2.

320A. Using *C. elegans* to explore the role of presenilin in the pathogenesis of Alzheimer's Disease. **Shaarika Sarasija**, Kenneth Norman.

321B. The rare sugar D-psicose extends *Caenorhabditis elegans* lifespan by increasing oxidative stress resistance. **M. Sato**, H. Sakoguchi, T. Shintani, K. Okuma, K. Izumori.

322C. DNA damage responses in development and ageing. Michael Mueller, Laia Castells-Roca, Maria Ermolaeva, Peter Frommolt, Sebastian Greiss, Jennifer Schneider, **Björn Schumacher**.

323A. Gut-specific regulation of transcription in long-lived *daf-2* mutants. Lamia M. Boukhibar, Zoja Nagurnaja, Nuria Vergara Irigaray, **Eugene F. Schuster**.

324B. Environmental Stress Resistance in *exl-1* and *dbl-1* Mutants of *C. elegans*. **Yakov Shaulov**, Tasmia Hoque, Jun Liang Rice, Cathy Savage-Dunn.

325C. Mitoflash is an Early Predictor of Lifespan in *C. elegans*. **Enzhi Shen**, Chunqing Song, Yuan Lin, Wenhong Zhang, Peifang Su, Wenyuan Liu, Pan Zhang, Jiejia Xu, Na Lin, Cheng Zhan, Xianhua Wang, Yu Shyr, Heping Cheng, Mengqiu Dong.

326A. The role of autophagy in lipid and mitochondrial homeostasis. **Melissa J. Silvestini**, Hannah Hong, Alicia Meléndez.

327B. Assessing drug induced mitochondrial toxicity using *C. elegans*. **Reuben L. Smith**, Richard de Boer, Winnok H. de Vos, Erik M. Manders, Stanley Brul, Hans van der Spek.

328C. Effect of chronic treatment with amphetamine in an experimental model using Caenorhabditis elegans. Tássia Fontana Lehmen, Bruna Puntel, Priscila Gubert, Roselei Fachinetto, **Félix A. A. Soares**.

329A. ROS and antioxidant interaction in *C. elegans*. **Paul Neal Stein**, Craig W. LaMunyon.

330B. A genetic screen for stress resistance combined with next-generation sequencing reveals new longevity candidates. **Nadia J. Storm**, Martin S. Denzel, Adam Antebi.

331C. Quantitative analysis of ionizing radiation-induced effects on locomotion in *Caenorhabditis elegans*. **Michiyo Suzuki**, Tetsuya Sakashita, Toshio Tsuji, Yuya Hattori, Yasuhiko Kobayashi.

332A. Mitochondrial dynamics in response to oxygen deprivation. **Alexandra L. Tabakin**, Piya Ghose, Eun Chan Park, Nathaly Salazar-Vasquez, Christopher Rongo.

333B. Quiescence of entomo-phoretic nematode *Caenorhabditis japonica*. **R. Tanaka**, Y. Hirooka, T. Kikuchi, N. Kanzaki.

334C. Fructose accelerates neuronal aging in *C. elegans*. **Marton Toth**, Leena Shah, Monica Driscoll.

335A. Calcium Exerts Critical Functions in Adult Neuronal Maintenance. **Marton Toth**, Ivana Ganihong, Khushboo Patel, Kelli Gaul, Camisha DuBose, Steven Kim, Saurabh Patel, Wenying Zhang, Jian Xue, Monica Driscoll.

336B. Regulation of organismal proteostasis by trans-cellular chaperone signaling. **Patricija van Oosten-Hawle**, Robert S. Porter, Richard I. Morimoto.

337C. Protein homeostasis dysregulation drives aberrant morphology of aging mechanosensory neurons. **Elena Vayndorf**, Courtney Nichols, Cyrena Parker, Skyler Hunter, Marton Toth, J. Alex Parker, Christian Neri, Monica Driscoll, Barbara Taylor.

338A. Genome-wide study of stress-responsive microRNA and mRNA transcriptomes in C. elegans. Maria C. Ow, **Isana Veksler-Lublinsky**, Victor Ambros.

339B. Investigating progranulin in aging and neurodegeneration. **Julie Vériène**, J. Alex Parker.

340C. Age-dependent Proteome Turnover Changes in C. elegans. **K. Vukoti**, X. Yu, J. Feng, A. Hsu, M. Miyagi.

341A. Direct Regulation of HIF-1 by the Metabolic Network in C. elegans. Robert J. Mishur, Maruf Khan, **Haley M. Willhelm**, Shane L. Rea.

342B. Intermittent hyperoxia-induced hormesis decreases aerobic respiration via ins/IGF-1 and p53/CEP-1 signalings in *C. elegans*. **Sumino Yanase**, Tetsuji Shoyama, Hitoshi Suda, Naoaki Ishii.

343C. Genetic mechanism of carotenoid nanoparticle-induced health promotion effects in *Caenorhabditis elegans*. **Ji Suk You**, Yeong Hun Kim, Sang Ho Koo, Shin Sik Choi.

344A. *C. elegans* SIRT6/7 homolog SIR-2.4 promotes stress response and longevity via distinct mechanisms. Wei-Chung Chiang, **Xiaokun Yu**, Daniel X. Tishkoff, Bo Yang, Tsui-Ting Ching, David B. Lombard, Ao-Lin Hsu.

345B. The *Rosmarinus officinalis* extract protects against oxidative stress and increase *C. elegans* lifespan. **D. C. Zamberlan**, G. P. Amaral, S. T. Stefanello, R. L. Puntel, F. A. A. Soares.

346C. Neutral cholesterol ester hydrolase 1 is protective against α-synuclein-induced toxicity in *C. elegans*. **S. Zhang**, K. A. Caldwell, G. A. Caldwell.

347A. Identification of genes that regulate the ribotoxic stress response in Caenorhabditis elegans. Yan Qi, **Xinrui Zhang**, Natalia Kirienko, Peter Breen, Holli Rowedder, Gary Ruvkun.

348B. Delineating AMPK and TOR longevity. **Yue Zhang**, Ianessa Morantte, William Mair.

349C. A quantitative proteomic analysis of aging in C. elegans. **Stephanie M. Zimmerman**, Izumi V. Hinkson, Joshua E. Elias, Stuart K. Kim.

Physiology: Dauer Larvae and Metabolism

350A. Analysis of the CRTC1 pathway in fat regulation of C. elegans. **Sravya Challa**, Rebecca Hintz, Alexander van der Linden.

351B. Multiple Insulin-like Ligands Regulate the Insulin/IGF Receptor Activity to Prevent Dauer Formation. **Jyothsna D. Chitturi**, Wesley L. Hung, Ying Wang, Mei Zhen.

352C. Regulation of Metabolism by TGF-β Signaling in C. elegans. **James F. Clark**, Vanessa Almonte, Cathy Savage-Dunn.

353A. Post-embryonic control of DAF-2 insulin-like signaling by the conserved dosage compensation protein DPY-21. **Kathleen Dumas**, Colin Delaney, Stephane Flibotte, Donald Moerman, Gyorgyi Csankovszki, Patrick Hu.

354B. A novel role for the p120RasGAP family member GAP-3 in dauer regulation. **Kathleen Dumas**, Joseph Kruempel, Stephane Flibotte, Donald Moerman, Patrick Hu.

355C. Synthetic cannabinoids influence dauer formation via insulin peptides. Neale Harrison, Pedro Reis-Rodrigues, Mark Lucanic, Jason M. Held, Gordon J. Lithgow, Thomas Gallagher, Young-Jai You, **Matthew S. Gill**.

356A. Metabolic effects of manganese in the nematode *Caenorhabditis elegans.*. **Priscila Gubert**, Bruna Bruna, Tassia Lehmen, Daiana Avila, Felix Soares, Michael Aschner.

357B. Glycosphingolipid mediates clozapine-induced developmental delay and lethality in C. elegans. **Limin Hao**, Bruce Cohen, Edgar Buttner.

358C. Regulation of fat and body growth by SIKs and class II HDACs.

Rebecca Hintz, Sravya Challa, Faye Schilkey, Alexander van der Linden.

359A. The Mediator subunit MDT-15 is required for maintenance of membrane lipid unsaturation and ER homeostasis. **Nicole S. Hou**, Stefan Taubert.

360B. The *C. elegans* Insulin Signaling Response to Glucose Stress Requires Unique Regulators. **Michael James Hoy**, Brian Ganley, John A. Hanover, Michael W. Krause, Michelle A. Mondoux.

361C. A CaMK signaling cascade modulates pheromone-mediated developmental plasticity. **In-sok Hwang**, Zhi Fang, Piali Sengupta, Kyuhyung Kim.

362A. Epigenetic regulation of stress response in C. elegans. **Moonjung Hyun**, Catherine Dumur, Young-jai You.

363B. Targeting Obesity: a new perspective from the worm's point of view. **Tom Janssen**, Lise Peeters, Kevin Van Calster, Nick Suetens, Liliane Schoofs.

364C. Unfolded protein response and *enpl-1* depletion sensitize *C. elegans* to the anti-cancer drug cisplatin. Balasubramanian Natarajan, Rahul Gaur, Oskar Hemmingsson, **Gautam Kao**, Peter Naredi.

365A. Bacterial fatty acids influence dauer recovery. **Tiffany Kaul**, Ifedayo V. Ogungbe, Frank C. Schroeder, Matthew S. Gill.

366B. FGT-1 is a mammalian GLUT2-like facilitative glucose transporter in *Caenorhabditis elegans*. **Shun Kitaoka**, Anthony Morielli, Feng-Qi Zhao.

367C. Neuronal endoplasmic reticulum stress promotes dauer entry through the activation of the Unfolded Protein Response. **Warakorn Kulalert**, Dennis H. Kim.

368A. ATGL-1 and LID-1 : Key Players in Fasting-Induced Lipolysis in Caenorhabditis elegans. **Junghyun Lee**, Jinuk Kong, Ju Yeon Jang, Junho Lee, Jae Bum Kim.

369B. Absolute Quantitation of *C. elegans* Dafachronic Acids during Development and in Daf Mutants. **Tie-Mei Li**, Jie Chen, Xiangke Li, Xiao-Jun Ding, She Chen, Xiaoguang Lei, Meng-Qiu Dong.

370C. Effect of Repeated Starvation on Fat Content in *C.elegans*. **Shinya Matsumoto**, Kosuke Kato, Yasuki Matsumura, Nao Sato, Yukari Yamamoto, Akari Sawanaga.

371A. Overexpression of an orphan gene in *Pristionchus pacificus* causes complete inhibition of dauer formation. **Melanie G. Mayer**, Ralf J. Sommer.

372B. Identifying transcription factors that regulate fat metabolism and body size. **Akihiro Mori**, Marian Walhout.

373C. Bis (2-ethylhexyl) phthalate regulates cytochrome P450 (DAF-9) and a specific C4 methylase (STRM-1) towards dauer formation in *C. elegans*. **S. Mukherjee**, T. Paul, M. Guria, A. Nag, J. Bandyopadhyay.

374A. Using Stable Isotope Tracers to Understand the Role of Membrane Maintenance in Aging. Shaw-Wen Chen, **Carissa Perez Olsen**.

375B. Quantity proteome analysis of dauer by using iTRAQ in *Caenorhabditis elegans*. **Takehiro Oshime**, Yukako Toshato, Toshiya Hayano, Masahiro Ito.

376C. Comparative metabolomics reveals endogenous ligands of the nuclear hormone receptor DAF-12 regulating *C. elegans* development and lifespan. Parag Mahanti, Neelanjan Bose, Joshua Judkins, Axel Bethke, Joshua Wollam, **Oishika Panda**, Kathleen Dumas, Anna Zimmerman, Patrick Hu, Adam Antebi, Frank Schroeder.

377A. Identification of a diet induced signaling pathway for mitochondrial adaptation during aging. **Shanshan Pang**, Sean P. Curran.

378B. Nematoil - a novel secreted lipid that coats the outer surface of the dauer larva of Pristionchus pacificus. **Sider Penkov**, Akira Ogawa, Ulrike Pässler, Margit Gruner, Hans-Joachim Knölker, Ralf Sommer, Teymuras Kurzchalia.

379C. Isolation of N-acylethanolamine resistant mutants using a forward genetic screen for resistance to synthetic cannabinoids. **Pedro R. Rodrigues**, Neale Harrison, Jitendra Mishra, Thomas Bannister, Matthew S. Gill.

380A. Folate metabolism and the rescue of folate deficiency by thiamine supplementation. **Jason A. Rothman**, Craig W. LaMunyon.

381B. Worms as chemical masterminds: complete control with small molecules. **Frank C. Schroeder**.

382C. Mechanistic studies on the regulation of fat accumulation by the TOR pathway in *C. elegans*. **Ming Sheng**, Josefin Friberg, Philip McQuary, Rahul Gaur, Staffan Lundstedt, Malene Hansen, Simon Tuck.

383A. EGL-8, a phospholipase C beta homolog, is a novel regulator of dauer arrest. **Hung-Jen Shih**, Andrew Polzin, Kathleen J. Dumas, Stephane Flibotte, Donald G. Moerman, Patrick J. Hu.

384B. Genetic requirements of the pentose phosphate pathway for the intestinal granule formation in *C. elegans*. **Hirohisa Shiraishi**, Takato Oikawa, Maya Ishibashi, Mari Tanabe, Yumi Asanuma, Reiko Aoyama, Kenji Nishikori, Takahiro Tanji, Ayako Ohashi-Kobayashi.

385C. Overexpression, purification and characterization of *C. elegans* glyceraldehyde-3-phosphate dehydrogenase isozymes. **Ruth L. Silimon**, Derek Schwabe-Warf, Valeria S. M. Valbuena, Justin W. Spengler, Megan K. Gautier, Katherine M. Walstrom.

386A. Diet Another Day: *agl-1* (glycogen debranching enzyme) embryonic lethality depends on maternal diet. **Jeff Simske**.

387B. Novel secreted proteases regulate systemic heme homeostasis in C. elegans. **J. Sinclair**, K. Meng, K. Pinter, I. Hamza.

388C. Metabolism of benzimidazole anthelmintics in Caenorhabditis elegans, and the ruminant parasite, Haemonchus contortus. **Susan J. Stasiuk**, Gillian MacNevin, Dae-Qyun Ro, John S. Gilleard.

389A. PAQR-2 Regulates Fatty Acid Desaturation During Cold

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390B. Functional analysis of the acylpyruvase FAHD1 in *C. elegans*. **A. Taferner**, H. Pircher, N. Tavernarakis, P. Jansen-Dürr.

391C. Biochemical and Genetic Analysis of Lipid Droplets in *C. elegans*. **Tracy L. Vrablik**, Vlad Petyuk, Olga Shiva, Jennifer L. Watts.

392A. Genome wide responses to methyl donor availability reveal effects on metabolic, stress response and germline function genes. Wei Ding, Michael Irwin, Malene Hansen, **Amy K. Walker**.

393B. New path to NAD^+: In addition to salvage biosynthesis, NRK and *de novo* NAD^+ synthesis contribute to NAD^+ recycling in *C. elegans*. **Wenqing Wang**, Matthew R. Lynes, Wendy Hanna-Rose.

394C. *natc-1* mediates stress resistance and dauer formation as a downstream effector of the insulin/IGF-1 signaling pathway. **Kurt Warnhoff**, John T. Murphy, Daniel L. Schneider, Michelle Peterson, Simon Hsu, Kerry Kornfeld.

395A. Screening receptor guanylyl cyclase genes for roles in fat regulation in *C. elegans*. **Emily Witham**, Supriya Srinivasan.

396B. ATGL-1 requires CGI-58 to Localize to Lipid Droplets Where it Controls Droplet Morphology by Regulating Lipid Exchange and Hydrolysis. **Meng Xie**, Richard Roy.

397C. Development of a Global-scale Metabolic Network Model of C. elegans. **Lutfu S. Yilmaz**, Albertha J. Walhout.

398A. Investigating the role of neuropeptides in regulating body fat levels in *C. elegans*. **Yorke Zhang**, Supriya Srinivasan.

399B. Biological function of PUFA-derived eicosanoids in *Caenorhabditis elegans*. **Yiwen Zhou**, Jingjuan Ju, Erik Nehk, Lihong Yin, Chrisitan Steinberg, Ralph Menzel.

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400C. Neuronal serotonin signaling through G proteins negatively regulates the *C. elegans* immune response. **Alexandra Anderson**, Henry Laurenson-Schafer, Rachel McMullan.

401A. Microsporidia infection regulates ubiquitin- and chromatin remodeling-associated gene expression in the *C. elegans* intestine. **Malina A. Bakowski**, Chistopher A. Desjardins, Tiffany L. Dunbar, Christina A. Cuomo, Emily R. Troemel.

402B. Genetic architecture underlying variation in Caenorhabditis elegans host resistance to a natural pathogen. **Keir M. Balla**, Erik C. Andersen, Leonid Kruglyak, Emily R. Troemel.

403C. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces mitochondrial and nuclear DNA damage in Caenorhabditis elegans. **Rakesh Bodhicharla**, Joel Meyer, Prasad G.L.

404A. Enhanced RNA virus susceptibility results from defects in lipid metabolism. **Gina Broitman-Maduro**, Saige Pompura, Stephanie Coffman, Yuanyuan Guo, Francisco Carranza, Shou-Wei Ding, Morris Maduro.

405B. Antagonistic cGMP Signaling Pathways Regulate a Heritable Developmental Response to Pathogens. **Nicholas Burton**, Bob Horvitz.

406C. Host-finding strategies of mammalian-parasitic nematodes. **Michelle Castelletto**, Ryo Okubo, Anastassia Tselikova, Elissa Hallem.

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408B. Role of OCTR-1 expressing neurons during pathogen infection. **Argenia L. Doss**, Alejandro Aballay.

409C. Tissue Tropism and Infection Characterization of Novel Viruses in Caenorhabditis Nematodes. **Carl J. Franz**, Hilary Renshaw, Lise Frezal, Yanfang Jiang, Marie-Anne Félix, David Wang.

410A. *Caenorhabditis elegans* as a Model to Study Parasite-Induced Alterations in Host and Gut Microbiota Interaction. **Teklu Gerbaba**, Xin Wang, Kevin Rioux, Dave Hansen, Andre Buret.

411B. A key role of mitochondrial dynamics, cytochrome c release and IP3R activity in muscular dystrophy. Jean Giacomotto, Nicolas Brouilly, Marie-Christine Mariol, Laurent Segalat, **Kathrin Gieseler**.

412C. Transcriptome analysis of ALS-associated Mutants *fust-1* and *tdp-1*. **Patrick K. Gonzales**.

413A. Identification of molecular networks that modulate intestinal *ilys-3* activity in response to danger signals and bacterial challenges. **Maria J. Gravato-Nobre**, Suzanne Jordan, Sophie Andrews, Jonathan Hodgkin.

414B. Whole genome expression analysis of *C. elegans* upon recovery from *Salmonella enterica* infection. **Brian P. Head**, Alejandro Aballay.

415C. Observation of *Legionella pneumophila* infectious cyst-like forms in the host model *Caenorhabditis elegans* . **Jacqueline Hellinga**, Ann Karen Brassinga.

416A. PTR-15/BUS-13: A patched-related protein affecting surface properties and susceptibility to multiple surface pathogens. Dave Stroud, Alexis Tchaconas, Patricia Kuwabara, **Jonathan Hodgkin**.

417B. Using *C. elegans* to identify the nematode phosphorycholine transferase. **Kevin J. Jenson**, Patricia M. Berninsone.

418C. Development of a reverse genetics system for a novel nematode virus. **H. Jiang**, D. Wang.

419A. Identification of disease-causing mechanisms and potential therapeutic targets of Hereditary Spastic Paraplegias using *C. elegans*. **Carl Julien**, Arnaud Tauffenberger, Dina Aggad, Patrick A. Dion, Guy A. Rouleau, Pierre Drapeau, J. Alex Parker.

420B. An integrative *D. discoideum*, *C. elegans* and *D. rerio* approach to

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421C. A C. elegans-P. aeruginosa Liquid Assay Identifies Novel Small Molecules with Anti-Infective Properties. **Daniel Kirienko**, Natalia Kirienko, Frederick Ausubel.

422A. Deciphering the function of the ALS/FTD causing genes TDP-43 and C09ORF72 in *C.elegans*. **P. Kratsios**, J. Kerk, A. Vidal-Gadea, J. Villarin, J. Pierce-Shimomura, O. Hobert.

423B. Shiga-like toxin 1 confers the full pathogenicity of Enterohaemorrhagic *Escherichia coli* and activation of the p38/MAPK pathway in *Caenorhabditis elegans*. **Cheng-Ju Kuo**, Ting-Chen Chou, Hao-Chieh Chiu, Wan-Jr Syu, Wen-Tai Chiu, Chang-Shi Chen.

424C. The canonical WNT pathway is an important regulator of *C. elegans* innate immunity against the pathogen *staphylococcus aureus*. **sid ahmed LABED**, Amanda WOLLENBERG, Anna ALVES, Javier IRAZOQUI.

425A. Olfactory Plasticity in Entomopathogenic Nematodes. **Joon Ha Lee**, Elissa Hallem.

426B. A non endosomal role for ESCRT-II proteins in neuron and muscle physiology. Emmanuel Culetto, Christophe Lefebvre, Xavier Manière, Ivan Matic, **Renaud Legouis**.

427C. Identification of TDP-43 modifying kinases in a *C. elegans* model of TDP-43 proteinopathy. **Nicole Liachko**, Pamela McMillan, Chris Guthrie, Thomas Bird, James Leverenz, Brian Kraemer.

428A. Searching for therapeutic compounds for Machado-Joseph disease: a *C. elegans*-based screening. A. Teixeira-Castro, A. Jalles, M. Araújo, A. Miranda, C. Bessa, R. Morimoto, **P. Maciel**.

429B. Role of natural genetic variation in the control of susceptibility to bacterial infections in Caenorhabditis elegans. **Natalia Martin**, Alejandro Aballay.

430C. *fshr-1* provides a connection between the oxidative stress response and innate immunity in *C.elegans*. **Elizabeth V. Miller**, Jennifer R. Powell.

431A. Update: Screening Potential Anthelmintic Compounds for Novel Activity. Megan Gross, Michael Smith, Aaron Monte, **Jennifer Miskowski**.

432B. The discovery of intestinal intracellular microbes in the soil nematode collected from the field of the Tohoku district, northeastern Japan. **Kenji Nishikori**, Eisuke Kuroda, Takahiro Tanji, Hirohisa Shiraishi, Ayako Ohashi-Kobayashi.

433C. Does the *C.elegans* glycosylation gene *bus-8* undergo translational frameshifting to generate different protein isoforms? **Delia M. O'Rourke**, Martin Cullen, Dave Stroud, Mark Pavlyukovskyy, Frederick A. Partridge, Jonathan Hodgkin.

434A. Pathogen induced diapause formation requires the RNAi machinery. **Maria Fernanda Palominos**, Lidia Verdugo, Francisco Chavez, Andrea Calixto.

435B. The Mitochondrial Unfolded Protein Response Regulates a Pathogen-Specific Innate Immune Pathway. **Mark W. Pellegrino**, Amrita Nargund, Cole M. Haynes.

436C. Suppressors of TDP-1 toxicity in *Caenorhadbitis elegans*. **I. Pena-Gonzalez**, CD. Link.

437A. From fungal spore adhesion to effector gene transcription. Olivier Zugasti, Shizue Omi, Guillaume Bordet, Vincent Rouger, Carole Couillault, Julien Soule, Jerome Belougne, Didier Marguet, Jonathan Ewbank, **Nathalie Pujol**.

438B. Functional analysis of candidate effector proteins from a natural, intracellular pathogen of C. elegans. **Aaron W. Reinke**, Emily Troemel.
439C. Epitope-tagging the G-Protein Coupled Receptor *fshr-1*. **Joseph D. Robinson**, Jennifer R. Powell.

440A. On methane seeps, worms, and parasitic fungi: microsporidia-infected nematodes reveal another secret of the deep sea. **Amir Sapir**, Adler Dillman, Benjamin Grupe, Jeroen Ingels, Stephanie Connon, Manuel Mundo-Ocampo, John DeModena, Lisa Levin, James Baldwin, Victoria Orphan, Paul W. Sternberg.

441B. A *C. elegans* genome-wide RNAi screen identifies modifiers of mutant TDP-43. **Aleen Saxton**, Nicole Liachko, Brian Kraemer.

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443A. Key residues of Cry5B structure and function: Mutagenesis by alanine scanning. **Jillian Sesar**, Yan Hu, Hui Fan, Partho Ghosh, Raffi Aroian.

444B. PUFA therapy ameliorates Parkinson disease like symptoms. **Shashikumar Shivaiah**, Rajanikant Golgodu Krishnamurty.

445C. Antimicrobial compound screens using *C. elegans* model system for periodontal pathogens -. **Shahid S. Siddiqui**, Fathy A. Faskhani, Mohammad Al-Beyari.

446A. Using Caenorhabditis elegans to investigate the genetic and mechanistic basis of cellular defense against bacterial pore-forming proteins. **Anand Sitaram**, Raffi Aroian.

447B. *C. elegans* and mammalian cell cultures: comparison of two different approaches to toxicological screening of silver nanoparticles. **C. Soria**, T. Coccini, S. Giorgetti, L. Marchese, I. Zorzoli, U. De Simone, V. Bellotti, M. Stoppini, L. Manzo.

448C. *C. elegans* expressing human β2-microglobulin: a novel model for studying the amyloid toxicity. **C. Soria**, L. Diomede, M. Romeo, S. Giorgetti, L. Marchese, PP. Mangione, I. Zorzoli, F. Romano, S. Ramat, M. Salmona, V. Bellotti, M. Stoppini.

449A. Toxicity of the fungal lectin CCL2 against *C. elegans*. **K. Stutz**, A.

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450B. Interplay of host and pathogen genetics upon RNA virus infection in *C. elegans*. **Melanie Tanguy**, Peter Sarkies, Jeremie Le Pen, Eric A. Miska.

451C. Role of wild-type ataxin-3 and valosin-containing protein/p97 in Machado-Joseph disease: a study in *C. elegans*. **A. Teixeira-Castro**, H. Brignull, D. Ribeiro, R. Morimoto, P. Maciel.

452A. Potential Roles of Peroxidases in C. elegans Innate Immunity. **George R. Tiller**, Danielle A. Garsin.

453B. The ABC transporter MRP-7 inhibits methylmercury-induced whole animal and dopamine neuron pathology. **Natalia VanDuyn**, Richard Nass.

454C. HLH-30/CeTFEB plays a central role in host defense against bacterial infection. **Orane Visvikis**, Nnamdi Ihuegbu, Anna-Maria Alves, Lyly Luhachack, Amanda Wollenberg, Gary Stormo, Javier Irazoqui.

455A. Genetic basis underlying differential susceptibility to bacteria in *Caenorhabditis elegans*. **Ziyi Wang**, Michael Herman, L. Basten Snoek, Jan Kammenga.

456B. Acyl-CoA synthase-3 and the nuclear receptor NHR-25 regulate innate immunity genes and promote pathogen resistance. **Jordan D. Ward**, Carol Couillault, Brendan Mullaney, Benjamin Schiller, Teresita Bernal, Sarah Petnic, Marc Van Gilst, Kaveh Ashrafi, Jonathan Ewbank, Keith Yamamoto.

457C. A unique *Stenotrophomonas maltophilia* strain that evades a major *Caenorhabditis elegans* defense pathway. **Corin Vashoun White**, Brian Darby, Michael Herman.

458A. The effects of bacteria from rotting fruit on *C. elegans* gene expression and lifespan. **Amanda C. Wollenberg**, Marie-Anne Félix, Javier E. Irazoqui.

459B. *Enterococcus* infection of *C. elegans* as a model of innate immunity. **Grace J. Yuen**, Frederick M. Ausubel.

460C. Effects of Diphenyl Diselenide on transgenic *Caernohabiditis elegans* Alzheimer's disease model. **D. C. Zamberlan**, L. P. Arantes, J. B. T. Rocha, F. A. A. Soares.

461A. UNC-119 interacts with ARL-13 and plays a role in ciliogenesis. **Qing Zhang**, Qing Wei, Yuxia Zhang, Jinghua Hu.

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462B. CED-10 Rac signaling pathway regulates axon regeneration via JNK MAPK pathway. **Tanimul Alam**, Kazuya Hirose, Strahil Pastuhov, Naoki Hisamoto, Kunihiro Matsumoto.

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464A. Postmitotic diversification of olfactory neuron types is mediated by differential activities of the HMG-box transcription factor SOX-2. **Amel Alqadah**, Yi-Wen Hsieh, Berta Vidal Iglesias, Chieh Chang, Oliver Hobert, Chiou-Fen Chuang.

465B. An Investigation into the Affect of Neuronal Activity on Proper Neural Connectivity in *C. elegans*. **Kristine E. Andersen**, Benjamin Barsi-Rhyne, Kristine Miller, Christopher Vargas, Joori Park, Emma Holdrich, Miri VanHoven.

466C. Dendritic arborization in dauer IL2 neurons: Genetic and bioinformatic analyses. **Rebecca J. Androwski**, Alina Rashid, Tom Ritter, Nathan E. Schroeder, Maureen M. Barr.

467A. Candidate modulators of tubulin and microtubule dynamics during *C. elegans* neural development. **Renee A. Baran**, Hyun S. Kim, Evan Choate, Mealani Kaiser.

468B. PLR-1, a novel E3 ligase, controls cell polarity and axonal extensions in *C. elegans*. **Jaffar M. Bhat**, Jie Pan, Harald Hutter.

469C. Gαq mediates effects of antipsychotic drugs on C. elegans developmental delay/lethality. Limin Hao, Afsaneh Sheikholfslami, Kristin Harrington, Bruce Cohen, **Edgar Buttner**.

470A. Mechanisms of age-related decline in axon regeneration. **Alexandra Byrne**, Trent Walradt, Austin Hubbert, Marc Hammarlund.

471B. Mechanisms of VD motor neuron differentiation: UNC-55 expression and repression is determined by isoforms of UNC-62. **R. Campbell**, W. Walthall.

472C. A role for muscle-skin interactions in shaping PVD sensory dendrites. **Kevin Celestrin**, Hannes Buelow, Zaven Kaprielian.

473A. Microtubules-Based Inhibition of RhoA and MAPK Signaling Promotes Synapse Maturation and Axon Branch Maintenance in *C. elegans*. **Chun-Hao Chen**, Yu-Hsien Lee, Chun-Liang Pan.

474B. Asymmetric Hox Expression by Two Opposing Wnt Signals Drives *C. elegans* Neuroblast Migration through Differential Cell Polarization. **Chung-Kuan Chen**, Gian Garriga, Chun-Liang Pan.

475C. Autonomous and nonautonomous regulation of Wnt-mediated neuronal polarity by the *C. elegans* Ror kinase CAM-1. **Shih-Chieh Chien**, Mark Gurling, Gian Garriga.

476A. Multi-layers of molecular mechanisms govern dendritic arborization in neurons. **H. Chiu**, Y. Zou, T. Ferreira, C.-F. Chuang, C. Chang.

477B. Neuronal fusion induced by *unc-70/β-spectrin* dependent axonal injury requires components important for clearance of apoptotic cells. **Sean Coakley**, Brent Neumann, Hengwen Yang, Ding Xue, Massimo Hilliard.

478C. Characterization of nde-5, a newly identified mutant displaying axon guidance and sprouting defects. **Zhao Hua Ding**, Cristina Slatculescu, Antonio Colavita.

479A. Early neural specification and the regulation of asymmetric

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480B. UNC-68/RyR channels modulate critical sub-cellular calcium signals during normal and optogenetically enhanced neuronal regeneration. Lin Sun, James Shay, Samuel Chung, Christopher Clark, Mark Alkema, **Christopher V. Gabel**.

481C. Lesion conditioned regeneration of *C. elegans* amphid sensory neurons is mediated through a reduction of sensory signaling and does not require DLK-1. Samuel H. Chung, **Christopher V. Gabel**.

482A. Menorah-menorah auto-fusion as a mechanism of PVD response to injury. **Tamar Gattegno**, Meital Oren, Benjamin Podbilewicz.

483B. Development and Function of RIS, a Caenorhabditis elegans GABAergic interneuron. **Marie Gendrel**, Oliver Hobert.

484C. Studying membrane fusion during development and maintenance of *C. elegans* neurons. **R. Giordano-Santini**, M. A. Hilliard.

485A. A transcriptional network controlling reciprocal homeotic transformations of ALM and BDU neurons. **P. Gordon**, O. Hobert.

486B. EGL-13/SoxD Specifies Distinct O_2 and CO_2 Sensory Neuron Fates. **Jakob Gramstrup Petersen**, Teresa Rojo Romanos, Vaida Juozaityte, Alba Redo Riveiro, Ingrid Hums, Lisa Traunmüller, Manuel Zimmer, Roger Pocock.

487C. How to Fix a Broken Neuron. **Julie E. Grimm**, Meital Oren, Benjamin Podbilewicz.

488A. Neuronal microtubule organization in C. elegans. **Martin Harterink**, Bart de Haan, Sander van den Heuvel, Casper Hoogenraad.

489B. Excluding membrane proteins from the cilia: A role for UNC-101 and LRK-1. **A. Holmes**, M. Doll, A. Gartner.

490C. A paired-like homeodomain protein UNC-42 specifies the SAA neuron fate in *C. elegans*. **Myeongjin Hong**, Leesun Ryu, Chris Li, Kyuhyung Kim.

491A. *tld-1* regulates the synaptic localization of the TIR-1 Ca^{2+} signaling scaffold protein in left-right neuronal asymmetry. **Yi-Wen Hsieh**, Chieh Chang, Chiou-Fen Chuang.

492B. Coordination of small GTPase Arls in cilia signaling in C.elegans. **Zeng Hu**, Yujie Li, Yuxia Zhang, Qing Zhang, Jinghua Hu.

493C. UNC-116/KHC Acts with UNC-6/Netrin and UNC-40/DCC to Maintain Sensory Neuron Position in Caenorhabditis elegans. Ben Barsi-Rhyne, Kristine Miller, Christopher Vargas, Anthony Thomas, Joori Park, Martina Bremer, **Jessica Jarecki**, Miri VanHoven.

494A. Transcriptome analysis reveals genes regulated by MAB-5/Hox in posterior migration of Q neuroblast descendants. **Matthew P. Josephson**, Joel Tamayo, Mahekta Gujar, Stuart Macdonald, Erik Lundquist.

495B. Patterning of sexually dimorphic neurogenesis in the ventral nerve cord by HOM-C/Hox and TALE homeodomain transcription factors. **Andrea K. Kalis**, Djem Kissiov, Breanna Tetreault, Emily Kolenbrander, Jennifer Ross Wolff.

496C. Region-specific control of ventral cord neuron fate revealed by *lin-39(ccc16)*. **Andrea K. Kalis**, Maria Carson Sterrett, Jennifer Ross Wolff.

497A. Non-autonomous regulation of neuronal migration by Insulin signaling, DAF-16/FOXO and PAK-1. **Lisa Kennedy**, Steven Pham, Alla Grishok.

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500A. Dissecting the role of CEBP-1 in axon regeneration. **Kyung Won Kim**, Phoenix Ying, Thijs Koorman, Mike Boxem, Yishi Jin.

501B. Characterizing Ca^{2+} dynamics in the M4 neuron using GCaMP3. **Alena Kozlova**, Sana Hussain, Peter Okkema.

502C. Diversification of motor neuron differentiation programs through a network of evolutionarily conserved transcription factors. **P. Kratsios**, J. Kerk, R. Mourao, O. Hobert.

503A. The effects of aging on dendritic plasticity and PVD-FLP branch coexistence. **V. Kravtsov**, M. Oren-Suissa, B. Podbilewicz.

504B. kin-20 is required for maintenance of neuronal architecture. **M. LaBella**, R. Rawson, C. Frøkjær-Jensen, N. Jorgensen, MW Davis, M. Bastiani, EM Jorgensen.

505C. A screen for regulators of anterior-posterior axon outgrowth in *C. elegans* identifies the DNC-1/$p150^{Glued}$ subunit. **Vi Leitenberger**, Angela Lee, Brian Ackley.

506A. A Neuron from Mesoderm: A Likely Case of *in vivo* Neuronal Reprogramming. **Shuo Luo**, Bob Horvitz.

507B. The *C. elegans* Microtubule Minus-end Capping Homolog, PTRN-1, Stabilizes Synapses and Neurites. **Jana Dorfman Marcette**, Jessica Jie Chen, Michael Nonet.

508C. Role of the histone demethylase RBR-2 in neuronal development. **Luca Mariani**, Julien Vandamme, Anna Elisabetta Salcini.

509A. A forward genetic screen identifies modifiers of a voltage- and Ca^{2+}-activated K^+ channel in left-right neuronal asymmetry. **Grethel Millington**, Chieh Chang, Chiou-Fen Chuang.

510B. Neuropeptides in neuronal development, maintenance and regeneration. **Ellen Meelkop**, Massimo A. Hilliard.

511C. Developing a cell contact sensor for tracking neuron-glia interactions in vivo. **Karolina Mizeracka**, Maxwell Heiman.

512A. Local and global inhibitory cues define the stereotyped synaptic

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513B. Genes Needed for Neuronal Ensheathment. **Emalick Njie**, Daniel Cabrera, Brian Colbitz, Xiaoyin Chen, Martin Chalfie.

514C. A Neomorphic Mutation of mec-12/alpha-Tubulin Redirects Synaptic Vesicle Transport in C. elegans by Enhancing Dynein Activity. Jiun-Min Hsu, Chun-Hao Chen, Yen-Chih Chen, Kent McDonald, Mark Gurling, Albert Lee, Gian Garriga, **Chun-Liang Pan**.

515A. Endocannabinoid AEA as injury signal in axon regeneration. **Strahil Iv Pastuhov**, Naoki Hisamoto, Kunihiro Matsumoto.

516B. Characterization of the VC class motor neurons in Caenorhabditis elegans: molecular mechanisms required for their differentiation. **Laura Pereira**, Oliver Hobert.

517C. Genetic analysis of cholinergic synaptogenesis in Caenorhabditis elegans. **Marie Pierron**, Bérangère Pinan-Lucarré, Jean-Louis Bessereau.

518A. CEH-28 activates *dbl-1* expression and signaling in the M4 pharyngeal neuron. **Kalpana Ramakrishnan**, Paramita Ray, Peter Okkema.

519B. KPC-1 protease activity regulates dendritic arborization. **Nelson J. Ramirez**, Yehuda Salzberg, Julius Fredens, Niels Færgeman, Hannes Buelow.

520C. Neuron-glia communication in the assembly of the C. elegans nerve ring. **Georgia Rapti**, Shai Shaham.

521A. Cutting Edge: Expression and function of KPC-1/furin in C. elegans. **A. Rashid**, R. Androwski, N. Schroeder, M. Barr.

522B. The role of the C. elegans Shugoshin homolog in sensory neurons. **Bryn Ready**, Tiffany Timbers, Kunal Baxi, Michel Leroux, Carlos Carvalho.

523C. *egl-46* is a novel BAG cell fate modulator. **Alba Redo Riveiro**, Roger Pocock.

524A. Hunting for new genes that function in neuronal maintenance. **James Ritch**, Avery Fisher, Andrea Thackeray, Claire Benard.

525B. Neurobehavioral defects in a C. elegans mutant for an intellectual disability-associated gene. **Ana-Joao Rodrigues**, Carlos Bessa, Filipe Marques, Bruno Vasconcelos, Filipa Pereira, Adriana Miranda, Patrícia Maciel.

526C. Deciphering the molecular mechanisms that regulate the specification of O_2 and CO_2 sensing neurons. **Teresa Rojo Romanos**, Jakob Gramstrup Petersen, Roger Pocock.

527A. Modular control of glutamatergic neuronal identity in *C. elegans* by distinct homeodomain proteins. **Esther Serrano Saiz**, Richard J. Poole, Felton Terry, Hobert Oliver.

528B. Methods for Studying Nerve Ring Assembly in C. elegans. **Anupriya Singhal**, Peter Insley, Shai Shaham.

529C. Regulation of sensory neuron architecture. **Aakanksha Singhvi**, Christine Friedman, Shai Shaham.

530A. Regulatory logic of pan-neuronal gene expression. Inés Carrera, **Nikolaos Stefanakis**, Oliver Hobert.

531B. The CDH-4 Fat-like cadherin is required for anterior-posterior Q neuroblast migrations. **Lakshmi Sundararajan**, Erik Lundquist.

532C. A PCP-like pathway acts to position or properly space (tile) embryonic motor neurons along the ventral nerve cord. **M. Tanner**, A. Ghadban, C. Slatculescu, B. Huang, T. Perkins, A. Colavita.

533A. Transmembrane Collagen COL-99 is Involved in Axon Guidance Along Major Longitudinal Axon Tracts and Ventral Nerve Cord Asymmetry in *C. elegans*. Thomas Unsoeld, **Jesse Taylor**, Harald Hutter.

534B. Mutations in *C9ORF72/F18A1.6* produce developmental and behavioral defects. **Xin Wang**, Edgar Buttner.

535C. Investigation of a novel transition fiber component DYF-20 in C.elegans. **Qing Wei**, Jinghua Hu.

536A. Transition fibers protein DYF-19 regulates the ciliary entry of assembled IFT complex. **Qing Wei**, Qingwen Xu, Kun Ling, Jinghua Hu.

537B. The SWI/SNF chromatin remodeling complex selectively affects multiple aspects of serotonergic neuron differentiation. **Peter J. Weinberg**, Nuria Flames, Hitoshi Sawa, Gian Garriga, Oliver Hobert.

538C. Developmental Specification of a Polymodal Nociceptor in C. elegans. **Jordan Wood**, Denise Ferkey.

539A. SYD-1 mediates ventral axon guidance in the HSN neuron of *C. elegans*. **Yan Xu**, Christopher Quinn.

540B. Understanding cellular mechanisms of selective fasciculation between dendrites. **Candice Yip**, Maxwell Heiman.

541C. Dendrite tiling as an emergent property of self-avoidance. **Candice Yip**, Maxwell Heiman.

542A. Suppression of microtubules dynamics by DHC-1 is required for an intact cytoskeleton and efficient cargo trafficking in C. elegans dendrites. **Shaul Yogev**, Kang Shen.

543B. Identification of APL-1 as a long-range or short-range signaling molecule in *C. elegans* . **Pei Zhao**, Chris Li.

544C. Genetic basis of subtype diversification of Touch Receptor Neurons in *C. elegans*. **Chaogu Zheng**, Margarete Diaz Cuadros, Martin Chalfie.

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545A. Sink or Swim: Identifying Novel Regulators of Presynaptic Dopamine Signaling. **Sarah Baas**, J. Andrew Hardaway, Shannon Hardie, Sarah Whitaker, Tessa Popay, Phyllis Freeman, Randy Blakely.

546B. Understanding the function of Cell Adhesion molecules in the *C.*

elegans nervous system. Pratima Sharma, Ashwani Bhardwaj, Pallavi Sharma, Vina Tikiyani, Shruti Thapliyal, Nagesh Kadam, **Kavita Babu**.

547C. Unraveling mechanisms for the establishment of synaptic connectivity in a simple neural circuit. **B. Barbagallo**, M. Francis.

548A. Understanding the mechanism of environmental stress induced nervous system plasticity. **Abhishek Bhattacharya**, Oliver Hobert.

549B. Serotonergic/Peptidergic Cotransmission in the C. elegans Egg-Laying Circuit. **Jacob Brewer**, Michael Koelle.

550C. Multiple dopamine signaling pathways antagonize RhoA signaling in the nervous system. **Kimberley H. R. Bryon-Dodd**, Clara Essmann, Andrew Porter, Rachel McMullan, Stephen Nurrish.

551A. Amphetamine and β-Phenylethylamine Activate an Amine-Gated Chloride Channel. Bryan Safratowich, Chee Lor, Laura Bianchi, **Lucia Carvelli**.

552B. Redundant mechanisms for modulation of the serotonergic HSNs by an environmental cue. **Jung-Hwan Choi**, Niels Ringstad.

553C. Understanding how neurotransmitter signaling drives two-state activity of the *C. elegans* egg-laying behavior circuit. **Kevin M. Collins**, Michael R. Koelle.

554A. Screening for Suppressors of Excitotoxic Neurodegeneration in *C. elegans*: Using Nematode Genetics to Understand the Process of Neurodegeneration in Brain Ischemia. **Anthony O. Edokpolo**, Itzhak Mano.

555B. VAV-1 acts cell autonomously to regulate cholinergic motor neuron presynaptic activity. **A. Fry**, J. Laboy, K. Norman.

556C. The AP2 clathrin adaptor subunit APM-2 regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. **Steven D. Garafalo**, Caroline L. Dahlberg, Emily Malkin, Peter Juo.

557A. Analysis of optogenetically evoked motor neuron activity by Ca^{2+} imaging using RCaMP in muscle. Sebastian Wabnig, **Caspar Glock**, Cornelia Schmitt, Alexander Gottschalk.

558B. Changes in Cellular Circuits for Chemosensation during Development May Underlie Maturation of Chemotaxis Behavior. **Laura A. Hale**, Sreekanth H. Chalasani.

559C. The C2A domain of synaptotagmin-1 drives synaptic vesicle fusion and endocytosis. **Robert J. Hobson**, Eric G. Bend, Shigeki Watanabe, Erik M. Jorgensen.

560A. The UNC-73/Trio Sec14 Domain Localizes to a Subcellular Compartment in *C. elegans* Neurons. Daniel Hoffman, **Alyssa Hoop**, Robert Steven.

561B. Combination of optogenetics and reverse genetics: novel behavior screening for regulators of neural differentiation. **S. Hori**, S. Oda, Y. Suehiro, Y. Iino, S. Mitani.

562C. Worm migraines: Characterization of a Gain-of-Function Mutation in the Voltage-Gated Calcium Channel, UNC-2/Ca_V2. **Yung-Chi Huang**, Jennifer K. Pirri, Diego Rayes, Yasunori Saheki, Cornelia I. Bargmann, Michael M. Francis, Mark J. Alkema.

563A. Regeneration of synaptic vesicles from large endocytic vesicles. **Edward J. Hujber**, Shigeki Watanabe, M. Wayne Davis, Erik M. Jorgensen.

564B. Mechanisms of C. elegans locomotion speed control during oxygen-chemotaxis. **Ingrid M. Hums**, Fanny Mende, Lisa Traunmüller, Michael Sonntag, Manuel Zimmer.

565C. VPS-39 promotes synaptic vesicle fusion in *C. elegans*. **Susan M. Klosterman**, Szi-chieh Yu, Anna O. Burdina, Janet E. Richmond.

566A. Single Cell Mass Spectrometry of Neuropeptides in Ascaris suum Motor Neurons. **Christopher J. Konop**, Jenny Knickelbine, Molly Seygulla, Martha Vestling, Antony O. W. Stretton.

567B. The Anaphase-Promoting Complex Regulates GABA Transmission at the *C. elegans* Neuromuscular Junction. **Jennifer R. Kowalski**, Hitesh Dube, Denis Touroutine, Patricia R. Goodwin, Marc Carozza, Zachary Didier, Michael M. Francis, Peter Juo.

568C. Ca^{2+} dynamics of a whole single neuron. **Sayuri Kuge**, Takayuki Teramoto, Takeshi Ishihara.

569A. Maintaining sensitivity: dissecting sensory adaptation using high-throughput *in vivo* calcium imaging. **Johannes Larsch**, Dirk R. Albrecht, Cori I. Bargmann.

570B. Pattern generation in the locomotory system by optogenetic stimulation of command neurons and sensory neurons. **J. Liewald**, C. Schmitt, S. Wabnig, C. Glock, J. Akerboom, L. Looger, N. Pokala, C. Bargmann, E. Ardiel, C. Rankin, A. Gottschalk.

571C. Postsynaptic current bursts instruct action potential firing at a graded synapse. **Ping Liu**, Bojun Chen, Zhao-Wen Wang.

572A. Multiple innexins contribute to electrical coupling of *C. elegans* body-wall muscle. **Ping Liu**, Bojun Chen, Zeynep Altun, Maegan Gross, Alan Shan, Benjamin Schuman, David Hall, Zhao-Wen Wang.

573B. Regulation of the nicotinic acetylcholine receptor ACR-16. **Ashley A. Martin**, Feyza Sancar, Janet E. Richmond.

574C. Chemical tuning of CO2-responsive BAG neurons. Ewan St. John Smith, **Luis Antonio Martinez-Velazquez**, Niels Ringstad.

575A. The DAF-7/TGF-β signaling pathway regulates abundance of the glutamate receptor GLR-1. **Annette McGehee**, Benjamin Moss, Peter Juo.

576B. Locating synaptic calcium channels. **Sean Merrill**, S. Watanabe, J. R. Richards, C. Frøkjær-Jensen, E. M. Jorgensen.

577C. A Novel UNC-43 (CaM Kinase II) Dense Core Vesicle Trafficking

Pathway Blocks UNC-31 (CAPS) - Dependent Secretion from Neuronal Cell Somas. Christopher Hoover, Stacey Edwards, Szi-chieh Yu, Maike Kittelmann, Stefan Eimer, Janet Richmond, **Kenneth Miller**.

578A. Does Cysteine String Protein contribute to *C. elegans* Nervous System Function? **Ben Mulcahy**, Paul Ibbett, Lindy Holden-Dye, Vincent O'Connor.

579B. Age-dependent changes at the *C. elegans* neuromuscular junction. **Ben Mulcahy**, Lindy Holden-Dye, Vincent O'Connor.

580C. A role for miRNA machinery at the neuromuscular junction? **Patrick J. O'Hern**, Anne Hart.

581A. An unconventional role of a conserved sterol biosynthetic protein ERG-28 in SLO-1 function. **Kelly H. Oh**, Hongkyun Kim.

582B. The molecular mechanisms of behavioral sexual dimorphism. **Meital Oren**, Oliver Hobert.

583C. Syntaxin Habc domain is required for synaptic function. **Leonardo Parra**, Jenna Whippen, Catherine Dy, Erik Jorgensen.

584A. Sexually dimorphic synaptic connectivity in the *C. elegans* tail. Matthew Johnson, Deborah Ryan, **Douglas Portman**.

585B. *unc-17* suppressors and subway crowding. **Jim Rand**, Ellie Mathews, Greg Mullen.

586C. Regulating Rho and neurotransmitter release. **K. R. Ryan**, S. J. Nurrish.

587A. A circuit for decision making in *C. elegans*: a computational approach. **Tom Sanders**, Gert Jansen, Netta Cohen.

588B. The computational role of the head navigation circuit in *C. elegans*: Exploring the interneuron layer *in silico*. **Tom Sanders**, Netta Cohen.

589C. *lgc-40* Encodes a Choline-Gated Chloride Channel Subunit Expressed in Neurons and Muscles. **Steve Sando**, Bob Horvitz.

590A. A gustatory neural circuit for salt concentration memory in *Caenorhabditis elegans*. **H. Sato**, H. Kunitomo, S. Oda, Y. Iino.

591B. Understanding the auto-receptor component of the DOP-2 signal transduction pathway in modulating dopamine release. **Jatinder Singh**, Roderick King, Crystal Clark, Ping Han, Singh Harbinder.

592C. Notch signaling regulates synaptic transmission at the *C. elegans* neuromuscular junction. **Altar Sorkac**, Michael DiIorio, Hannah Graham, Komudi Singh, Anne Hart.

593A. Role of serotonin signaling in *C. elegans* fat metabolism. **T. Noble**, S. Srinivasan.

594B. Integration of Sensory Perception, Lipid Metabolism and Food Intake in *C. elegans*. **J. Stieglitz**, S. Srinivasan.

595C. Dopamine regulates acetylcholine signaling and body size via octopamine and CREB signaling in *C. elegans*. **Satoshi Suo**, Eitaro Oami, Midori Yoshida, Shoichi Ishiura.

596A. Neuropeptides Function in a Homeostatic Manner to Modulate Excitation-Inhibition Imbalance in *C. elegans*. **Seika Takayanagi-Kiya**, Tamara M. Stawicki, Keming Zhou, Yishi Jin.

597B. 4-D Ca^{2+} imaging of the multiple neurons in a local circuit regulating behavioral choice. **Takayuki Teramoto**, Yuta Yamamoto, Takeshi Ishihara.

598C. Neurexin and Neuroligin Mediate Retrograde Synaptic Inhibition in C. elegans. Zhitao Hu, Sabrina Hom, Tambudzai Kudze, **Xiajing Tong**, Seungwon Choi, Gayane Aramuni, Weiqi Zhang, Joshua Kaplan.

599A. Identifying molecules involved in dense-core vesicle biology. **Irini Topalidou**, Brooke Jarvie, Jill Hoyt, Michelle Giarmarco, Angela L. Barr, Michael Ailion.

600B. A computational model of the intracellular signaling pathway for odor receptor neuron in *C. elegans*. **Mamoru Usuyama**, Yuishi Iwasaki, Chisato Ushida, Ryuzo Shingai.

601C. A Genetic Resource for Assaying Neuropeptide Function *in vivo*. **Amy B. Vashlishan Murray**, Edward Pym, Joshua Kaplan.

602A. Does local protein synthesis in the sensory dendrites of the AFD thermosensory neuron play a role in long-term memory? **V. Venkatachalam**, S. Yogev, J. Calarco, A. Calvo, J. Hawk, M. Klein, D. Colon-Ramos, K. Shen, A. Samuel.

603B. *Track-A-Worm*, an open-source system for quantitative assessment of C. elegans locomotory and bending behaviors. Sijie Wang, **Zhao-Wen Wang**.

604C. DAF-19 acts as a negative regulator to modulate environment-dependent GABA phenotypes. **Yusu Xie**, Mustapha Moussaif, Ji Ying Sze.

605A. A role for T-type calcium channels in serotonin signaling. **Kara Zang**, Niels Ringstad.

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606B. The Pristionchus pacificus obi-3 mutant lacks attraction to beetle host pheromone and shows increased turning frequency. **Georgina Aguilar-Portillo**, Jimmy Escobedo, Neomal Muthumala, Ray Hong.

607C. Insights into the molecular mechanisms of memory rewriting in Caenorhabditis elegans. **Ichiro Aoki**, Ikue Mori.

608A. Response to repeated activation of ASH requires glutamate, dopamine, and neuropeptide signaling. **Evan L. Ardiel**, Andrew C. Giles, Theodore Lindsay, Ithai Rabinowitch, William Schafer, Shawn Lockery, Catharine H. Rankin.

609B. NLP-7 peptide modulation of the egg-laying circuit. **Navonil Banerjee**, Raja Bhattacharya, Michael Francis.

610C. Molecular regulators of male sex-drive. Scott W. Emmons,

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611A. Conserved neuropeptidergic regulation of associative learning by vasopressin/oxytocin-related peptides. **I. Beets**, L. Temmerman, T. Janssen, E. Meelkop, L. Frooninckx, G. Jansen, L. Schoofs.

612B. Notch DSL ligand *lag-2* is required for *C. elegans* lethargus quiescence. **Heather L. Bennett**, Huiyan Huang, Komudi Singh, Anne C. Hart.

613C. A Mutant in Another cGMP-dependent Protein Kinase, PKG-2, is defective in short and long-term odor adaptation. **Eduardo Bernal**, Neomal Muthumala, Ray L. Hong.

614A. Effects of mutations in the C. elegans presenilin homologue, sel-12, on tap habituation. **T. Bozorgmehr**, C. Rankin.

615B. The voltage-gated chloride channels encoded by *clh-3* regulate the excitability of the HSN neurons. **Robyn Branicky**, Hiroaki Miyazaki, Kevin Strange, William R. Schafer.

616C. A quantifiably complete repertoire of *C. elegans* locomotion. **Andre E. X. Brown**, Roland Schwarz, Robyn Branicky, William Schafer.

617A. Acute odor recognition in AWC neuron of *C. elegans* after adaptation. **Chantal Brueggemann**, Damien O'Halloran, Noelle L'Etoile.

618B. The AIB interneuron is required for thermotaxis. **Ana C. Calvo**, Josh Hawk, Nathan Cook, Vivek Venkatachalam, Aravinthan D. T. Samuel, Daniel A. Colon-Ramos.

619C. Oxygen sensing neurons control carbon dioxide response in C. elegans. **M. A. Carrillo**, M. L. Guillermin, S. Rengarajan, R. Okubo, E. A. Hallem.

620A. Natural polymorphisms in HECW-1 E3 ubiquitin ligase affect C. elegans pathogen avoidance behavior. **Howard Chang**, Dennis Kim.

621B. Pleiotropic genes affecting touch sensitivity in *C. elegans*. **Xiaoyin Chen**, Martin Chalfie.

622C. Neuropeptides can regulate feeding behavior in the absence of MC activity. **Mi Cheong Cheong**, Young-Jai You, Leon Avery.

623A. Elucidation of a neuronal mechanism of nictation, a dispersal behavior, in *C. elegans*. **Myung-kyu Choi**, Harksun Lee, Daehan Lee, Dongjun Park, Junho Lee.

624B. Single-cell transcriptomic analysis identifies quiescence-inducing neuropeptides. **Elly S. Chow**, Erich M. Schwarz, Paul W. Sternberg.

625C. Investigating the role of polycystins in sex pheromone chemotaxis of male *C. elegans*. Ching-Ki Li, **King-Lau Chow**.

626A. SRD-1 is required for *C. elegans* males to respond to the *Caenorhabditis remanei* female sex-pheromone. Hainan Yang, Yuan Zhou, **King-Lau Chow**.

627B. AWC Neurons Mediate Navigation in a DC Electric Field. **Steven D. Chrisman**, Christopher B. Waite, Eric P. Foss, Lucinda Carnell.

628C. Dopamine down-modulates the activity of a re-current circuit via D2-like signaling during male mating. **Paola Correa**, Luis Rene Garcia.

629A. Uncovering the molecular basis for ethanol action on the BK channel using genetic screens. **Scott Davis**, Kevin Hu, Jon Pierce-Shimomura.

630B. Genetic sex alters the logic of sensory behavior. **Kelli A. Fagan**, Jessica R. Bennett, Frank C. Schroeder, Douglas S. Portman.

631C. HLH-17 Dependent Regulation of the Dopamine Transporter Gene, *dat-1* and the Dopamine Receptor Gene, *dop-3* Can Be Tied to the Oxidative Stress Response in *C. elegans*. **Chaquettea M. Felton**, Casonya Johnson.

632A. Caenorhabditis-in-Drop (CiD) method to measure worm behavior and longevity. **Benjamin L. Freedman**, Samy Belfer, Han-Sheng Chuan, Jinzhou Yuan, Michael Norton, Haim Bau, David Raizen.

633B. ASI regulates satiety quiescence. **Thomas L. Gallagher**, Leon Avery, Young-jai You.

634C. Neuropeptide modulation of C. elegans light avoidance circuitry. **D. Dipon Ghosh**, Michael R. Koelle, Michael N. Nitabach.

635A. Modular genetic architecture shapes individual variation in innate avoidance behavior in *C. elegans*. **Rajarshi Ghosh**, Aylia Mohammadi, William Ryu, Leonid Kruglyak.

636B. Toward the identification of behavioral strategies underlying *C. elegans* thermotaxis using the Multi-Worm Tracker. **Andrew C. Giles**, Yuki Tsukada, Shunji Nakano, Ikue Mori.

637C. The neuronal basis of food choice behavior in *Caenorhabditis elegans*. Brian Conroy, Maria Morabe, Lillian Haynes, Melissa Chambers, Rachel Macfarlane, **Elizabeth Glater**.

638A. Unbiased optogenetic circuit mapping: AVK interneurons, a case study. C. Schultheis, K. Erbguth, S. Wabnig, M. Brauner, **A. Gottschalk**.

639B. Monoamines amplify and focus global peptidergic signaling cascades to modulate nociceptive responses in *Caenorhabditis elegans*. **V. Hapiak**, A. Stein, W. J. Law, A. Ortega, R. W. Komuniecki.

640C. How do worms choose the right food? - Dissecting the signaling mechanisms underlying preference of food odors. **Gareth Harris**, Yu Shen, Heon-ick Ha, Alessandra Donato, Xiaodong Zhang, Yun Zhang.

641A. Exploring the low-dose activating effects of ethanol in *C. elegans*. **E. G. Hawkins**, J. C. Bettinger, A. G. Davies.

642B. A Common Behavioral Model Underlies the Motility of a Diverse Set of Nematodes. **Stephen J. Helms**, Leon Avery, Greg J. Stephens, Thomas S. Shimizu.

643C. A genetic screen for Notch downstream targets regulating *C. elegans* sleep. **Huiyan Huang**, Chen-Tsen Zhu, Anne Hart.

644A. A calcium-rich breakfast: physiological activity in C. elegans

serotonergic neurons during the enhanced slowing response and upon emergence from lethargus. **Shachar Iwanir**, Adam Brown, Dana Najjar, Meagan Palmer, Ivy Fitzgerald, David Biron.

645B. ASE and ASH neuron sensitivities determine NaCl attraction or avoidance behaviour. Oluwatoroti Umuerri, Martijn Dekkers, **Gert Jansen**.

646C. Regulation of motivational states in *C. elegans*. **Changhoon Jee**, L. René Garcia.

647A. Studying the neural circuits of food choice imprinting in C. elegans. **Xin Jin**, Navin Pokala, Cori Bargmann.

648B. Identification of novel roles of the ETS-5 transcription factor in sensory neuron specification. **Vaida Juozaityte**, Roger Pocock.

649C. Pheromones regulate nematode dispersal. **F. Kaplan**, H. Alborn, S. von Reuss, F. Schroeder.

650A. Chemotaxis follows the nose. **Rex A. Kerr**.

651B. The regulation of nictation, a dispersal behavior in *C. elegans*, by insulin-like molecules. **Nari Kim**, Harksun Lee, Myung-kyu Choi, Daehan Lee, Junho Lee.

652C. Identification of regulatory factors for forgetting in *C. elegans*. **Tomohiro Kitazono**, Akitoshi Inoue, Takeshi Ishihara.

653A. The *C. elegans* cGMP-dependent Protein Kinase EGL-4 Regulates Nociceptive Behavioral Sensitivity. **Michelle C. Krzyzanowski**, Chantal Brueggemann, Meredith J. Ezak, Jordan F. Wood, Kerry L. Michaels, Christopher A. Jackson, Bi-Tzen Juang, Kimberly D. Collins, Michael C. Yu, Noelle D. L'Etoile, Denise M. Ferkey.

654B. Thermoreceptor neurons regulate the temperature-dependence of motor programs. **S. Lasse**, V. Y. Wang, M. B. Goodman.

655C. Food signals modulate sensory integration behavior. **Hiu Lau**, Sreekanth Chalasani.

656A. Analyses of *C. elegans* male ray neuron activity during mating. Olivia Philpot, May Boggess, Rene Garcia, **Robyn Lints**.

657B. A neuronal flip-flop generates random search behavior in the nematode *C. elegans*. W. Roberts, S. Augustine, T. Lindsay, K. Lawton, T. Thiele, N. Pokala, R. Anderson, M. Britton, C. Bargmann, **S. Lockery**.

658C. Different Stressors, Same Sleep: heat, cold, salt, alcohol all trigger ALA-dependent behavioral quiescence. **Jessie M. Lopez**, Cheryl Van Buskirk.

659A. Characterizing the *hsf-1*-Independent Behavioral Response to Heat Shock. **Richard Mansfield**, Cheryl Van Buskirk.

660B. The SWI/SNF chromatin remodeling complex modifies ethanol-responsive behaviors. **L. Mathies**, G. Blackwell, L. Hack, A. Adkins, T. Webb, K. Kendler, B. Riley, A. Davies, J. Bettinger.

661C. The Role of Heterotrimeric G-protein Signaling Pathways in Habituation. **Andrea McEwan**, Andrew Giles, Catharine Rankin.

662A. Acute Laser Dissection of Mechanosensory Circuitry in *C. elegans*. **Pavan Mehat**, Lin Sun, Samuel Chung, Christopher Gabel.

663B. Serotonergic and peptidergic signaling interact to modulate aversive behavior. **Holly Mills**, Amanda Ortega, Richard Komuniecki.

664C. Alkaline pH sensation mediated by GCY-14, a transmembrane guanylyl cyclase. **T. Murayama**, M. Fujiwara, J. Takayama, I. Maruyama.

665A. A longitudinal study of *C. elegans* larvae reveals a novel locomotion switch, regulated by $G_{\alpha s}$ signaling. **Stanislav Nagy**, Charles Wright, Nora Tramm, Nicholas Labello, Stanislav Burov, David Biron.

666B. Identification of New Genes Involved in *C. elegans* Thermotaxis Behavior. **Shunji Nakano**, Jiang Tianyu, Takamasa Suzuki, Tetsuya Higashiyama, Ikue Mori.

667C. The CMK-1 CaMKI protein integrates food signals to regulate sensory neuron state. **Scott J. Neal**, Kyuhyung Kim, Piali Sengupta.

668A. A new imaging system for high-throughput C. elegans analysis of temperature-entrained rhythmic gene expression. **Dru Charles Nelson**, Ari Winbush, Alexander van der Linden.

669B. NLP-22 is a Neuromedin S-like neuropeptide which regulates behavioral quiescence. **Matthew Nelson**, David Raizen.

670C. The effect of sex difference on olfactory learning in *Caenorhabditis elegans*. **Julia Nguyen**, Shane Smith*, Gareth Harris, Yun Zhang.

671A. Isoform-specific axonal translocation of a novel DAF-2 isoform regulates synaptic and behavioral plasticity. **Hayao Ohno**, Shinya Kato, Yasuki Naito, Hirofumi Kunitomo, Masahiro Tomioka, Yuichi Iino.

672B. Genetically imposed dietary restriction makes normally appetitive food repulsive by altering odor-sensing circuits. **Birgitta Olofsson**.

673C. The Role of Post-Translational Modifications in the Regulation of Serotonin Signalling. **Andrew C. Olson**, Michael R. Koelle.

674A. Remote Control and Observation for more meaningful Behavioral Experiments. **Andy Papp**, John Biondo.

675B. Genetic screens for IL2 lineage-specific regulators in *Caenorhabditis elegans*. **Dongjun Park**, Peter Swoboda, Junho Lee.

676C. Regulation of the Egg-Laying Behavioral Response to Hypoxia. **Corinne Pender**, Bob Horvitz.

677A. A male-specific neuropeptide, FLP-23, is necessary for sperm transfer in *C. elegans*. Renee Miller, Inna Hughes, Teigan Ruster, Andrew Spitzberg, Steven Husson, Tom Janssen, Liliane Schoofs, **Douglas Portman**.

678B. A Potential Role for Palmitoylation in the Acute Response to

Ethanol. **R. Raabe**, A. G. Davies, J. C. Bettinger.

679C. PKC-1 mediates responses to 5-HT-dependent behavioral adaptation in *C. elegans*. **Seth Ronk**, Eric Foss, Lucinda Carnell.

680A. Internal metabolic status modulates pheromone-mediated neural plasticity in *C. elegans*. **L. Ryu**, K. Kim.

681B. Identification of molecules interacting with the insulin/PI3K pathway involved in salt chemotaxis learning. **N. Sakai**, M. Tomioka, T. Adachi, T. Jiang, Y. Iino.

682C. Regulation of Behavioral Suspended Animation and Mitochondrial Dynamics in Response to Oxygen Deprivation. **N. Salazar-Vasquez**, P. Ghose, E. C. Park, A. Tabakin, C. Rongo.

683A. The *C. elegans* interneuron ALA is a nociceptor. **Jarred Sanders**, Stanislav Nagy, Graham Fetterman, Charles Wright, David Biron.

684B. G protein coupled receptor SRTX-1 is a key component for thermosensation in AFD ensuring temperature sensation range. **Hiroyuki Sasakura**, Hiroko Ito, Kyogo Kobayashi, Keita Suzuki, Ikue Mori.

685C. Control of sleep-like behavior by the G-alpha(q) gene *egl-30*. **Juliane Schwarz**, Henrik Bringmann.

686A. Identifying Novel BK Channel Modulators. **Luisa Scott**, Sangeetha Iyer, Scott Davis, Ashley Philpo, Angela Shen, Sarah Nordquist, Jon Pierce-Shimomura.

687B. The *C. elegans* male regulates movement direction during mating through cholinergic control of the sex-shared command cells. **Amrita Laxman Sherlekar**, Abbey Janssen, Meagan Siehr, Laura Caflisch, May Boggess, Robyn Lints.

688C. Is sleep conserved? Making the case in *D. melanogaster* and *C. elegans*. **Komudi Singh**, Jennifer Y. Ju, Melissa B. Walsh, Michael A. DiIorio, Anne C. Hart.

689A. Expanding the Spectrum of Dopamine Regulators: Swip-10, the (m)BLAC Sheep of the Family. **C. Snarrenberg**, S. Whitaker, Q. Han, E. Pohl, J. A. Hardaway, R. D. Blakely.

690B. Quantitative trait loci mapping of temperature-dependent behaviour in *Caenorhabditis briggsae*. **Gregory W. Stegeman**, Asher D. Cutter, William S. Ryu.

691C. Odorant choice behavior and systematic reverse genetics approach to reveal molecular mechanisms underlying the behavior. **Y. Suehiro**, S. Mitani.

692A. Sensory inputs are centrally integrated to modulate nociception in *Caenorhabditis elegans*. **Philip Summers**, Amanda Ortega, Richard Komuniecki.

693B. Exploring the role of *rapsyn-1* in regulating *C. elegans* behavior. **Ada Tong**, Sreekanth Chalasani.

694C. Dopaminergic control of gait switching in C. elegans. **Stephen M. Topper**, Sara Aguilar, Layla Young, Jonathan Pierce-Shimomura.

695A. Ethanol induces state-dependent behavioral transition. **Stephen M. Topper**, Sara Aguilar, Layla Young, Andres Vidal-Gadea, Jonathan Pierce-Shimomura.

696B. Identification of genes involved in the pheromone signaling that regulates olfactory plasticity. **H. Toriyabe**, K. Yamada, Y. Iino.

697C. Why do sleeping worms look like hockey sticks? **Nora Tramm**, Naomi Oppenheimer, Efraim Efrati, Stanislav Nagy, David Biron.

698A. The roles of biogenic amines on feeding state-dependent thermotactic behavior in *C. elegans*. **Satomi Tsukamoto**, Shunji Nakano, Ikue Mori.

699B. Caenorhabditis elegans can detect and avoid from rare earth ions, which have toxic effects on the worm locomotion, growth, and development. **Tokumitsu Wakabayashi**, Yuta Nakano, Yui Nojiri, Miwa Watanabe, Hiroshi Tomita.

700C. Small Molecule Communication: C. elegans and bacterial chemical signals. **Kristen Werner**, Lark Perez, Martin Semmelhack, Bonnie Bassler.

701A. The monoamine neurotransmitter serotonin shows evolutionarily divergent effects on feeding behavior in *Pristionchus pacificus*. **Martin Wilecki**, James W. Lightfoot, Ralf J. Sommer.

702B. Root-knot Nematode Behavior, Pheromones, and Genetics. **Valerie Williamson**, George Bruening, Jacinta Gimeno, Sylwia Fudali, Frank Schroeder.

703C. Genetic analysis of dopamine signaling for repulsive odor learning. **Shuhei Yamazaki**, Kotaro Kimura.

704A. A forward screen to identify genes involved in the blockage of olfactory adaptation by food in *C. elegans*. Amanda Cha, Ghazal Ghafari, Laine Janzen, Stephanie Summers, Pilar Stinson, Marie Engelhardt, Kelsi Kettellapper, Jamie Knight, Noelle L'Etoile, **Jared Young**.

705B. Investigating the neural mechanisms underlying a hypertonic response in Caenorhabditis elegans. **Jingyi Yu**, Yun Zhang.

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706C. Neurotoxic unc-8 mutants encode constitutively active DEG/ENaC channels that are blocked by divalent cations. Ying Wang, Lu Han, Cristina Matthewman, Tyne Miller, David Miller, **Laura Bianchi**.

707A. A circuit for working memory in *C. elegans*. **Adam Calhoun**, Tatyana Sharpee, Sreekanth Chalasani.

708B. Protein with tau-like repeats regulates neuronal integrity and lifespan in *C. elegans.*. **Yee Lian Chew**, Xiaochen Fan, Jürgen Götz, Hannah Nicholas.

709C. Regulation of Coincident Activity is an Efficient Strategy for

Reversible Modulation of Arousal in *C. elegans* Sleep. **Julie Cho**, Paul Sternberg.

710A. *In vivo* optical recording of action potentials in *C. elegans* body wall muscles using the voltage sensitive fluorescent protein ArcLight. Liping He, Julian Wooltorton, Brian Salzberg, **Chris Fang-Yen**.

711B. Transport mechanisms involved in pH regulation of *C. elegans* amphid sheath glia. **Jeff Grant**, Rachele Sangaletti, Laura Bianchi.

712C. Imaging the brain. Wafa Amir, Nicholas Swierczek, **Rex A. Kerr**.

713A. Temperature experience-inducing cold tolerance is regulated by insulin signaling in intestine and neuron. Akane Ohta, Satoru Sonoda, Tomoyo Ujisawa, Yuko Kobayashi, Hayato Nakamoto, **Atsushi Kuhara**.

714B. Mechanosensitive innexin channels in *C. elegans* touch neurons. **R. Sangaletti**, L. Bianchi.

715C. A Genome Wide Analysis of Na^{+}/Ca^{2+} Exchanger Genes in *C. elegans*. **Vishal Sharma**, Chao He, Julian Sacca-Schaeffer, Eric Brzozowski, Damien O'Halloran.

716A. Isolating genes for temperature experience-dependent cold tolerance. **Satoru Sonoda**, Yukari Kinoshita, Shoko Furukawa, Mikiko Endo, Yushuke Uehara, Akane Ohta, Atsushi Kuhara.

717B. A single neuron class with contrasting sensory tuning curves enables sex-specific attraction in *C. elegans*. **Jagan Srinivasan**, Anusha Narayan, Omer Durak, Neelanjan Bose, Frank C. Schroeder, Paul W. Sternberg.

718C. Screening a Million Mutations to Identify Novel Ciliary Proteins. **Tiffany A. Timbers**, Victor L. Jensen, Katherine Lee, Stephanie Garland, Mark Edgley, Donald G. Moerman, Michel R. Leroux.

719A. System identification for thermosensory neuron encoding thermal environment. **Y. Tsukada**, N. Honda, A. Murase, T. Shimowada, O. Noriyuki, A. Kuhara, S. Ishii, I. Mori.

720B. Photo and pheromone sensoryneuron regulates temperature experience-dependent cold tolerance. **Tomoyo Ujisawa**, Satoru Sonoda, Tomohiro Ishiwari, Akane Ohta, Atsushi Kuhara.

721C. BBS-4 and BBS-5 function redundantly to regulate IFT recycling in cilia. **Yuxia Zhang**, Qingwen Xu, Yan Hang, Qing Wei, Qing Zhang, Yujie Li, Zeng Hu, Kun Ling, Jinghua Hu.

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722A. Identification and characterisation of novel genes involved in the development of the *C. elegans* stem-like seam cells. **Peter J. Appleford**, Alison Woollard.

723B. Systematic quantification of developmental phenotypes at single-cell resolution during embryogenesis. Julia Moore, Zhuo Du, **Zhirong Bao**.

724C. Robustness of the vulval cell fate pattern to pathway dosage modulation and cryptic evolution of *lin-3* regulatory sequences. **Michalis Barkoulas**, Alexandre Peluffo, Marie-Anne Félix.

725A. Exploring the genetic regulation of a stochastic cell decision using Mutation Accumulation lines in C. elegans and C. briggsae. **Fabrice BESNARD**, Marie-Anne Félix.

726B. Environmental flexibility of C. elegans vulval signalling pathways. Stephanie Grimbert, **Christian Braendle**.

727C. Muscles from Ectoderm: Possible Cases of *in vivo* Reprogramming. **Kirk B. Burkhart**, Nick Burton, Shuo Luo, Bob Horvitz.

728A. Y39G10AR.7 is a putative MPK-1 Erk target during excretory duct cell fate specification. **Preston Chin**, Phil Cheng, Christian Rocheleau.

729B. An RNAi screen for maternal factors influencing endoderm specification. **Hailey H. Choi**, Morris F. Maduro.

730C. Characterization of a BMP negative regulator in the extracellular matrix. Y. J. Ang, **King-Lau Chow**.

731A. The role of the claudin-like gene *nsy-4* in *C. elegans* sensory ray development. **King L. Chow**, Kei C. Fan.

732B. Transcriptional Regulation of the Hox gene *lin-39* by LIN-31, a Winged-Helix Transcription Factor Involved in C. elegans Cell Fate Specification. **A. Dewey**, F. Meza Gutierrez, C. Morris-Singer, L. Miller.

733C. Elucidating the role of *nmy-2* in seam cell division patterns. **Siyu S. Ding**, Peter J. Appleford, Alison Woollard.

734A. Imaging developmental landscape of *C. elegans* embryos. **Zhuo Du**, Anthony Santella, Fei He, Michael Tiongson, Zhirong Bao.

735B. The *let-7* microRNA is dispensable for early vulva cell fate specification, but is required for later stages of vulva development. **Matyas Ecsedi**, Helge Grosshans.

736C. Suppressors of *pos-1* identify a novel function for GLP-1 and new players, *gld-3*, cyclin E and *spos-1*, involved in endoderm specification. **Ahmed Elewa**, Masaki Shirayama, Sandra Vergara, Takao Ishidate, Craig Mello.

737A. Defining genetic pathways of disease through genetic suppression screening in *C. elegans*. **Amy Fabritius**, Andy Golden.

738B. Asymmetric regulation of the human VAX ortholog homeobox gene ceh-5 during early neurogenesis in Caenorhabditis elegans. **Umesh Gangishetti**, Lois Tang, Johan Henriksson, Thomas R. Burglin.

739C. Investigating the role of SEM-4/SALL in development of the postembryonic mesoderm. **Vikas Ghai**, Chenxi Tsian, Jun Liu.

740A. Defining the role of the *Caenorhabditis elegans* homeobox protein, PAL-1, in the development of the stem-like seam cells. **Sophie P. R. Gilbert**, Charles Brabin, Peter J. Appleford, Alison Woollard.

741B. When two become one: Sperm-egg fusion during *C. elegans* fertilization. **Boaz Gildor**, Meital Oren, Benjamin Podbilewicz.

742C. The Mediator subunit CDK-8 is a dual negative/positive regulator of EGFR-Ras-MAPK signaling. **Jennifer M. Grants**, Lisa TL Ying, Stefan Taubert.

743A. Genetic analysis of vulval development in *C. briggsae*. **Bhagwati P. Gupta**, Devika Sharanya, Bavithra Thillainathan, Cambree J. Fillis, Kelly A. Ward, Edward M. Zitnik, Molly E. Gallagher, Helen M. Chamberlin.

744B. A role for UNC-55 in AS motor neuron subtype specification. **Michael Hart**, Oliver Hobert.

745C. Identification of *hcf-1* as a *dsh-2* suppressor. Kyla Hingwing, Tammy Wong, Jack Chen, **Nancy Hawkins**.

746A. HAM-1: An asymmetrically localized transcriptional regulator? **Khang Hua**, Amy Leung, Maria Wu, Nancy Hawkins.

747B. The small GTPase Ral signals via an Exocyst-GCK-2/MAP4K-p38-MAPKAPK cascade. **Rebecca E. W. Kaplan**, Channing J. Der, David J. Reiner.

748C. MEX-5 positively regulates *mex-3* mRNA at anterior blastomere in early *C. elegans* embryo. **Hiroyuki Konno**, Koki Noguchi, Yuji Kohara.

749A. Structure-function of SYS-1/β-catenin, an effector of Wnt-directed asymmetric cell divisions. **Koon Yee Lam**, Michael Molumby, Jennifer Hutchinson, Lori Adams, Bryan Phillips.

750B. Towards sequencing key cells of the developing Caenorhabditis elegans vulva. **James Lee**, Pei Shih, Paul Sternberg.

751C. UNC-40 positively modulates BMP signaling independent of netrin signaling. Chenxi Tian, Herong Shi, Shan Xiong, Fenghua Fu, Wen-Cheng Xiong, **Jun Liu**.

752A. Regulation of serotonergic neuron patterning in *C. elegans* by Wnt signaling genes. **Curtis M. Loer**, Erin Williams.

753B. The role of the *C. elegans* Jarid1 histone lysine demethylase RBR-2 in vulva cell fate determination. **Yvonne C. Lussi**, Toshia R. Myers, Anna Elisabetta Salcini.

754C. Animal to Animal Variability During Vulval Cell Fate Specification. **Sabrina Maxeiner**, Daniel Roiz, Alex Hajnal.

755A. Dynein Heavy Chain-1: a novel negative regulator of LET-23 EGFR induced vulva induction. **Jassy Meng**, Olga Skorobogata, Christian Rocheleau.

756B. Control of LET-23 localization by PRMT-1 during vulval development. **Sabrina Kathrin Merkle**, Juan Miguel Escobar-Restrepo, Tobias Schmid, Fabienne Largey, Alex Hajnal.

757C. The role of Wnt and FGF signaling in *C. elegans* vulval cell lineage polarity. **Paul Minor**, Paul Sternberg.

758A. Role of the $CRL2^{LRR-1}$ ubiquitin ligase in regulating LIN-12/Notch signaling in *C. elegans* vulva development. **Madhumati Mukherjee**, Edward T. Kipreos.

759B. Tousled-like Kinase Is Required to Generate a Bilateral Asymmetry in the *C. elegans* Nervous System. **Shunji Nakano**, Bob Horvitz, Ikue Mori.

760C. TORC2 Signaling Antagonizes SKN-1 to Induce C. elegans Mesendodermal Embryonic Development. Vanessa Ruf, Christina Holzem, Tobias Peyman, Gerd Walz, T. Keith Blackwell, **Elke Neumann-Haefelin**.

761A. Identification of mechanisms by which the expression of *lim-4* homeodomain gene is regulated to specify the SMB motor neuron fate. **Jisoo Park**, Jihye Yeon, Kyuhyung Kim.

762B. SOS-1 is required for remodeling epithelial junctions during the G1 excretory pore cell's transition from epithelial tube to neuroblast. **Jean Parry**, Meera Sundaram.

763C. Cell Fate Restriction and Reprogramming in *C. elegans*. **Tulsi Patel**, Oliver Hobert.

764A. Regulation of Post-embryonic Seam Cell Proliferation and Identity by the Non-Receptor Tyrosine Kinase, FRK-1. **Aaron Putzke**, Danielle Mila, Katherine Genzink, McLane Watson, Caroline Askonas, Kelsey Moore.

765B. Multiple Aspects of C. elegans Germ Cell Development are Regulated by XND-1. **Mainpal Rana**, Judith Yanowitz.

766C. Netrin expression in *P. pacificus*. Brent Wyatt, Kelly Mahoney, **David Rudel**.

767A. A *C. elegans* FerT Kinase Regulates Developmental Cell-Cell Fusion to Direct Cell Identity. **R. Mako Saito**, David Tobin, Sarah Roy.

768B. Differential regulation of HLH-2/E2A stability during gonadogenesis in *C. elegans* . **Maria Sallee**, Iva Greenwald.

769C. Quantitative proteome analysis of maternal gene *spn-4* mutant in *Caenorhabditis elegans*. **Aimi Tomita**, Yukako Toshato, Toshiya Hayano, Masahiro Ito.

770A. Role of *sox-2* in postembryonic lineage progression. **Berta Vidal Iglesias**, Oliver Hobert.

771B. Centrosomal localization of SYS-1/beta-catenin is required for proper expression patterns during asymmetric cell division. **Setu M. Vora**, Bryan T. Phillips.

772C. ceh-36 regulates cell fate patterning during embryogenesis. **Travis Walton**, John Murray.

773A. Wnts and VANG-1/Van Gogh control cell fates in the Q lineage. **Falina J. Williams**, Jerome Teuliere, Gian Garriga.

774B. Glucose 6-phosphate dehydrogenase (G6PD) deficiency impairs early embryogenesis in C. elegans. **Hung-Chi Yang**, Meng-Hsin Ou, Szecheng J. Lo, Daniel Tsun-Yee Chiu.

775C. Single-blastomere transcriptome profiling after the first embryonic division. Erin Osborne Nishimura, **Jay C. Zhang**, Adam Werts,

Bob Goldstein, Jason D. Lieb.

776A. Genome-wide landscape of hybrid incompatibility (HI) between *Caenorhabditis briggsae* and *C.* sp.9 . **Zhongying Zhao**, Cheung Yan, Yu Bi, Xiaoliang Ren, Dongying Xie.

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777B. Perturbation of NAD+ salvage biosynthesis causes a distinct death program in a neuroendocrine cell. **Awani Awani**, Matt Crook, Wendy Hanna-Rose.

778C. A *C. elegans* model for TDP-43-induced motor neuron pathology. **J. C. Chaplin**, M. Mangelsdorf, R. Narayanan, R. Wallace, M. A. Hilliard.

779A. Elucidating the mechanism by which C.elegans KRI-1 regulates damaged-induced germline apoptosis. **Eric M. Chapman**, W. Brent Derry.

780B. Using *Caenorhabditis elegans* to Fight Human Neurodegenerative Diseases. **Xi Chen**, Brian C. Kraemer, Jeff Barclay, Robert D. Burgoyne, Alan Morgan.

781C. C. elegans peroxidasin, pxn-1 is essential for epidermal attachment of muscles and neurons. **Jeong H. Cho**, Juyeon Lee.

782A. Analysis of the Function and Dysfunction of the Human Amyotrophic Lateral Sclerosis Gene *C9ORF72* Using *C. elegans*. **Anna Corrionero**, Bob Horvitz.

783B. Blocking NAD+ salvage biosynthesis sensitizes specific mechanosensory neurons to nutritional conditions and predisposes them to death. **Matt Crook**, Wendy Hanna-Rose.

784C. EGF and phosphocholine; a novel mechanism to prevent necrosis. **Matt Crook**, Wendy Hanna-Rose.

785A. BRAP-2 is necessary for the regulation of DNA damage induced germ line apoptosis in *C. elegans*. **Dayana R. D'Amora**, Terrance J. Kubiseski.

786B. The Possible Role of Autophagic Cell Death in the Regulation of Excitotoxicity in *C. elegans*. **John S. Del Rosario**, Towfiq Ahmed, JunHyung An, Tauhid Mahmud, Itzhak Mano.

787C. Investigation of SMN1 structure-function relationship in neuronal degeneration. **A. Donato**, I. Gallotta, G. Battaglia, M. A. Hilliard, P. Bazzicalupo, E. Di Schiavi.

788A. *ced-11* is Required for the Morphological Appearance of Apoptotic-Cell Corpses. **Kaitlin B. Driscoll**, Gillian Stanfield, Bob Horvitz.

789B. Diapause protects neurons from degeneration and promotes axonal regrowth. **Andres Fuentes**, Andrea Calixto.

790C. UNC-105 activation causes mitochondrial dysfunction and CED-4 dependent caspase-mediated protein degradation in terminally differentiated *C. elegans* muscle. **C. J. Gaffney**, F. Shephard, J. Chu, D. L. Baillie, A. Rose, D. Constantin-Teodosiu, P. L. Greenhaff, N. J. Szewczyk.

791A. Neurodegeneration and death induced by neuron-specific knock-down of *smn-1*, the homolog of the gene responsible for Spinal Muscular Atrophy. **I. Gallotta**, A. Donato, N. Mazzarella, P. Bazzicalupo, M. Hilliard, E. Di Schiavi.

792B. Chronic alcohol exposure induces toxicity and neurodegeneration in C. *elegans*. **Lina Gomez**, Sangeetha Iyer, Ashley Crisp, Jesse Cohn, Jon Pierce-Shimomura.

793C. Functional analysis of VPS41-mediated protection from β-Amyloid cytotoxicity. **Edward F. Griffin**, Christopher Gilmartin, Kim A. Caldwell, Guy A. Caldwell.

794A. SGK-1 promotes germline apoptosis by a cell non-autonomous mechanism. **Madhavi Gunda**, W.Brent Derry.

795B. The Sodium-Potassium ATPase alpha subunit EAT-6 promotes programmed cell death. **Tsung-Yuan Hsu**, Meng- I. Lee, Yi-Chun Wu.

796C. Innate immune signaling protects against patterned neurodegeneration in Alzheimer's disease. **Sangeetha V. Iyer**, Ashley Crisp, Anushri Kushwaha, Jon Pierce-Shimomura.

797A. Sirtuin Mediated Neuroprotection and its Association with Autophagy and Apoptosis: Studies Employing Transgenic C. elegans Model. **Pooja Jadiya**, Aamir Nazir.

798B. LIN-3/EGF promotes programmed cell death by transcriptional activation of the pro-apoptotic gene *egl-1*. **Hang-Shiang Jiang**, Yi-Chun Wu.

799C. Assisted Suicide: a Caspase- and Engulfment-Dependent Cell Death. **Holly Johnsen**, Bob Horvitz.

800A. Modulation of *mec-10(d)*-induced necrosis by ER chaperone NRA-2. **Shaunak Kamat**, Shrutika Yeola, Monica Driscoll, Laura Bianchi.

801B. Mutations in progranulin and cell death genes confer organismal stress resistance. M. Judy, A. Nakamura, H. McCurdy, A. Huang, H. Grant, C. Kenyon, **A. Kao**.

802C. A C. elegans model of Adult-onset Neuronal Ceroid Lipofuscinosis reveals a sir-2.1 independent protective effect of resveratrol. **Sudhanva Kashyap**, James Johnson, Mimi Ayala, Jeff Barclay, Bob Burgoyne, Alan Morgan.

803A. Post-transcriptional control of C. elegans germ cell apoptosis by RNA-binding proteins. **Martin Keller**, Michael O. Hengartner.

804B. Investigating neuroprotecive genes on *S. venezuelae* toxicity in *C. elegans*. **H. Kim**, G. A. Caldwell, K. A. Caldwell.

805C. Using artificial insemination to identify genes involved in linker cell death and corpse removal. **Lena M. Kutscher**, Nima Tishbi, Shai Shaham.

806A. Identification of interacting partners of a poly-glutamine protein involved in non-apoptotic cell death. **Lena M. Kutscher**, Shai Shaham.

807B. Necrotic Cells Share a Similar Mechanism with Apoptotic Cells in being Recognized by Engulfing Cells in *C. elegans*. **Zao Li**, Victor Venegas, Prashant Raghavan, Yoshinobu Nakanishi, Zheng Zhou.

808C. A small metabolite isolated from *Streptomyces venezuelae* enhances age-dependent proteotoxic stress in *C. elegans* models of neurodegenerative diseases. **B. A. Martinez**, A. Ray, G. A. Caldwell, K. A. Caldwell.

809A. Unraveling the role of MOAG-4 in protein aggregation. A. T. van der Goot, **A. Mata-Cabana**, E. Stroo, E. A. Nollen.

810B. Neuronal expression of wild-type and A152T mutant tau cause distinct patterns of toxicity in *C. elegans*. **Helen L. McCurdy**, D. Cox, B. Bliska, Aimee W. Kao.

811C. Neuronal Exophers: a Novel Mechanism for Removal of Neurotoxic Cytoplasm Components. **Ilija Melentijevic**, Marton Toth, Christian Neri, Monica Driscoll.

812A. Progressive degeneration of dopaminergic neurons through TRP channel-induced necrosis. **Archana Nagarajan**, Ye Ning, Oliver Hobert, Maria Doitsidou.

813B. MEC-17 protects from axonal degeneration, maintaining mitochondrial organization and axonal transport. **Brent Neumann**, Massimo Hilliard.

814C. Axonal degeneration in *C. elegans* proceeds independently from the WldS pathway. **Annika L. A. Nichols**, Brent Neumann, Ellen Meelkop, Massimo A. Hilliard.

815A. Cell-Nonautonomous Inhibition of Radiation-Induced Apoptosis by Dynein Light Chain 1 in *Caenorhabditis elegans*. Tine H. Møller, Anna Dippel Lande, **Anders Olsen**.

816B. TPPP/p25α causes degeneration of dopaminergic neurons in *C.elegans*. Katrine Christensen, Lotte Vestergaard, Rikke Kofoed, **Anders Olsen**.

817C. Bacterial metabolite causes mitochondrial dysfunction and oxidative stress in a *C. elegans* Parkinson's disease model. **A. Ray**, B. A. Martinez, G. A. Caldwell, K. A. Caldwell.

818A. A Small-Molecule Screen for Linker Cell Death Inhibitors. **Andrew Schwendeman**, Shai Shaham.

819B. *C. elegans* clathrin and its adaptor epsin promote apoptotic-cell engulfment through regulating cytoskeleton remodeling. **Qian Shen**, Bin He, Nan Lu, Barbara Conradt, Barth D. Grant, Zheng Zhou.

820C. Glucose influences aging, proteotoxicity and stress response in *C. elegans*. **Arnaud Tauffenberger**, Alexandra Vaccaro, J. Alex Parker.

821A. Cortical HAM-1 positions the cleavage furrow in myosin-dependent asymmetric neuroblast divisions that generates apoptotic cells. **Jerome Teuliere**, Nancy Hawkins, Gian Garriga.

822B. The DNA binding protein *dpff-1* is required to trigger starvation-induced germ cell apoptosis. **Angel E. Villanueva Chimal**, Rosa E. Navarro.

823C. Characterization of a Chloride-Mediated Cell Death Pathway in *C.elegans* PLM Neurons. **Claudia M. Wever**, Aamna Kaul, Miles Byworth, Joseph A. Dent.

824A. Genetic modifiers of amyloid-beta toxicity in *C. elegans* Alzheimer's disease models. **Xiaohui Yan**, Adam L. Knight, Kim A. Caldwell, Guy A. Caldwell.

825B. Comparisons of three *C. elegans* DNase II activities *in vitro* and *in vivo*. **Hsiang Yu**, Szecheng J. Lo.

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826C. Functional characterization of *lin-41* and its targets. **Hrishikesh Bartake**.

827A. Activity and functional interactions of the leucine-rich protein PAN-1 during larval development. **Derrick L. Cardin**, Chris R. Gissendanner.

828B. Roles of *C. elegans* LIN-28 in hermaphrodite fertility and embryonic viability. **Sungwook Choi**, Anna Zinovyeva, Victor Ambros.

829C. Significant Transcription in Zygote Pronuclei and 1-4 Cell Embryos Drives Early Development in the Nematode, *Ascaris suum*. **Richard E. Davis**, Julianne Roy, Jianbin Wang.

830A. Regulation of Developmental Timing and Cell-Fate Determination by MAB-10 and LIN-29. **Akiko Doi**, Bob Horvitz.

831B. A *lin-42* null allele: highly penetrant defects in developmental timing and molting. **Theresa L. B. Edelman**, Katherine A. McCulloch, Angela Barr, Christian Frøkjaer-Jensen, Erik M. Jorgensen, Ann E. Rougvie.

832C. Hypodermis integrates nutrient signaling to regulate blast cell quiescence. **M. Fukuyama**, K. Kontani, A. Rougvie, T. Katada.

833A. Dynamically-expressed prion-like proteins form a cuticle in the pharynx of *Caenorhabditis elegans*. **J. B. George-Raizen**, K. R. Shockley, A. L. Lamb, D. M. Raizen.

834B. LEP-2/Makorin represses LIN-28 to keep nematode tail tip differentiation on schedule. **R. Antonio Herrera**, Karin Kiontke, David Fitch.

835C. The *C. elegans* plasminogen/HGF-like protein SVH-1 is required for larval developmental growth. **Naoki Hisamoto**, Motoki Yoshida, Chun Li, Kunihiro Matsumoto.

836A. BLMP-1/BLIMP1 - a novel substrate of the DRE-1/FBXO11 SCF complex that regulates *C. elegans* developmental timing. **Moritz Horn**, Christoph Geisen, Adam Antebi.

837B. Ascaroside signals suppress heterochronic phenotypes of the *daf-12(rh61)* mutant. **Orkan Ilbay**, Zhiji Ren, Jagan Srinivasan, Frank C.

Schroeder, Victor Ambros.

838C. Post-dauer regulation of developmental timing. Stephen Domingue, Benjamin Prout, **Xantha Karp**.

839A. Heterochronic gene *lin-46*: protein expression and interaction with HBL-1. **Kevin Kemper**, Bhaskar Vadla, Eric G. Moss.

840B. A comparative analysis of the genetic basis of molting in the necromenic nematode Pristionchus pacificus. **Victor Lewis**, Maryn Cook, Justin Alonso, Ray Hong.

841C. Low population density increases lifespan and delays egg laying of *C. elegans* hermaphrodites. **Andreas H. Ludewig**, Frank C. Schroeder, Frank Doering.

842A. The *C. elegans period* homolog *lin-42* regulates the timing of heterochronic miRNA expression. **K. A. McCulloch**, A. E. Rougvie.

843B. Insulin-regulated nutritional checkpoints in post-dauer *C. elegans* larval development. **Adam J. Schindler**, L. Ryan Baugh, David R. Sherwood.

844C. Transcription factors involved in dauer recovery. **Pei-Yin Shih**, Paul W. Sternberg.

845A. What is *lin-28*'s *let-7* independent mechanism? **Jennifer Tsialikas**, Bhaskar Vadla, Kevin Kemper, Eric Moss.

846B. A Quantitative Approach Reveals the Conditional Role of *elt-7* in the *C. elegans* Intestinal Specification Network. **Allison Wu**, Scott Rifkin.

847C. A high-throughput genetic screen for lethargus mutants. **C. Yu**, M. Churgin, D. Raizen, C. Fang-Yen.

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848A. CYP35A3-GFP induction and reproductive toxicity of environmental samples in *C.elegans*. **A. Abbas**, L. Valek, J. Oehlmann, M. Wagner.

849B. GLD-1 Expression During Germline Development. **Jennifer R. Aleman**, Sudhir B. Nayak.

850C. Role of autophagy genes in *C.elegans* germline proliferation. **K. Ames**, A. Meléndez.

851A. Anillin promotes syncytial organization and maintenance of the *C. elegans* germline. **Rana Amini**, Sara Labella, Monique Zetka, Amy S. Maddox, Nicolas T. Chartier, Jean-Claude Labbé.

852B. Structural characterization of the P-granule protein scaffold. **Scott Takeo Aoki**, Judith Kimble.

853C. FOG-1 and FOG-3, their mRNA targets and the sperm/oocyte fate decision. Daniel Noble, Marco Ortiz, **Scott Aoki**, Kyung Won Kim, Judith Kimble.

854A. Homeodomain interacting protein kinase (HPK-1) is required in the soma for robust germline proliferation in *C. elegans*. **S. Berber**, E. Llamosas, P. Boag, M. Crossley, H. Nicholas.

855B. Identification of direct targets of the *Caenorhabditis elegans* global sexual regulator TRA-1 by ChIP-seq. **Matt Berkseth**, Kohta Ikegami, Jason Lieb, David Zarkower.

856C. The Role of Sperm Specific PP1 Phosphatase GSP-3/4 in Kinetochore Localization and Function During Spermatogenesis. **Joseph Beyene**, Diana Chu, PhD.

857A. Exploring miRNA function in the proliferation versus meiosis decision in the *C. elegans* germline. **John L. Brenner**, Gavriel Y. Matt, Tim Schedl.

858B. Distal tip cell processes provide extensive contact between the germline stem cell pool and its cellular niche. **Dana T. Byrd**, Karla Knobel, Katharyn Schmitt, Sarah L. Crittenden, Judith Kimble.

859C. PUF-8 controls mitochondrial biogenesis and apoptosis in the germline. **A. Chaturbedi**, G. Anil Kumar, M. Ariz, K. Subramaniam.

860A. Timing is everything: dissecting the male sperm activation pathway. **Daniela Chavez**, Joseph Smith, Angela Snow, Gillian Stanfield.

861B. The Nucleosome Remodeling Factor complex controls germ cell fates in *C. briggsae*. **Xiangmei Chen**, Ronald E. Ellis.

862C. An importin β controls the sperm/oocyte decision in *C. briggsae*. **Xiangmei Chen**, Greg Minevich, Yongquan Shen, Alexander Boyanov, Oliver Hobert, Ronald E. Ellis.

863A. Control of cell cycling speed to minimize mutation accumulation. Michael Chiang, Amanda Cinquin, **Olivier Cinquin**.

864B. Progress on developing the Q system to study GSCs and their control. **Sarah L. Crittenden**, Ipsita Mohanty, Judith Kimble.

865C. Giving Light to Sperm-Specific Phosphatases. **Tyler S. Curran**, Leslie Mateo, Diana Chu.

866A. Signaling pathways that mediate the deleterious effects of dietary fatty acids in the germ line. **Marshall Deline**, Jennifer L. Watts.

867B. A Quality Control Mechanism Coordinates Meiotic Prophase Events. **Alison J. Deshong**, Alice L. Ye, Piero Lamelza, Needhi Bhalla.

868C. GLD-4, a cytoplasmic poly(A) polymerase, is part of a translational feedback loop regulating stem cell pool size and meiotic entry in the adult *C. elegans* germ line. Sophia Millonigg, Ryuji Minasaki, Marco Nousch, **Christian R. Eckmann**.

869A. Gamma Secretase Function During Germline Development. **Cassandra Farnow**, Ipsita Agarwal, Caitlin Greskovich, Caroline Goutte.

870B. Nuclear Envelope Components and Dynein act Coordinately with MEL-28 to Promote Post-Embryonic Development. **Anita G. Fernandez**, Allison Lai, Carly Bock, Angela Quental, Mike Mauro, Emily Mis, Fabio

Piano.

871C. SPCH-1/2/3 localize to mature sperm chromatin and may play a role in fertility and genome stability. **Jennifer Gilbert**, Dana Byrd, Jordan Berry, Diana Chu.

872A. Differential expression of germline genes in the presence/absence of H3K9me2. **Yiqing Guo**, Eleanor Maine.

873B. MRG-1 and RFP-1 regulate proliferation in the germline. **Pratyush Gupta**, Lindsay Leahul, Katie Jasper, David Hansen.

874C. The RNA binding protein TIAR-1 is essential for *C. elegans* fertility. **Gabriela Huelgas Morales**, Carlos G. Silva García, Rosa E. Navarro.

875A. Exploring the contribution of chromosomal context in shaping the *C. elegans* high-resolution recombination rate landscape. **Taniya Kaur**, Matthew Rockman.

876B. RNA recognition by OMA-1, a *C. elegans* oocyte maturation determinant. **Ebru Kaymak**, Sean P. Ryder.

877C. Characterization of non-SMC elements of the SMC-5/6 complex in *C. elegans*. **Jayshree Khanikar**, Jaclyn Fingerhut, Jeremy Bickel, Raymond Chan.

878A. Novel spermatogenesis-defective gene candidates. **Takashi Koyama**, Megumi Endo, Yusuke Hokii, Chisato Ushida.

879B. Identifying regulators of sex-specific gonadal development in *C. elegans* by cell-specific RNA-seq. **Mary B. Kroetz**, David Zarkower.

880C. *Y23H5A.4* is a sperm gene that encodes a mitochondrially-associated protein involved in spermatid activation. **Craig W. LaMunyon**, Ubaydah Nasri, Nicholas Sullivan, Jessica Clark.

881A. DNA damage response and spindle assembly checkpoint collaborate to elicit cell cycle arrest in response to replication defects in the *C. elegans* male germ line. **Kate Lawrence**, JoAnne Engebrecht.

882B. Elucidating how TRA-1 promotes spermatogenesis in *C. briggsae*. **Shin-Yi Lin**, Yiqing Guo, Ronald E. Ellis.

883C. Molecular analysis of *ego-3*, an enhancer of *glp-1*. **Jim Lissemore**, Elyse Connors, Ying Liu, Eleanor Maine.

884A. DAF-2 and ERK regulate C. elegans oogenesis as a physiological adaptive response to nutrient availability. **Andrew Lubin Lopez**, Jessica Chen, Hyoe-Jin Joo, Melanie Drake, Miri Shidate, Cedric Kseib, Swathi Arur.

885B. C. elegans p53/p63 protein CEP-1 promotes meiotic recombinational repair. **Abigail Rachele Mateo**, Kristine Jolliffe, Alissa Nicolucci, Bin Yu, Olivia McGovern, Zebulin Kessler, Judith Yanowitz, W. Brent Derry.

886C. Acetylation of H2AK5 and genome instability in *xnd-1* mutants. **Brooke McClendon**, Judith Yanowitz.

887A. Mechanism of Germ Cell Loss by Ionizing Radiation in a *C. elegans* Tumor Model. **David Michaelson**, Xinzhu Deng, Diana Rothenstein, Regina Feldman, Simon Powell, Zvi Fuks, E. Jane Albert Hubbard, Richard Kolesnick.

888B. Temperature Sensitive Fertility of *lin-35* Mutants. **Brian P. Mikeworth**, Lisa N. Petrella.

889C. Spermiogenesis regulation involves multiple sperm cell compartments as revealed through a suppressor screen of *spe-27*. **Ubaydah Nasri**, Misa Austin, Nicholas Sullivan, Craig LaMunyon.

890A. Deletion of ccm-3 in C. elegans promotes increased accumulation of reactive oxygen species resulting in apoptosis of germline cells. **Swati Pal**, Bin Yu, W. Brent Derry.

891B. Selective elimination of male-producing sperm by apoptosis in a nematode. **Manish Parihar**, Sarah Smith, Andre Pires da Silva.

892C. Visualizing dynamics of meiotic prophase chromosome structures. **Divya Pattabiraman**, Baptiste Roelens, Marc Presler, Grace Chen, Anne Villeneuve.

893A. Live imaging reveals active infiltration of mitotic zone by its stem cell niche. **Adrian Paz**, Brandon Wong, Amanda Cinquin, Elliot Hui, Olivier Cinquin.

894B. Regulation of TGFβ signaling in germline stem cell development in *C. elegans*. **O. Pekar**, E. J. A. Hubbard.

895C. Localization dynamics of SPE-6, a sperm-specific CK1 in *C. elegans*. **Jackson Peterson**, Brianna Waller, Diane Shakes.

896A. Ascaroside-mediated sex determination in a nematode with three genders. Vikas Kache, Stephan H. von Reuss, Joshua Yim, Jyotiska Chaudhuri, Christine Bateson, Frank Schroeder, **Andre Pires da Silva**.

897B. Effect of Synapsis Challenges on Meiotic Progression. **Baptiste Roelens**, Susanna Mlynarczyk-Evans, Anne Villeneuve.

898C. Elucidating the role of S6K-Notch interactions in cell fate specification in the *C. elegans* germ line. **Debasmita Roy**, E. Jane Albert Hubbard.

899A. Assembly of RNP granules in *C. elegans* oocytes promotes oocyte quality and is regulated by the cytoskeleton. Megan Wood, Angela Hollis, Kevin Gorman, Joseph Patterson, Ashley Severance, Gregory Davis, Peter Boag, **Jennifer Schisa**.

900B. Nutritional Control of Germline Stem Cells in *Caenorhabditis elegans*. **Hannah S. Seidel**, Judith Kimble.

901C. IFET-1 an eIF4E-binding protein is required for normal P-granules formation and translational regulation of mRNAs. **Madhu S. Sengupta**, Peter R. Boag.

902A. PQN-94 regulates hermaphrodite development by interacting with SHE-1. **Yongquan Shen**, Ronald E. Ellis.

903B. Characterization of SYGL-1, a novel regulator of germline stem cells. **Heaji J. Shin**, Kimberly Haupt, Aaron M. Kershner, Judith Kimble.

904C. The Role of Condensin I during Meiosis. **M. Sifuentes**, K. Colette, G. Csankovszki.

905A. UBC-25 promotes Ras/MAPK signaling to regulate oocyte growth and embryonic morphogenesis in *C. elegans*. **Mideum Song**, Kevin Cullison, Phil Cheng, Meera Sundaram, Christian Rocheleau.

906B. GLP-1/Notch signaling in germline stem cell maintenance. **Erika B. Sorensen**, Amy C. Groth, Judith Kimble.

907C. Loss of UNC-84 in the C. elegans germ line activates the recombination and synapsis checkpoints. **Erin Tapley**, Kate Lawrence, K. C. Hart, JoAnne Engebrecht, Daniel Starr.

908A. The SACY-1 DEAD-box RNA helicase genetically interacts with components of the spliceosome. Seongseop Kim, **Tatsuya Tsukamoto**, David Greenstein.

909B. The Torsin Homolog OOC-5 is Required for Normal Nucleoporin Localization. **Michael J. W. VanGompel**, Sumati Hasani, Lesilee S. Rose.

910C. Cellular machinery promoting FBF-2 regulatory activity. Xiaobo Wang, Dominique Rasoloson, Elle Johnson, **Ekaterina Voronina**.

911A. Asymmetric segregation of P granules requires granule remodeling by two novel serine-rich proteins. **Jennifer T. Wang**, Geraldine Seydoux.

912B. Investigating the cellular mechanisms of skewed sex ratios in non-*C. elegans* nematodes. **Ethan S. Winter**, Diane C. Shakes.

913C. Regulation of Apoptosis by Meiotic Checkpoint Proteins: New Roles for *egl-1* and *ced-13*. **Alice L. Ye**, Matt Ragle, Barbara Conradt, Needhi Bhalla.

914A. MRX/N Commits Homologous Recombination of Meiotic Double-Strand-Breaks by Promoting Resection, Antagonizing Non-Homologous End Joining, and Stimulating EXO-1 in *Caenorhabditis elegans*. **Yizhi Yin**, Sarit Smolikove.

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915B. If we have children together, will they be less fit? Hybrid incompatibilities in *Caenorhabditis* species. Piero Lamelza, Jerome Cattin, Vanessa Wilson, Irini Topalidou, **Michael Ailion**.

916C. Gene movement between X and autosomes and its effect on transcription. **Sarah E. Albritton**, Anna-Lena Kranz, Sevinc Ercan.

917A. Revisiting the effects of spontaneous mutations on the (micro)environmental variance in Caenorhabditis. **Charles F. Baer**, Erik C. Andersen, Reza Farhadifar, Daniel Needleman.

918B. Evolution of a Higher Intracellular Oxidizing Environment in Caenorhabditis elegans Under Relaxed Selection. Joanna Joyner-Matos, Kiley A. Hicks, Dustin Cousins, Michelle Keller, Dee R. Denver, **Charles F. Baer**, Suzanne Estes.

919C. Natural variation and sensory biology of *C. elegans* hermaphrodite control of mating. **Adam K. Bahrami**, Yun Zhang.

920A. The hunt for quantitative trait nucleotides: a near-isogenic line based approach in *C. elegans*. **Max Bernstein**, Matthew Rockman.

921B. Evolution and plasticity of embryo retention in Caenorhabditis nematodes. Paul Vigne, **Christian Braendle**.

922C. Evolution of ZIM proteins in *Caenorhabditis*. **Victoria Cattani**, Matthew Rockman.

923A. Population genomic variation in the outcrossing species *Caenorhabditis remanei*. Cristel G. Thomas, Janna L. Fierst, John H. Willis, Wei Wang, Richard Jovelin, Patrick C. Phillips, **Asher D. Cutter**.

924B. RhabditinaDB: online database for wild worms. **D. Fitch**, K. Kiontke.

925C. Microevolution and coexistence of Santeuil and Le Blanc viruses in *Caenorhabditis briggsae.* . **Lise Frézal**, Marie-Anne Félix.

926A. Evolution of avermectin resistance in *C. briggsae*. **Rajarshi Ghosh**, Cristel Thomas, Wei Wang, Richard Jovelin, Asher Cutter, Leonid Kruglyak.

927B. Strong outbreeding depression and low genetic diversity in the selfing *Caenorhabditis* sp. 11. **Clotilde Gimond**, Richard Jovelin, Shery Han, Celine Ferrari, Asher D. Cutter, Christian Braendle.

928C. Fainting towards Necromeny: Anesthesia caused by a Beetle Pheromone is Mediated by a Lipid-Binding Protein in *Pristionchus pacificus*. **James L. Go**, Jessica K. Cinkornpumin, Margarita Valenzuela, Roonalika D. Wisidagama, Ray L. Hong.

929A. Cross Species NILs - A Resource for Speciation, Evolution and Development. **Jeremy C. Gray**, Joanna Bundus, Asher D. Cutter.

930B. Widespread pleiotropic Bateson-Dobzhansky-Muller incompatibilities between *C. elegans* isolates. Helen E. Orbidans, L. Basten Snoek, Jana Stastna, Jan E. Kammenga, **Simon C. Harvey**.

931C. Comparative mapping of dauer larvae development in growing populations of *C. elegans* and *C. briggsae*. James W. M. Green, **Simon C. Harvey**.

932A. Co-evolution of Mitochondrial and Nuclear Genomes in *Caenorhabditis*. **Emily E. King**, Scott E. Baird.

933B. The latest update on *Caenorhabditis* species, their ecology, phylogeny, and morphology. **Karin C. Kiontke**, Marie-Anne Félix, David H. A. Fitch.

934C. Evidence for at least two ancient duplications of presenilin genes in the nematode phylum. **Bernard Lakowski**.

935A. The Role of Pharyngeal Glands in Nematode Feeding and Diet. **James W. Lightfoot**, Ralf J. Sommer.

936B. Genomic Analysis of Hox Genes in Five Steinernema Genomes.

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937C. Interactions of *C. elegans* with its gut microbiota: from sampling in the wild to molecular genetic studies. **Sarah E. Marsh**, Marie-Anne Félix.

938A. From locus to nucleotide to phenotype: mapping the genetic architecture of quantitative traits. **Luke M. Noble**, Matthew V. Rockman.

939B. C. elegans harbors pervasive cryptic genetic variation for embryogenesis. **Annalise B. Paaby**, Amelia White, Kris Gunsalus, Fabio Piano, Matt Rockman.

940C. Natural Variants of C. elegans demonstrate defects in both sperm function and oogenesis at elevated temperatures. **Lisa N. Petrella**, Thomas Buskuskie, Susan Strome.

941A. The evolution of nematode operons. **Jonathan Pettitt**, Henrike Goth, Debjani Sarkar, Lucas Phillippe, Bernadette Connolly, Berndt Muller.

942B. Elucidating the cellular and genetic basis of hybrid dysfunction between wild isolates of *Caenorhabditis briggsae*. **Kevin Pham**, Joseph Ross.

943C. Environmental sensitivity and evolution of *Caenorhabditis* germline proliferation and differentiation. **Nausicaa Poullet**, Anne Vielle, Christian Braendle.

944A. Survey of *C. elegans* local polymorphism in an apple orchard by RAD-sequencing. **Aurélien Richaud**, Marine Stefanutti, Marie-Anne Félix.

945B. The rate and spectrum of spontaneous mutations in experimental populations of the nematode *Caenorhabditis remanei*. **Matthew P. Salomon**, Chikako Matsuba, Dejerianne G. Ostrow, Charles F. Baer.

946C. Evolution of Nematode Spliced Leader *trans*-splicing. **Debjani Sarkar**, Berndt Müller, Bernadette Connolly, Jonathan Pettitt.

947A. Embryogenesis of nematodes: traveling through transcriptomes. Christopher Kraus, **Einhard Schierenberg**.

948B. Major changes in the core developmental pathways of nematodes: *Romanomermis culicivorax* reveals the derived status of the *Caenorhabditis elegans* model. Philipp Schiffer, Michael Kroiher, Christopher Kraus, Georgios Koutsovoulos, Sujai Kumar, Julia Camps, Ndifon Nsah, Dominik Stappert, Krystalynne Morris, Peter Heger, Janine Altmüller, Peter Frommolt, Peter Nürnberg, Kelley Thomas, Mark Blaxter, **Einhard Schierenberg**.

949C. Assortative Fertilization in the Elegans-Group of *Caenorhabditis*. **Sara R. Seibert**, Blaine E. Bittorf, Scott E. Baird.

950A. Complex regulation of *C. briggsae tra-1*. **Yongquan Shen**, Yiqing Guo, Ronald E. Ellis.

951B. Inferring the order of mutational changes responsible for mechanistic divergence of a functionally constrained promoter. **Mohammad Siddiq**, Antoine Barriere, Ilya Ruvinsky.

952C. Gene-environment interactions drive genomic and transcriptomic diversity in wild *Caenorhabditis elegans* populations. R. J. M. Volkers, **L. B. Snoek**, C. J. van Hellenberg hubar, R. Coopman, W. Chen, M. G. Sterken, H. Schulenburg, B. P. Braekman, J. E. Kammenga.

953A. From *C. elegans* to parasitic nematodes: *Strongyloides* spp. and *Onchocerca* spp. **Adrian Streit**.

954B. Genetic and developmental mechanisms underlying sperm size variation in Caenorhabditis nematodes. **Anne Vielle**, Nuno Soares, Nicolas Callemeyn-Torre, Nausicaa Poullet, Christian Braendle.

955C. Testing for non-Mendelian assortment of chromosomes in *Caenorhabditis*. Son Tho Le, Chia-Yi Kao, **John Wang**.

956A. Using TALENs to create new genetic model systems. **Qing Wei**, Yongquan Shen, Xiangmei Chen, Yelena Shifman, Ronald E. Ellis.

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957B. BAR-1/beta-catenin regulates expression of a subset of collagen genes that are necessary for normal adult cuticle integrity. B. Jackson, **P. Abete Luzi**, D. Eisenmann.

958C. Long non-coding RNAs have conserved developmental gene expression patterns. **Gal Avital**, Michal Levin, Itai Yanai.

959A. Pervasive cis-regulatory divergence despite functional conservation in Caenorhabditis nematodes. **Antoine Barriere**, Ilya Ruvinsky.

960B. Multiple HRG-1 paralogs regulate heme homeostasis in *C. elegans*. **Haifa B. Bensaidan**, Iqbal Hamza.

961C. Antibiotics can modulate the immune system to enhance survival of Caenorhabditis elegans during Yersinia pestis infection. **Yun Cai**, Alejandro Aballay.

962A. An RNA-Seq based, longitudinal study of root-knot nematode parasitism. **Soyeon Cha**, Peter DiGennaro, Dahlia Nielsen, David Mck. Bird.

963B. Function of APL-1, a protein related to human APP, which has been implicated in Alzheimer's Disease. **Shah Nawaz Chaudhary**, Adanna Alexander, Christine Li.

964C. Reverse genetics and functional analysis of an uncharacterized cysteine rich gene family in Caenorhabditis elegans. **Poulami Chaudhuri**, Dr Stephen Sturzenbaum.

965A. Deciphering a genetic regulatory network of the ALA neuron. **Elly S. Chow**, Erich M. Schwarz, Cheryl Van Buskirk, Paul W. Sternberg.

966B. Systematic analysis of *cis*-acting elements of a key transcription factor regulating *C. elegans* ray assembly -*tbx-2*. Kelvin K. K. Ip, **King-Lau Chow**.

967C. Identification of essential genes that alter L1-diapause recovery. **Shu Yi Chua**, Jeffrey S. C. Chu, Robert Johnsen, Ann M. Rose, David L. Baillie.

968A. A prime and boost mechanism drives left/right asymmetric expression of the miRNA *lsy-6* resulting in neuronal functional asymmetry. **Luisa Cochella**.

969B. Structural and functional analysis of a *daf-16* homolog in the parasitic nematode *Brugia malayi*. **Kirsten Crossgrove**, Katherine Stanford, Alexius Folk, Chris Veldkamp.

970C. Identification of Phorbol Ester Responsive Genes in *C. elegans* Using Genome-wide Expression Analysis. **Ana DePina**, Xiugong Gao, Piper Hunt, Nicholas Olejnik, Andriy Tkachenko, Renate Reimschuessel, Jeffrey Yourick, Robert Sprando.

971A. Transcription of the cadmium-responsive genes *numr-1* and *numr-2* is neuronally regulated. **Queying Ding**, Jonathan H. Freedman.

972B. Functional interplay of two SWI/SNF chromatin-remodeling subunits during *C. elegans* development. **Iris Ertl**, Montserrat Porta-de-la-Riva, David Aristizabal-Corrales, Eva Gomez-Orte, Laura Fontrodona, Eric Cornes, Simo Schwartz, Juan Cabello, Julian Ceron.

973C. The *C. elegans* ATPase inhibitors MAI-1 and MAI-2 are localized in different cellular compartments. **Laura P. Fernández-Cárdenas**, L. S. Salinas-Velázquez, L. T. Agredano-Moreno, L. F. Jiménez-García, M. Tuena de Gómez Puyou, R. E. Navarro.

974A. Actively Transcribed Reverse Transcriptases are Correlated with Hookworm Larval Development. **Xin Gao**, Sahar Abubucker, John Hawdon, Makedonka Mitreva.

975B. The role of non-coding RNAs in gene regulation. **Kah Yee Goh**, Takao Inoue.

976C. Characterization of the *Caenorhabditis elegans* REF-1 Family Member, HLH-25. **Raymarie Gomez-Vazquez**, Casonya Johnson.

977A. Metallothionein transcriptional regulation, ROS and aging. **J. Hall**, JH Freedman.

978B. Function of the *C. elegans* T-box factor TBX-2 depends on SUMOylation. **Paul Huber**, Tanya Crum, Lynn Clary, Tom Ronan, Adelaide Packard, Peter Okkema.

979C. The role of *C. elegans* Histone H2A Variants in Transcription during Spermatogenesis. **Londen C. Johnson**, Liezl Madrona, Margaret Jow, Diana Chu.

980A. The homeobox transcription factors, CEH-14 and TTX-1 regulate the AFD neuron specific gene expression of *gcy-8* and *gcy-18* in *C. elegans*. **Hiroshi Kagoshima**, Yuji Kohara.

981B. Affecting gene expression through nucleosome positioning. **Colton E. Kempton**, Steven M. Johnson.

982C. Hunting for the causes of inter-individual variation in chaperone expression. **Adam Klosin**, Kadri Reis, Ben Lehner.

983A. Genetic analysis of *pry-1/Axin* regulation and Wnt signaling in nematode vulva development. **Jessica Knox**, Philip Cumbo, Bhagwati P. Gupta.

984B. Towards Unrestricted Direct Reprogramming: A genome-wide RNAi screen to identify cell fate reprogramming-inhibiting factors. **Ena Kolundzic**, Oktay Kaplan, Martina Hajduskova, Andreas Ofenbauer, Alina Schenk, Baris Tursun.

985C. X chromosome dosage compensation in the early C. elegans embryo. **Maxwell Kramer**, Sevinc Ercan.

986A. A quantitative system to define the role of transcription factor binding affinity in transcriptional activation. **Brett Lancaster**, James McGhee.

987B. *C. elegans* CCM-3 may affect excretory canal development through endosome recycling. **Benjamin Lant**, W. Brent Derry.

988C. *In silico* identification and functional analysis of genes responsive to dietary restriction in *Caenorhabditis elegans* . **Andreas H. Ludewig**, Meike Bruns, Maja Klapper, Thomas Meinel, Frank Doering.

989A. Altered-function mutations of the U2AF large subunit are protective modifiers of the *C. elegans SMN* mutant defects. X. Gao, **L. Ma**.

990B. An *in vivo C. elegans* gene regulatory network unveils post-developmental role of intestinal transcription factors. **Lesley T. MacNeil**, H. Efsun Arda, Lauren D'Elia, A. J. Marian Walhout.

991C. DBL-1 Target Gene Regulation By SMA-2, SMA-3, and SMA-4. **Uday Madaan**, Jianghua Yin, Edlira Yzeiraj, Cathy Savage-Dunn.

992A. The Receptor Tyrosine Phosphatase MOA-1 shows a temperature-dependent induction and affects *C. elegans* viability and development. **Vanessa Marfil**, Chris Li.

993B. Inverted repeat sequences required for alternative splicing of the *unc-17 - cha-1* cholinergic locus. **Ellie Mathews**, Greg Mullen, Jim Rand.

994C. Poising and pausing of Pol II during starvation. **Colin S. Maxwell**, William S. Kruesi, Nicole Kurhanewicz, Leighton J. Core, Colin T. Waters, Caitlin L. Lewarch, Igor Antoshechkin, John T. Lis, Barbara J. Meyer, L. Ryan Baugh.

995A. Alternative 3'UTRs: A Mechanism for Post-transcriptional Regulation in *C. elegans* Germline and Early Embryo. **D. Mecenas**, R. Ahmed, M. Gutwein, J. Reboul, J. Polanowska, K. Gunsalus, F. Piano.

996B. In vivo spatiotemporal analysis of mRNA alternative splicing during C.elegans neural development. **Jonathan R. M. Millet**, Denis Dupuy.

997C. A high-throughput EMS screen to identify direct reprogramming regulating factors. **Andreas Ofenbauer**, Oktay Kaplan, Martina Hajduskova, Ena Kolundzic, Stefanie Seelk, Selman Bulut, Alina Schenk, Baris Tursun.

998A. A global genetic screen for the identification of factors involved in *C. elegans* spliced leader *trans*-splicing. **L. Philippe**, B. Connolly, B. Müller, J. Pettitt.

999B. LPR-1 facilitates LIN-3/EGF signaling during the development of the excretory system. **Pu Pu**, D. Freed, M. Lemmon, M. Sundaram.
1000C. *In vivo* reporters for spatiotemporal regulation of genes by microRNA. **Cecile A. L. Quere**, Denis Dupuy.

1001A. The transcriptional repressor CTBP-1 functions in the nervous system to regulate gene expression. **Anna E. Reid**, Duygu Yücel, Estelle Llamosas, Sashi Kant, Hannah Nicholas.

1002B. Complex expression dynamics and robustness in C. elegans insulin networks. **Ashlyn D. Ritter**, Yuan Shen, Juan Fuxman Bass, Sankarganesh Jeyaraj, Bart Deplancke, Arnab Mukhopadjay, Jian Xu, Monica Driscoll, Heidi Tissenbaum, A. J. Marian Walhout.

1003C. Uncovering genotype specific variation of Wnt signaling in C. elegans. **M. Rodriguez**, LB Snoek, T. Schmid, N. Samadi, L. van der Bent, A. Hajnal, JE Kammenga.

1004A. The sudden transcriptional switch to adulthood in L4 stage *C. elegans*. **L. B. Snoek**, M. G. Sterken, R. J. M. Volkers, M. Klatter, K. Bosman, R. P. J. Bevers, J. A. G. Riksen, J. E. Kammenga.

1005B. Toxicogenomic responses of *Caenorhabditis elegans* to silver nanomaterials. **Daniel L. Starnes**, C. Starnes, J. Smith, E. Oostveen, J. Unrine, B. Collin, P. Bertsch, O. Tsyusko.

1006C. Cadmium exposure affects insulin signaling in *Caenorhabditis Elegans*. **Y. Sun**, J. Freedman.

1007A. Ribosomal Protein L1 regulates alternative splicing of its own pre-mRNA. **Satomi Takei**, Hidehito Kuroyanagi.

1008B. High throughput EMS mutagenesis screen for cadmium response genes in *C. elegans*. **Yong-Guang Tong**, Jonathan H. Freedman.

1009C. In search of genes necessary for the identity or specification of the gonadal sheath. **Laura G. Vallier**.

1010A. Molecular genetic deciphering of the reproductive pathway in *Caenorhabditis elegans*. **Liesbeth Van Rompay**, Lotte Frooninckx, Isabel Beets, Liesbet Temmerman, Tom Janssen, Liliane Schoofs.

1011B. Exploring Gene Expression and Transcriptional Regulation Data in WormBase. **Xiaodong Wang**, Wen Chen, Daneila Raciti.

1012C. Determining the time and tissue specific expression of genes during embryogenesis. **Adam D. Warner**, Chau Huynh, Robert H. Waterston.

1013A. Identification of *cis*-regulatory elements that confer zinc-responsive transcription in intestinal cells of *C. elegans*. Hyun Cheol Roh, Ivan Dimitrov, Krupa Deshmukh, Guoyan Zhao, **Kurt Warnhoff**, Daniel Cabrera, Wendy Tsai, Kerry Kornfeld.

1014B. Integration of metabolic and gene regulatory networks governs the transcriptional response to diet in *C. elegans*. **Emma Watson**, Lesley MacNeil, H. Efsun Arda, Lihua Julie Zhu, Albertha J. M. Walhout.

1015C. Transcriptional regulation in the intestine. **T. Wiesenfahrt**, J. Berg, E. Osborne Nishimura, J. McGhee.

1016A. Phenotype analyses and expression of the sphingomyelin synthase genes in *Caenorhabditis elegans*. **Haruka Yamaji**, Yukako Tohsato, Kenji Suzuki, Masahiro Ito.

1017B. Investigating the expression and function of a C. elegans chemosensory receptor. **Jinzi Yang**, Harleen Basrai, Alisha Anderson, Stephen Trowell, Carolyn Behm.

1018C. Independent Regulation of Metabolism but Coordinated Control of Tissue Development by Epidermis Specific Proteins in *Caenorhabditis elegans* . Jiaofang Shao, Kan He, Hao Wang, Vincy Ho, Xiaoliang Ren, Xiaomeng An, Ming-Kin Wong, Bin Yan, Dongying Xie, John Stamatoyannopoulos, **Zhongying Zhao**.

1019A. Knock-down of nuclear pore subunit NPP-11 suppresses the germline apoptosis and differentiation defects of *C41G7.3* mutants in *C. elegans*. **Xue Zheng**, Ataman Sendoel, Deni Subasic, Anneke Brümmer, Shivendra Kishore, Mihaela Zavolan, Michael Hengartner.

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1020B. Heat shock activates a miRNA-dependent response pathway in *Caenorhabditis elegans*. **Antti P. Aalto**, Ian A. Nicastro, Amy E. Pasquinelli.

1021C. Analysis of microRNA regulation of defecation behavior. Benedict J. Kemp, Adele Gordon, Carmela Rios, Megan Mohnen, Spencer Agnew, Julien Aoyama, **Allison L. Abbott**.

1022A. The Visual Detection of odr-1 22G RNAs via a MosSCI Sensor System. **Adriel-John Ablaza**, Bi-Tzen Juang, Noelle L'Etoile, Maria Gallegos.

1023B. CEY-1 attenuates *let-7* microRNA-mediated silencing in *C. elegans*. **Amelia F. Alessi**, Vishal Khivansara, Sang Young Chun, James J. Moresco, John R. Yates III, John Kim.

1024C. LIN-28-dependent repression of *let-7* miRNA is required for oogenesis. **Yoshiki Andachi**, Yuji Kohara.

1025A. Cytoplasmic versus nuclear RNAi mechanisms in transgene-induced gene silencing in Caenorhabditis elegans. **Nadeem Asad**, Laticia Rivera, Arthur Ankeney, Raeann Whitney, Lisa Timmons.

1026B. Identification of endogenous *let-7* miRNA target sites by iCLIP. **James P. Broughton**, Michael T. Lovci, Gene W. Yeo, Amy E. Pasquinelli.

1027C. *Mir-34* and *mir-83* protect *C. elegans* gonad morphogenesis against temperature fluctuations. **Samantha Burke**, Molly Hammell, Victor Ambros.

1028A. Receptor of Activated C Kinase RACK-1 may regulate the *Caenorhabditis elegans* heterochronic gene pathway at the larva-to-adult transition. **Shih-Peng Chan**, Yu-De Chu, We-Chieh Wang, Shi-An Chen, Frank Slack.

1029B. Characterizing the individual roles of CSR-1 isoforms across development in *Caenorhabditis elegans*. **V. H. W. Cheung**, J. M. Claycomb.

1030C. An RNAi-based screen for the DExD/H-box RNA helicases involved in *Caenorhabditis elegans* microRNA function. **Yu-De Chu**, Tao Huang, Guan-Rong Chen, Shin-Kai Chen, Shih-Peng Chan.

1031A. Functional Characterization of the CSR-1 Small RNA Pathway in *C. briggsae*. Monica Wu, Jie Wang, Shikui Tu, Zhiping Weng, **Julie M. Claycomb**.

1032B. Identification of genes required for RNAi-mediated antiviral immunity by a genome-wide genetic screen in *C. elegans*. **Stephanie R. Coffman**, Yuanyuan Guo, Zhihuan Gao, Gina Broitman-Maduro, Morris Maduro, Shou-wei Ding.

1033C. NHL-2 Influences 22G RNAs to Maintain Germline Mediated Chromosomal Integrity. **Gregory M. Davis**, Wai Y. Low, Julie M. Claycomb, Peter R. Boag.

1034A. Identifying Zrt, Irt-like proteins that promote resistance to zinc toxicity in *C. elegans*. **Nicholas K. Dietrich**, Kerry Kornfeld.

1035B. Dissecting the role of NAP-1 in small RNA-mediated chromatin modulation. **M. A. Francisco**, J. M. Claycomb.

1036C. *C. elegans* as a model for fatty acid oxidation disorders. **Wen Gao**, Ronald J. Wanders, Riekelt H. Houtkooper.

1037A. The Virus Sensing Domains of RIG-I Functionally Replace the Corresponding Domains of DRH-1 in Antiviral RNA Silencing in *Caenorhabditis elegans*. **Xunyang Guo**, Rui Zhang, Jeffery Wang, Rui Lu.

1038B. Analysis of ok2951, a mutation found in F56D2.6, a putative homologue of the yeast PRP43 protein. **Jonathan E. Karpel**, Miranda Roland.

1039C. Direct Reprogramming of Distinct Cell Types in *C. elegans* into GABAergic Motor Neurons. **Marlon Kazmierczak**, Ena Kolundzic, Baris Tursun.

1040A. Investigating how an autoregulatory loop enhances let-7 biogenesis. **Sarah A. Lima**, Dimitrios G. Zisoulis, Zoya S. Kai, Vanessa Mondol, Amy E. Pasquinelli.

1041B. *C. elegans* RNA Helicase A genetically interacts with genes involved in two different germline RNAi pathways. **Penelope L. Lindsay**, Megan K. Gautier, Karen J. Muschler, Sarah K. O'Connor, Katherine M. Walstrom.

1042C. The mir-35 family of microRNAs regulates hermaphrodite fecundity, male development, and genetically interacts with the sex determination pathway. **Katherine McJunkin**, Victor Ambros.

1043A. Orsay virus replication kinetics in *C. elegans* strain Bristol N2. **Mark G. Sterken**, Kobus J. Bosman, L. Basten Snoek, Jikke Daamen, Joost A. G. Riksen, Jaap Bakker, Gorben P. Pijlman, Jan E. Kammenga.

1044B. Nicotine Exposed Chronically During the Post-embryonic Stages Systematically Altered the MicroRNA Expression Profiles in *C. elegans*. **Faten A. Taki**, Baohong Zhang.

1045C. TEG-1 regulates the stability of miRISC components and the levels of microRNAs. **Chris Wang**, Dave Hansen.

1046A. 3'LIFE: A functional assay to detect *C. elegans* miRNA targets in high-throughput. **JM Wolter**, K. Kotagama, AC Pierre-Bez, M. Firago, M. Tennant, M. Mangone.

1047B. Analysis of PUF-9 and miRNA interactions in *C. elegans*. **Danny Yang**, Sang Chun, Ting Han, James Moresco, John Yates III, John Kim.

1048C. The SNARE protein SEC-22 is a negative regulator of RNAi. **Y. Zhao**, B. Holmgren, A. Hinas.

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1049A. The first 100 nematode genomes: towards a genomic biology of Nematoda. **Mark L. Blaxter**, Georgios Koutsovoulos, Sujai Kumar, Michael Clarke, Martin Jones, Alex Marshall, Benjamin Makepeace, Philipp Schiffer, Einhardt Schierenberg, Simon Babayan, Nick Gray.

1050B. 959.nematodegenomes.org. **Mark L. Blaxter**, Georgios Koutsovoulos, Sujai Kumar, Philipp Schiffer.

1051C. Advancing and Refining the *C. elegans* 3'UTRome. **SM Blazie**, AC Pierre-Bez, CE Otto, CA Lynch, M. Mangone.

1052A. A pair of RNA binding proteins shape alternative splicing regulatory networks in distinct neuronal subtypes. Adam D. Norris, Mei Zhen, **John A. Calarco**.

1053B. A genome-wide network of genetic interactions in embryonic development. **Patricia G. Cipriani**, Amelia White, Huey-Ling Kao, Eliana Munarriz, Katherine Erickson, Jessica Lucas, Indrani Chatterjee, Jerome Reboul, Kristin Gunsalus, Fabio Piano.

1054C. A draft genome assembly of *Caenorhabditis* sp. 9. and its use in characterizing genome shrinkage in self-fertile nematodes. Da Yin, Erich M. Schwarz, Caitlin M. Schartner, Edward J. Ralston, Barbara J. Meyer, **Eric S. Haag**.

1055A. Mapping transcriptional regulatory networks in the nematode Caenorhabditis elegans. **Margaret Ho**, Paul Sternberg.

1056B. Analysis of developmental RNA-Seq libraries reveals signature profile for cilia-related genes. **Victor L. Jensen**, Tiffany A. Timbers, Chunmei Li, Ryan D. Morin, Michel R. Leroux.

1057C. Characterisation of genomic instability and interstrand crosslink sensitivity associated with mutation of *dog-1*, the functional ortholog of human Fanconi Anemia protein *FancJ*. **Martin R. Jones**, Jeffrey S. Chu, Ann M. Rose.

1058A. Genome-wide binding characteristics of the dosage compensation complex in *C. elegans*. **Anna-Lena Kranz**, Chen-Yu Jiao, Lara Winterkorn, Sarah Albritton, Sevinç Ercan.

1059B. The use of C.elegans to identify novel mutations that confer benzimidazole resistance. **Sharmilah L. J. Latheef**, Susan S. Stasiuk, John S. Gilleard.

1060C. The involvement of the Caenorhabditis elegans EPE1 homolog in DNA interstrand crosslink repair. Sang-Yong An, **Changrim Lee**, Hyeon-Sook Koo.

1061A. Using Next-Generation Sequencing to Determine Gene Identity in Temperature-Sensitive, Embryonic Lethal Mutants. **Josh Lowry**, Amy Connolly, Bruce Bowerman.

1062B. Genomic analysis of Steinernema: Insights into insect parasitism, intragenus and intergenus evolution. **Ali Mortazavi**, Marissa Macchietto, Adler Dillman, Alicia Rogers, Brian Williams, Igor Antoshechkin, Camille Finlinson, Zane Goodwin, Xiaojun Lu, Patricia Stock, Edwin Lewis, Heidi Goodrich-Blair, Byron Adams, Paul Sternberg.

1063C. Selenocysteine incorporation in metazoa: the peculiar case of the nematode lineage. **Lucía Otero**, Laura Romanelli, Vadim N. Gladyshev, Antonio Miranda-Vizuete, Gustavo Salinas.

1064A. Molecular characterization of mitocycin C-induced lethal mutations in *Caenorhabditis elegans*. **Annie Tam**, Jeffrey SC. Chu, Ann M. Rose.

1065B. Combining genomic approaches to characterize alternative splicing events in *C. elegans*. **June Tan**, Arun Ramani, Hong Na, Debashish Ray, Timothy Hughes, Andrew Fraser.

1066C. Determining Fragile Nucleosome Distribution Bias Within the Chromosome. **Ashley Wright**, Steven Johnson.

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1068B. Analysis of Histone methylation in germ cells using C. elegans as model system. **Pier Giorgio Amendola**, Toshia Myers, Anna Elisabetta Salcini.

1069C. Functional analysis of H3K79 methylation. **Fanelie Bauer**, Alex Appert, Julie Ahringer.

1070A. Roles of histone demethylation in germline maintenance. **Sara E. Beese-Sims**, Monica P. Colaiacovo.

1071B. Forward Genetic Screen for Induced Conversion of Germ Cells. **Idris Selman Bulut**, Oktay Ismail Kaplan, Baris Tursun.

1072C. O-GlcNAcylation of a conserved chromatin factor, ZFP-1(AF10), as a possible glucose-sensing mechanism. **Ainhoa Ceballos**, Germano Cecere, Daphne Avgousti, Grishok Alla.

1073A. "Who wants to live forever?"- chromatinome RNAi screen for longevity in *C. elegans*. **Karolina Chocian**, Hayley Lees, Helena Cantwell, Gino Poulin, Jane Mellor, Alison Woollard.

1074B. synMuv B regulation of chromatin states at high temperature. **Meghan Elizabeth Costello**, Andreas Rechtsteiner, Thea Egelhofer, Susan Strome, Lisa N. Petrella.

1075C. Epigenetic regulation of fertility in *C. elegans* males depends on the gamete source and chromatin history of the X chromosome. **Laura Gaydos**, Andreas Rechtsteiner, Wenchao Wang, Susan Strome.

1076A. CEC-4 is a novel chromodomain protein involved in perinuclear chromatin anchoring. **Adriana V. Gonzalez Sandoval**, Veronique Kalck, Benjamin D. Towbin, Teddy Yang, Kehao Zhao, Susan M. Gasser.

1077B. An extended RNAi sub-library to uncover chromatin factors implicated in direct cell-type conversion. **M. Hajduskova**, M. L. Beato del Rosal, E. Kolundzic, B. Tursun.

1078C. Nucleosome organization in *C. elegans* gamete chromatin. **Tess E. Jeffers**, Jason D. Lieb.

1079A. Conversion of epithelial cells into a neuron like cell in *C. elegans*. **Oktay Ismail Kaplan**, Idris Selman Bulut, Baris Tursun.

1080B. Examining the Role of Histone Acetyltransferases in Targeting the *C. elegans* DCC to the X Chromosomes. **Alyssa C. Lau**, Gyorgyi Csankovszki.

1081C. Dao-5/CeNopp140 modulates rDNA chromatin epigenetic status and transcription to sustain oogenesis. **Chi-Chang Lee**, Yi-Tzang Tsai, Li-Wei Lee, Chih-Wei Kao, Huey-Jen Lai, Tien-Hsiang Ma, Yu-Sun Chang, Ning-Hsin Yeh, Szecheng J. Lo.

1082A. Epigentic regulation of L1 longevity. **Inhwan Lee**, Young-jai You.

1083B. Spatial gene positioning in the *Caenorhabditis elegans* embryo. **Darina Majovská**, Christian Lanctôt.

1084C. Dynamics of nuclear compartments in *C. elegans*. Rahul Sharma, Georgina Gomez-Saldivar, Jop Kind, Bas van Steensel, Peter Askjaer, **Peter Meister**.

1085A. Towards understanding the role of histone demethylation in replication-induced DNA damage repair. **Toshia Myers**, Pier Giorgio Amendola, Anna Elisabetta Salcini.

1086B. The *C. elegans* acetylome identifies genes and molecular pathways involved in dopamine neuron vulnerability. **Richard M. Nass**, Jonathan Trinidad, Natalia VanDuyn.

1087C. Starving chromosome; reshaping the chromatin to survive. **Ehsan Pourkarimi**, Mark Larance, Angus Lamond, Anton Gartner.

1088A. *usp-48* encodes a deubiquitinating enzyme involved in cell fate restriction during development. **Dylan Rahe**, Tulsi Patel, Oliver Hobert.

1089B. SET-2, ASH-2 and WDR-5 regulate distinct sets of genes in the *C. elegans* germline. **Valérie J. P. Robert**, Marine Mercier, Lucie Kozlowski, Cécile Bedet, Diyavarshini Gopaul, Stéphane Janczarski, Francesca Palladino.

1090C. The characterization of the histone-chaperone lin-53. **Stefanie Seelk**, Baris Tursun.

1091A. HTZ-1/H2A.z maintains cell fates through transcriptional repression in an H3K27me-independent manner. **Yukimasa Shibata**, Hitoshi Sawa, Kiyoji Nishiwaki.

1092B. Turnover of the H3K9me2 Mark During Late Spermatogenesis. **Matthew P. Snyder**, Xia Xu, Eleanor Maine.

1093C. Molecular Characterization of Epigenetic Inheritance Factors in *C.elegans*. **G. Spracklin**, S. Kennedy.

1094A. Expression of MET-2, a H3K9 methyltransferase, in the C. elegans germ line. **Bing Yang**, Xia Xu, Eleanor Maine.

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1095B. Using a glo-2 enhancer screen to investigate BLOC-1 function in protein trafficking to gut granules. **Alec Barrett**, Daniel Saxton, Greg Hermann.

1096C. The *Caenorhabditis elegans* UDP-Glc:glycoprotein glucosyltransferase homologue CeUGGT-2 is an essential protein that does not function as a glycoprotein conformation sensor. Lucila Buzzi, Sergio Simonetta, Armando Parodi, **Olga Castro**.

1097A. An RNAi screen for LRP-1 trafficking regulators reveals a role for EPN-1 epsin in endocytosis of LDL receptor superfamily. Yuan-Lin Kang, John Yochem, Leslie Bell, Erica Sorensen, **Lihsia Chen**, Sean Conner.

1098B. A microtubule minus-end binding protein and minus-end directed transport function in the C. elegans epidermis. **Marian Chuang**, Tiffany I. Hsiao, Amy Tong, Shaohe Wang, Karen Oegema, Andrew Chisholm.

1099C. Polarity proteins regulate the localization of a spindle-positioning mediator, LET-99. **Eugenel B. Espiritu**, Jui-Ching Wu, Kari Messina, Lesilee Rose.

1100A. Networks regulating pharyngeal development and morphogenesis. **David S. Fay**, Stanley Polley, Jujiao Kuang, Jon Karpel, Evguenia Karina, Aleksandra Kuzmanov, John Yochem.

1101B. Disparate endocytic recycling of the TGFβ signaling receptors, Sma-6 and Daf-4, regulates signaling of the Sma/Mab pathway. **Ryan Gleason**, Adenrele (Dee) Akintobi, Ying Li, Nanci Kane, Barth Grant, Richard Padgett.

1102C. The EXC-1 RAS-Domain Protein Mediates Vesicle Movement in the Excretory Canal. **Kelly A. Grussendorf**, Alexander T. Salem, Christopher J. Trezza, Matthew Buechner.

1103A. A screen for mislocalization of the LET-23 EGF receptor during vulval development. **Andrea Haag**, Juan M. Escobar Restrepo, Alex Hajnal.

1104B. Identification and characterization of conserved factors mediating gut granule protein trafficking. **Greg Hermann**, Jared Delahaye, Olvia Foster, Annalise Vine, Thomas Curtin.

1105C. *C. elegans* Rag genes are involved in endosome / lysosome biogenesis in a TORC1-independent fashion. **K. Iki**, Y. Ito, Y. Shimomura, H. Kajiho, M. Fukuyama, K. Kontani, T. Katada.

1106A. Identification of genes important for excretory system function and maintenance using Whole Genome Sequencing. **Michelle Kanther**, Jennifer Cohen, Jean Parry, Meera Sundaram.

1107B. Starvation-responsive behavioral plasticity is tuned by tubulin polyglutamylation in sensory cilia. **Yoshishige Kimura**, Alu Konno, Koji Tsutsumi, Saira Hameed, Mitsutoshi Setou.

1108C. Instructive polarization of early embryonic cells by the cadherin-catenin complex and the RhoGAP PAC-1. **Diana Klompstra**, Dorian Anderson, Jeremy Nance.

1109A. Unraveling the interactome underlying cell polarity. **Thijs Koorman**, Monique van der Voet, Sander van den Heuvel, Mike Boxem.

1110B. An ABC transporter regulates systemic heme homeostasis in *C. elegans*. **Tamara Korolnek**, Iqbal Hamza.

1111C. Regulation of TBC-2, an endosomal Rab5 GAP. **Fiona Law**, Laëtitia Chotard, Farhad Karbassi, Christian Rocheleau.

1112A. Depletion of *mboa-7*, an enzyme that incorporates polyunsaturated fatty acids into phosphatidylinositol (PI), impairs PI 3-phosphate signaling. **Hyeon-Cheol Lee**, Takuya Kubo, Nozomu Kono, Eriko Kage-Nakadai, Keiko Gengyo-Ando, Shohei Mitani, Takao Inoue, Hiroyuki Arai.

1113B. Suppressors of the *pam-1* aminopeptidase and the role of centrosome-cortical contact in one-cell anteroposterior polarity. Margaret Williams, Ashley Kimble, Zachary Klock, Jessica Meeker, Kevin Kozub, Eva Jaeger, **Rebecca Lyczak**.

1114C. Sequential roles of Atg8 homologs during autophagosome formation. **Marion Manil-Segalen**, C. Lefebvre, C. Jenzer, C. Boulogne, B. Satiat-Jeunemaitre, V. Galy, R. Legouis.

1115A. LET-99 regulates G protein signaling and spindle positioning during asymmetric division. **Jennifer A. Milan**, Dae Hwi Park, Lesilee S. Rose.

1116B. Patched family member PTR-2 is required for permeability barrier formation in the *C. elegans* zygote. Jade P. X. Cheng, **Sara K. Olson**, Alexander Soloviev, Olivier Zugasti, Karen Oegema, Patricia E. Kuwabara.

1117C. Screen for endocytic genes required for dauer development and autophagy. **Nicholas J. Palmisano**, David Jimenez, Alicia Meléndez.

1118A. Role of fibroblast growth factor receptor in regulation of

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1119B. Several ArfGEFs regulate the apoptotic fate in Q neuroblast asymmetric cell divisions. **Jerome Teuliere**, Shaun Cordes, Gian Garriga.

1120C. Regulatory effect of MAGUK/LIN-2 on kinesin-3-based transport in the neuronal system of C. elegans. **Gong-Her Wu**, Oliver Wagner.

1121A. VANG-1, one of the PCP core components, is involved in asymmetric divisions of seam cell. **M. Yokoo**, H. Sawa.

1122B. Amphiphysin 2 binds nesprin and regulates nuclear positioning. **M. D'Alessandro**, K. Hnia, C. Koch, C. Gavriilidis, S. Quintin, N. B. Romero, Y. Schwab, M. Labouesse, J. Laporte.

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1123C. EXC-2 and Maintenance of excretory canal tube structure. **Hikmat Al-Hashimi**, Robyn Harte, Jenny Hackett, Stuart Macdonald, Matthew Buechner.

1124A. Microtubules and Fertilization: The Meiosis to Mitosis Transition. **Sarah M. Beard**, Ben G. Chan, Paul E. Mains.

1125B. The molecular genetics of epithelial cell morphogenesis-functional dissection of *C. elegans* homologue zyxin. **Keliya Bai**, Jonathan Pettitt.

1126C. Dissecting the mechanistic insights through which EGL-26 controls *C. elegans* vulva tubulogenesis. **Nagagireesh Bojanala**, Avni Upadhyay, Hongliu Sun, Matt Crook, Ariana Detwiler, Nishat Seraj, Sarah Chang, Jimmy Goncalves, Ryan Fine, Nick Serra, Wendy Hanna-Rose.

1127A. Characterizing regulators of the *C. elegans* embryonic elongation pathway. **Benjamin Chan**, Simon Rocheleau, Paul Mains.

1128B. The RhoGEF ECT-2 is Required for Ventral Enclosure During C. elegans Embryogenesis. **Y. Chen**, A. Marte, G. Stylianopoulos, A. Piekny.

1129C. The Regulation of Bone Morphogenetic Protein Pathway by LON-1 in Extracellular Matrix using a novel BMP readout. **King-Lau Chow**, Ho-Tsan Wong, Kwok-Hei Wong.

1130A. A genome-wide RNAi screen to identify new players of a muscle-to-epidermis mechanotransduction pathway essential for embryonic elongation. **Christelle Gally**, Agnès Aubry, Michel Labouesse.

1131B. Regulation of *C. elegans* MCAK by Aurora Kinase Phosphorylation. **Xue Han**, Martin Srayko.

1132C. Mechanisms of Sperm Competition in *C. elegans*. **Jody Hansen**, Daniela Chavez, Gillian Stanfield.

1133A. Actin-based cell motility in developing *C. elegans*: dissecting actin assembly factors. **Svitlana Havrylenko**, Philippe Noguera, Julie Plastino.

1134B. A heterogeneous mixture of F-Series prostaglandins promotes sperm guidance in the *Caenorhabditis elegans* reproductive tract. **Hieu D. Hoang**, Jeevan K. Prasain, Michael A. Miller.

1135C. Anatomic Expression of the Tubulin Superfamily. **Daryl D. Hurd**.

1136A. ROL-3, the ortholog of the human proto-oncogene ROS1, is required to orchestrate the morphogenesis and development of the seam syncytium and interacts with the Bicaudal-C homolog *bcc-1*. **Martin R. Jones**, Ann M. Rose, David L. Baillie.

1137B. A novel protein complex required for the collective migration of the male somatic gonad. **Mihoko Kato**, Tsui-Fen Chou, Collin Z. Yu, Wen Chen, Paul W. Sternberg.

1138C. The role of SYM-3 and SYM-4 in tissue integrity and organogenesis. **Pushpa Khanal**, John Yochem, Anna Justis, David Fay.

1139A. Molecular mechanism of *egl-15*/FGFR and *ina-1*/α-integrin in gland cell migration during the development of *Caenorhabditis elegans* pharynx. **S. Kim**, J. Kormish.

1140B. Using genetics and proteomics to identify substrates of a PP2A phosphatase required for mitotic spindle assembly. **Karen I. Lange**, Martin Srayko.

1141C. Identification of DMD-3 targets in the *C. elegans* male tail tip. **H. Littleford**, R. A. Herrera, K. Kiontke, A. Mason, J.-S. Yang, S. Ercan, D. Fitch.

1142A. Analysis of tissue-to-tissue signaling and its effects on cytoskeletal polarity during embryonic cell migrations. **Sailaja Mandalapu**, Martha Soto.

1143B. pix-1 differential expression along the anterio-posterior axis of the embryos controls early elongation in parallel to mel-11 and let-502 in Caenorhabditis elegans. **Emmanuel Martin**, Sharon Harel, Bernard Nkengfac, Karim Hamiche, Mathieu Neault, Sarah Jenna.

1144C. Three distinct Wnt signaling mechanisms act sequentially to position the migrating QR neuroblasts of C. elegans. **Remco A. Mentink**, Chung Y. Tang, Marco C. Betist, Hendrik C. Korswagen.

1145A. "Ultrastructure analysis of the sarcomeres in worms that lack Z-line formins". **Lei Mi-Mi**, David Pruyne.

1146B. dpy-19 and mig-21 control the persistent directionality of migrating Q neuroblasts in Caenorhabditis elegans. **Teije C. Middelkoop**, Thijs Koorman, Mike Boxem, Hendrik C. Korswagen.

1147C. The role of LIN-3 during morphogenesis of the dorsal lumen in the vulva. **Louisa Mueller**, Matthias Morf, Alex Hajnal.

1148A. Analysis of novel pathways for nuclear migration in C. elegans. **Shaun P. Murphy**, Yu-Tai Chang, Daniel A. Starr.

1149B. Mitochondria-type GPAT is required for mitochondrial fusion. **Y. Ohba**, T. Inoue, T. Sakuragi, N. H.Tomioka, A. Inoue, N. Ishihara, J. Aoki, E. Kage-Nakadai, S. Mitani, H. Arai.

1150C. Genomic analysis of the duct and pore cells reveals novel effectors and regulators of morphogenesis. **Gregory Osborn**, Travis Walton, Meera Sundaram, John Murray.

1151A. What is tubulin glutamylation good for? **Nina Peel**, Zach Barth, Ruchi Shah, Jessica Lee, Kevin O'Connell.

1152B. The in vivo Dynamics of IFT Motors and Axoneme Microtubules in Cilia Signaling. **Jay Pieczynski**, Patrick Hu, Kristen Verhey.

1153C. The Secretory Protein Calcium ATPase PMR-1 is essential for cell migration during gastrulation. **V. Praitis**, J. Simske, S. Kniss, R. Mandt, L. Imlay, C. Feddersen, M. B. Miller, J. Mushi, W. Liszewski, R. Weinstein, A. Chakravorty, D.-G. Ha, A. Schacht Farrell, A. Sullivan-Wilson, T. Stock.

1154A. LAWD-1, a potential scaffold protein with a WD40 domain involved in epithelial morphogenesis. **Mengmeng Qiao**, Jonathan Hodgkin, Patricia Kuwabara.

1155B. Structure-function analysis of the cell-fusion protein EFF-1. **Hadas Raveh-Barak**, C. Valansi, O. Avinoam, T. Krey, J. Perez-Vargas, FA. Rey, B. Podbilewicz.

1156C. Formins Play a Role in the C. elegans Embryonic Elongation. **Osama M. Refai**, Christopher A. Vanneste, David Pruyne, Paul E. Mains.

1157A. Towards the Complete Embryonic Cell Lineage. **Anthony Santella**, Zhuo Du, Zidong Yu, Yicong Wu, Hari Shroff, Zhirong Bao.

1158B. A Complex Issue: Understanding vMSP Receptor Heteromeric Complexing Behavior. **Jessica L. Schultz**, Sung Min Han, Se-Jin Lee, Michael Miller.

1159C. DBL-1 TGF-β localization and the physiological basis of body size regulation in *C. elegans*. **Robbie D. Schultz**, E. Ann Ellis, Tina L. Gumienny.

1160A. An In Vivo Analysis of Critical Functional Domains of α-catenin in *C. elegans*. **Xiangqiang Shao**, Jeffrey Simske, Anjon Audhya, Jeff Hardin.

1161B. LET-653, a secreted ZP-domain and mucin-related protein, functions in the excretory duct/pore, and not the excretory canal cell. Corey Poggioli, Kevin Bickard, **Meera V. Sundaram**.

1162C. SPV-1, a RhoGAP and F-BAR domain protein, regulates spermatheca contractility. **Pei Yi Tan**, Ronen Zaidel-Bar.

1163A. Regulation of the cadherin-catenin complex by the ULP-2 SUMO protease. **Assaf Tsur**, Ulrike Bening-Abu-Shach, Orit Adir, Limor Broday.

1164B. UNC-54 and Y54E5B.2 work in concert to inhibit ectopic membrane extensions away from the nerve cord in *C. elegans* body wall muscle. **Ryan Viveiros**, Ralf Schnabel, Robert Barstead, Donald Moerman.

1165C. Mechanical forces in *C. elegans* embryo elongation. **Thanh TK Vuong**, Michel Labouesse.

1166A. NOCA-1 isoforms regulate non-centrosomal microtubule array formation in different C. elegans tissues. **Shaohe Wang**, Arshad Desai, Karen Oegema.

1167B. Searching for regulators of LIT-1 localization in the amphid sensory compartment. **Wendy M. Wang**, Shai Shaham.

1168C. LIN-29/EGR1, a zinc-finger transcription factor, controls the depth of anchor cell invasion in C. elegans. **Zheng Wang**, Shelly McClatchey, Lara Linden, Qiuyi Chi, David Sherwood.

1169A. Anillin non-autonomously regulates epidermal morphogenesis during *C. elegans* embryogenesis. **Wernike D.**, Fotopoulos N., Piekny A.

1170B. FAX-1 and PROMININ function in migration and morphogenesis. **Bruce Wightman**, Emily Bayer.

1171C. DYF-7 prevents rupture of a sensory epithelium made of neurons and glia. **Claire R. Williams**, Maxwell G. Heiman.

1172A. Role of integrin in neuronal cell migration. **Jing Wu**, Richa Manglorkar, Myeongwoo Lee.

1173B. The Ezrin/Radixin/Moesin protein ERM-1 controls actin-mediated cell shape changes during vulval invagination. **Qiutan Yang**, Juan Restrepo, Alex Hajnal.

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1174C. Study of Aurora-B Kinase Regulators in *C. elegans* Meiosis. **Elisabeth Altendorfer**, Saravanapriah Nadarajan, Monica Colaiacovo.

1175A. Main chromosome abberations found amount 4617 pediatric patients at a third level children Mexican Hospital. **Juan M. Aparicio**, Maria de L. Hurtado H., Margarita Barrientos P., Hortencia Chavez O., Sergio Chatelain M.

1176B. Spindle assembly checkpoint proteins regulate and monitor meiotic synapsis in *C. elegans*. **T. Bohr**, P. Lamelza, N. Bhalla.

1177C. Characterization and comparative profiling of the mitotic spindle proteome reveals a glycosylation factor, OSTD-1 as being necessary for cell division and ER morphology. **Mary Kate Bonner**, Ahna Skop.

1178A. SMCL-1 interacts with condensin proteins and modulates their function in chromosome dynamics. **Lucy Fang-I Chao**, Meha Singh, John Yates III, Kirsten Hagstrom.

1179B. Suppressor mutations for the CAND-1 regulator of cullin-RING ubiquitin ligases. **Snehal N. Chaudhari**, Edward T. Kipreos.

1180C. Investigating loss-of-function suppressors of C. elegans centrosomal defective mutants. **Chien-Hui Chuang**, Sean O'Rourke, Bruce Bowerman.

1181A. CHL-1 is required for DNA replicative integrity in *Caenorhabditis elegans*. **George Chung**, Ann M. Rose.

1182B. PP1 phosphatases, GSP-3 and GSP-4, are required for

chromosome segregation in sperm meiosis. **Thais G. Cintra**, Joseph Beyene, Jui-Ching Wu, Diana Chu.

1183C. Oocyte meiotic spindle assembly. **Amy Connolly**, Valerie Osterberg, Sara Christensen, Chenggang Lu, Kathy Chicas-Cruz, Shawn Lockery, Paul Mains, Bruce Bowerman.

1184A. Dissection of the temporal requirements for cell division proteins. **Tim Davies**, Shawn Jordan, Vandana Chand, Kimberley Laband, Mimi Shirasu-Hiza, Julien Dumont, Julie Canman.

1185B. Developmental regulation of telomere anchoring in *C. elegans*. **Helder C. Ferreira**, Benjamin Towbin, Thibaud Jegou, Susan M. Gasser.

1186C. Characterizing the role of ATX-2, the C. elegans ortholog of Ataxin-2, in cell division. **Megan Gnazzo**, Ahna Skop.

1187A. Transcriptional Regulation of Centrosome Duplication in *C. elegans*. **Jacqueline Goeres**, Kevin O'Connell.

1188B. Functional dissection of MEL-28, a chromatin-binding protein with essential roles in nuclear envelope function and chromosome segregation. **Georgina Gómez-Saldivar**, Anita G. Fernandez, Allison Lai, Carly Bock, Cristina González-Aguilera, Fabio Piano, Peter Askjaer.

1189C. ZTF-15 is required for the meiotic synapsis checkpoint in *C. elegans*. **Tom Hwang**, Matt Ragle, Needhi Bhalla.

1190A. Intertwined Functions of Separase and Caspase in Chromosome Separation and Programmed Cell Death. **Pan-Young Jeong**, Ashish Kumar, Pradeep Joshi, Joel H. Rothman.

1191B. Systematic characterization and positional cloning of temperature-sensitive, embryonic-lethal *C. elegans* mutants. **Reza Keikhaee**, Chien-Hui Chiang, Amy Connolly, Josh Lowry, John Yochem, Bruce Bowerman.

1192C. Condensin depletion licenses an alternate meiotic DSB repair pathway. **Teresa W. Lee**, Barbara J. Meyer.

1193A. Suppression of Cell Cycle Defects through Knockdown of Tumor Suppressor Genes. **Y. Liu**, D. Tobin, M. Saito.

1194B. Cell cycle uncoupling and centriole elimination in the endoreduplicating intestinal cells of *C. elegans*. **Yu Lu**, Richard Roy.

1195C. A forward genetic screen for suppressors of an allele of microtubule-bundling factor, *spd-1*, crucial for central spindle formation during cytokinesis. E. Pablo-Hernando, B. Esmaeili, **M. Mishima**.

1196A. Methods to Study Toxic Transgenes: Analysis of Protease-Dead Separase in Membrane Trafficking. **Diana Mitchell**, Lindsey Uehlein, Joshua Bembenek.

1197B. The nuclear envelope protein LEM-2 is critical for nuclear positioning and centrosome attachment. **Adela Morales Martinez**, Agnieszka Dobrzynska, Cristina Ayuso, Peter Askjaer.

1198C. Mechanistic Insights Into The Recruitment of the Spindle Checkpoint Protein MDF-1 To Unattached Kinetochores. **Mark Moyle**, Karen Oegema, Arshad Desai.

1199A. Regulation of meiotic recombination by the MAP kinase cascade. **Christian R. Nelson**, Tom Hwang, Needhi Bhalla.

1200B. Centriole Copy Number Control Is Mediated by Protein Phosphatase 1-Beta in *C. elegans*. **Nina Peel**, Jyoti Iyer, Michael Dougherty, Kevin O'Connell.

1201C. The NR4A Orphan Nuclear Receptor NHR-6 Plays an Important Role in Cell Cycle Progression and Cell Differentiation during Spermatheca Development. **Brandon Praslicka**, Chris R. Gissendanner.

1202A. Evolutionary comparisons reveal a positional switch for spindle pole oscillation, and divergent regulation of GPR in Cænorhabditis embryos. **Soizic Riche**, Melissa Zouak, Françoise Argoul, Alain Arnéodo, Jacques Pécréaux, Marie Delattre.

1203B. Chromosome bi-orientation in the first spermatocyte meiotic division prevents abnormal spindle organization in the second division. **Mara Schvarzstein**, Anne Villeneuve.

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1205A. RNA-binding Protein ATX-2 Interacts with SZY-20 to Regulate Centrosome Assembly in C. elegans Embryos. Michael Bobian, Madeline Topitzes, John Ross, Jake Crumb, Abigail Meisel, Dongyan Zhang, **Mi Hye Song**.

1206B. Characterization of *lin-5* mRNA localization in the early embryo. **Zoltán Spiró**, Pierre Gönczy.

1207C. Identification and characterization of *mel-43*, a gene required for the meiosis-to-mitosis transition in *C. elegans*. Maryam Ataeian, **J. Tegha-Dunghu**, Martin Srayko.

1208A. Critical targets of CYD-1/CDK-4 in the control of cell cycle entry. **Inge The**, Suzan Ruijtenberg, Javier Muñoz, Martine Prinsen, Albert Heck, Sander van den Heuvel.

1209B. The *C. elegans* UBE2Q2 homolog, UBC-25, Promotes Cell Cycle Quiescence by Inhibiting Cyclin E Expression. **David V. Tobin**, Sarah H. Roy, Nadin Memar, Barbara Conradt, R. Mako Saito.

1210C. A splice mutation in pat-3 β integrin reveals genetic interactions between the extracellular matrix and cki-1/p27^{KIP1}. **Eun-Jeong Yu**, Lena Al-Rashed, Myeongwoo Lee.

1211A. The lipid binding and GAP domains of CYK-4 are essential for cytokinesis. **Donglei Zhang**, Andy Loria, Michael Glotzer.

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1213C. A Functional Genomic Screen for the Telomerase RNA in *C. elegans*. **Robert D. Cohen**, Christopher Smith, Diana Chu.

1214A. Exploring the role of mechanosensory extracellular matrix components in the structure and function of primary cilia. **Deanna Michele De Vore**, Karla Knobel, Maureen Barr.

1215B. Study of arrhythmogenic mutations in the pharynx using electrophysiological and optogenetic approaches. **E. Fischer**, C. Schüler, S. Wabnig, K. Erbguth, P. Hegemann, L. L. Looger, A. Gottschalk.

1216C. MiniMos and Universal MosSCI sites - new methods for *C. elegans* transgenesis. **C. Frokjaer-Jensen**, MW Davis, M. Sarov, X. Liu, K. Rebora, J. Taylor, S. Flibotte, A. Pozniakovski, SK Kim, D. Dupuy, DG Moerman, EM Jorgensen.

1217A. A conditional knockout system based on the single/low-copy integration of transgenes in *C. elegans*. E. Kage-Nakadai, **R. Imae**, O. Funatsu, S. Hori, Y. Suehiro, S. Yoshina, S. Mitani.

1218B. Developing a high-throughput approach for identifying genetic interactions in *C. elegans*. **Calvin A. Mok**, O. A. Thompson, M. Edgley, L. Gevirtzman, C. Huynh, D. G. Moerman, R. H. Waterston.

1219C. Systematic comparison of bacterial feeding strains for increased yield of C. elegans males by RNA interference induced non-disjunction. **Vaishnavi Nagarajan**, Nadeem Asad, Hayley Luna, Jordan Martinez, Zachary Moore, Lisa Timmons.

1220A. Characterization of the *hmgr-1* mutant, which lacks the *C. elegans* Homolog of HMG-CoA reductase. **Parmida Ranji**, Marc Pilon.

1221B. Expanding the repertoire of mutations amenable to identification by whole-genome sequencing. Sijung Yun, Michael Krause, **Harold E. Smith**.

1222C. Acute, High-throughput RNAi in *C. elegans*. **Elizabeth J. Thatcher**, Victor Ambros.

1223A. Genomic variation data in WormBase. **Mary-Ann Tuli**, Paul Davis, Michael Paulini, Gary Williams, Kevin Howe.

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1224B. A cGMP reporter for *C. elegans*. **Mary Bethke**, Chantal Brüggemann, O. Scott Hamilton, Damien O'Halloran, Bi-Tzen Juang, Klaus Kruttwig, Ben Barsi-Rhyne, Mihn Pham, Dominique Glauser, Miriam Goodman, Piali Sengupta, Miri Van Hoven, Noelle L'Etoile.

1225C. Endrov, a general imaging framework, to visualize C. elegans gene expression, 4D models, and lineage. Johan Henriksson, Jurgen Hench, Martin Luppert, Akram Abou-Zied, Lois Tang, Yong-Guang Tong, David Baillie, **Thomas R. Burglin**.

1226A. A non-trapping method for live imaging of specific neuronal connections. **Muriel Desbois**, Hannes Buelow.

1227B. *In vivo* calcium imaging of motor circuit during spontaneous locomotion using improved G-CaMPs. **K. Gengyo-Ando**, Y. Kagawa-Nagamura, M. Ohkura, X. Fei, M. Suzuki, K. Hashimoto, J. Nakai.

1228C. Mapping the entire connectome of C. elegans L1 larvae. Valeriya Laskova, Quan Wen, Richard Shalek, Daniel Berger, Maria Lim, Bobby Kasthuri, Verena Kaynig-Fittkau, Hanspeter Pfister, Jeff Lichtman, Aravi Samuel, Mei Zhen, **Sihui Guan**.

1229A. Development of a comprehensive image analysis software package for the analysis of lifespan, locomotion, body length, and egg laying of *C. elegans*. **S. K. Jung**, B. Aleman-Meza, C. M. Riepe, W. Zhong.

1230B. WormView: a library of modular Matlab functions for static and dynamic image analysis. **Gunnar Kleemann**, Lance Parsons, Coleen Murphy.

1231C. Development and applications of TEM approaches adapted for *C. elegans* research. **Irina Kolotueva**.

1232A. Probing intercellular lipoprotein transport in Caenorhabditis elegans by fluorescent nanodiamond imaging. **Yung Kuo**, Tsung-Yuan Hsu, Yi-Chun Wu, Huan-Cheng Chang.

1233B. 3-D Worm Tracker for *C. elegans*. **Namseop Kwon**, Ara B. Hwang, Seung-Jae Lee, Jung Ho Je.

1234C. WormSizer: Image-based analysis of nematode size and shape. **Brad T. Moore**, James M. Jordan, L. Ryan Baugh.

1235A. Investigation of Simplified Dual-fluorophore Dissecting Stereomicroscopes. **Andy Papp**, Chris Aldrich.

1236B. High-throughput approaches to motility analysis in *C. elegans* and parasitic nematodes. **Frederick A. Partridge**, David B. Sattelle.

1237C. *C. elegans* imaging by combined, selective plane illumination microscopy and optical projection tomography in a microfluidic device. **M. Rieckher**, G. Zacharakis, J. Ripoll, N. Tavernarakis.

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1239B. Imaging lipid depositions with third harmonic generation microscopy. George Tserevelakis, Evgenia Megalou, George Filippidis, Barbara Petanidou, Costas Fotakis, **Nektarios Tavernarakis**.

1240C. High-speed, high-magnification tracking system for calcium imaging of neurite during free moving. **Y. Tsukada**, C. Min, X. Fei, K. Hashimoto, I. Mori.

1241A. DiSPIM: time to leave your confocal behind. Hari Shroff, Yicong Wu, Peter Wawrzusin, Justin Senseney, Robert Fischer, Ryan Christensen, Anthony Santella, Andrew York, **Peter Winter**, Clare Waterman, Zhirong Bao, Daniel Colón-Ramos, Matthew McAuliffe.

1242B. Lipid droplets distribution in different developmental stages of C. elegans by using Coherent Anti-stoke Raman Scattering (CARS) microscopy. **Yung-Hsiang Yi**, Cheng-Hao Chien, Wei-Wen Chen, Tian-Hsiang Ma, Kuan-yu Liu, Yu-sun Chang, Ta-Chau Chang, Szecheng J. Lo.

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1246C. Cell type-specific profiling of the transcriptome in *Caenorhabditis elegans*. **Meenakshi K. Doma**, Igor Antoshechkin, Paul W. Sternberg.

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ABSTRACTS

1. Feedback and Self-Organization in Meiosis. **Anne Villeneuve**. Stanford University.

2. WormBase - still growing upwards and outwards. **Kevin Howe**[1], The WormBase Consortium[1,2,3,4]. 1) EMBL-EBI, Hinxton, Cambridge, UK; 2) California Institute of Technology, Pasadena, CA, USA; 3) Ontario Institute For Cancer Research, Toronto, Ontario, Canada; 4) Washington University, St. Louis, MO, USA.

Since its first release in 2001, WormBase (http://www.wormbase.org) has grown from a small database serving the specific research community of a single species, to a resource encompassing the breadth of the nematode phylum and serving as a fundamental tool for broader biomedical and agricultural research. We now include the genome sequences of over twenty nematode species, around half of which are parasitic worms implicated in animal or plant disease. We have begun to engage directly with parasitic nematode research communities, and have recently collaborated on the annotation of a new version of the *Brugia malayi* reference genome, manually curating around one fifth of the gene models. We also continue to scale-up our literature curation workflows and develop our phenotype, life-stage and anatomy ontologies to apply them beyond the *Caenorhabditis* genus. In parallel with this diversification, we continue to increase the depth and detail of information for *C.elegans*. We have adopted new approaches to how we represent and analyse genomic variation data in response to continued growth in whole-genome sequencing of mutant and wild isolate strains; we have enriched our data sets of gene expression, transcriptional regulation, pathways, and interactions; and we now provide both predicted and experimentally-confirmed associations between *C. elegans* genes and human disease genes, collaborating with other model organism databases in the adoption of a common ontology for human disease. Finally, continued expansion of the resource, both in breadth and depth, has motivated us to completely rethink how data is presented to and accessed by users. The new WormBase web-site, demonstrated in 2011, is now in full production, resulting in greater speed, stability and flexibility. We are now pleased to introduce our new platform for data mining, *WormMine*. Conceptually similar to our previous data-mining tool (WormMart), WormMine allows custom queries to be composed from pre-prepared query templates, greatly improving the speed and ease with which users can obtain the precise data they need.

3. Tactile Toy, Teaching Tool, or Transformative Technology? WormGUIDES EmbryoAtlas 1.0. **William A. Mohler**[1,5], Zhirong Bao[2], Daniel Colon Ramos[3], Hari Shroff[4], Jim Schaff[5], Ion Moraru[5], wormguides.org. 1) Dept of Genetics & Dev Biology, Univ Connecticut Health Ctr, Farmington, CT; 2) Developmental Biology Program, Sloan-Kettering Institute, New York, NY; 3) Dept. of Cell Biology, Yale University, New Haven, CT; 4) NIBIB, NIH, Bethesda, MD.NIBIB, NIH, Bethesda, MD; 5) Center for Cell Analysis and Modeling, Univ Connecticut Health Ctr, Farmington, CT.

Over the past 50 years, the *C. elegans* community has excelled at cataloguing and distilling vast quantities of data into succinct yet comprehensive summaries: the cell lineage, the anatomical parts list, the neural wiring diagram, the genome database. Out of technological necessity, these cornerstones of the worm knowledge base are generally accessed in text-based or 2-dimensional still-image formats. Correlations among members of these sets and details of their spatio-temporal arrangements are often left to the individual researcher's intuition, imagination, or further experimentation. The WormGUIDES EmbryoAtlas integrates a variety of cell-specific data into a visually intuitive multidimensional exploration/understanding interface that, by definition, can only be conveyed poorly in this abstract. Nuclei of embryonic cells are plotted within a freely rotatable, time-animated model of the developing embryo. Each nucleus can be queried by the user for its identity and for information about its ancestry, fate, and function. Conversely, the viewer can easily compose scenes in which specific sublineages and/or cells with common fates and/or precursors of specific organs and/or cell-by-cell gene-expression patterns are simultaneously highlighted within the interactive 4-D model. Moreover, any scene from a single user's screen can be instantly shared with collaborators or published as a URL web-link. And there's an app for that: Android (available April 2013). https://play.google.com/store/apps/details?id=org.wormguides.embryoatlas iOS (coming Spring 2013). https://itunes.apple.com/us/app/embryoatlas/id635126084?ls=1&mt=8 Java for Windows/Mac/Linux (coming Summer 2013) http://www.wormguides.org/launch.

4. WormAtlas Update. L. A. Herndon, Z. F. Altun, C. A. Wolkow, K. Fisher, C. Crocker, **D. H. Hall**. Ctr C Elegans Anatomy, Albert Einstein Col Med, Bronx, NY.

Since its inception in 2001, WormAtlas has added new features and become more user friendly each year. New additions since the last International Meeting include: 1) A new Handbook on the anatomy of the Dauer Larva consisting of 6 chapters, with more on the way. Many Handbook chapters for the adult Hermaphrodite have been updated to include recent findings, new references, illustrative movies, and more links. For the future, we are planning new Handbooks that will feature the anatomy of the Embryo and of Aging worms. 2) An updated version of the Pharynx Atlas uses a rollover cell identification function to allow users to link to pages with detailed descriptions of each pharyngeal cell in the terminal bulb. New movies have been added to illustrate patterning during embryonic pharynx development. 3) Many Individual Neuron pages have been added or substantially revised, adding more recent data on gene expression patterns. 4) The Neurons and Circuits section is expanding, with a comprehensive new Table of Neurotransmitter and Neuropeptide Receptors for *C. elegans*, plus links to new initiatives such as the WormWiring website. 5) The Anatomical Methods section has been updated and now includes new techniques and detailed protocols for high resolution EM studies and “connectomics”. 6) SlidableWorm has a new interface and has many more sections available. Users can now access transverse cross-sections of *C. elegans* by moving a cursor along the length of the worm to select which slice to visualize. There are four different ways to view the electron micrograph sections: with no annotation, with labels and with tissue highlighting displayed as either a transparent or an opaque overlay. 7) WormImage 2.0 has a new look and new search interface making data more easily accessible, first introduced at the last meeting. WormImage now includes virtually every well-annotated animal received from the MRC EM archive of Brenner, White and colleagues. We welcome further contributions from the community to this archive. 8) WormAtlas offers mobile users better access to its content. All Handbooks are now available in a format optimized for browsing from mobile devices. This work is supported by NIH OD 010943 to DHH.

5. WormBook News. **Jane E. Mendel**[1], Qinghua Wang[1], Todd Harris[2], Paul Sternberg[1,3], Oliver Hobert[4], Martin Chalfie[5]. 1) Div Biol, California Inst Tech, Pasadena, CA; 2) Hi-Line Informatics, LLC. Livingston, MT; 3) Howard Hughes Medical Institute, Pasadena CA; 4) Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University, New York NY; 5) Department of Biological Sciences, Columbia University, New York, NY.

WormBook (http://www.wormbook.org/) is a comprehensive, open-access collection of original peer-reviewed chapters covering the biology of C.

elegans and other nematodes. We are currently undertaking a major revision and updating of the older chapters in WormBook as well as adding chapters on new topics. New features include the launching of a new section, WormHistory, on the history of nematode research, and we are also adding links to review articles from other sources that may be useful to WormBook readers. Changes are also coming to WormMethods, a section of WormBook containing protocols ranging from the basics of C. elegans culture to advanced imaging techniques. We are reorganizing and updating the current content of WormMethods to make it easier to find relevant protocols, and we are also adding many new chapters. WormBook also hosts The Worm Breeder's Gazette, which is an on-line informal newsletter. Since the re-launch of the Gazette in December of 2009, we have published 7 issues with 124 articles. Gazette articles are brief and include research results, new or improved methods, web resources and anything generally of interest to worm researchers. New features of the Gazette include lists of notable recent papers organized by topic, links to and brief descriptions of recently published methods, and announcements of new labs. We also feature artwork as 'covers' for the journal. We welcome your feedback and comments on how to improve WormBook, WormMethods and The Worm Breeder's Gazette.

6. Caenorhabditis Genetics Center. Aric Daul, Theresa Stiernagle, Julie Knott, Brittany Werre, **Ann E. Rougvie**. Dept Gen, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN.

The Caenorhabditis Genetics Center (CGC), supported by the National Institutes of Health - Office of Research Infrastructure Programs (NIH-ORIP) and housed at the University of Minnesota, supplies Caenorhabditis strains and information to researchers throughout the world. The CGC has also initiated a small research component aimed at enhancing the genetic tool-kit available to *C. elegans* researchers. The CGC continues its duties of acquiring, maintaining and distributing worm stocks. There are now over 17,512 different strains in the collection. We strive to have at least one allele of every published gene and all chromosome rearrangements, duplications and deficiencies. Selected multiple-mutant stocks and transgenic strains are also available including strains that express various fluorescent protein reporter fusions. Greater than two thousand whole-genome sequenced strains, obtained from the Million Mutation Project and other sources, are available and have proved popular. The CGC also has stocks of nematode species closely related to *C. elegans* and bacterial strains necessary for nematode growth. A searchable strains list, including information about CGC stocks, is accessible either through the CGC website (www.cbs.umn.edu/CGC/) or through WormBase. Requests for strains should be made via the on-line ordering system available through our website. As mandated by NIH-ORIP, a small yearly user fee and charge per strain is assessed with each order. The CGC strongly encourages use of credit cards for payments. We provide yearly reports to the NIH with statistics that reflect our services to the worm community. A key tracked parameter is the number of published papers that acknowledge the CGC for providing strains. **Please remember to acknowledge the CGC in your publications!**.

7. The viral 2A peptide technology to express multiple functional proteins from a single ORF in Caenorhabditis elegans. **Arnaud AHIER**, Sophie JARRIAULT. Development & Stem cells program, IGBMC-Institut de Genet. et de Bio. Moleculaire, Illkirch, Alsace, France.

Caenorhabditis elegans is a powerful in vivo model for which transgenesis is highly developed. However, the expression of more than one protein of interest is often required but no reliable tool exists to ensure efficient concomitant and stoichiometric expression of more than 2 polypeptides from a single promoter. Solutions such as the IRES (internal ribosomal entry sites) result in expression of 2 polypeptides with very different expression levels. In C. elegans, intergenic sequences from operons have been used to express two products from a single promoter but the expression of more proteins remains difficult. For these reasons we decided to develop the 2A viral peptides technology for C. elegans. 2As have been shown to deliver multiple proteins from a single ORF in several vertebrate models and very recently in drosophila. 2As are viral peptides that trigger a "ribosomal-skip" or "STOP&GO" mechanism during translation. Four 2A peptides, isolated from different viruses, have been characterised: F2A, T2A, E2A and P2A. We showed here that these four canonical 2A peptides are working in several tissues of the worm (i. e. epithelia, neurons, intestin and muscles) and all developmental stages. Comparison of their efficiency pointed to T2A, E2A and F2A. P2A, although described as the most efficient in vertebrates, seems to be qualitatively less efficient for the worm. We then used several 2As to express five different functional proteins addressed to different cell compartments (nucleus, nucleus memb., cytoplasm and cell memb.) while rescuing a single cell biological process (the Y-to-PDA TransDifferentiation event). Finally, to facilitate the emergence of this new technology for C. elegans we built a 2A-based toolkit for the expression of multiple proteins and to generate 2A-tagged fosmids. 2A based vectors allow efficient and comparable expression of proteins in the worm and thus constitute an invaluable tool to visualize proteins in vivo in C. elegans. They can also be considered as a tool of choice to deliver cocktails of factors or even reconstitute sub-unit-composed proteins.

8. A genome-scale resource for *in vivo* tag-based protein function exploration in *C. elegans.*. **Mihail S. Sarov**[1], John I. Murray[2], Susanne Ernst[3], Andrei Pozniakovski[3], Elisabeth Loester[1], Stephan Janosch[1], Wadim Kapulkin[1], Siegfried Schloissnig[4], Anthony A. Hyman[3], The ModENCODE C. elegans TF binding sites group.. 1) TransgeneOmics, MPI-CBG, Dresden, Germany; 2) Department of Genetics, University of Pennsylvania School of Medicine, Philadelphia, USA; 3) Hyman lab, MPI-CBG, Dresden, Germany; 4) Computational Biology, HITS, Heidelberg, Germany.

Our understanding of biological systems at all levels of organization depends on our ability to probe the molecular function of their building blocks *in vivo*. To enable systematic protein function interrogation in a multicellular context, we have built a genome-scale transgenic platform for *in vivo* expression of fluorescent- and affinity-tagged proteins in *Caenorhabditis elegan*s under endogenous *cis* regulatory control. The resource covers 14,637 protein coding genes, or 73% of the proteome. The function of the tagged proteins can be interrogated with various standard, tag-based techniques - *in vivo* imaging, AP-MS, ChIP, PAR-CLIP. The resource not only simplifies the work required to characterize individual genes but opens up the way towards systematic function discovery on a proteome-wide scale. The resource is now available to the community and hundreds of researchers have already started to generate lines at a rate far exceeding what could be achieved by any single lab. We are providing web based tools that enable the exploration of the resource, simplify sample tracking throughout the transgenesis pipeline and the storage and collaborative annotation of imaging data. We will present the results of the project so far and our ongoing efforts to improve the available resources and to extend them to other nematode species as a tool to explore the evolution of developmental regulation.

9. The Million Mutation Project and Beyond. Owen Thompson[1], **Mark Edgley[2]**, Pnina Strasbourger[1], Stephane Flibotte[2], Brent Ewing[1], Ryan Adair[2], Vinci Au[2], Iasha Chaudhry[2], Lisa Fernando[2], Harald Hutter[3], Joanne Lau[2], Angela Miller[2], Greta Raymant[2], Bin Shen[2], Jay Shendure[1], Jon Taylor[2], Emily Turner[1], LaDeana Hillier[1], Donald G. Moerman[2], Robert H. Waterston[1]. 1) Department of Genome Sciences, University of Washington, Seattle, WA; 2) Department of Zoology and Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada; 3) Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada.

We have created a library of 2,007 mutagenized C. elegans strains, each sequenced to a target depth of 15X coverage, to provide the research community with mutant alleles for each of the worm's more than 20,000 genes. The library contains over 820,000 unique SNVs with an average of eight non-synonymous changes per gene and more than 16,000 indel and copy number changes, providing an unprecedented genetic resource for C. elegans. We supplemented this collection with 40 sequenced wild isolates, identifying more than 630,000 unique SNVs and 220,000 indels. Comparison of the two sets shows that the mutant collection has a much richer array of both nonsense and missense mutations than the wild isolate set. Comparison with expected events suggests that very few missense alleles are incompatible with life but more than 40% are subject to long term selection. All the strains are available through the CGC; all the sequence changes have been deposited in WormBase and our own interactive web site (http://genome.sfu.ca/mmp/about.html). Loss-of-function alleles are now available for 13,760 of 20,514 protein coding genes. To identify mutations in the remaining genes, including essential genes, we use the following strategy. F1 progeny of mutagenized P0s are minimally propagated before sequencing and analysis. We use gain or loss of restriction sites to facilitate following the mutations through subsequent steps to isolate homozygous viable animals, or to create heterozygous balanced lethals. Pilot experiments have demonstrated the feasibility of this approach; we have identified new nonsense alleles of known essential genes, as well as nonsense and splicing mutations in several genes with no previous null mutations.

10. Advances in Targeted Genome Editing Across Species: Heritable Designer "Knock-In" and "Knock-Out" Modifications. **Te-Wen Lo**, Catherine Pickle, Mark Gurling, Caitlin Schartner, Erika Anderson, Ed Ralston, Barbara J. Meyer. HHMI/UC Berkeley.

We have achieved targeted genome editing across nematode species diverged by 300 million years. These methods have proven to be invaluable for evolutionary studies across species that lack reverse genetic tools but have sequenced genomes. Our editing protocols work in parasitic nematodes (*P. pacificus*), male/female species (*C. sp.9*), and hermaphroditic species (*C. elegans* and *C. briggsae*). Our approach uses engineered nucleases made of fusions between the DNA cleavage domain of FokI and a custom-designed DNA-binding domain of transcription activator-like effector (TALE) repeats. Our protocols permit not only the isolation of "knock-out" mutations, but also the recovery of multiple different custom-designed "knock-in" mutations in the genomic location of choice. The various "knock-in" and "knock-out" modifications can be recovered from the progeny of a single injected animal. The entire process from TALEN design to isolation of DNA-sequence-verified homozygous mutants can be completed in three weeks, and the cost is minimal.

11. Heritable genome editing in C. elegans via CRISPR-Cas systems. **Ari E. Friedland[1]**, Yonatan B. Tzur[1], Kevin M. Esvelt[2], Monica P. Colaiacovo[1], George M. Church[1,2], John A. Calarco[3]. 1) Genetics, Harvard Medical School, Boston, MA; 2) Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA; 3) FAS Center for Systems Biology, Harvard University, Cambridge MA.

Clustered, regularly interspaced, short palindromic repeats (CRISPR) and CRISPR-associated (Cas) systems are adaptive defense mechanisms evolved by bacteria and archaea to repel invading viruses and plasmids. These Cas systems have recently been used with single guide RNAs (sgRNAs) for accurate editing of target genomic sequences in multiple biological systems. Here we report the use of a novel U6 small nuclear RNA promoter to drive expression of sgRNAs that guide the endonuclease Cas9 to specific genomic sequences in the C. elegans germline. The double stranded breaks that are induced there by Cas9 can be repaired through the process of non-homologous end joining, generating insertions and deletions (indels) in the vicinity of the cleavage site and leading to truncated proteins. We independently targeted multiple genes and recovered several loss-of-function mutant lines, confirming the presence of indels at our target site by sequencing and finding no other indels at candidate "off-target" sites in these lines. Our results demonstrate that heritable genetic alterations can be achieved in vivo in multicellular animals using engineered CRISPR-Cas systems, and provide a convenient and effective approach for generating loss of function mutant animals in C. elegans.

12. CRE-LoxP mediated gene inactivation to study the coordination between proliferation and differentiation. **Suzan Ruijtenberg**, Sander van den Heuvel. Developmental Biology, Utrecht University, The Netherlands.

The proper balance between proliferation and differentiation is critical for the formation and function of somatic cells, tissues and organs. During development, terminal differentiation usually coincides with permanent cell-cycle exit, while sustained proliferation is a hallmark of cancer cells. Despite their importance, the mechanisms that accomplish cell-cycle arrest in a developmental context are poorly understood. Proliferation and differentiation are tightly coordinated in *C. elegans*, as illustrated by its reproducible cell lineage and lack of mutants with severe over-proliferation phenotypes. Inactivation of *lin-35* Rb leads to substantial hyperplasia only when combined with loss of other negative regulators of the cell cycle, such as *fzr-1* Cdh1 or *cki-1/2* Cyclin-dependent Kinase Inhibitors. Importantly, even *lin-35;cki-1* and *lin-35;fzr-1* double mutants form apparently normal post-mitotic differentiated cells. While this points to redundant control of cell-cycle arrest, essential functions in embryogenesis and maternal contribution complicate the genetic analysis of combined mutations.

To be able to study cell-cycle arrest in post-embryonic cell lineages, we developed a CRE-LoxP based recombination system. This system combines two features: tissue-specific expression of the CRE recombinase induces lineage-specific inactivation of a gene of interest and, at the same time, causes a switch in fluorescent protein expression. This latter aspect visualizes recombination events and helps lineage tracing. After optimization and dependent on the promoter used, CRE induced recombination in many different tissues, with nearly 100% efficiency in the intestine and mesoblast lineages. Combination of *lin-35* knock down and intestine-specific inactivation of *fzr-1* Cdh1 by CRE-LoxP recombination resulted in overproliferation in the intestine to a similar extent as the *lin-35;fzr-1* double mutant. Importantly, other tissues were unaffected and the knock-out animals were viable and fertile, in contrast to the double mutant. We currently focus on the mesoblast lineage to obtain a comprehensive understanding of the regulatory mechanisms that coordinate cell-cycle exit with muscle differentiation.

13. Magnetotaxis in *C. elegans*. **Andrés G. Vidal-Gadea**, Kristi A. Ward, Jonathan T. Pierce-Shimomura. Section of Neurobiology, The University of Texas at Austin, Austin, TX.

The magnetic field of the Earth provides an ancient and continuous source of directional information to organisms able to detect it. While the last half-century has seen the list of species capable of this feat grow steadily (from bacteria to mammals), the mechanisms by which magentosensation is accomplished in animals has remained elusive. Magnetotactic bacteria are known to harvest iron from their environment to build nanometer-sized "compasses" and evidence of magnetite has been reported in many magnetotactic animals. It remains unclear whether and how these putative "biological compasses" are used. This is partly due to the fact that a magnetosensory neuron has never been identified in any animal. We show that the nematode *C. elegans* readily orients to common magnets in addition to the Earth's magnetic field. Using a custom-built magnetic cage, we tested worms in magnetic fields controlled along three dimensions. Worms migrated at an angle to the field, in what would be the "down" direction. Our findings suggests that the Earth's magnetic field may serve as a better cue than gravity for these tiny animals to orient while burrowing. Through behavioral analysis of mutants as well as cell-specific rescue and ablation, we next identified a pair of amphid sensory neurons as necessary and sufficient to mediate magnetotaxis in *C. elegans*. Functional calcium imaging performed on these neurons revealed calcium transients induced by magnetic fields. Genes and neurons required for magnetotaxis are distinct from those previously reported to be essential for electrotaxis. Magnetic orientation in *C. elegans* appears to take place through a light-independent mechanism, and to rely on a cGMP-dependent transduction pathway. To our knowledge, this represents the first report of sensory neurons required for magnetic orientation in any species.

14. FLP-13 neuropeptides released from the ALA neuron signal through FRPR-4 to regulate behavioral quiescence. **Matthew Nelson[1]**, Tom Janssen[2], Liliane Schoofs[2], David Raizen[1]. 1) Dept Neurology, University of Pennsylvania, Philadelphia, PA; 2) Functional Genomics and Proteomics lab, University of Leuven, Leuven, Belgium.

Activating the peptidergic interneuron ALA by the epidermal growth factor LIN-3 causes quiescence of locomotion and feeding (Van Buskirk and Sternberg, 2007). While synaptic transmission is required for this effect, the neurotransmitter released by ALA to induce quiescence has been unknown. A proteomics study of the ALA neuron performed in the nematode *Ascaris* found peptides encoded by an *Ascaris* gene homologous to C. elegans *flp-13* (Jarecki et al, *ACS Chem. Neurosci*, 2010). We therefore hypothesized that *flp-13* is expressed in *C. elegans* ALA, and that *flp-13* derived neuropeptides promote behavioral quiescence induced by ALA. In support of this hypothesis, we found that 5 kb upstream of the *flp-13* start site drives expression in the ALA neuron; that over-expression of *flp-13* using a heat-shock promoter induces behavioral quiescence; that *flp-13* mutants are defective in feeding quiescence induced by EGF; and that this defect is rescued by restoring *flp-13* expression in ALA. To identify a receptor for FLP-13, we postulated that a gene encoding a FLP-13 receptor might show cyclical expression with the larval cycle, since behavioral quiescence occurs naturally during larval transitions. The gene *frpr-4*, which encodes a predicted G-protein coupled neuropeptide receptor, fits this criterion. Transgenic multi-copy expression of *frpr-4* expressed under its endogenous regulatory elements induces spontaneous bouts of feeding and locomotion quiescence, which were reduced in the presence of a *flp-13* null mutation. Peptides encoded by *flp-13* potently activate FRPR-4 in a heterologous mammalian cell culture system. Based on this combination of *in vivo* and *in vitro* data, we conclude that FLP-13 neuropeptides released from the ALA neuron signal through FRPR-4 to regulate behavioral quiescence.

15. Counterbalance between BAG and URX neurons via guanylate cyclases controls lifespan homeostasis in *C. elegans*. **T. Liu**, D. Cai. Dept Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

Lifespan of *C. elegans* is known to be affected by the nervous system; however, the underlying neural integration still remains unclear. In this study (*EMBO J*, 2013 in press), we targeted an antagonistic neural system consisting of BAG neurons and URX neurons that are known to mediate low versus high oxygen sensing, and found that these two neuronal groups work to counteractively regulate lifespan of *C. elegans*. Our results demonstrated that *C. elegans* mean lifespan increases 27.7% by BAG neuron ablation ($P < 0.0001$) and decreases 21.8% by URX neuron ablation ($P < 0.0001$). Consistently, genetic loss-of-function of low-oxygen sensor GCY-31/GCY-33 of BAG neurons extends mean lifespan by 20.4-27.3% ($P < 0.0001$), whereas genetic loss-of-function of high-oxygen sensor GCY-35/GCY-36 of URX neurons shortens mean lifespan by 13.9-16.1% ($P < 0.01$). Cell-specific genetic rescues and epistasis analyses show that these different GCYs act in BAG versus URX neurons to counteractively control lifespan. Moreover, the lifespan-modulating effects of these GCYs are independent of the actions from insulin/IGF-1 signaling, germline signaling, sensory perception, or dietary restriction. Given the known gas-sensing properties of these neurons, we profiled that *C. elegans* lifespan is promoted under moderately-low (4-12%) oxygen or moderately-high (5%) carbon dioxide but inhibited under high-level (40%) oxygen; however, these pro-longevity and anti-longevity effects are independent of and counteracted by BAG or URX neurons via different GCYs. In conclusion, BAG and URX neurons antagonistically control lifespan balance independently of canonical pathways in *C. elegans*, and oxygen and carbon dioxide sensing mediated by GCYs in these neurons act as a previously unappreciated neural system that integrates lifespan control with physiological homeostasis.

16. PQM-1: the missing "DAE Factor" and key regulator of DAF-2-mediated development, longevity, and homeostasis. **Ronald G. Tepper[1]**, Jasmine Ashraf[2], Rachel Kaletsky[2], Gunnar Kleemann[2], Coleen T. Murphy[2], Harmen J. Bussemaker[1,3]. 1) Department of Biological Sciences, Columbia University, New York, NY 10027, USA; 2) Lewis-Sigler Institute for Integrative Genomics and Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544, USA; 3) Center for Computational Biology and Bioinformatics, Columbia University Medical Center, New York, NY 10027, USA.

Reduced insulin/IGF-1-like signaling (IIS) extends C. elegans lifespan by upregulating stress response (Class I) and downregulating development (Class II) genes through a mechanism that depends on the conserved transcription factor DAF-16/FOXO. By integrating a genomewide analysis of gene expression responsiveness to DAF-16 with genomewide *in vivo* binding data for a compendium of transcription factors, we discovered that the transcriptional activator PQM-1 directly controls Class II genes by binding to the DAF-16 associated element (DAE). DAF-16 directly regulates Class I genes only, through the DAF-16 binding element (DBE). Loss of PQM-1 suppresses *daf-2* longevity and thermotolerance and further slows development. The nuclear presence of PQM-1 and DAF-16 is controlled by IIS in opposite ways, and, surprisingly, was found to be mutually exclusive. We observe progressive loss of nuclear PQM-1 with age, explaining declining expression of PQM-1 targets. Together, our data suggest an elegant mechanism for balancing stress response and

development.

17. Analysis of mutation accumulation by large scale C. elegans whole genome mutation profiling. B Meier[1], S Cook[1], J Weiss[2], A BAilly[3], P Campbell[2], **Anton Gartner[2]**. 1) Centre for Gene Regulation/Expression, Dundee, UK; 2) Wellcome Trust Sanger Institute, Cambridge, UK; 3) CRBM, CNRS Montpellier,.

Tumorigenesis is driven by the accumulation of mutations. Nevertheless, we know little about the responsible mutagens, and about how DNA damage response (DDR) pathways prevent mutatagenesis. Tumor therapy largely depends on chemotherapeutic agents that generate DNA lesions, such as DNA adducts, DNA crosslinks and DNA double strand breaks. However, the actual nature of the resulting lesions or the mutant profiles are largely unknown. We initiated a systematic study to assay mutation patterns of a large number of known or suspected carcinogens and chemotherapeutic agents in wild-type and ~45 highly backcrossed DDR mutants. The worm provides a clonal system for mutagenizing single germ cells, for assaying mutation rates and spectra by whole genome sequencing (WGS). Up to now we have sequenced and analyzed ~300 worm genomes. We investigated the accumulation of mutations in a set of DDR mutants propagated for 20 generations without being challenged with mutagens. This analysis revealed increased rates of point mutations or altered mutation profiles in some strains, the most severe mutant showing evidence for gross chromosomal instability. We are also using DNA crosslinking agents such as the chemotherapy drug cisplatin. Base substitution patterns observed in wild-type and DDR mutants are consistent with cisplatin's ability to induce 1,2-intrastrand d(GpG) and d(ApG) crosslinks. In contrast, increased di-nucleotide substitutions and genomic rearrangements are detectable in only a subset of mutants in response to drug treatment. We also observe (small) insertion/deletion events, with a frequency and type greatly varying among different DNA repair mutants. In addition, a small number of complex rearrangements occur, the severity and incidence of which increase in several repair mutants. We are currently analyzing breakpoints and sequence micro-environments for insertions-deletions profiles to determine possible mechanisms by which they arise. We are also confident to get general and important insights into DNA damage processing and repair in vivo.

18. TORC2 regulates SGK-1 in two opposing longevity pathways. **M. Mizunuma[1,2]**, E. Neumann-Haefelin[1,3], N. Moroz[1,4], K. Blackwell[1]. 1) Joslin Diabetes Center, Harvard Stem Cell Institute, and Dept of Genetics, Harvard Medical School , Boston, MA; 2) Dept of Molec Biotech, Hiroshima Univ, Higashi-Hiroshima, Japan; 3) Renal Division, University Hospital Freiburg, Freiburg, Germany; 4) Dept of Genetics and Complex Diseases, Division of Biological Sciences, Harvard School of Public Health, Boston, MA.

The mechanistic target of rapamycin (mTOR) kinase, which is critical for growth, is present in the mTORC1 and mTORC2 complexes. mTORC1 stimulates protein synthesis and inhibits autophagy, but mTORC2 functions are less well understood. Rapamycin or mTORC1 inhibition increases lifespan in organisms as diverse as yeast, C. elegans, and mice. Analyses of the mTORC2 component Rictor (RICT-1) in C. elegans indicate that mTORC2 also affects lifespan, but in a manner that is dependent upon the food source. RICT-1 regulates growth and lipid metabolism by activating the kinase SGK-1. It is not clear whether SGK-1 might mediate longevity effects of RICT-1, in part because of SGK-1 data that appear conflicting: it has been reported that lifespan is increased by loss of SGK-1 function, but also that sgk-1 mutants are short-lived and that SGK-1 acts in a pathway that increases lifespan at lower temperature. Here, we reconcile published and new findings within a model whereby RICT-1 acts through SGK-1 to exert two opposing effects on lifespan. RICT-1 limits longevity by directing SGK-1 to inhibit the stress defense transcription factor SKN-1/Nrf. This mechanism is food source-dependent, but does not seem to involve differences in nutrient consumption. At the same time, RICT-1 is required for SGK-1 to promote lifespan at lower temperature. The balance between these two pathways determines whether RICT-1 and SGK-1 accelerate or oppose aging. The data show that SKN-1/Nrf is a key biological target of mTORC2 and SGK-1 with respect to stress resistance and longevity, and delineate complex effects of mTORC2 on pathways that influence aging.

19. A CREB-Dependent Neuropeptide Signal from the Thermosensory AFD Neuron Regulates *C. elegans* Life Span at Warm Temperatures. **Yen-Chih Chen**, Wei-Chin Tseng, Chun-Liang Pan. Institute of Molecular Medicine, National Taiwan University, Taiwan.

Ambient temperature is one of the diverse environmental stimuli that influence life span. Previous studies indicate that in *C. elegans*, thermosensory input from the AFD neuron activated the DAF-9 sterol hormone pathway and promoted longevity at warm temperatures. However, how thermosensation is translated into a neuronally derived signal that communicates with the DAF-9 endocrine system remains elusive. We found that *crh-1*, which encodes the *C. elegans* CREB, was required for a normal life span at warm, but not at cold temperatures. *crh-1* was expressed in the thermosensory neuron AFD, as well as in the AWC, the ASE, the interneuron SIA, and the intestine. *crh-1* acted in the AFD for temperature-dependent longevity at warm temperatures, and its activity was dependent on phosphorylation by the CaMKI/IV CMK-1. Elimination of *crh-1* functions in the short-living *daf-9* mutants did not further reduce life span, and DAF-9 level was decreased in the hypodermal tissues of the *crh-1* mutant, suggesting that DAF-9 mediates the effects of CRH-1 on longevity. Interestingly, we found that mutations in the FMRFamide-like neuropeptide gene *flp-6* showed temperature-dependent life span reduction similar to that in the *crh-1* or the *cmk-1* mutant. We demonstrated that *flp-6* acted in a common pathway with *crh-1*, was expressed in the AFD neuron, and its expression was dependent on a CRE motif in its promoter. Our preliminary results suggested that the interneuron AIY, which does not express CRH-1, may be the downstream cell that FLP-6 targets to regulate longevity. Together these experiments identify a CREB-dependent neuronal circuit that integrates information on ambient temperature into a synaptic neuropeptide signal, which then promotes longevity at warm temperatures by positively regulating the sterol hormone endocrine system. This work is supported by a National Health Research Institute Career Development Grant to C.-L. Pan (NHRI-EX101-10119NC).

20. FGT-1 is the sole glucose transporter in C. elegans and is central to aging pathways. Ying Feng[1], B. Williams[1,2], F. Koumanov[1], **A.J. Wolstenholme[2]**, G.D. Holman[1]. 1) Dept of Biology & Biochemistry, University of Bath, Bath, UK; 2) Dept of Infectious Diseases, University of Georgia, Athens, GA.

Caenorhabditis elegans has been widely used as a model for investigation of the relationships between nutrition, signalling and aging. Remarkably, while glucose metabolism is implicated in aging, in worms the identity of the genuine glucose transporters is unknown. By homolog searching eight candidate genes have been proposed to fulfil this role. We cloned cDNAs from all eight genes annotated as having a possible glucose transport function and expressed them in Xenopus oocytes. We find that only the two splice variants of one gene, H17B01.1, encode a functional glucose transporter. This gene we rename fgt-1 (Facilitated Glucose Transporter, isoform 1). We show both that FGT-1 (the FGT-1a and b proteins) not only transports glucose in an

oocyte expression system, but that knockdown of fgt-1RNA in the worms leads to loss of glucose transport and reduced glucose metabolism in wild type worms. As glucose utilization strongly influences the aging process we investigated the role of fgt-1 in aging. Aging in C. elegans is influenced both by signalling via the DAF-2 receptor for insulin-like peptides and by nutrient deprivation. We find that knockdown of fgt-1RNA in the worms leads to loss of glucose transport and reduced glucose metabolism in daf-2 and age-1 worms. Importantly, knockdown of fgt-1 also leads to an extension of lifespan equivalent to that observed in the daf-2 and age-1 mutant worms. This extension is not seen in worms cultured in high concentrations of glucose (20mM). We suggest that DAF-2 and AGE-1 signal to glucose transport, mediated by FGT-1, in C. elegans and that this process partly determines the longevity phenotype in daf-2 and age-1 mutant worms. The FGT-1 glucose transporters of C. elegans thus play a key role in glucose energy supply to C. elegans. We propose that fgt-1 constitutes a common axis for the life-span extending effects of nutrient calorie restriction and reduced insulin-like peptide signalling.

21. Sensory neuronal regulation of lifespan through modulating insulin-like peptides in *C. elegans*. **Murat Artan**[1], Dae-Eun Jeong[2], Dongyeop Lee[2], Young-Il Kim[1,2], Joy Alcedo[4], Seung-Jae Lee[1,2,3]. 1) WCU ITCE; 2) Department of Life Sciences; 3) IBIO, Pohang University of Science and Technology, Pohang, Kyungbuk, South Korea; 4) Department of Biological Sciences, Wayne State University, Detroit, MI, USA.

Sensory neurons regulate the lifespan of several organisms, including *C. elegans* and *Drosophila*. In *C. elegans*, insulin/IGF-1 and steroid signaling pathways have been shown to mediate the longevity response by sensory neurons. Although it is established that perturbation of chemosensory neurons promotes longevity by activating DAF-16, upstream mediators of this activation are poorly understood. Here, we show that mutations in *tax-2* and *tax-4*, which encode subunits of neural cGMP-gated channels, increase lifespan at low temperature via modulating the expression of several insulin-like peptides. After confirming *daf-16*-dependency of longevity by *tax-2* mutations, we determined the tissues crucial for the action of DAF-16. We found that pan-neuronal, intestinal or hypodermal expression of DAF-16::GFP partially rescued the short lifespan of *tax-2 daf-16* double mutants, whereas muscle-specific expression did not. In addition, *tax-2* mutations increased nuclear localization of DAF-16 in non-neuronal tissues, including the intestine. Because the expression of *tax-2* and *tax-4* is restricted to neurons, these data suggest a tissue non-autonomous signaling between sensory neurons and other tissues. In addition, the expression of *sod-3*, *dod-8* and *mtl-1*, downstream target genes of DAF-16, was elevated in *tax-2; tax-4* mutants, suggesting a transcriptional activation of DAF-16. Next, we reasoned that neuroendocrine signaling via insulin-like peptides may participate in the longevity of *tax-2* and *tax-4* mutants. Among 34 insulin-like peptide genes we examined, *daf-28* and *ins-6* were significantly repressed in *tax-2; tax-4* mutants. We demonstrated the functional significance of this down-regulation by showing that overexpression of *ins-6* or *daf-28* suppressed the long lifespan of *tax-2* mutants. Together, we propose that sensory neurons modulate lifespan via neuroendocrine signaling mediated by insulin-like peptides that regulate the activity of DAF-16.

22. HLH-30/TFEB is a conserved regulator of autophagy and modulates longevity in *C. elegans*. **Louis R. Lapierre**[1], C. Daniel De Magalhaes Filho[2], Philip R. McQuary[1], Chu-Chiao Chu[1], Orane Visvikis[3], Jessica T. Chang[1], Sara Gelino[1], Binnan Ong[1], Andrew Davis[1], Javier E. Irazoqui[3], Andrew Dillin[2], Malene Hansen[1]. 1) Del E. Webb Neuroscience, Aging and Stem Cell Research Center, Program of Development and Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA, USA; 2) The Howard Hughes Medical Institute, The Glenn Center for Aging Research, The Salk Institute for Biological Studies, La Jolla, CA, USA; 3) Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School, MA, USA.

The cellular recycling process of autophagy is emerging as an important conserved modulator of aging. Autophagy is critical for lifespan extension induced by selected genetic mutations or by nutrient deprivation in *C. elegans*, but the mechanism(s) by which this occurs remains unclear. Although several transcription factors such as DAF-16/FOXO and PHA-4/FOXA are known to promote longevity, no single transcription factor has been shown to regulate autophagy in these long-lived *C. elegans* models. Here we show that the *C. elegans* helix-loop-helix transcription factor HLH-30, a predicted ortholog of the mammalian transcription factor EB (TFEB), not only regulates autophagy but also plays a critical role in lifespan extension. We found that, similar to TFEB, HLH-30 translocates to the nucleus via a mechanism dependent on the conserved autophagy regulator and longevity determinant TOR, where it modulates the expression of multiple autophagy-related genes. Consistent with a role for autophagy in aging, overexpression of HLH-30 extends lifespan and hlh-30 is required for the long lifespan of all known autophagy-dependent longevity models in *C. elegans*, including dietary restriction. Similarly, we see Tfeb expression upregulated and nuclear localized in liver of dietary-restricted mice, raising the possibility that HLH-30/TFEB influences lifespan in a conserved manner by inducing autophagic flux. Our results thus demonstrate a conserved role for TFEB in regulating autophagy and identify HLH-30 as a novel longevity-modulating transcription factor.

23. Regulation of SKN-1/Nrf by the germline longevity pathway. **Michael J. Steinbaugh**[1,2], Sri Devi Narasimhan[1,2], Stacey Robida-Stubbs[1,2], Prashant Raghavan[1,2], Theresa Operana[1,2], T. Keith Blackwell[1,2]. 1) Islet Cell & Regenerative Biology, Joslin Diabetes Center, Boston, MA; 2) Harvard Stem Cell Institute, and Department of Genetics, Harvard Medical School, Boston, MA.

In *C. elegans*, a reduction in germline stem cell (GSC) number increases lifespan and stress resistance through a longevity pathway that seems to be distinct from those related to nutrient sensing and metabolism (e.g. insulin/IGF-1 signaling, the TOR pathway, mitochondrial function, and dietary restriction). The GSC pathway is a tissue non-autonomous regulator of longevity that is known to involve the transcription factors DAF-16, DAF-12, and NHR-80 in the intestine.

We recently determined that the transcription factor SKN-1 (Nrf in mammals) is also regulated by this pathway. The germline can be removed through laser ablation or genetic disruption of the Notch ortholog GLP-1. Using *skn-1(zu135)*, *skn-1* RNAi, and temperature-sensitive *glp-1(bn18)* mutants, we determined that *skn-1* is required for lifespan extension associated with GSC removal. In contrast to DAF-16, SKN-1 is also required for the associated increase in oxidative stress resistance (e.g. sodium arsenite, tert-butylhydroperoxide). We found that SKN-1 accumulates in intestinal nuclei when GSC number is reduced, and that this is partially dependent upon KRI-1, an ankryin repeat protein implicated in regulation of DAF-16 by this pathway. Using qRT-PCR and analysis of transcriptional reporters, we determined that multiple SKN-1 target genes (e.g. *gst-4*, *gcs-1*, *nit-1*, and *F20D6.11*) are transcriptionally upregulated in germline-deficient animals in a SKN-1-dependent manner. This distinctive longevity pathway provides an exciting example of environmental sensing and signaling from the reproductive stem cell niche. Current efforts are aimed at understanding how the GSC pathway regulates SKN-1, and identifying processes controlled by SKN-1 in this context.

24. Importance of Growth, Stress Defense, and NAD+-related Pathways for Dietary Restriction Longevity. **Natalie Moroz**[1,2], Juan J. Carmona[3], Edward Anderson[3,4], Anne Hart[4], David A. Sinclair[3], T. Keith Blackwell[2]. 1) Department of Genetics and Complex Diseases, Division of Biological Sciences, Harvard School of Public Health, Boston, MA; 2) Section on Islet Cell and Regenerative Biology, Joslin Diabetes Center, Department of Genetics, Harvard Medical School, Harvard Stem Cell Institute, Boston, MA; 3) Department of Genetics, Harvard Medical School and Glenn Labs for Aging Research, Boston, MA; 4) Department of Neuroscience, Brown University, 185 Meeting Street, Providence, RI.

The reduction of food consumption without malnutrition, called dietary restriction (DR), increases lifespan in essentially all eukaryotes. In *C. elegans* genetic requirements for lifespan extension vary among DR protocols, suggesting that *C. elegans* will be valuable for elucidating and analyzing processes involved in DR. Using a simple liquid DR protocol that minimizes maintenance and produces robust lifespan extension, we identified several mechanisms involved in DR. Some of these have been implicated by other DR protocols: the transcription factors Nrf/SKN-1, FoxA/PHA-4, and FoxO/DAF-16, and the low-energy sensing kinase AMPK/AAK-2. These factors have been linked to nutrient availability or growth, and implicated in stress defense. The importance of FoxO/DAF-16 is of particular interest because its role in DR has been controversial, and because this suggests a possible link between DR and insulin/IGF-1-like signaling. We also observed a partial but significant involvement of the conserved Sirtuin and NAD-dependent deacetylase SIR-2.1, for which a role in DR and aging has also been a subject of debate. The nicotinamidase NAMPT/PNC-1, which is required for nicotinamide metabolism and NAD+ synthesis through the salvage pathway, was largely required for DR to benefit lifespan but not two healthspan indicators: stress resistance and movement. Our findings support the view that growth- or nutrient-regulated defense mechanisms are of central importance in DR lifespan extension, that NAD+-dependent pathways also play an important role, and that some benefits of DR can be uncoupled from lifespan.

25. Nonsense-mediated decay as a novel modulator of toxic CUG repeats in *C. elegans*. **Susana M. Garcia**[1,2], Yuval Tabach[1,2], Guinevere Lourenço[1,2]. 1) Dept Molecular Biology, Massachusetts Gen Hosp, Boston, MA, USA; 2) Department of Genetics, Harvard Medical School, Boston, MA, USA.

An increasing number of dominantly inherited neuromuscular disorders are associated with expanded nucleotide repeats. RNAs containing expanded CUG repeats in non-coding regions cause cellular dysfunction and are associated with myotonic dystrophy. These toxic CUG RNAs are known to cause abnormal regulation of alternative splicing resulting in cellular toxicity. To better understand the mechanisms that regulate CUG pathogenesis and are responsible for either protecting or damaging cells from CUG toxic RNAs, we identified and characterized a complement of factors that modulates expanded CUG toxicity. We used transgenic *C. elegans* expressing RNAs containing CUGs in their 3'UTRs and took a combined genetic screen and computational approach to identify modifiers of expanded CUG cellular toxicity. We identified 15 conserved genes that functioned as either suppressors or enhancers of CUG-induced toxicity, corresponding to distinct functional classes. These genes modulated CUG-induced toxicity through distinct mechanisms including: RNA export, RNA clearance, among others, supporting a model where CUG cellular toxicity is regulated by a complex network of pathways. In addition, we reveal that the nonsense-mediated (NMD) decay pathway is a key suppressor of CUG pathogenesis by regulating toxic RNA transcript levels. We show that NMD recognition of CUG RNA transcripts is dependent on the GC nucleotide content, in the 3'UTR. We propose a previously unknown function for CG-rich sequences in 3'UTRs as a signal for NMD degradation, and underscore the potential of the NMD pathway to influence myotonic dystrophy 1 disease outcomes.

26. A high throughput chemical screen identifies a novel activator of dietary restriction. **Mark S. Lucanic**, Ravi Shah, Ivan Yu, Bob Hughes, Gordon Lithgow. Buck Institute for Research on Aging, Novato, CA.

We have used small molecule screens in C. elegans to identify new chemical structures that can modulate physiological pathways that contribute to aging. Aging is the single largest risk factor for chronic disease in developed countries and is consequently responsible for an enormous social and economic burden. Chemicals that can slow aging are highly sought after due to their potential for treating age related diseases. In addition to being possible leads on potential drug interventions, characterization of new biologically active chemicals can also function to inform on the overall biology of aging by helping to describe the endogenous systems that are altered by these exogenous agents. Through drug discovery we can therefore identify new biologically active chemical structures, as well as molecular pathways that modulate organismal aging. Here we describe one such novel chemical structure and a physiological pathway that modulates the lifespan of C. elegans. We have screened 30,000 structurally diverse chemicals by testing their ability to extend the lifespan of C. elegans. One of these, a novel biological chemical, named NPP1, robustly extends lifespan of well fed animals, but not in animals undergoing strong dietary restriction (DR). Additionally, it requires functional PHA-4 for lifespan extension, further suggesting that NPP1 acts through a DR pathway. Interestingly, NPP1 subtly decreases feeding rates but does not lead to animals having the starved appearance indicative of eat mutants. We have determined that the decrease in feeding behavior is due to NPP1 blocking inhibitory glutamatergic signaling in the pharyngeal muscles, and further that functional glutamate signaling is required for NPP1's extension of lifespan. We have also identified a putative G-protein coupled receptor which is required for NPP1's extension of lifespan but not for its decrease in feeding behavior, indicating the two chemical's effects can be de-coupled. Collectively, our results indicate that NPP1 acts by inhibiting glutamate signals to the pharyngeal muscle and results in inhibition of a G protein coupled receptor pathway involved in nutrient perception that initiates a DR response.

27. Mating-induced somatic collapse reveals a novel soma-germline interaction. **Cheng Shi**, Coleen Murphy. Lewis-Sigler Institute for Integrative Genomics and Dept. of Molecular Biology, Princeton University, Princeton, NJ 08544.

Interactions between the germline and the soma are important to optimize reproduction and to influence somatic aging. The prevailing germline-soma model suggests that the lifespan shortening signal from the germline antagonizes the lifespan lengthening signal in the somatic gonad in *C. elegans*. Previous studies focused on removal of germline cells and its longevity-repressing activity to reveal the somatic gonad lifespan-extending signal. We have identified a new phenomenon linking reproductive status to longevity and have illustrated the elusive lifespan-shortening signal for the first time: in *C. elegans*, mating leads to "post-mating somatic collapse" (PMSC), a state in which mated worms shrink pronouncedly and die early, compressing the post-reproductive life span. The post-mating shrinking and lifespan phenomena are genetically separable, and the DAF-12 steroid receptor and DAF-16/FOXO transcription factor are involved in regulating different aspects of PMSC. Components from males contribute differently to PMSC. Likewise,

hermaphrodites have germline-dependent and germline-independent responses that process the corresponding male-initiated signals. Post-mating somatic collapse may be an extreme version of the influence that males in many species exert on female behavior to maximize their own reproductive success. Our study provides new insight into the communication between males and the female germline and soma to regulate reproduction and longevity.

28. Neuromodulation of *C. elegans* mechanosensation. **Xiaoyin Chen**, Martin Chalfie. Biological Sciences, Columbia Univ, New York, NY.

Sensory perception is modified by cues detected by the sensory cells themselves and from signals initiated by other sensory cells. We show that mechanosensation in the touch receptor neurons (TRNs), which receive no synaptic input, is modulated by both mechanical and non-mechanical cues. These signals alter touch sensitivity through two signaling pathways that modulate touch sensitivity by converging on a common mechanism. Sustained mechanical signals, such as continuous vibration, increase TRN sensitivity to force. This long-term sensitization requires the activation of a secondary mechanosensory system involving the integrins and is independent of the MEC-4 mechanotransduction channels. Non-mechanical stress cues, such as dauer formation, hypoxia, and high salt, reduce touch sensitivity by reducing the expression of two insulin-like peptides, INS-10 and INS-22, which act as long-range neuromodulators to activate insulin signaling in the TRNs. Both insulin and integrin signaling compensate for each other and converge by using AKT-1 and DAF-16. Loss of AKT-1 activity increases the transcription of *mfb-1*, which reduces the amount of MEC-4 mechanotransduction channel available on the plasma membrane, thus changing the TRN sensitivity to force. These alterations in TRN sensitivity adjust the acuity and priority of the touch response to benefit the animal under diverse conditions. During sustained background stimulation, habituation reduces the response to background stimuli, but long-term sensitization counters habituation to maintain TRN response to strong stimuli, thus distinguishing background stimulation from stronger signals. The reduction of touch sensitivity under non-mechanical stress conditions increases the efficiency of the animal's ability to do non-mechanosensory tasks. For example chemotaxis is more efficient in the presence of mechanical distractions. Thus, modulation of touch can alter the balance between senses to prioritize sensory responses that can facilitate an escape from the stress conditions. Our findings demonstrate a non-synaptic neuromodulatory network that integrates multi-modal signals and alters behavior to optimize the animal's response to multiple conditions.

29. Humidity sensation requires a conserved DEG/ENaC complex in multi-dendritic FLP neurons. **Josh Russell**[1], Jonathan Pierce-Shimomura[2]. 1) Department of Cellular and Molecular Biology, University of Texas at Austin, Austin, TX; 2) Department of Neuroscience, University of Texas at Austin, Austin, TX.

Moisture is essential for life. Thus, it is surprising that the molecular basis for how most animals, including humans, sense humidity levels (hygrosensation) is unknown. Through development of a novel assay we discovered that C. elegans is exquisitely sensitive to humidity gradients as shallow as 0.03% relative humidity per 1 mm. A series of control experiments demonstrated that the worm detects changes in moisture in the air rather than cues specific to the assay apparatus. Next, by analyzing mutants we found several genes that may encode a conserved complex of mechanosensitive DEG/ENaC channels required for hygrosensation. Finally, though cell-specific ablation and rescue studies we have determined that hygrosensation requires the mechanosensitive neuron pair FLP, but not other mechanosensory neurons. FLP is unique for its extensive dendritic branching just beneath the cuticle in the head. These findings raise the possibility that humidity levels may be encoded by the extent to which the FLP dendritic field is stretched by local skin hydration. Clear orthologs of this mechanosensitive complex have also been identified in the multi-dendritic mechanosensory neurons in mouse and human those also terminate just below the epidermis. The similar receptive fields, morphology and mechanosensitive functions of these mammalian neurons to FLP neurons suggests C. elegans may help uncover the molecular basis for this elusive sensory modality in other animals including humans.

30. CEPsh glia modulate a sleep-related neuronal circuit in *C. elegans*. **Menachem Katz**[1], Francis Corson[3], Shachar Iwanir[2], Elena Dragomir[1], David Biron[2], Shai Shaham[1]. 1) Lab Developmental Genetics, Rockefeller Univ, New York, NY; 2) Department of physics, The University of Chicago, Chicago, IL; 3) Unit Physics of Biological Systems, Institute Pasteur, Paris, France.

Glia are essential components of the nervous system. However, their precise roles in the regulation of neuronal circuit activities and animal behavior are poorly understood. In 1895, Ramon y Cajal postulated that astrocytes regulate the transition between sleep and waking states. Remarkably, recent studies in mice and flies hint that Cajal's ideas may be more than theoretical musings. Yet, the mechanisms by which glia influence sleep are not fully resolved. Sleep in *C. elegans* is defined by behavioral lethargy that coincides with molting, linking development and locomotion. The CEPsh glia, which envelope the *C. elegans* nerve ring, are reminiscent of astrocytes, and extend processes that abut specific nerve-ring synapses. L1 ablation of CEPsh glia results in molting-independent short duration locomotory pausing, episodes of longer immobility immediately preceding molting, and developmental delay. The ALA neuron was previously implicated in sleep control. Interestingly, synapses between ALA and the backward command interneuron AVE are ensheathed by CEPsh glia, suggesting functional roles for these glia in modulating ALA-AVE synaptic activity. Indeed, loss of ALA function suppresses the locomotory defects, developmental delay, and precocious lethargus of CEPsh glia-ablated animals. Furthermore, inactivation of AVE in adults induces pausing reminiscent of CEPsh ablation. In animals lacking CEPsh glia, the duration of spontaneous calcium transients in AVE is prolonged and calcium spikes are decorrelated from backward locomotion. Correlation is restored upon ALA inactivation. These results as well as our functional epistasis studies of ALA, AVE and CEPsh glia are consistent with a model in which glia attenuate a sleep-promoting inhibitory connection between ALA and AVE. We propose that glia play key roles in *C. elegans* sleep control and that this role may be conserved.

31. A neuronal mechanism for navigation along a repulsive odor gradient. Akiko Yamazoe[1], Yuki Tanimoto[1], Kosuke Fujita[1], Yuya Kawazoe[1], Yosuke Miyanishi[1], Shuhei Yamazaki[1], Xianfeng Fei[2], Karl Emanuel Busch[3], Keiko Gengyo-Ando[4], Junichi Nakai[4], Yuichi Iino[5], Yuishi Iwasaki[6], Koichi Hashimoto[2], **Kotaro Kimura**[1]. 1) Osaka Univ; 2) Tohoku Univ., Japan; 3) MRC, UK; 4) Saitama Univ; 5) Univ. Tokyo; 6) Ibaraki Univ., Japan.

For survival and reproduction, animals navigate toward or away from certain stimuli, which requires the coordinated transformation of sensory information into motor responses. In worms, the pirouette and the weathervane strategies are considered the primary navigation strategies for responding chemosensory stimuli. We found, however, that worms use a novel navigation strategy in odor avoidance behavior: In a gradient of the repulsive odor 2-nonanone, worms efficiently avoid the odor, and ~80% of initiation of long, straight migrations ("runs") were away from the odor source,

which cannot be simply explained by the two known major strategies. Direct measurement of local odor concentration suggested that pirouettes are efficiently switched to runs when worms sense negative *dC/dt* of 2-nonanone. To test whether runs are indeed caused by negative *dC/dt*, we established an integrated microscope system that tracks a freely moving worm during stimulation with a virtual odor gradient and simultaneously allows for calcium imaging and optogenetic manipulations of neuronal activity (Tanimoto et al., this meeting). Using this system, we found that a realistic temporal decrement in 2-nonanone concentration (~ 10 nM/sec) caused straight migration by suppressing turns. We also found that a pair of AWB sensory neurons were continuously activated during the odor decrement and that optogenetic activation or inactivation of AWB neurons suppressed or increased turning frequency, respectively. In addition, we found that ASH nociceptive neurons increased turning frequency during odor increment. Taken together, our data indicate that the counteracting turn-inducing and turn-suppressing sensory pathways can effectively transform temporal sensory information into spatial movement to select the right path leading away from potential hazards.

32. Multiple cholinergic pathways for excitation of the *Caenorhabditis elegans* pharynx. **Nicholas Trojanowski**[1], Olivia Padovan-Merhar[2], David Raizen[3], Christopher Fang-Yen[4]. 1) Neuroscience Graduate Group; 2) Department of Physics; 3) Department of Neurology; 4) Departments of Bioengineering and Neuroscience; University of Pennsylvania, Philadelphia, PA.

C. elegans feeds through rhythmic contractions of its pharynx, a neuromuscular pump innervated by a network of 20 neurons. Laser ablation studies have provided key insights into how the pharyngeal nervous system regulates feeding. However, these experiments only permit unidirectional and permanent manipulation of circuit function. To overcome these limitations and study the function of the pharyngeal nervous system, we developed a technique in which individual pharyngeal neurons in immobilized worms are optogenetically manipulated using selective illumination by a laser beam shaped by a digital micromirror device, while machine vision is used to quantify behavioral changes. As expected from ablation data, we found that excitation of the two cholinergic MC neurons causes rapid pumping. Activation of the cholinergic M2 neurons, for which no role has been reported, also causes rapid pumping. This effect is decreased but not abolished when MC is ablated, suggesting that M2 can act independently of MC. Stimulation of cholinergic I1 neurons, which connect the somatic and pharyngeal nervous systems and are anatomically defined to synapse on the MC and M2 neurons, also induces pumping. I1 excitation after ablation of both MC and M2 does not increase pump rate, demonstrating that I1 excites pumping through these two neurons. Previous work indicates that MC stimulates pumping via nicotinic neurotransmission. Surprisingly, in worms lacking the nicotinic receptor subunit EAT-2, excitation of MC or M2 still increases pumping rate. This effect persists in *eat-18* mutants, which lack pharyngeal nicotinic neurotransmission, but not *unc-17* mutants, which are defective in acetylcholine release, suggesting that MC and M2 can stimulate muscle though a non-nicotinic cholinergic mechanism. MC and M2 can each still excite pumping when the other is ablated in an *eat-18* background, suggesting they both act through this cholinergic non-nicotinic pathway. We are identifying other cholinergic receptors involved in feeding and exploring the function of I1 in connecting the pharyngeal and somatic nervous systems.

33. Pathogen-induced changes of neuronal TGF-b signaling promote avoidance behavior and survival. **Joshua D. Meisel**, Dennis H. Kim. Biology Department, MIT, Cambridge, MA.

In *C. elegans* the TGF-b ligand *daf-7* is expressed in the ASI pair of chemosensory neurons and functions in the neuroendocrine regulation of diverse aspects of organismal development and physiology. We observe that upon exposure to the gram-negative bacterial pathogen *Pseudomonas aeruginosa* *daf-7* transcription is rapidly activated in the ASJ pair of chemosensory neurons. *daf-7* mutants display enhanced susceptibility to infection by *P. aeruginosa* due to a failure to avoid the bacterial lawn. Through cell-specific rescue and ablation experiments we show that the induction of *daf-7* expression in ASJ is necessary for a complete avoidance response to *P. aeruginosa*. We determined that DAF-7 promotes pathogen avoidance behavior by activating the *daf-1*/TGF-b Type I receptor in the RIM/RIC interneurons, which are adjacent to the ASJ chemosensory neurons on the far ventral side of the amphid, suggesting that spatial control of DAF-7 secretion may be a key mechanism for responding to changes in the microbial environment. To understand the mechanism by which the ASJ neurons respond to *P. aeruginosa* we carried out a forward genetic screen for mutants that maintained *daf-7* expression in the ASI neurons but failed to induce *daf-7* expression in ASJ upon exposure to pathogen. We identified components of conserved signaling pathways, such as the G protein alpha subunit *gpa-3*, which act cell autonomously in ASJ to activate *daf-7* expression in response to *P. aeruginosa*. Our data demonstrate that the sites of *daf-7* neuromodulator expression are not invariant, but are determined by interactions with the microbial environment, and that this dynamic expression is required for the survival of *C. elegans* on pathogenic bacteria.

34. Nematophagous fungi eavesdrop on nematode pheromones, and lure their prey with attractive volatile organic compounds. **Yen-Ping Hsueh**[1], Erich Schwarz[2], Weihua Zeng[3], Zhaoying Xian[1], Parag Mahanti[4], Matthew Gronquist[5], Frank Schroeder[4], Ali Mortazavi[3], Paul Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA; 2) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853, USA; 3) Department of Developmental and Cell Biology, University of California, Irvine, CA 92697, USA; 4) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA; 5) Department of Chemistry, SUNY Fredonia, Fredonia, NY 14063, USA.

Nematophagous fungi are natural predators of soil-dwelling nematodes, and this predator-prey relationship makes them an attractive model to study co-evolution. How do microorganisms detect their metazoan prey, and how do prey respond to predators? We set out to investigate the cues that nematophagous fungi use to trigger morphogenesis of their nematode-trapping devices and how C. elegans responds to nematophagous fungi. We found that nematophagous fungi can detect and respond to ascarosides, small molecules produced by many nematodes that regulate nematode development and behavior. In response to ascarosides, Arthrobotrys oligospora and closely related nematophagous fungi induce morphogenesis of their nematode traps. Ascarosides thus represent a conserved molecular pattern used by nematophagous fungi to detect prey. Through RNA-seq analysis, we identified A. oligospora genes regulated by ascarosides and nematode exposure. On the other hand, C. elegans are attracted to A. oligospora. This attraction is, at least in part, mediated by volatile compounds. Gas chromatography and mass spectrometry revealed that volatile organic compounds produced by A. oligospora could attract nematodes. Genetic analysis and cell-specific laser ablation showed that AWC neurons are required for this behavior. To find genes involved in the AWC-mediated A. oligospora attraction, we performed single-cell RNA-seq of the AWCon neuron. We detected expression of 6,608

genes in AWC neurons, with 1,278 being AWC-enriched. Preliminary mutant screening revealed genes that have a function in AWC-mediated chemosensation.

35. Sensory neurons override recurrent motor programs to induce ejaculation during mating. **Brigitte L. LeBoeuf**[1,2], L. Rene Garcia[1,2]. 1) Dept Biol, Texas A&M Univ, College Station, TX; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

Siring progeny requires sustained spicule insertion into the hermaphrodite vulva coupled to sperm transfer. Coordinated signaling maintains the male's copulatory spicules over the vulva and couples genital position with repetitive spicule thrusts necessary to breach the vulva. Breaking out of this loop requires penetration, but this alone is insufficient to promote sperm transfer, as ectopic protraction does not lead to ejaculation. Males readily reverse ectopic spicule protraction and return to their interest in mating. However, after intromission and ejaculation, males are not immediately interested in mating again. To elucidate the circuit that controls ejaculation and how it is integrated with the circuits promoting vulva location and spicule insertion, we used optogenetics, cell ablation, and calcium imaging in free-moving males during mating. To achieve consistent ectopic ejaculation, we had to photo-stimulate using channelrhodopisin2 in a large circuit including muscles, tail and head neurons. Playing a vital role are neurons previously identified to promote vulva location and spicule insertion behaviors, but also synapse the somatic gonad. G-CaMP3 calcium imaging revealed that although gonadal activity and sperm initiation is coordinated with spicule insertion, it is not enough to allow sperm release. Efficient exit of the sperm out of the cloaca requires SPV and SPD sensory neurons that send their processes through the spicules and are exposed at the spicule tips. Following insertion and sperm initiation, calcium transients occur throughout the intestines and sex and body wall muscles. The transients could be a result of the seizure-like state that occurs in the posterior third of the male following insertion, allowing for sperm movement. After retraction, the male takes several minutes to recover, a period that is shortened when the male's ability to transfer sperm is reduced. Integrating one additional component to the circuitry that maintains spicule location at the vulva and facilitates insertion allows male mating behavior to be completed.

36. *eol-1*, the homolog of mammalian *Dom3z*, is a novel genetic regulator of *C. elegans* olfactory learning. **Yu Shen**, Jiangwen Zhang, John Calarco, Yun Zhang. Harvard University, Cambridge, MA.

Neural plasticity, a remarkable feature of the nervous system, allows animals to adjust their behavior based on previous experience. *C. elegans* is able to modify its olfactory preference to avoid the odor of pathogenic bacteria after ingesting the pathogens [1]; this learning is regulated by a recently identified neural circuit as well as multiple signaling pathways [2-4]. The genetic accessibility and well-characterized nervous system of *C. elegans* provide a unique opportunity to study the molecular and cellular mechanisms of olfactory learning, which are often conserved from the nematode to higher organisms.

To characterize novel molecular regulators of *C. elegans* olfactory learning, we conducted a forward genetic screen for mutants with altered learning abilities. Using a high-throughput behavioral assay, we have identified several candidate mutants, among which one recessive allele displays significantly enhanced olfactory learning. This phenotype is not due to any detectable deficiency in innate immunity or olfactory preference under the naive condition. By whole genome sequencing and trangenic rescue, we have mapped the gene of interest *eol-1* (enhanced olfactory learning-1) to a predicted protein-coding sequence on Chromosome V. GFP reporter driven by the endogenous promoter of *eol-1* is expressed in the reproductive system and several identified neurons; neuron-specific expression of *eol-1* in the URX sensory neuron restores the learning phenotype in the mutant background. The protein encoded by *eol-1* is conserved in different *Caenorhabditis* species and other eukaryotes; its yeast ortholog Rai1 is involved in RNA metabolism, whereas the function of the mammalian ortholog DOM3Z remains unclear. Our ongoing work to identify genetic interaction of *eol-1* and to evaluate its function in *C. elegans* may contribute to better understanding the function of this conserved gene in more complex systems.

References: 1. Zhang et al. Nature 438, 179-184. 2. Ha et. al. Neuron 68, 1173-86. 3. Zhang and Zhang, Proc Natl Acad Sci U S A 109(42):17081-6. 4. Chen et. al. Neuron 77, 572-585.

37. Membrane phospholipids that contain arachidonic acid regulate touch receptor neuron mechanics and touch sensation. **V. Vásquez**, M. Krieg, D. Lockhead, M.B. Goodman. Stanford School of Medicine, Department of Molecular and Cellular Physiology.

Touch, proprioception, and blood pressure regulation rely on the ability of mechano-electrical transduction (MeT) channels to convert mechanical stimuli into electrochemical signals. The protein subunits that form MeT channel complexes are known only in a small set of mechanosensory neurons, including the touch receptor neurons (TRNs) responsible for gentle body touch in *C. elegans*. Little is known about the interplay between these channel complexes and the surrounding membrane environment. However, three proteins in the complex are believed to bind lipids: MEC-2, UNC-24, and MEC-6. The TRNs are ideal to test the effect of lipids in MeT because electrical responses to touch depend only on one kind of MeT channel (the ASH nociceptors have two MeT channels) and because connections to downstream neurons are well-characterized. We tested a model in which lipids regulate the mechanical response of TRNs by defining the membrane environment around the MeT channel complex. To this end, we combined genetic dissection (*fat* and *mboa* mutants) with behavioral studies, chemical complementation, optogenetic, and biophysical approaches to determine the role of membrane lipids in the response to gentle touch. We demonstrate that disrupting arachidonic acid (AA) or its incorporation into phospholipids impairs TRN-dependent behavioral responses. Thus, membrane phospholipids containing AA are critical for touch sensitivity. AA is likely synthesized within TRNs *in vivo*, since we show that *fat-4*, the enzyme needed for its synthesis, is expressed in TRNs. We used optogenetics to show that the defect in touch sensation likely reflects a loss of mechanotransduction rather than downstream signaling. Finally, we found that loss of long polyunsaturated fatty acids, including AA, increases bending stiffness of TRN membranes, as determined by atomic force microscopy. Our findings demonstrate that when AA is part of membrane phospholipids it helps to fine-tune the mechanical properties of TRN membranes and is crucial for MeT. These findings provide a framework for understanding eukaryotic mechanotransduction as a process that relies on a cellular machine composed of both proteins and membrane lipids.

38. Serotonin and PDF are opposing neuromodulators that control a bistable foraging behavior in C. elegans. **Steven W Flavell**, Navin Pokala, Evan Z Macosko, Dirk A Albrecht, Johannes Larsch, Cornelia I Bargmann. Laboratory of Neural Circuits and Behavior, The Rockefeller University, New York, NY.

Foraging animals have distinct exploration and exploitation behaviors that are organized into discrete, long-lasting behavioral states. Here we characterize a neuromodulatory circuit that generates such long-lasting roaming and dwelling states in Caenorhabditis elegans. We find that two opposing

neuromodulators, serotonin and the neuropeptide pigment dispersing factor (PDF), each initiate and extend one behavioral state. Serotonin promotes dwelling states through the MOD-1 serotonin-gated chloride channel. The spontaneous activity of serotonergic neurons correlates with dwelling behavior, and optogenetic modulation of the critical MOD-1-expressing targets induces long-lasting dwelling states. PDF promotes roaming states through the Gas-coupled PDFR-1 receptor; optogenetic activation of cAMP production in PDFR-1-expressing cells induces long-lasting roaming states. The neurons that produce and respond to each neuromodulator form a distributed circuit orthogonal to the classical wiring diagram, with several essential neurons that express each molecule. The slow temporal dynamics of the neuromodulatory circuit supplement fast motor circuits to give rise to long-lasting behavioral states.

39. Adenosine signaling in *C. elegans*: does skin rule the brain? **Hsiao-Fen Han**[1], Michael Ailion[1], Mary Beckerle[2], Erik Jorgensen[1]. 1) Dept Biol, HHMI, Univ Utah, Salt Lake City, UT; 2) Dept Onc Sci, Huntsman Cancer Institute, Univ Utah, Salt Lake City, UT.

Behavioral plasticity is crucial to promote survival of animals in response to environmental change. Nevertheless, how the activity of neural circuits reconfigures and alters behavior remains poorly understood. In our study, we demonstrate that adenosine, an endogenous purine metabolite, functions as a neuromodulator to regulate behaviors in *C. elegans*. First, we found that excess adenosine reduces feeding and increases locomotion speed. These effects are mediated by the adenosine receptor ador-1 in the nervous system. Further, we performed tissue-specific rescue experiments to define the cellular focus of the change in adenosine levels. Intriguingly, restoration of adenosine metabolism in epidermis rescued the phenotype resulting from altered adenosine signaling. Finally, wild-type *C. elegans* displays altered feeding and locomotion when food is deprived. We showed that adenosine signaling functions to regulate this plasticity. Together, our results suggest that adenosine serves as a hunger signal to modulate feeding and locomotion behaviors. The identification of adenosine signaling also suggests that there are channels of communication between the epidermis and the nervous system that can regulate behaviors.

40. Chemosensing a predator: *Pristionchus pacificus* and *C. elegans*. **Kevin Curran**[1], Ada Tong[1], Matthew Joens[1], James Fitzpatrick[1], Jagan Srinivasan[2], Sreekanth Chalasani[1]. 1) Salk Institute, La Jolla, CA; 2) Biology Department, Worcester Polytechnic Institute, Worcester, MA.

A current challenge in neuroscience is to bridge the connections between genes, neurons, neural circuits, and behavior in a single animal model. We are approaching this convergence by exploring the mechanisms governing a social interaction between a predator and its prey. A starving predator, *Pristionchus pacificus*, an omnivorous nematode, will attack and kill its prey, *Caeonrhabditis elegans*, when the two species share an agar plate. Certain *Pristionchus* strains exhibit carnivorous mouth morphology allowing them to slice open *C. elegans* cuticular exoskeleton and consume the underlying tissue. We have employed a novel imaging technique, Helium Ion Microscopy (HIM), to image the mouth cavities and teeth of several *Pristionchus* isolates. The tooth morphology revealed via HIM imaging correlates with observed feeding behaviors of the *Pristionchus* strains. We also find that, upon exposure to *Pristionchus*, *C. elegans* exhibit an increase in avoidance behavior. Moreover, we have identified ASI, ASJ and ASH as the specific chemosensory neurons that drive this avoidance behavior. We next performed mutant analysis coupled with cell specific rescue experiments and concluded that *ocr-2*, a TRPV channel, is required in ASH, while *tax-4*, a cGMP channel, is required in ASI and ASJ, in order to properly execute *C. elegans* predator avoidance behavior. Finally, this behavior is modulated by anti-anxiety drugs, suggesting that predator avoidance may serve as a model for complex human behaviors, such as fear and anxiety.

41. ZTF-8 interacts with the 9-1-1 complex and is required for DNA damage response and double-strand break repair in the C. elegans germline. **Hyun-Min Kim**, Monica Colaiacovo. Dept of Genetics, Harvard Medical School, Boston, MA.

Germline mutations in DNA repair genes are linked to tumor progression. Furthermore, failure in either activating a DNA damage checkpoint or repairing programmed meiotic double-strand breaks (DSBs) can impair chromosome segregation. For this reason, understanding the molecular basis for DNA damage response and DSB repair within the germline is highly important. Here we define ZTF-8, a previously uncharacterized protein conserved from worms to humans, as a novel factor involved in the repair of both mitotic and meiotic DSBs as well as in meiotic DNA damage checkpoint activation in the C. elegans germline. We show that ZTF-8 partially co-localizes with the 9-1-1 DNA damage response complex and interacts with MRT-2/Rad1, a component of this complex. In the absence of ZTF-8, mitotic nuclei arrest at S-phase and both ATL-1 and CHK-1 DNA damage checkpoint kinases are activated. Moreover, both mitotic and meiotic recombination intermediates accumulate in ztf-8 mutants, and these exhibit sensitivity to g-IR and HU, but not UV, nitrogen mustard or camptothecin, implicating ZTF-8 in DSB repair. However, impaired meiotic DSB repair progression partially fails to trigger the CEP-1/p53-dependent DNA damage checkpoint in late pachytene, also supporting a role for ZTF-8 in meiotic DNA damage response. We propose that ZTF-8 is involved in promoting repair at stalled replication forks and meiotic DSBs in part by transducing DNA damage checkpoint signaling via the 9-1-1 pathway.

42. Interplay between structure-specific endonucleases for crossover control during meiosis. **Takamune T. Saito**, Doris Y. Lui, Hyun-Min Kim, Katherine Meyer, Monica P. Colaiácovo. Department of Genetics, Harvard Medical School, Boston, MA.

The number and distribution of crossover events are tightly regulated at prophase of meiosis I. The resolution of Holliday junctions by structure-specific endonucleases, including MUS-81, SLX-1, XPF-1 and GEN-1, is one of the main mechanisms proposed for crossover formation. However, how these nucleases coordinately resolve Holliday junctions is still unclear. Here we identify both the functional overlap and differences between these four nucleases regarding their roles in crossover formation and control in the *C. elegans* germline. We show that MUS-81, XPF-1 and SLX-1, but not GEN-1, can bind to HIM-18/SLX4, a key scaffold for nucleases. Analysis of synthetic meiotic defects revealed that MUS-81 and SLX-1, but not XPF-1 and GEN-1, have overlapping roles with the Bloom syndrome helicase ortholog, HIM-6, supporting their in vivo roles in processing recombination intermediates. Taking advantage of the ease of genetic and high-resolution imaging afforded by *C. elegans*, we analyzed crossover designation, frequency, distribution and chromosomal morphology in single, double, triple and quadruple mutants of the structure-specific endonucleases. This revealed that XPF-1 functions redundantly with MUS-81 and SLX-1, respectively, in promoting crossover formation, but not crossover designation. Analysis of crossover distribution revealed that SLX-1 is required for crossover suppression at the center region of the autosomes. Finally, analysis of chromosome morphology in oocytes at late meiosis I stages uncovered that SLX-1 and XPF-1 promote meiotic chromosomal stability by preventing formation of chromosomal abnormalities. We

propose a model in which coordinate action between structure-specific nucleases at different chromosome domains, namely MUS-81, SLX-1 and XPF-1 at the arms and SLX-1 at the center region, exerts positive and negative regulatory roles, respectively, for crossover control during *C. elegans* meiosis.

43. Sperm-derived TRP-3 channel specifies the onset of the fertilization Ca^{2+} wave in the oocyte of *C. elegans*. **J. Takayama**, S. Onami. Lab. for Developmental Dynamics, RIKEN QBiC, Kobe, Japan.

Fertilization induces a Ca^{2+} wave in the egg to start embryogenesis. How sperm triggers the Ca^{2+} wave is not well understood. Here we show that sperm-derived TRP-3 channel specifies the onset of the Ca^{2+} wave in the oocyte upon plasma membrane fusion in *C. elegans*.

First, by live confocal imaging, we found that sperm entry triggered a biphasic Ca^{2+} response: a fast local rise and a following slow global wave. The fast local rise emerged near the sperm entry point immediately after sperm entry, whereas the slow global wave traveled from that point to the opposite pole. This biphasic waveform was reproduced by simulations assuming Ca^{2+}-induced Ca^{2+} release and local Ca^{2+} influx. Next, to understand how sperm triggers the Ca^{2+} wave, we examined Ca^{2+} responses in sperm mutants. In *spe-9* mutants, whose sperm cannot enter the oocyte, no Ca^{2+} responses were observed. On the other hand, in *spe-11* mutants, whose sperm cannot activate but can enter the oocyte, a wild type-like response was observed. In *trp-3/spe-41* mutants, which lack the sperm-specific Ca^{2+}-permeable channel TRP-3, fertilized oocytes generated no local Ca^{2+} rise but a slow global wave with delayed onset. Approximately half of the fertilized *trp-3* embryos were arrested during embryogenesis. Finally, we investigated how the sperm plasma membrane channel mediates the local Ca^{2+} rise in the oocyte. By high-speed imaging, we found that Ca^{2+} concentration in the fused sperm cytoplasm increased from a resting level rather than decreased from a high level equivalent to the local rise. In addition, visualization of the plasma membrane of the oocyte revealed that sperm entered the oocyte by fusion at the plasma membrane, which contains the sperm channels.

Altogether, these results suggest that sperm-derived plasma membrane that contains TRP-3 channels acts as a signaling membrane domain to specify the onset of the Ca^{2+} wave in the oocyte. TRP-3 channels might ensure successful embryogenesis by timely triggering the Ca^{2+} wave to coordinate with other events occurring during oocyte-to-embryo transition.

44. Gap junctions between soma and germline regulate germ cell proliferation, meiotic maturation, and early embryogenesis. **Todd Starich**[1], David Hall[2], David Greenstein[1]. 1) Dept GCD, 6-160 Jackson, Univ. Minnesota, Minneapolis, MN; 2) Albert Einstein College of Medicine, Yeshiva University, Bronx, NY.

Gap junctions between proximal sheath and oocytes are known negative regulators of meiotic maturation. We show that gap junctions between germline and soma are formed in the primordial gonad, continue throughout development, and are required for germ cell proliferation. We used specific antibodies and GFP fusions to decipher a complex co-dependency for localization of innexins to gap junction-like puncta. In germ cells, INX-14 requires either INX-21 or INX-22 to localize; INX-21 and INX-22 likewise require INX-14 for localization. In the soma, *inx-8* and *-9* are co-dependent with the germline innexins for localization. In addition, *inx-14(0)* animals produce few germ cells, a phenotype mimicked in *inx-22(0) inx-21(RNAi)* animals and in *inx-8(0) inx-9(0)* double mutants (isolated in a screen anticipating this phenotype), supporting functional co-dependence. Consistent with a role in germ cell proliferation, expression of innexins is detected in their respective Z1-Z4 progenitors and continues into adulthood. In adults, soma:germline gap junctions are detected by IF in distal and proximal arms, a finding supported by TEM analysis of freeze fracture samples. *inx-8(0) inx-9(0)* mutants avg. <4 germ cells per gonad arm. *inx-8(0) inx-9(0)* is epistatic to *glp-1(oz112gf)*, an activated-Notch mutation leading to tumorigenic germ cell proliferation; therefore soma:germline gap junctions are required, in either a permissive or instructive role, for competence of germ cells to proliferate in response to Notch signaling. Genetic mosaic and expression analyses indicate that DTC expression of *inx-8* (*Plag-2::inx-8::gfp*), or expression in other somatic gonad cells, is sufficient to rescue germ cell proliferation, even at later larval stages. *Plag-2::inx-8::gfp* rescue of germ cell proliferation, but not formation of sheath:oocyte gap junctions, in *inx-8(0) inx-9(0)* confirmed that these junctions act as inhibitors of meiotic maturation; additionally, fertilized embryos arising from these animals often exhibit early mutant phenotypes resembling those of genes implicated in eggshell formation, such as *syn-4*.

45. Evidence for a meiotic crossover surveillance system. T. Machovina[1], O. McGovern[1], A. Woglar[2], D. Paouneskou[2], V. Jantsch[2], **J. Yanowitz**[1]. 1) Magee-Womens Research Institute and U. Pittsburgh, 204 Craft Avenue, Pittsburgh, PA 15213; 2) Dept. of Chromosome Biology, Max F. Perutz Laboratories, U. Vienna, Dr. Bohrgasse 9, A-1030 Vienna, Austria.

During meiosis, each chromosome must receive a crossover (CO) to ensure its proper segregation into developing gametes or risk producing aneuploid progeny. Due to the critical importance of attaining this obligate crossover, it has long been speculated that a system exists to monitors that a CO has been made on each chromosome. Nevertheless, experimental support for such a system has been lacking, in part because most meiotic defects affect all chromosomes, and not one or two. Over the last several years, we have been studying the *him-5* and *xnd-1*, genes that are required to ensure that a meiotic double strand break (DSB) is made on the X chromosome. Thus, in the mutants, CO frequency is dramatically reduced on the X. In both *him-5* and *xnd-1* mutants, the failure to receive the DSB on the X activates a delay in progression through early pachytene that alters SUN-1 phosphorylation and retains the chromosomes in a clustered morphology at the nuclear periphery. It also causes a premature dismantling of the synaptonemal complex (SC) on the X chromosome. Adding DSBs into the nucleus with gamma irradiation restores normal meiotic progression, SC morphology and crossover formation on the X, indicating that it is indeed the lack of a DSB which is responsible for the delay. In an effort to identify the signaling components responsible for this delay, we screened all the germline expressed kinases and phosphatases using RNAi knockdown. We show that components of the spindle assembly checkpoint have been co-opted for this meiotic checkpoint, and suppress the delay and SC phenotypes. We also have made double mutants with many of the known repair mutants and have shown that homolog engagement appears critical for checkpoint activation. Lastly, we show that the phenotypes we observe are not specific to defects in crossover on the X chromosome, but that defects in crossover formation on autosomes are all sufficient to activate this checkpoint. Thus, we believe that there exists a surveillance system that is monitoring crossover formation in a chromosome by chromosome specific manner.

46. Interchangeable a-kleisin subunits specify meiotic cohesin function in *C. elegans*. **Aaron F. Severson**[1,2], Barbara J. Meyer[2]. 1) BGES Dep't and GRHD Center, Cleveland State University, Cleveland, OH; 2) HHMI and MCB Dep't, University of California, Berkeley, CA.

Faithful transmission of the genome through sexual reproduction requires reduction of genome copy number during meiosis to produce haploid sperm and eggs. To achieve this, homologous chromosomes become linked through crossover recombination, the two sister chromatids of each homolog attach to microtubules from the same spindle pole (co-orient) in meiosis I and from opposite spindle poles (bi-orient) in meiosis II, and sister chromatid cohesion (SCC) is released in two steps to allow separation of homologs before sisters. In yeast, reducing ploidy during meiosis requires that Scc1, the "kleisin" subunit of cohesin complexes that mediate mitotic SCC, is replaced by the meiosis-specific paralog Rec8. We have shown that REC-8 is not the sole meiotic kleisin in *C. elegans*, and we predicted that this was also true in plants and mammals. This has now been proven. In *C. elegans*, REC-8 and two functionally redundant kleisins called COH-3 and COH-4 (henceforth, COH-3/4) perform specialized roles, indicating that interchangeable kleisin subunits determine cohesin function during meiosis. For example, only REC-8 cohesin can co-orient sister chromatids and mediate SCC that persists after anaphase I. Kleisin identity also influences the mechanism by which cohesin loads onto chromosomes and establishes cohesion between sisters. The axial element protein HTP-3 is required for loading of REC-8, but not COH-3/4. Once loaded, COH-3/4 cohesin is triggered to become cohesive by SPO-11-dependent double strand DNA breaks, while REC-8 cohesin generates SCC independently of SPO-11. Finally, REC-8 and COH-3/4 become asymmetrically distributed on meiotic chromosomes late in prophase of meiosis I: COH-3/4 becomes enriched where SCC is released at anaphase I and REC-8 becomes enriched where sister chromatids co-orient and SCC persists until anaphase II. Because REC-8 alone can co-orient sisters and mediate SCC that persists following anaphase I, we are testing whether achieving this reciprocal pattern of cohesin localization facilitates the stepwise separation of homologs and sister chromatids.

47. A spatial and temporal transcriptomic survey of gene expression in the C. elegans embryo reveals organizing principles in cell fate specification. Tamar Hashimshony, Martin Feder, David Silver, Avital Polsky, Michal Levin, **Itai Yanai**. Technion, Haifa, Israel.

Multicellular development results from the execution of gene regulatory networks operating in distinct cell lineages - both autonomous and responsive to signaling - which specify the temporal and spatial gene expression required for cell-type differentiation. Embryonic development in the nematode C. elegans consists of the cell lineages of five somatic founder cells, encoding the three germ layers. Here, we determine, for the first time, the transcriptomes of the five somatic founder cell lineages as they develop in vitro and independently from each other throughout ten stages and compare with the gene expression in the whole embryo. All five somatic lineages reach tissue differentiation with transcript levels that overall match that of the whole embryo, indicating the integrity of the cells throughout the experiment. For many known regulators, expression is consistent in both lineage specificity, timing and expression levels suggesting a broad mode of autonomous expression. However, while endoderm, muscle and neuronal differentiation occurs in the expected lineages, a lack of appropriate signaling to the AB lineage resulted in its lack of specification of the pharynx. Surprisingly, genes with early expression are less spatially restricted than those with later expression, suggesting that the process of cell fate specification encompasses both specific and non-specific components. Analysis of lineage specific transcription factors recapitulates the known endoderm gene network and further leads to a prediction of the neuronal transcriptional specification pathway which we validated by coupling transcriptomics analysis with RNAi perturbation. This work has revealed organizing principles of metazoan cell fate specification and is readily applicable to the elucidation of gene pathways in diverse systems.

48. LIN-41 and OMA-1/2 spatially control M-phase entry during oogenesis. **Caroline Spike**, Donna Coetzee, David Greenstein. GCD Department, University of Minnesota, Minneapolis, MN.

The oocytes of most animals arrest at diplotene or diakinesis, but resume meiosis (meiotic maturation) in response to hormones. M-phase entry, triggered by CDK1-cyclin B activation, is a hallmark of meiotic maturation. In *C. elegans*, maturation of the -1 oocyte requires the presence of sperm, Ga_s-adenylate cyclase-PKA signaling in the gonadal sheath cells, and germline function of two Tis11-like CCCH zinc-finger proteins, OMA-1 and OMA-2. Here we show that the OMA proteins function with the TRIM-NHL protein LIN-41 to spatially regulate M-phase entry during oogenesis. Whereas oocytes in *oma-1; oma-2* double mutants fail to undergo meiotic maturation, oogenic cells precociously enter M phase in *lin-41(null)* mutants. Precocious M-phase entry occurs late in pachytene and is CDK-1-dependent. Proteomic analysis identified LIN-41 and translational regulators, including translation initiation factors and subunits of the GLD-2 poly(A) polymerase and CCR4-NOT deadenylase complexes as core components of purified OMA ribonucleoprotein particles (RNPs).

To test the hypothesis that OMA RNPs and LIN-41 control oogenesis in part through translational regulation, we identified approximately 400 germline-expressed mRNAs that are enriched in OMA RNPs, some of which have critical functions in oogenesis or early embryogenesis. LIN-41 and the OMA proteins are both required for the translational repression of several OMA RNP-associated mRNAs in oocytes, based on 3'-UTR reporters. Importantly, one shared mRNA target is the CDK1 activator, CDC-25.3. *cdc-25.3(ok358)* is dispensable for oogenesis, but smFISH indicates that *cdc-25.3* mRNA is present in oocytes. Furthermore, genetic epistasis analysis suggests that *cdc-25.3* contributes to the precocious M-phase entry of oogenic cells in a *lin-41(null)* mutant. We present a model in which translational regulation by LIN-41 and the OMA RNPs plays a key role in the spatial control of M-phase entry in oogenesis.

49. Molecular Antagonism between X-Chromosome and Autosome Signals Determines Sex. **Behnom Farboud**, Paola Nix, Margaret Jow, John Gladden, Barbara Meyer. HHMI and UC Berkeley.

Sex is determined in *C. elegans* by the ratio of X chromosomes to the sets of autosomes. X-signal elements (XSEs) communicate X-chromosome dose by repressing the masculinizing switch gene *xol-1* in a dose-dependent manner. Autosomal signal elements (ASEs) act in a cumulative, dose-dependent manner to counter XSEs by stimulating *xol-1* transcription. We identified new ASEs and cofactors of XSEs, and explored the biochemical basis by which XSEs counter ASEs to specify sex. Multiple antagonistic interactions carried out on a single promoter explain how the X:A signal elicits different sexual fates. XSEs (nuclear receptors and homeobox proteins) and ASEs (T-box and zinc-finger proteins) bind directly to several sites on *xol-1* to counteract each other's activities and thereby regulate *xol-1* transcription. Disrupting ASE and XSE binding sites *in vivo* recapitulated the mis-regulation of *xol-1* caused by disrupting cognate signal element genes. XSE and ASE binding sites are non-overlapping, suggesting that direct competition for *xol-1* binding is not how

XSEs counter ASEs. Instead, XSEs likely antagonize ASEs by recruiting cofactors with reciprocal activities that induce opposite transcriptional states. Most ASE and XSE binding sites overlap *xol-1*'s -1 nucleosome, which has activating chromatin marks only when *xol-1* is on. Coactivators and corepressors tethered by proteins similar to ASEs and XSEs deposit and remove such marks. We identified numerous SEX-1 interacting proteins. Many are transcription factors that likely bind DNA with SEX-1 as a heterodimer. Association of SEX-1 with some factors causes transcriptional repression while association with others causes transcriptional activation in a heterologous system. A coordinated exchange of SEX-1 partners, causing a switch of corepressors for coactivators, could transform SEX-1 from a potent repressor to an activator. Another class of SEX-1 interacting proteins includes CPSF complex subunits, suggesting SEX-1 acts via a corepressor complex to regulate mRNA processing. The concept of a sex signal having competing XSEs and ASEs arose as a theory for fruit flies a century ago. Ironically, while the fly signal does not fit this simple paradigm, the worm signal does.

50. The role of an SLC6 family transporter in *C. elegans* sperm activation. **Kristin Fenker**, Angela Hansen, Conrad Chong, Molly Jud, Gillian Stanfield. University of Utah, Salt Lake City, UT.

Many types of cells must change their morphology to function properly. In *C. elegans* sperm, morphological changes occur during a regulated process called sperm activation, which culminates in mature sperm that are motile and competent for fertilization. Sperm activation is regulated differently in male and hermaphrodite worms, and we are interested in the signals regulating male sperm activation. Our lab previously identified TRY-5, a serine protease in seminal fluid, as a key regulator. We have identified additional factors, including *snf-10*, a member of the solute carrier 6 (SLC6) transporter family.

Like *try-5*, *snf-10* is not required for fertility and acts in parallel to the *spe-8* group of genes that regulate hermaphrodite sperm activation. Males transfer an activator in their seminal fluid during mating; unlike *try-5*, *snf-10* is not required for this transfer. However, without *snf-10*, sperm are unable to respond to the male activator. These data suggest *snf-10* is in the same pathway as *try-5*, but is unlikely to regulate it. Thus, *snf-10* likely acts downstream of *try-5* in the male activation pathway. Consistent with this model, SNF-10 functions in sperm, and is polarized to the cell body in activated sperm.

We are using *in vitro* sperm activation assays to address the function of SNF-10. Wild-type sperm can be activated by protease treatment, suggesting a protease may act on a target on the sperm plasma membrane. *snf-10* mutant sperm do not activate in response to protease, demonstrating that SNF-10 is biochemically downstream of protease activity. *snf-10* mutant sperm do activate in response to treatment with other known activators, such as TEA and DIDS. We also have shown *snf-10* mutant sperm are more sensitive than wild-type to treatment with the ionophore monensin.

Our results indicate SNF-10 is a good candidate for a target of TRY-5 cleavage. We continue to investigate this relationship, and are pursuing experiments to characterize SNF-10's transporter activity and the role of SNF-10 in transducing signals that mediate changes in cell morphology.

51. Regulation of lineage-specific transcription factors by Wnt signaling in *C. elegans* embryogenesis: more than one way to regulate expression. **Amanda L. Zacharias**, Travis Walton, Joshua T. Burdick, Elicia Preston, John I. Murray. University of Pennsylvania, Philadelphia, PA.

The invariant *C. elegans* lineage robustly generates diverse cell fates, with Wnt and other signals regulating expression of lineage-specific transcription factors (TFs). We previously used lineage tracing to map spatiotemporal expression of reporters for 127 embryonic TFs. This identified many "lineally repetitive" (LR) TFs expressed in multiple posterior branches of the lineage, suggesting they are potential targets of Wnt signaling and that they may act combinatorially to specify cell fates. We tested whether 15 of these posterior LR genes, *ceh-6*, *ceh-13*, *ceh-27*, *ceh-36*, *elt-6*, *mir-57*, *nhr-25*, *nhr-67*, *nob-1*, *pal-1*, *pax-3*, *tlp-1*, *tbx-11*, *unc-130*, and *vab-7*, are regulated by Wnt signaling by analyzing expression patterns in embryos treated with RNAi against the Wnt effector TF, *pop-1*, or its export factor, *lit-1*. We found all 15 genes had altered expression patterns indicating they are regulated by Wnt signaling. All of the genes' regulatory regions harbor candidate POP-1 binding sites suggesting they could be direct targets, which we are working to test. While previously identified targets are both repressed by POP-1 in unsignaled anterior cells and activated by POP-1 in Wnt-targeted posterior cells, we instead observed two other types of responses. Some genes required POP-1 function only for repression in anterior cells, while others required POP-1 only for activation in posterior cells. To explore the importance of these patterns, we tested the requirement of LR TFs by lineage tracing of mutant embryos, which identified many incorrectly specified cells based on aberrant position, division timing, death, or survival. We identified target genes for LR TFs by RNA-seq of embryos globally overexpressing TFs under a heat-shock promoter and flow-sorted embryonic cells expressing TF reporters. We also used RNA-seq of *pop-1* or *lit-1* RNAi embryos to identify new potential Wnt targets, some of which are new posterior LR TFs. Together, our results support a general mechanism by which Wnt regulates expression of LR TFs that then combine to ensure correct terminal fates across the lineage.

52. Two molecularly distinct 3'end-directed translational control mechanisms establish two identical protein gradients in germ cells. **Ryuji Minasaki**, Nick Jourjine, Anfisa Solovyeva, Beate Kuechler, Christian R. Eckmann. MPI-CBG, Dresden, Germany.

Post-transcriptional mRNA regulation is widely used in animal development and physiology to control the right amount of protein in time and space, which is especially prevalent in germ cells, early embryos and neurons. To study gene-specific translational control, we use the *C. elegans* germ line as our model tissue to dissect the molecular mechanism of mRNA 3'end-directed regulation. Previously, GLD-1 protein synthesis served us as a paradigm of translational control in the adult female germ line. We reported that, following FBF/Pumilio-mediated translational repression, *gld-1* mRNA is subject to translational activation by two distinct cytoplasmic poly(A) polymerases (cytoPAPs), GLD-2 and GLD-4. By introducing sPAT, an improved method of measuring poly(A) tail lengths, we now demonstrate that these conserved enzymes promote *gld-1* mRNA stability and translation via poly(A) tail length extension *in vivo*. Using this technique, we also identified *cpb-3* mRNA as a novel target of GLD-2 cytoPAP. CPB-3 is an ortholog of the conserved cytoplasmic polyadenylation element-binding protein family and, therefore, a predicted RNA-binding protein. While the *cpb-3* expression pattern is analogous to that of *gld-1* at the mRNA and protein level, our additional genetic, molecular and biochemical data suggest that different translational control mechanisms are at works to achieve a similar protein expression gradient during female germline development. Our *in vivo* translational reporter analyses revealed several active cytoplasmic polyadenylation elements in the 3'UTR of *cpb-3* mRNA. Together with CPB-3's ability to form a CPB-3/GLD-2/*cpb-3* mRNP complex that regulates *cpb-3* mRNA's polyA tail activity, our combined data suggest that a direct autoregulatory feedback loop underlies the translational regulation of *cpb-3* mRNA. This molecular difference also appears to be strongly influenced by several FBF-binding elements. Hence, the combination of different *cis*-regulatory elements may organize a unique mRNP composition that guides cytoPAP-mediated poly(A) tail regulation to

translationally activate mRNAs for a timely increase in protein abundance during germ cell development.

53. The hazards of love: Sterilization and lethality in interspecies crosses. JJ Ting[1], **GC Woodruff[2]**, T Maugel[2], N Kanzaki[3], RJ Sommer[4], AD Cutter[1], ES Haag[2]. 1) Dept. of Ecol. & Evol. Biol., Univ. of Toronto, ON; 2) Dept. of Biol., Univ. of Maryland, College Park MD; 3) Forestry & Forest Prod. Res. Inst., Tsukuba, Japan; 4) Dept. for Evol. Biol., Max-Planck Institute for Dev. Biol., Tübingen, Germany.

Because sexual selection is weak in selfing species, hermaphrodites may evolve greater susceptibility to the harmful effects of mating. In addition, more specific 'gene-for-gene' mismatches might also be evident in interspecies crosses, even between species with the same reproductive mode. We examined heterospecific matings between and within a given sexual mode. Hermaphrodites of *C. briggsae, C. elegans*, and *C*. sp. 11 mated to gonochoristic males produce far fewer self-progeny than controls. In *C. briggsae*, one mating with a gonochoristic male is sufficient for sterilization, and this is likely due to premature oocyte maturation. *C.* sp. 9 males also greatly accelerate the mortality of *C. briggsae* hermaphrodites. Dying hermaphrodites often have sperm outside of the uterus and spermatheca ("sperm metastasis"). Germline-feminized *C.* sp. 9 males deposit copulatory plugs, but fail to sterilize or reduce the lifespan of *C. briggsae* hermaphrodites, indicating sperm are necessary for both effects. These patterns of asymmetrical sterilization and mortality are also evident in crosses between selfing and gonochoristic Pristionchus species. Matings between different hermaphroditic or gonochoristic species also reduce brood sizes, but to a lesser extent. Collectively, our findings are consistent both with a general 'weak inbreeder' susceptibility of hermaphrodites in matings with males of outcrossing species, and with mating system-independent accumulation of reproductive incompatibilities. The latter may represent resolutions of species-specific conflicts that do not 'complement' each other in interspecific crosses. Sperm size differences between sexes and species may also contribute. The relaxation of the evolutionary arms race between males and females in hermaphrodites may make them especially susceptible to harm by gonochoristic males. This could accelerate and/or reinforce reproductive isolation between hermaphroditic species and outcrossing relatives.

54. A pathway for unicellular tube extension depending on the lymphatic vessel determinant Prox1 and on osmoregulation. **Irina Kolotueva[1,2]**, Vincent Hyenne[1], Yannick Schwab[1], David Rodrigues[1], Michel Labouesse[1]. 1) IGBMC, 1 rue Laurent Fries - BP 10142; F-67400 Illkirch, France; 2) Current address: BIOSIT - UMS 3480, Université de Rennes 1, 2 avenue du Pr Léon Bernard, CS 34317, Rennes, France.

Biological tube formation and extension is a universal, yet still poorly understood process. Excretory canal in *C. elegans* is a unicellular tube that runs alongside the body. It was previously assigned an osmoregulatory role and served as a model for kidney. Using genetics, light and electron microscopy we characterized and identified several steps in excretory canal growth and lumen extension. We propose a model whereby the basal and apical extensions of canal membranes grow sequentially: basal is followed by apical, fed by cytoplasmic vesicles (canaliculi) fusion to the central lumen. The process of basal process extension resembles the paradigm of the axon growth cone. The internal lumen of the canal, as indicated by our results extends as a result of an osmoregulatory activity that triggers the fusion of peri-apical vesicles. Intermediate filaments and actin crosslinking proteins in the apical cytoskeletal web provide straight lumen growth. We have characterized a *rdy-3 /ceh-26* mutant (now *pros-1*) that we found essential for excretory canal formation. Expression of several genes encoding proteins mediating excretory lumen extension, such as the osmoregulatory STE20-like kinase GCK-3, aquaporin *aqp-8* and the intermediate filament IFB-1, are regulated either directly or indirectly by *pros-1*. PROS-1 is homologous to vertebrate Prox1, a transcription factor controlling lymphatic vessel growth. Our findings have potential evolutionary implications for the origin of fluid-collecting organs, and provide a reference for lymphangiogenesis.

55. NHR-67 mediates cell cycle arrest and promotes the differentiation of the invasive phenotype. **David Q. Matus[1]**, Laura C. Kelley[1], Michalis Barkoulas[2], Adam J. Schindler[1], Qiuyi Chi[1], Marie-Anne Félix[2], David R. Sherwood[1]. 1) Biology, Duke University, Durham, NC; 2) Institute of Biology of the Ecole Normale Supérieure, Paris, France.

There is a well-established link between cell cycle arrest and the transcriptional regulation of cell differentiation. Emerging data suggests that the complex cell biological movements orchestrated during morphogenesis (*e.g.* epithelial to mesenchymal transition (EMT) and convergent extension) also require cell cycle arrest to be properly executed. Cell invasion through basement membrane (BM) is a poorly understood morphogenetic behavior that occurs during development, immune surveillance and is mis-regulated during cancer metastasis. To functionally dissect cell invasive behavior at single-cell resolution, we are using the simple *in vivo* model of anchor cell (AC) invasion during *C. elegans* larval development. The AC, a specialized somatic gonadal cell, undergoes a BM transmigration event to connect the developing uterine and vulval tissues. In order to identify transcriptional regulators of AC invasion, we performed a uterine-specific transcription factor based RNAi screen, which identified the conserved nuclear hormone receptor, NHR-67/TLX. Strikingly, loss of NHR-67 results in multiple ACs that fail to invade. Through laser ablation, GFP reporter analyses and single molecule fluorescence *in situ* hybridization we show that NHR-67-depleted ACs arise from a single mitotic AC that expresses markers of an active cell cycle. Halting cell cycle progression by inducing a G1/G0 arrest in NHR-67-depleted animals rescues the invasion defect, demonstrating that cell cycle arrest is required for invasion. Blocking the cell cycle in the S or G2 phase failed to rescue AC invasion, indicating a specific requirement for a G1/G0 arrest. Time-lapse microscopy shows that mitotic ACs lack invadopodia, subcellular structures within the AC that function to breach the BM. Mitotic ACs also show reduced expression of downstream markers of invasive differentiation, including matrix metalloproteinases (MMPs). Our data indicates the requirement for the precise transcriptional control of a genetic program that links a G1/G0-specific cell cycle arrest to the differentiation of the invasive phenotype.

56. UNC-84 spans the nuclear envelope and connects the nucleoskeleton to KASH proteins at the outer nuclear membrane. Natalie Cain[1], Courtney Bone[1], Erin Tapley[1], Ben Lorton[1], Kent McDonald[2], **Daniel Starr[1]**. 1) UC Davis; 2) UC Berkeley.

Nuclear positioning is central to many cellular and developmental events. A bridge of inner nuclear membrane (INM) SUN proteins and outer nuclear membrane (ONM) KASH proteins spans the nuclear envelope. In *C. elegans*, the SUN protein UNC-84 recruits the KASH protein UNC-83 to the ONM where it targets dynein and kinesin-1 to move nuclei. However, it remains unknown how SUN proteins span the perinuclear space of the nuclear envelope and how forces transferred across the bridge are dissipated at the nucleoskeleton. To elucidate mechanisms of how mechanical forces are transferred from the bridge to the nucleoskeleton, we performed a yeast two-hybrid screen with the nucleoplasmic domain of UNC-84 as bait and identified an interaction with the lamin B gene LMN-1. *lmn-1(RNAi)* animals had a hyp7 nuclear migration defect. Moreover, the LMN-1 interaction was significantly weakened by an

UNC-84 P91S mutation that was previously identified in genetic screens with a partial loss-of-function hyp7 nuclear migration defect. These data support a model where UNC-84 interacts with LMN-1 to dissipate force from the bridge to the nucleoskeleton. We identified a new player in this network, the integral INM protein SAMP-1 (T24F1.2); *samp-1(RNAi)* animals had a weak nuclear migration defect. To test the hypothesis that UNC-84 spans and regulates the space between the INM and ONM, we examined the morphology of the nuclear envelope by electron microscopy in *unc-84(null)* mutants. We observed extreme separation of the ONM from the INM in *unc-84(null)* L1 muscle nuclei. Surprisingly, a deletion of ~300 residues between the trans-membrane and SUN domains of UNC-84 remained functional, thus, the majority of this linker domain is not required for nuclear migration. However, a 60 amino acid span immediately upstream of the SUN domain, and the SUN domain itself are required for function. Additionally, mutations in the SUN domain of UNC-84 predicted to interact with KASH peptides from human SUN structures disrupt hyp7 nuclear migrations. In our model the nucleoplasmic domain of UNC-84 directly binds lamin to transfer forces from the bridge to the nucleoskeleton. UNC-84 is also required for even nuclear envelope morphology.

57. Endocytosis controls EFF-1 mediated cell fusion. **K. Smurova**, B. Podbilewicz. Biology Dept, Technion, Haifa, Israel.

Developmental cell fusion is an important morphogenetic force in tissue and organ formation in multicellular organisms. In C. elegans most embryonic and postembryonic hypodermal cell fusions are mediated by eff-1 (epidermal fusion failure). Overexpression of EFF-1 leads to abnormal cell fusion and causes embryonic lethality. Numerous transcription factors repress eff-1 expression; however, mechanisms that repress EFF-1 activity and surface expression to prevent cells from hyperfusion are poorly understood. When EFF-1 is expressed in heterologous insect or mammalian cells, it localizes to the plasma membrane and mediates cell-cell fusion. In contrast, we found that EFF-1 is not localized to the plasma membrane of cells fated to fuse in C. elegans embryos. Surprisingly, the steady state localization of EFF-1 revealed by monoclonal antibodies showed that EFF-1 is expressed in intracellular puncta and not on the plasma membrane. We have found that during and shortly after fusion EFF-1 mainly colocolizes with RAB-5, the GTP-ase responsible for endocytic trafficking of early endosomes. rab-5 mutants showed abnormal and excessive cell fusion caused by EFF-1 mislocalization on cell junctions. eff-1(oj55) hypomorphic mutation resulted in the loss of EFF-1/RAB-5 colocalization suggesting the importance of the extracellular Domain III of EFF-1 in EFF-1/RAB-5 interaction. Membrane-associated proteins of the vacuolar ATPase (v-ATPase) complex VHA-17 and VHA-5 were also involved in the regulation of cell fusion (Kontani et al., 2005). Mutants of VHA-17 and VHA-5 showed excessive cell fusion and increased amount of EFF-1 vesicles in the cytoplasm of cells. We hypothesize that parts of the v-ATPase are involved in EFF-1 trafficking and degradation. We propose that after transient trafficking of EFF-1 to the plasma membrane, it is endocytosed back to the early endosome to keep EFF-1 inactive and to protect cells from hyperfusion. RAB-5 and membrane-associated subunits of the v-ATPase regulate EFF-1 fusogen activity by governing EFF-1 trafficking. Live imaging of active EFF-1 reporters showed the dynamic exchange of EFF-1 between cytoplasm and plasma membrane. Thus, recycling of the EFF-1 membrane glycoprotein controls cell-cell fusion in C. elegans.

58. A new model system for studying cell shape change: identifying the molecular mechanisms necessary for *C. elegans* uterine seam cell development. **Srimoyee Ghosh**, Paul Sternberg. Biology, California Inst of Technology, Pasadena, CA.

Understanding the mechanisms necessary for proper cell shape change can shed light on the pathologies of numerous diseases, including metastatic cancer. The *C. elegans* uterine seam cell (UTSE) is a system that undergoes cell outgrowth during its development. We hope to identify novel complexes involved in cell shape change by determining the molecular mechanisms necessary for this cell's development. The uterine seam cell (UTSE) connects the uterus to the body wall and is a syncytium comprised of nine nuclei that move outward in a bidirectional manner. Through a candidate RNAi screen, we have identified several different complexes that are involved in UTSE development. These include proteins that are solely affect UTSE nuclear migration, such as the KASH protein UNC-83, the SUN protein UNC-84, and its corresponding nuclear anchoring protein, ANC-1, as well as proteins that are involved in UTSE cell outgrowth, such as the alpha integrin INA-1 and its corresponding beta subunit PAT-3, the astacins NAS-21 and NAS-22, the laminin EPI-1, the FGF receptor EGL-15, as well as several Rab GTPases. We have also looked for cues from other parts of the uterus that influence UTSE behavior. Using ablation experiments, we saw that two types of epithelial cells that line the uterine lumen, uterine toroid 1 and uterine toroid 2, are necessary for proper UTSE development. This led us to identify another gene involved in UTSE development, *tag-312*, which is expressed in the uterine toroids. Since we have identified two sets of genes with previously uncharacterized roles in cell shape change: the astacins *nas-21* and *nas-22* and uterine toroid marker *tag-312*, and we are now focusing on identifying the mechanisms that these genes are using to control UTSE cell outgrowth.

59. Dynamic interaction between hemidesmosomes and actin cytoskeleton regulated by RNA alternative splicing in elongating *C.elegans* epidermis. **H Zhang**[1], R Fu[1], H Zahreddine[2], M Labouesse[2]. 1) IBMS, Soochow University, Suzhou, Jiangsu, China; 2) IGBMC,Strasbourg, France.

C.elegans hemidesmosomes (CeHDs) share similar structure and composition with their mammalian counterparts, making C. elegans an ideal model for studying hemidesmosomes. Despite its importance, how the dynamics of hemidesmosomes are regulated at the mRNA level is poorly understood. Here we focus on the role of transcription factor lst-3, identified in a previous screen searching for VAB-10A enhancers, in CeHD biogenesis. Loss of LST-3 function in a vab-10(e698) background resulted in CeHD disruption and muscle detachment from the epidermis. Quantitative RT-PCR data showed that the transcription levels of the CeHD-related genes were unchanged upon loss of LST-3 function. However, the alternative splicing of exon 17 in unc-52, the predicted basement ECM ligand of CeHDs, was particularly enhanced. Expression analysis further revealed that the mRNA level of splicing factor SMU-2 was upregulated in lst-3 mutants. Overexpression of SMU-2 in embryos resulted in increased amount of the UNC-52 isoform with exon 17, as well as CeHD disruption and embryonic lethality siminar as what caused by lst-3 loss of function. It suggests that LST-3 may regulate CeHD biogenesis mainly through repressing smu-2 expression. To further explore the mechanisms of alternative splicing on CeHD biogenesis, we examined the CeHD pattern as relative to the circumferential actin bundles (CFBs) in the epidermis. In wildtype situation, newly formed CeHD stripes and CFBs are positioned in a perfectly alternating fashion. During late elongation, however, the CeHD stripes and actin bundles gradually close in upon each other and eventually overlap. Interestingly, in mutants with elevated UNC-52 isoform with exon-17, there are significantly more overlapped CeHD /CFBs compared to the wildtype situation. On the other hand, in unc-52(e669) mutants that are devoid of UNC-52 isoform with exon-17, the phenotype was reversed. These results further suggested the constant crosstalk between the CeHDs and the actin bundles during epidermal elongation, and the active roles of alternative splicing in

regulating such crosstalk.

60. Excretory canal development requires conserved kinases and *exc-6/INF2*, a formin implicated in kidney disease. **Daniel Shaye**[1], Iva Greenwald[1,2]. 1) HHMI; 2) Dept. of Biochemistry. Columbia University. New York, NY.

The excretory canal cell, which is required for osmoregulation, is a simple model to study tubulogenesis. It extends long processes first dorsally, then anterior and posteriorly, each with an intra-cellular lumen. Previous genetic analysis revealed that canal outgrowth requires some genes that also mediate neuronal outgrowth[1-3]; lumen formation is driven by regulation of fluid and ion transport [4,5,6]; and lumen maintenance depends on the apical actin cytoskeleton[7,8].

To identify new conserved genes involved in tubulogenesis, we used feeding RNAi in a sensitized background to test 243 conserved kinases[9], identifying 9 that caused excretory canal phenotypes. We have initially focused on the kinase PIG-1/MELK. Loss of *pig-1* causes shortening of anterior and posterior canal arms, a mild cystic phenotype and regions with multiple lumens, similar to phenotypes seen in the uncloned mutant *exc-6*[10]. We cloned *exc-6*, and found that it encodes an ortholog of the human formin *INF2*, a kidney disease gene[11]. INF2 promotes actin polymerization, de-polymerization and microtubule stability[12]. We have evidence that residues that regulate actin dynamics are required for EXC-6 function. Unexpectedly, EXC-6 may not predominantly co-localize with apical actin, instead accumulating with structures resembling microtubules. Thus, EXC-6 may coordinate actin and microtubule cytoskeletons in the excretory canal. Disease-causing *INF2* mutations are dominant and have been proposed to result in constitutive activity. Using rescue of *exc-6(0)*, we have found functional evidence supporting this model. We are currently investigating the functional relationship between *pig-1* and *exc-6* in canal development. Given the conservation of *INF2* function suggested by our rescue experiment, such a relationship might have potential relevance for disease.

1) Stringham et al., 2002. **2)** Katidou et al., 2012. **3)** Marcus-Gueret et al., 2012. **4)** Khan et al., 2013. **5)** Kolotuev et al., 2013. **6)** Berry et al., 2003. **7)** Tong and Buechner, 2008. **8)**Buechner, 2002. **9)** Shaye and Greenwald, 2011. **10)** Buechner et al. 1999. **11)** Brown et al. 2010. **12)** Gaillard et al., 2011.

61. A Rho-specific GAP functions in response to axonal guidance signals to regulate embryonic morphogenesis. **Andre Wallace**, Sanese Brown, Martha Soto. Dept. of Pathology, Robert Wood Johnson Medical School, UMDNJ, 675 Hoes Lane, Piscataway, NJ.

Axonal guidance signals are proposed to regulate the movements of neurons by reorganizing the actin cytoskeleton. Recent work from our lab has shown that three axonal guidance receptors, UNC-40/DCC, SAX-3/Robo, and VAB-1/Eph, regulate embryonic epidermal enclosure, and modulate the actin cytoskeleton through effects on the CED-10 GTPase and the WAVE/SCAR complex. Therefore, we predicted that regulators of these axonal guidance receptors function upstream of WAVE/SCAR, through a pathway that activates Rac-GTPases to control actin dynamics during embryonic morphogenesis. We identified HUM-7, a protein that contains both a Myosin IX and a Rho GAP domain, as a regulator of C. elegans embryonic morphogenesis in a screen for enhancers of unc-40 embryonic defects. While loss of hum-7 in an unc-40 genetic mutation enhanced embryonic defects, loss of hum-7 in a sax-3 genetic mutation suppressed embryonic defects. These results suggested that HUM-7 is functioning in a pathway with SAX-3 and in parallel to UNC-40. Loss of HUM-7 alone results in a low percent of embryos with embryonic morphogenesis defects, similar to mild Gex defects. Since HUM-7 contains a Rho-GAP domain, we predicted that HUM-7 would function as a GAP for CED-10 during embryogenesis. To test this hypothesis, we analyzed genetic and RNAi doubles of hum-7 and hypomorphic alleles of three C. elegans GTPases, CDC-42, RHO-1 and CED-10 or their regulators. Surprisingly, loss of hum-7 enhanced embryonic lethality and morphogenesis defects in ced-10 hypomorphs. In contrast, RNAi depletion of hum-7 in a hypomorphic allele of ect-2, a RHO-1-specific GEF, suppressed embryonic lethality and morphogenesis defects. Similarly, loss of hum-7 was able to suppress strong embryonic morphogenesis defects and embryonic lethality caused by cdc-42 RNAi. These results indicate that HUM-7 is likely functioning as a GAP for RHO-1 and/ or CDC-42 during embryonic morphogenesis. Overall, these results suggest that in addition to CED-10, there is a role for RHO-1 and CDC-42 in controlling the actin cytoskeleton during the epidermal enclosure step of C. elegans morphogenesis.

62. ZEN-4/MKLP1 and the establishment of epithelial polarity in the *C. elegans* foregut. **Stephen E. Von Stetina**, Susan E Mango. Molecular and Cellular Biology, Harvard University, Cambridge, MA.

How are polarized epithelia established and maintained? This question is of critical importance, as the loss of epithelial polarity is associated with human diseases, such as metastasis. For some cells, E-cadherin is the major initiator of cell polarity and epithelium formation via cell-cell adhesion. However, recent studies have discovered E-cadherin- and integrin-independent polarity pathways. These observations suggest that there are additional pathways to establish epithelial polarity, but little is known about the molecular mechanism. *C. elegans* offers a powerful system to study this non-canonical process, as E-cadherin and b-integrin are dispensible for epithelial polarity in nematodes.

We have begun an analysis of non-canonical epithelium formation using the foregut (pharynx) as a model system. To identify new regulators of epithelial polarity, our lab has undertaken a genetic screen for mutants that fail to form the arcade cell epithelium (Portereiko and Mango, unpublished). This screen revealed that the centralspindlin component ZEN-4/MKLP1 (mitotic kinesin-like protein) is critical to form the arcade cell epithelium in the foregut; *zen-4* mutants express at least some epithelial markers, but these fail to localize to the expected apical or junctional regions at the cell cortex. Surprisingly, our current results suggest that ZEN-4 acts prior to or at the birth of the arcade cells and not during polarization. This means that ZEN-4 is acting in the arcade cells ~100m prior to the onset of any visible signs of polarity (e.g. PAR-3, DLG-1). To elucidate its role in polarity, we performed structure-function analysis of this mitotic kinesin, which is thought to migrate toward microtubule plus ends and to bundle antiparallel microtubules to generate the central spindle during cytokinesis. Our studies revealed that the motor functions of ZEN-4 are dispensable for polarity, but not conserved residues important for binding its obligate partner in cytokinesis, CYK-4/GAP. Thus, our data have revealed an intriguing link between mitosis and the establishment of epithelial polarity hours later.

63. A Pre-Stressed UNC-70 b-Spectrin Network Governs the Sense of Touch. **Michael Krieg**[1], Alexander R Dunn[2], Miriam B Goodman[1]. 1) Molecular & Cellular Physiology, Stanford University, Stanford, CA; 2) Chemical Engineering, Stanford University, Stanford, CA.

Many somatosensory neurons have evolved specialized molecular sensors that convert mechanical stress into behavioral responses. The genetics,

development and physiology of the touch receptor neurons (TRNs) in Caenorhabditis elegans nematodes are especially well characterized and this animal has the particular advantage that the TRNs can be studied both in living animals and dissociated in culture. Like other somatosensory neurons, the TRNs use ion channels to convert mechanical stress into electrical signals and ultimately appropriate behaviors. Whereas the protein partners that form these mechanosensitive channels have been known for some time, the nature of the molecular machine important for efficient force transmission from skin to touch receptor neurite is essentially unknown. Here we show that sensation of mechanical forces depends on a continuous, pre-strained spectrin cytoskeleton inside neurons. We observed that mutations in the tetramerization domain of C. elegans b-spectrin (UNC-70), an actin-membrane crosslinker, lead to defective neuron morphologies under compressive stresses in moving animals. We performed AFM force spectroscopy experiments on isolated neurons, laser axotomy and FRET imaging to measure force across single cells and molecules. Our data indicate that spectrin is held under constitutive tension in living animals, which contributes to an elevated pre-stress in TRNs. Based on these results and data obtained from optogenetic and mechanical stimulation on b-spectrin mutants, we suggest that b-spectrin-dependent pre-tension is required for efficient responses to external mechanical stimuli.

64. Understanding the Role of MMPs In Basement Membrane Breaching *In Vivo*. **Laura C Kelley**, David Q Matus, Qiuyi Chi, David R Sherwood. Department of Biology, Duke University, Durham, NC.

Basement membrane (BM) is a dense sheet-like extracellular matrix that encapsulates and separates tissue compartments. The invasive ability of cells to cross BM barriers is required for normal and disease processes. Matrix metalloproteinases (MMPs) are overexpressed in cells responsible for tissue remodeling, wound healing, and cancer and are hypothesized to enzymatically facilitate BM removal. Due to the high number of MMPs expressed in vertebrates, the relevance and potential function MMPs in movement through BM is unclear. Our laboratory has combined high-resolution 4D imaging with the visually accessible and genetically tractable model of anchor cell (AC) invasion in *C. elegans* to analyze BM breaching *in vivo*. We have previously shown that the c-fos oncogene homologue, FOS-1A, promotes BM penetration during AC invasion (Sherwood and Sternberg, 2005). In *fos-1a* mutants, the AC extends invasive protrusions that are blocked at the BM, indicating that FOS-1A regulates the transcription of genes that mediate BM removal. Recently, we identified that FOS-1A regulates the expression of three MMPs in the AC during the time of invasion. In addition, two of the three remaining MMPs in the *C. elegans* genome are expressed in the tissues surrounding the invasive cell. Individual knockdown of these MMPs fails to cause defects in invasion suggesting that they act redundantly to promote invasion. To test this we genetically derived animals that lack all five MMPs expressed within the gonad prior to, and during the invasive process. Unexpectedly, AC invasion persists in the quintuple-MMP mutant animals, indicating that these proteases are not absolutely required for BM removal. Live-cell imaging of the BM, however, revealed distinct differences in the BM dynamics and architecture in MMP-deficient animals. In the MMP (-) mutants BM removal under the AC occurred at a slower rate. Further, BM clearing was disorganized and less uniform in the absence of MMPs. Thus, it appears that MMPs play a modulatory role during the invasive process, functioning to ensure more precise and rapid breaching of BM. These results also suggest that other transcriptional targets of FOS-1A must play a role alongside the MMPs in clearing BM.

65. The PAF1 complex is essential for epidermal morphogenesis in *C. elegans* embryos. **Yukihiko Kubota**[1], Yusuke Takabayashi[1], Kenji Tsuyama[1], Nami Haruta[1], Rika Maruyama[2], Asako Sugimoto[1]. 1) Developmental Biology and Neurosciences, Tohoku University, Sendai, Hyogo, Japan; 2) RIKEN Center for Developmental Biology, Kobe.

During morphogenesis, cells undergo dynamic changes including polarization, migration and rearrangement of cell groups. To identify new genes involved in epidermal morphogenesis, we performed an RNAi screen of ~800 embryonic lethal genes by live imaging, and identified *B0464.2* whose depletion caused highly penetrant defects in body elongation in late embryogenesis. *B0464.2* encodes the *C. elegans* ortholog of Ctr9 (Ce-Ctr9), a component of the Polymerase-Associated Factor 1 (PAF1) complex. The PAF1 complex consists of five conserved proteins (Paf1, Ctr9, Cdc73, Leo1 and Rtf1), and is implicated in diverse transcription related processes including transcriptional elongation, 3'-terminal end processing, and histone modification. We found by RNAi that, in addition to the Ce-Ctr9, orthologs of all other four components of the PAF1 complex were required for epidermal morphogenesis. Time-lapse analyses by DIC and with DLG-1::GFP (an epidermal junction maker), mCherry::TBB-2 (a microtubule marker) and VAB-10(ABD)::mCherry (an F-actin marker) revealed that, while depletion of components of the PAF1 complex did not affect the number of epithelial cells, it affected cell shape changes and cell positioning during epidermal enclosure and body elongation. Abnormalities in muscles and neurons were not detected in these embryos. Genomic translational GFP/mCherry-fusion constructs of Ce-Paf1, Ce-Leo1 and Ce-Rtf1 revealed that these proteins were localized to the nuclei of virtually all embryonic cells. They appeared not tightly associated with chromatins, because these proteins were diffused to the cytoplasm during NEBD. A deletion mutant of *Ce-Leo1* exhibited maternal effect embryonic lethality with epidermal defects similar to the RNAi embryos, which were rescued by mCherry::Leo1 expression under an epidermis-specific promoter. These results indicate that the PAF1 complex regulates the epidermal cell shape change and migration during embryonic morphogenesis in a cell-autonomous manner.

66. Nuclear membrane proteins act in transport of the Netrin receptor, UNC-5 in cell migration in *C. elegans*. **Hon-Song Kim**, Kiyoji Nishiwaki. Dept of Biosci, Kwansei-Gakuin Univ, Sanda, Hyogo, Japan.

The KASH protein interacts with the SUN protein in the nuclear membrane and recruits kinesin-1 to regulate nuclear migration along microtubules. In *C. elegans*, UNC-83/KASH and UNC-84/SUN act in nuclear migration of the distal tip cells (DTCs), the leader cells of gonadal development. We found that *unc-83(e1408)* and *unc-84(e1410)* mutants exhibited abnormal gonad formation due to the precocious dorsal turn of DTCs, in addition to the nuclear migration defect. The dorsal migration of DTCs is regulated by the repulsive action of the UNC-5 receptor in response to the guidance molecule UNC-6/Netrin. Using UNC-5::GFP (a functional fusion), we visualized the distribution of UNC-5 during DTC migration. UNC-5::GFP was first detected as vesicles associated with the nuclear membrane at the beginning of dorsal turn of DTCs and was gradually spread to the plasma membrane during the turn. Interestingly, however, UNC-5::GFP was not detected in the nuclear periphery, but detected in the plasma membrane of DTCs from the beginning of the turn in *unc-83* and *unc-84* mutants. We speculated that precocious distribution of UNC-5 in the plasma membrane could cause the precocious dorsal turn of DTCs. We also found that knockdown of *unc-116/kinesin-1 heavy chain* caused precocious turn of DTCs, suggesting the involvement of *unc-116* in controlling the timing of DTC turn. Furthermore, the precocious DTC turn in *unc-83(e1408)* and *unc-84(e1410)* mutants was suppressed either by introducing *unc-6* mutations or reducing the gene dosage of *unc-5*. These results suggest that the UNC-6 signal is hyper-activated in the *unc-83* and *unc-84* mutants. We propose that

UNC-83 and UNC-84 function with UNC-116 in tethering the UNC-5 vesicles to the nuclear envelope, thereby transporting them to the plasma membrane in proper timing and amount during the dorsal turn of DTCs.

67. No title at time of print. **Tony Hyman**. Max Planck Institute.

68. *In vivo* forced reprogramming and remodeling of differentiated somatic cells and organs by brief expression of a single transcription factor. Misty R. Riddle[1], Ken K.C.Q Nguyen[2], David H. Hall[2], **Joel H. Rothman[1]**. 1) MCD Biology, Univ California, Santa Barbara, CA; 2) Center for C. elegans Anatomy, Albert Einstein College of Medicine, Bronx, NY 10461, USA.

Early embryonic cells in *C. elegans* are pluripotent and can be forced to adopt alternative fates by ectopic expression of key regulators of endoderm, mesoderm, or ectoderm development. Postmitotic differentiated somatic cells in larvae and adults are generally considered locked in fate. We found that brief ectopic expression of ELT-7, a GATA transcription factor that regulates endoderm differentiation, reprograms fully differentiated somatic cells into intestine-like cells without the removal of inhibitory factors. Cells that form the pharynx and somatic gonad appear specifically competent to reprogramming by ELT-7. The reprogrammed cells express intestine-specific genes, paralleling loss of expression of the original cell-fate specific genes. Reprogrammed cells undergo dramatic remodeling at the ultrastructural level to resemble intestinal cells. Cells that form the somatic gonad become reorganized after ectopic ELT-7 expression to form a second intestinal lumen with remarkable similarity to the endogenous intestine. Thus, we may have observed forced “trans-organogenesis” of one organ into another. Our results show that terminally differentiated postmitotic cells can be remodeled to cells of another germ layer in the absence of cell division or prior removal of the original cell fate.

69. Evolution and genetic architecture of the first mitotic spindle in *C. elegans*. **R. Farhadifar[1]**, C. Baer[2], E. Andersen[3], G. Fabig[4], T. Müller-Reichert[4], M. Delattre[5], D. Needleman[1]. 1) Dep MCB, Harvard Uni, Cambridge, MA; 2) Dep Biology, Uni of Florida, Gainesville, FL; 3) Dep Mol. Biosci., Northwestern Uni, Evanston, IL; 4) MTZ, TU Dresden, Germany; 5) LBMC-ENS, Lyon, France.

The architecture and dynamics of sub-cellular structures show remarkable variations between species, but little is known about the evolutionary or mechanistic basis of this diversity. Examining intraspecies variation can provide valuable evolutionary insights because differences between species arise from differences between individuals of species, but the extent of intraspecific variation of sub-cellular traits is unknown. Mutations are the ultimate source of variation between individuals. Understanding how spontaneous mutations affect these structures shows what phenotypes are evolutionary accessible and provides a “baseline” of how these traits would change in the absence of selection. We are using the first embryonic division in *C. elegans* to study the evolution of the mitotic spindle; the sub-cellular structure that segregates chromosomes during cell division. We developed a high-throughput microscopy platform and automated image analysis software that allows us to obtain quantitative information on the structure and dynamics of spindles from thousands of embryos in hundreds of lines. We found extensive standing genetic variation among natural isolates of *C. elegans* for all traits we studied. We are studying the genetic architecture of these traits by performing a genome wide association analysis and by characterizing a panel of recombinant inbred advanced intercross lines. We also studied how spontaneous mutations modify the spindle by examining a panel of mutation accumulation (MA) lines. Comparing the spectrum of variations in MA lines to those we observe among natural isolates allows us to draw inferences about how selection and population dynamics combine with raw mutational inputs to shape the spindle in *C. elegans*. Our preliminary results indicate that most cellular traits evolved under weak stabilizing selection and suggest that combining cell biology, biophysics, and quantitative genetics will produce novel evolutionary and mechanistic insights.

70. Meiotic chromosome structures constrain and respond to designation of crossover sites. **Diana E. Libuda[1]**, Satoru Uzawa[2], Barbara J. Meyer[2], Anne M. Villeneuve[1]. 1) Dept of Developmental Biology, Stanford Univ, Stanford, CA; 2) Dept of Molecular and Cellular Biology, UC Berkeley/HHMI, Berkeley, CA.

Proper meiotic chromosome segregation requires processes that promote and constrain the formation of crossovers (COs). CO interference, a process that constrains the number of COs along a chromosome pair, was originally observed a century ago but is still poorly understood. *Caenorhabditis elegans* exhibits particularly robust CO interference, with only a single CO forming between each pair of homologous chromosomes. Here, we use a cytological marker of CO sites to reveal relationships between COs and the synaptonemal complex (SC), a meiosis-specific structure that assembles between aligned homologous chromosome pairs. We develop a system to assess interference strength quantitatively during wild-type meiosis, demonstrating that interference operates over distances that exceed the length of a normal *C. elegans* chromosome. Further, we show that partial depletion of the SC central region proteins attenuates CO interference, elevating COs and reducing the effective distance over which interference operates, indicating a role for SC central region proteins in limiting the formation of cytologically-differentiated CO sites. Finally, our analyses reveal a linear relationship between CO number and chromosome axis length, demonstrating that each CO causes a 0.4-0.5 mm increase in axis length. Moreover, we provide additional evidence that this CO-dependent increase in chromosome axis length is a local effect around the CO site. We propose that meiotic chromosome structures establish an environment that promotes CO formation, which in turn alters chromosome structure to inhibit other COs at additional sites.

71. A sulfatase encodes a developmental switch for a feeding-structure dimorphism and controls micro- and macroevolutionary patterns in *Pristionchus*. **Erik J. Ragsdale**, Manuela R. Müller, Ralf J. Sommer. Max Planck Institute for Developmental Biology, Tuebingen, Germany.

Developmental plasticity is of importance to both developmental biology and evolutionary ecology, with nematode dauer formation providing inroads into the underlying mechanisms. In *Pristionchus pacificus*, a feeding dimorphism provides a second example of developmental plasticity and highlights the importance of plasticity as a facilitator of evolutionary novelty. However, the genetic mechanisms underlying the regulation and evolution of plasticity, particularly as they promote novelty, have been largely out of reach. *P. pacificus* is dimorphic in its novel, predatory mouthparts, which comprise a stenostomatous (St) and a eurystomatous (Eu) form, the latter bearing a claw-like dorsal tooth and an opposing subventral tooth. We use a model-systems approach to test the relationship between developmental plasticity and evolution at a mechanistic level. To study the genetic basis of the dimorphism, we produced Eu-form-defective (*eud*) mutants. A mutant with several alleles, *eud-1*, is haploinsufficient, and overexpression of *eud-1* resulted in saturation of

the Eu form. Extra copies of this X-linked gene also induced the Eu form in otherwise highly St males, indicating a role for *eud-1* in sexual dimorphism. *eud-1* thus acts as a dose-dependent developmental switch gene for the dimorphism. Further experiments revealed that *eud-1* is not only necessary and sufficient for the mouth-form decision in the laboratory but is also a key determinant of micro- and macroevolutionary diversification. A survey of over 100 isolates of *P. pacificus* revealed natural variation in dimorphism phenotypes. Variation correlated with *eud-1* expression, and genetic transformation of highly St strains showed the role of *eud-1* as a dimorphism switch. Use of the recently discovered sister species to *P. pacificus*, *P. exspectatus*, revealed that *eud-1* also acts as a mouth-form determinant in macroevolution. *eud-1* encodes a novel sulfatase and acts downstream of pheromone and DAF-12 signaling, suggesting that a switch controlling micro- and macroevolution of developmental plasticity can evolve by terminal addition of new genes.

72. Axons degenerate in the absence of mitochondria. **Randi Rawson**[1], Lung Yam[2], Robby Weimer[1], Eric Bend[1], Erika Hartwieg[3], H. Robert Horvitz[3], Scott Clark[2,4], Erik Jorgensen[1]. 1) Dept of Biology and HHMI, University of Utah, Salt Lake City, UT; 2) Dept of Pharmacology, Skirball Institute, NYU School of Medicine, New York; 3) Dept of Biology and HHMI, MIT, Cambridge, MA; 4) current address: Dept of Biology, University of Nevada, Reno, NV.

Many neurodegenerative disorders are associated with mitochondrial defects. However, it is still debated whether mitochondria play a destructive or protective role in axonal pathologies. Mitochondria can play an active role in neurodegeneration by releasing reactive oxygen species and apoptotic factors. Alternatively, mitochondria could be involved in neurodegeneration via the loss of a protective role, such as calcium buffering. Compromised mitochondria might be unable to sustain axons or to protect the cell from stress and insult. Recent studies manipulating mitochondria lend support to both of these models. We have found that axons die if they lack mitochondria, but survive if mitochondria are present. We have identified a C. elegans mutant, ric-7, in which mitochondria are unable to exit the neuron cell bodies. When axons lacking mitochondria are cut with a laser they rapidly degenerate. To confirm that mitochondria are protective, wild-type axons were cut into sections containing or lacking mitochondria. The majority of axon sections containing a mitochondrion survived, while those lacking mitochondria degenerated. Thus, mitochondria have a neuroprotective role and are not required for degeneration.

73. MATH-33, a conserved DUB required for DAF-16/FOXO stabilization and function. **Thomas Heimbucher**[1,4], Zheng Liu[1], Carine Bossard[1], Andrea Carrano[8], Richard McCloskey[6], Christian G. Riedel[7], Bryan R. Fonslow[5], Christian Klammt[1], Celine Riera[2], Kenneth Kemphues[6], Björn F. Lillemeier[1], John R. Yates III[5], Clodagh O'Shea[1], Tony Hunter[1], Andrew Dillin[2,3]. 1) Salk Institute for Biological Studies, Molecular and Cell Biology Laboratory, La Jolla, CA; 2) Li Ka Shing Center, Molecular and Cell Biology Department, Berkeley, CA; 3) Howard Hughes Medical Institute, Molecular and Cell Biology Department, Berkeley, CA; 4) Glenn Center for Aging Research, Molecular and Cell Biology Laboratory, La Jolla, CA; 5) The Scripps Research Institute, Department of Chemical Physiology, La Jolla, CA; 6) Cornell University, Department of Molecular Biology and Genetics, Ithaca, NY; 7) University of Gronigen, European Research Institute for the Biology of Aging, Groningen, Netherlands; 8) University of California-San Diego, Division of Biological Sciences, La Jolla, CA.

The FOXO transcription factor DAF-16, a downstream effector of Insulin/IGF-1 signaling (IIS), is a critical longevity determinant in diverse organisms. However, the molecular basis of its regulation is still largely unknown. By mass spectrometry of affinity-purified DAF-16-associated factors, we have identified the deubiquitylating enzyme MATH-33 as a co-regulator for DAF-16 activity. We show that MATH-33 stabilizes DAF-16 protein levels in *C. elegans* under conditions when IIS is downregulated and DAF-16 is transcriptionally active. In addition, MATH-33 is required for maintaining DAF-16 function to regulate early developmental decisions and life span. Disruption of *math-33* expression in *C. elegans* significantly reduces DAF-16 protein levels under low IIS conditions without affecting *daf-16* mRNA levels. Genetic ablation of both *math-33* and *rle-1*, a previously identified DAF-16 ubiquitin E3 ligase, is able to partially rescue DAF-16 activity, indicating an epistatic relationship between both factors for regulating DAF-16 levels. Finally, we demonstrate that expression of a catalytically inactive form of MATH-33 in cultured mammalian cells results in an increase of ubiquitylated DAF-16. Our data support a model in which MATH-33 acts as a deubiquitylating enzyme to stabilize DAF-16 protein levels when IIS is downregulated.

74. DAF-16(FOXO) employs the chromatin remodeller SWI/SNF to promote stress resistance and longevity. **Christian G. Riedel**[1,2,3], Robert H. Dowen[2,3], Guinevere F. Lourenco[2,3], Natalia V. Kirienko[2,3], Thomas Heimbucher[4], Jason A. West[2,3], Sarah K. Bowman[2,3], Robert E. Kingston[2,3], Andrew Dillin[4], John M. Asara[5,6], Gary Ruvkun[2,3]. 1) European Research Institute for the Biology of Ageing, University Medical Center Groningen, Groningen, Netherlands; 2) Deptartment of Molecular Biology, Massachusetts General Hospital, Boston, MA; 3) Department of Genetics, Harvard Medical School, Boston, MA; 4) Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA; 5) Division of Signal Transduction, Beth Israel Deaconess Medical Center, Boston, MA; 6) Department of Medicine, Harvard Medical School, Boston, MA.

Organisms are constantly challenged by stresses and privations and require adaptive responses for their survival. The transcription factor DAF-16(FOXO) is central nexus in these responses, but despite its importance little is known about how it regulates its target genes. Proteomic identification of DAF-16(FOXO) binding partners in Caenorhabditis elegans and their subsequent functional evaluation by RNA interference (RNAi) revealed several candidate DAF-16(FOXO) cofactors, most notably the chromatin remodeller SWI/SNF. DAF-16(FOXO) and SWI/SNF form a complex and globally colocalize at DAF-16(FOXO) target promoters. We show that specifically for gene-activation, DAF-16(FOXO) depends on SWI/SNF, facilitating SWI/SNF recruitment to target promoters, in order to activate transcription by presumed remodelling of local chromatin. For the animal, this translates into an essential role of SWI/SNF for DAF-16(FOXO)-mediated processes, i.e. dauer formation, stress resistance, and the promotion of longevity. Thus we give insight into the mechanisms of DAF-16(FOXO)-mediated transcriptional regulation and establish a critical link between ATP-dependent chromatin remodelling and lifespan regulation.

75. Life in the hot seat: Comparing aging and stress resistance. **Nicholas Stroustrup**, Zachary Nash, Javier Apfeld, Walter Fontana. Systems Biology Department, Harvard Medical School, Boston, MA.

To address the current limitations of manual survival assays with regard to throughput, statistical power, and reproducibility, we developed an automated system for the acquisition and analysis of *C. elegans* lifespan data. Our technology -- dubbed the “Lifespan Machine” - provides a standardized method for accurate measurement of lifespan, while being low-cost and scalable to any desired statistical resolution. Our lab’s installation uses an array of fifty flatbed scanners to monitor up to 30,000 individuals across 2.5 square meters of agar lawn distributed across 800 plates every fifteen minutes at 8 mm optical resolution. We have developed software that analyzes this large volume of image data to identify the death times of individual worms based

on their spontaneous movement, with a focus on subtle, late-life postural changes. Our toolset permits the rapid visual validation of automatic measurements, which constitutes a crucial step for the routine production of rigorous mortality statistics. By using standard agar Petri plates, the lifespan machine can automate a variety of assays including feeding RNAi experiments, stress- and pathogen-resistance assays, as well as compound testing.

The statistical and temporal resolution afforded by automation allowed us to take a first look at the time-dependent hazard rate of *C. elegans* populations dying across the full temperature range from 20°C to 36.5°C at fraction of degree intervals. We identified striking similarities between the hazard functions of animals dying over 14 hours at 33°C and those dying over two weeks at 25°C. This calls into question the separation between stress resistance (time to death at high temperatures) and aging (time to death at room temperature), widely considered as qualitatively distinct phenomena. Our finding that the shape of the hazard function is universal across temperature is especially surprising when considering that the observed scaling relationship (mean lifespan vs temperature) suggest that distinct physiological transitions occur as temperature increases.

76. The functional and regulatory organization of the *C. elegans* insulin-like peptide network. **D. A. Fernandes de Abreu**[*1], A. Caballero[*1], P. Fardel[2,3], N. Stroustrup[4], Z. Chen[5], K. Lee[6], W. D. Keyes[6], Z. M. Nash[4], I. F. López Moyado[4], F. Vaggi[7], A. Cornils[2], M. Regenass[2], A. Neagu[2,3], I. Ostojic[2], C. Liu[1], D. Sifoglu[8], W. Fontana[4], A. Csikasz-Nagy[7,9], C. Murphy[6], A. Antebi[+10], E. Blanc[+1], J. Apfeld[+4], Y. Zhang[+5], J. Alcedo[+2,8], Q. Ch'ng[+1] * Shared first author + Corresponding author. 1) MRC Centre for Developmental Neurobiology, King's College London, London; 2) Friedrich Miescher Institute for Biomedical Research, Basel; 3) Biozentrum, University of Basel, Basel; 4) Dept of Systems Biology, Harvard Medical School, Boston, MA; 5) Dept of Organismic and Evolutionary Biology, The Center for Brain Science, Harvard University, Cambridge, MA; 6) Lewis-Sigler Institute for Integrative Genomics and Dept of Molecular Biology, Princeton University, Princeton, NJ; 7) Research and Innovation Center, Fondazione Edmund Mach, San Michele all'Adige; 8) Dept of Biological Sciences, Wayne State University, Detroit, MI; 9) Institute for Mathematical and Molecular Biomedicine, Randall Division of Cell and Molecular Biophysics, King's College London, London; 10) Max Planck Institute for Biology of Ageing, Koeln.

C. elegans has 40 insulin-like peptide (ILPs) that regulate several processes, including longevity, dauer formation, pathogen resistance and thermotolerance. But how multiple ILPs interact to control physiology is not fully understood. To understand their function, we systematically assessed the effect of 35 different ILP deletion mutants in 8 physiological processes. Furthermore, to delineate the genetic interactions amongst ILPs, we tested the dauer entry phenotype of 53 different double mutants. We found that the ILP family has undergone functional and regulatory diversification where different ILPs regulate specific sets of processes and where each process is controlled by different combinations of ILPs, thus detailing the specifics of a graded combinatorial ILP code. We also analyzed the expression of each ILP in the 35 ILP mutants. The results revealed that the ILPs are organized into a network with specific ILP-to-ILP regulation. This ILP network has "small world" properties that are associated with efficient communication and computation. Connectivity motifs within this network suggest that the ILPs cooperate not only via compensation, expected for duplicated gene families, but also via feedback, feed-forward and cross-talk functionality.

Integrative analysis of our systematic datasets revealed network mechanisms that contribute to robustness against gene perturbations and functional differences between ILPs. This analysis also mapped out the flow of information among the ILPs in the network, indicating how information from different parts of the network is processed and integrated during dauer entry.

77. Succinylated octopamine ascarosides and a new pathway of biogenic amine metabolism in C. elegans. Alexander B. Artyukhin[1,2], Joshua J. Yim[1], Jagan Srinivasan[3], Yevgeniy Izrayelit[1], Neelanjan Bose[1], Stephan H. von Reuss[1], James M. Jordan[4], L. Ryan Baugh[4], Paul W. Sternberg[3], Leon Avery[2], **Frank C. Schroeder**[1]. 1) Boyce Thompson Inst, Cornell Univ, Ithaca, NY; 2) Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA 23298, USA; 3) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, California 91125, USA; 4) Department of Biology, Duke Center for Systems Biology, Duke University, Durham, NC 27708, USA.

The ascarosides, small-molecule signals derived from combinatorial assembly of primary metabolism-derived building blocks, play a central role in C. elegans biology as regulators of development and behavior. Using HPLC-MS/MS-based targeted metabolomics, we identified novel ascarosides incorporating a side chain derived from succinylation of the neurotransmitter octopamine. These compounds, named osas#2, osas#9, and osas#10, are produced predominantly by L1 larvae, where they serve as part of a dispersal signal, whereas octopamine ascarosides are largely absent from the metabolomes of other life stages. Investigating the biogenesis of the octopamine ascarosides, we found that succinylation represents a previously unrecognized pathway of biogenic amine metabolism. At physiological concentrations, the neurotransmitters serotonin, dopamine, and octopamine are converted to a large extent into the corresponding succinates, in addition to the previously described acetates. Chemically, bimodal deactivation of biogenic amines via acetylation and succinylation parallels posttranslational modification of proteins via acetylation and succinylation of L-lysine. Our results reveal a small-molecule connection between neurotransmitter signaling and interorganismal regulation of behavior and suggest that ascaroside biosynthesis is based in part on co-option of degradative biochemical pathways.

78. Discovering Conserved Mechanisms of Protection against Ischemia-reperfusion Injury Using a Novel *C. elegans* Behavioral Model. **Dengke K. MA**, Bob Horvitz. MIT, Cambridge, MA.

Ischemia-reperfusion-related disorders (e.g., strokes and heart attacks) are the most common causes of adult deaths. How ischemia-reperfusion causes disease and how organisms protect themselves from ischemia-reperfusion injury are fundamental and unanswered questions. The *C. elegans* gene *egl-9* defines a highly conserved family of O_2-sensing hydroxylases (EGL-9 in *C. elegans* and EGLN2 in mammals) that regulate HIF transcription factors. Exposure to chronic low levels of O_2 (hypoxic preconditioning) or direct inhibition of EGLN2 strongly protects from stroke, heart attack and ischemia-reperfusion injury in mammals.

By quantitatively measuring O_2-modulated behaviors of *C. elegans*, we discovered a robust behavior called the O2-ON response, which is characterized by a rapidly increased locomotion speed triggered by reoxygenation (20% O_2) following brief exposure of animals to anoxia (0% O_2) (Ma et al., 2012). The O2-ON response requires the EGL-9/HIF-1 pathway and models key aspects of mammalian tissue responses to ischemia-reperfusion, as (1) reoxygenation is the major pathological aspect of reperfusion, (2) hypoxic preconditioning can both suppress the O2-ON response in *C. elegans* and protect from reperfusion injury in mammals, and (3) the central regulators (EGL-9/HIF-1) of the O2-ON response and ischemia-reperfusion injury are evolutionarily

conserved. From an *egl-9* suppressor screen, we identified *cyp-13A12*, which encodes a cytochrome P450 oxygenase that acts with the EGL-9/HIF-1 pathway to facilitate the O2-ON response. A *cyp-13A12(gf)* mutation restores the defective O2-ON response of *egl-9* mutants, whereas loss of *cyp-13A12* causes a defective O2-ON response in wild-type animals. CYP-13A12 promotes oxidation of lipids into eicosanoids, inflammatory signaling molecules that in mammals can potently affect ischemia-reperfusion injury responses. We suggest that the molecular pathway from EGL-9/HIF-1 to lipid signaling in regulating the O2-ON response and ischemia-reperfusion is conserved from nematodes to mammals. Further elucidating molecular and neural mechanisms of how the O2-ON response is controlled might help identify new conserved modulators and mechanisms of hypoxia/reperfusion injury.

79. Apoptotic hyperfunction causes gonadal atrophy in aging *C. elegans*. **Yila de la Guardia**, Ann Gilliat, Josephine Hellberg, David Gems. Institute of Healthy Ageing, University College London, United Kingdom.

The mechanisms of aging remain mysterious. The many findings arguing against the oxidative damage theory have led to doubts about the central premise that aging is a function of damage and maintenance. The recently proposed hyperfunction theory suggests that aging is caused not by damage but by deleterious run-on in late life of processes that promote fitness in early life. Such *quasi-programmed hyperfunction* [1] generates hypertrophy, atrophy and dysplasia, leading to age-related pathologies which cause death. We are testing this theory in *C. elegans*, in which many dysplastic pathologies do occur during aging [2], including atrophy and disintegration of the hermaphrodite distal gonad [3]. During normal hermaphrodite reproduction, germ cells undergo apoptosis, probably to provide cytoplasm for oocyte growth. We postulated that distal gonad atrophy is caused by quasi-programmed germline apoptosis, i.e. germline apoptosis is not switched off in post-reproductive worms, and so eats away the gonad. Consistent with this, blocking apoptosis by mutation of *ced-3* suppressed atrophy. Moreover, in males, where germline apoptosis does not occur, gonad atrophy does not occur either. A range of other genetic tests also supported our model, e.g. *gld-1(op236)*, which increases germline apoptosis, accelerated gonad atrophy, and *ced-9(n1950)*, which suppresses somatic but not germline apoptosis, did not suppress gonad atrophy. By contrast, treatments that increase damage levels (e.g. extra iron or mutation of *sod-2*) did not accelerate gonad atrophy, nor did mutation of *spo-11*, which blocks formation of DNA double-strand breaks during meiosis, have any effect on gonad atrophy. These findings provide a mechanistic explanation for a major pathology of aging in *C. elegans*, and its sex specificity: not molecular damage accumulation, but hyperfunction in the form of sex-limited quasi-programmed germline apoptosis. This supports a radical reinterpretation of the nature of aging in *C. elegans.* 1. Blagosklonny, *Cell Cycle* **7**: 3344 (2008). 2. Gems, de la Guardia, *Antiox. Redox Signal*. Sep 24. [Epub ahead of print] (2012). 3. Garigan et al., *Genetics* **3**: 1101 (2002).

80. Molecular Outsourcing: Reproductive Signals Deploy NHR-49/PPARa to Reorganize Lipid Homeostasis and Alter Lifespan. **Ramesh Ratnappan**[1], Jordan Ward[2], Francis RG Amrit[1], Hasreet Gill[1], Kyle Holden[1], Keith Yamamoto[2], Arjumand Ghazi[1]. 1) Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Department of Cellular and Molecular Pharmacology, University of California, San Francisco.

Aging is an inherently entropic but remarkably plastic phenomenon. The rate of aging can be altered by modifications to an energy-intensive process such as reproduction. In worms, removal of the Germline-Stem Cells (GSCs) results in increased fat accumulation and the activation of a transcriptional network in intestinal cells that extends lifespan. Two key regulators in this network are DAF-16/FOXO and TCER-1/TCERG1. In a screen designed to identify nuclear hormone receptors (NHRs) that impact DAF-16/FOXO and TCER-1/TCERG1 function, one of the NHRs we isolated was NHR-49/PPARa, a conserved and crucial modulator of energy and fat metabolism. Independently, we also identified *nhr-49* as a gene jointly upregulated by DAF-16/FOXO and TCER-1/TCERG1 through RNA-Seq transcriptomics. Predictably, *nhr-49* mutation suppressed the longevity of GSC(-) worms, but did not appear to change the overall elevated fat content. A GFP-tagged translational reporter showed expression in the cytoplasm and nuclei of all somatic cells and rescued *nhr-49(-)* phenotypes. NHR-49/PPARa overexpression increased the lifespan of *nhr-49* mutants substantially without a concomitant loss of fertility. NHR-49/PPARa regulated the expression of fatty-acid desaturases, specific mitochondrial b-oxidation pathway genes and acted in a feedback loop to increase DAF-16/FOXO and TCER-1/TCERG1 activity. At least part of these transcriptional changes may be brought about in co-operation with NHR-71, another NHR identified in our screen that interacted with both NHR-49/PPARa and TCER-1/TCERG1 in yeast two-hybrid experiments. Our results suggest that upon GSC loss, NHR-49/PPARa is recruited to fundamentally remodel the lipid metabolic profile of the worm. This reorganization not only allows the mobilization of fat that would otherwise have been transported to the oocytes, but also induces transcriptional and signaling events that direct the allotment of cellular resources towards somatic maintenance and longevity.

81. Serotonergic signaling modulates the heat shock response in *C. elegans*. **Veena Prahlad**[1], Richard Morimoto[2]. 1) Department of Biology, University of Iowa, Iowa City, IA; 2) Department of Molecular Biosciences, Northwestern University, Evanston, IL.

The mechanisms that allow for stable physiology, despite the temperature sensitivity of metabolic reactions, are poorly understood. Many organisms, like *C. elegans* possess neurosensory circuits dedicated to seeking out optimal temperatures. In addition cells within the animal possess conserved mechanisms such as the heat shock response (HSR) mediated by the transcription factor HSF-1, to maintain protein homeostasis despite temperature fluctuations. We discovered that in *C. elegans*, circuits formed by thermosensory AFD neurons that control behavioral responses to temperature change also control the activation of HSF-1 within all cells throughout the organism, linking sensation of temperature fluctuations to the regulation of metabolic homeostasis. Here we present evidence that the thermosensory control of HSF-1 may occur through the modulation of serotonin signaling by the AFD neurons. Specifically, we show that acute heat shock results in a change in serotonin localization, consistent with its release from the NSM neurons, within minutes after temperature increase. This is concomitant with activation of HSF-1 visualized by changes in its nuclear localization. Serotonergic signaling is necessary for HSF-1 activation and the subsequent induction of the protective heat shock proteins (HSPs): loss of tryptophan hydroxylase or the serotonergic receptors (*ser-1/ser-4*) prevents HSP induction upon heat shock. In animals harboring loss-of-function mutations in the guanylyl cyclase *gcy-8/23* genes required for AFD neuronal response to temperature, serotonin is not released from NSM neurons and HSF1 is not activated after heat shock. HSF-1 activation can be rescued in these thermosensory mutants by the delivery of exogenous serotonin. We are currently investigating how the AFD neurons affect serotonin release by the NSM neurons, and how serotonin influences HSF-1 activity. Serotonergic signaling regulates core body temperature and energy metabolism in mammals. We propose that neuronal control of HSF-1 through serotonin signaling is a conserved mechanism that allows multicellular organisms to adapt to their environment by linking their sensory response to temperature fluctuations with their metabolic state.

82. The conserved SKN-1/Nrf2 stress response pathway regulates synaptic function in *Caenorhabditis elegans*. **Trisha Staab**, Trevor Griffen, Connor Corcoran, Oleg Evgrafov, James Knowles, Derek Sieburth. Univ Southern California, Los Angeles, CA.

The Nrf family of transcription factors plays a critical role in mediating adaptive responses to cellular stress and defends against neurodegeneration, aging and cancer. We report a novel role for the *Caenorhabditis elegans* Nrf homolog SKN-1 in regulating synaptic transmission at neuromuscular junctions (NMJs). Activation of SKN-1, either by acute pharmacological treatment with the toxin sodium arsenite or by mutations that cause constitutive SKN-1 activation, results in defects in neuromuscular function. Additionally, elimination of the conserved WD40 repeat protein WDR-23, a principal negative regulator of SKN-1, results in impaired locomotion and synaptic vesicle and neuropeptide release from cholinergic motor axons. Mutations that abolish *skn-1* activity restore normal neuromuscular function to *wdr-23* mutants and animals treated with toxin. We show that negative regulation of SKN-1 by WDR-23 in the intestine, but not at neuromuscular junctions, is necessary and sufficient for proper neuromuscular function. WDR-23 isoforms differentially localize to the outer membranes of mitochondria and to nuclei, and the effects of WDR-23 on neuromuscular function are dependent on its interaction with cullin E3 ubiquitin ligase. Finally, whole transcriptome RNA sequencing of *wdr-23* mutants reveals an increase in the expression of known SKN-1/Nrf2-regulated stress-response genes, as well as neurotransmission genes not previously implicated in SKN-1/Nrf2 responses. Together, our results indicate that SKN-1/Nrf2 activation may be a mechanism through which cellular stress, detected in one tissue, affects cellular function of a distal tissue through endocrine signaling. These results provide insight into how SKN-1/Nrf2 might protect the nervous system from damage in response to oxidative stress.

83. Worms That Exercise Age Better. Daniel Burke, **Mary Anne Royal**, Leo Gefter, Christina Chang, Monica Driscoll. Molecular Biology & Biochemistry, Rutgers University, Piscataway, NJ.

Exercise has been identified as a powerful maintenance promoter, with anti-cancer, anti-diabetes, anti-sarcopenia, anti-cognitive decline, and possibly pro-immune consequences in humans. Nonetheless, the molecular, cellular, and systems-wide changes by which exercise extends healthspan remain poorly understood, limiting exploitation of molecular exercise pathways for therapeutic application. We have developed a swim training regimen for the nematode Caenorhabditis elegans. Adult and juvenile worms “exercised” by swimming in M9 on successive days exhibit an exercise benefit, as measured by CELEST swimming analysis software developed by Dr. C. Restif together with the Driscoll lab. We show that like in mammals, exercise can increase physical performance after repeat training, exercise benefits diminish if training is stopped, and too much exercise is deleterious. Importantly, two independent mutants for aak-2, a conserved subunit of an AMP kinase homologue that acts upstream of the transcriptional activator PGC-1a to increase mitochondrial density in response to physical activity in higher organisms, do not gain a training benefit, even though they swim train like wild type worms. We have also found that increased mitochondrial biogenesis is associated with improved swimming prowess. Our data suggest that molecular mechanisms by which C. elegans gain an exercise benefit may be conserved from nematodes to humans. Interestingly, animals that exercise exhibit some systemic health benefits such as extended period of pharyngeal pumping and longer median lifespans. In sum, our results suggest that C. elegans can be used as a model system for elucidating the molecular, cellular, and systems-wide benefits that occur as a consequence of exercise.

84. Perturbations of Glycolytic Flux Differentially Impact Healthspan via the Insulin Signaling and Dietary Restriction Pathways. **Brian Onken**, Monica Driscoll. Rutgers, The State University of New Jersey, Piscataway, NJ.

In *Caenorhabditis elegans*, adding excess glucose to the growth medium shortens lifespan [1, 2, 3], while inhibiting the glycolytic enzyme hexokinase with the glucose analog 2-deoxyglucose increases lifespan [1]. We have shown that disrupting genes encoding two other glycolytic enzymes that catalyze unidirectional, irreversible reactions in glycolysis lengthens *C. elegans* median lifespan, induces large gains in youthful locomotory ability, and triggers a fluorescent biomarker that distinguishes a healthy metabolic state. Conversely, disrupting counterpart unidirectional gluconeogenic genes *decreases* nematode healthspan. In investigating potential longevity-related pathways that might impinge upon glucose metabolism, we found that disrupting glycolytic genes increases healthspan through the FOXO transcription factor DAF-16, which is also required for the increased lifespan seen with lowered levels of insulin signaling, and which is downregulated by increased glucose availability [2]. Strikingly, we also found that gluconeogenic activity is specifically required for increased healthspan under dietary restriction, and that the SKN-1 transcription factor, which is required for the beneficial effects of dietary restriction [4], is also needed for the healthspan effects seen with decreased gluconeogenesis. In addition, we found that a transcriptional reporter for gluconeogenic gene *pck-2* is induced by several dietary restriction regimens in a SKN-1-dependent manner. These results provide evidence for an intriguing new paradigm: breakdown of glucose via glycolysis negatively impacts healthy aging through insulin signaling and DAF-16, while dietary restriction engages the reciprocal gluconeogenic pathway to promote healthspan via SKN-1. Our observations support that healthspan might be optimized via dietary, pharmacological, or genetic interventions that increase gluconeogenic activity or decrease glycolysis. 1. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, et al. (2007). Cell Metab 6: 280-293. 2. Lee SJ, Murphy CT, Kenyon C (2009). Cell Metab 10: 379-391. 3. Schlotterer A, et al. (2009). Diabetes 58: 2450-2456. 4. Bishop NA, Guarente L (2007). Nature 447: 545-549.

85. Regulation of *C. elegans* Reproductive Aging by a Novel Gene-Environment Signaling Mechanism. **Jessica Sowa**[1,2], Meng Wang[1,2]. 1) Dept. of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX; 2) Huffington Center on Aging, Baylor College of Medincine, Houston ,TX.

Reproductive senescence is a hallmark of aging, the onset of which can be modulated by both genetic and environmental factors. However, the molecular mechanisms that integrate environmental and genetic signals to regulate the onset and progression of reproductive aging remain largely unknown. Here we report the first known instance of a gene-environment signaling mechanism functioning to regulate reproductive senescence in *Caenorhabditis elegans*. We found that *C. elegans* fed the standard lab diet of OP50 *E. coli* reproduce significantly longer than *C. elegans* fed the alternate diet of HB101 *E. coli*. This effect is mediated by the AWB olfactory neurons, which perceive a volatile odorant signal produced by HB101 *E. coli*. The presence or absence of this sensory cue specifically affects germline proliferation and maintenance, ultimately contributing to the timing of reproductive senescence. The effect of the HB101 odorant on reproductive span was found to be independent of all previously identified pathways for the regulation of reproductive aging in *C. elegans*, indicating that it acts through a novel regulatory mechanism. Furthermore, we have found that serotonin signaling plays a role as a downstream effector of this response. Together, these studies describe a previously unknown regulatory mechanism for reproductive

senescence, and suggest the significance of gene-environment interactions in the regulation of reproductive aging.

86. Molecular determinants of longevity in *C. elegans* and the relationship between lifespan, "health span", and the rate of aging. **Zachary Pincus**, Frank Slack. Molecular, Cellular, & Developmental Biology, Yale University, New Haven, CT.

Lifespans are surprisingly variable across individuals of the same species, even in genetically identical animals reared in identical environments. To determine the mechanisms of such inter-individual variability, we used a novel single-animal culture system to identify predictive markers of future longevity in individual *C. elegans*. As proof of concept, we found various molecular and physiological predictors of lifespan, such as a buildup of autofluorescent species and decreases in movement rates (respectively). We further identified several microRNAs that determine a substantial amount of inter-individual variation in lifespan early in adult life, in part by modulating well-known "aging pathways" including insulin signaling.

Next, we sought to quantitatively characterize the process of senescent decline in long-lived versus short-lived animals using these markers. Surprisingly, we found that longer-lived animals do not stay "healthier" (as measured by our multivariate markers) for a larger fraction of their lives than short-lived animals. That is, lifespan appears uncoupled from relative health span (the fraction of life before senescent decline); further, we find that longer-lived animals do not appear to have additional molecular or physiological endowments. Instead, long-lived animals age along a health trajectory similar to that of short-lived animals, but at a slower pace. This suggests that differences in the rate of aging, rather than qualitative differences in individual biology, drive lifespan variability.

87. The conserved PBAF nucleosome remodeling complex mediates the response to stress in C. elegans. Aleksandra Kuzmanov, Evguenia Karina, Natalia Kirienko, **David Fay**. Dept Molec Biol, Univ Wyoming, Laramie, WY.

The ability to respond rapidly to environmental challenges ensures proper development, function and survival of an organism. To adapt to stress, cells undergo major changes in gene expression profiles. We have previously identified a largely uncharacterized stress-response pathway in C. elegans that acts through an evolutionarily conserved motif termed ESRE for ethanol and stress-response element. The ESRE pathway regulates the expression of hundreds of genes under a variety of stress conditions. The network includes SLR-2, a C2H2 zinc finger protein, and its downstream target JMJC-1, a jumonji-C domain-containing protein that functions as a histone demethylase. We have undertaken a detailed genetic and biochemical characterization of the ESRE-response pathway. This has led us to the discovery of a conserved chromatin remodeling factor that mediates stress response through the ESRE termed PBAF. PBAF, along with the related BAF complex, belongs to the SWI/SNF family of chromatin remodeling complexes. Although these two complexes share 6 subunits in C. elegans, we have shown that only PBAF is necessary to facilitate expression through the ESRE following stress. Depletion of PBAF subunits results in decreased transcription of ESRE genes and increased sensitivity to thermal stress. When overexpressed, SWSN-7 and PBRM-1 lead to induction of ESRE transcription and enhanced stress resistance. ESRE binding activity, almost absent under non-stressed conditions is readily detectable by gel mobility shift assays in strains overexpressing these two PBAF subunits. Our findings have uncovered a previously unrecognized function of the PBAF chromatin remodeling complex and to our knowledge is the first report of SWI/SNF complexes mediating an acute stress response in vivo in metazoa.

88. The tune of Insulin/IGF-1 signaling pathway set by lincRNAs. Pengpeng Liu, Min Liu, Li Zhang, Zhenglin Yang, Kai Xiong, Wei Dong, Wenxia Zhang, Zuoyan Zhu, Qichang Fan, **DONG LIU**. Peking University, Beijing, China.

The long non-coding RNAs transcribed from the intergenic regions, also named lincRNAs, remain mysterious, although a few in vitro studies implied that lincRNAs modulate target genes through modifying the chromatin. We have developed a strategy to screen and characterize worm lincRNAs, to learn their biological functions in vivo. We report here that four tissue/cell specific lincRNAs participate in the Insulin/IGF-1 signaling (IIS) pathway regulation, and two of them each regulate a conserved protein-coding gene in their neighborhood. Unexpectedly, these lincRNA-regulated protein-coding genes are also modulators of IIS pathway. Our study unveils the functions of lincRNAs in a multi-cell organism, and how a complex regulatory pathway is controlled by interplay of tissue/cell specific lincRNAs and their neighboring genes.

89. Mitochondrial ROS promote longevity and innate immunity via a feedback loop involving HIF-1 and AMPK. **Ara B. Hwang**[1,3], Eun-A Ryu[1,3], Murat Artan[1], William Mair[2], Seung-Jae Lee[1]. 1) Department of Life sciences/ IBIO/ and WCU ITCE, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Korea; 2) Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts 02115, USA; 3) These authors contributed equally.

Mild inhibition of mitochondrial respiration promotes longevity across phyla. Recently we reported that reduced respiration lengthens lifespan by increasing reactive oxygen species (ROS) that activate the hypoxia-inducible factor 1 (HIF-1) in *C. elegans*. Here we elucidated a feedback-regulatory mechanism and functional significance of the ROS-induced longevity. We initially found that mutations in *aak-2* (catalytic a subunit of AMP-activated protein kinase [AMPK]) suppressed the longevity induced by an ROS-generating chemical, paraquat (0.25 mM). In addition, we showed that mitochondrial ROS activated AMPK, and that paraquat did not further increase the longevity of gain-of-function AAK-2 transgenic animals. These data suggest that AMPK mediates the longevity induced by mitochondrial ROS. We then investigated the mechanisms by which AMPK contributes to the ROS-induced longevity. We demonstrated that AMPK acts as a negative feedback regulator of internal ROS levels, because *aak-2* mutants were hyper-sensitive to ROS and contained increased ROS levels upon paraquat treatment. In contrast, HIF-1 was required for increasing internal ROS levels, suggesting that HIF-1 acts as a positive regulator of ROS. These data imply that feedback regulation by AMPK and HIF-1 ensures balance between the benefit and toxicity of ROS. Next, we determined the relationship between the ROS-induced longevity and innate immunity, because infection by *E. coli* is the major cause of death of aged *C. elegans* in laboratory. We found that feeding non-pathogenic bacteria increased the lifespan of wild type but not that of the long-lived mitochondrial *isp-1* mutants, which display high levels of ROS. Moreover, *isp-1* mutants were resistant to several pathogens, and both *aak-2* and *hif-1* were required for the enhanced pathogen resistance. Taken together, we propose that the feedback circuit involving AMPK and HIF-1 regulates ROS levels to maintain an optimal immunity and longevity.

90. How to live without water: Molecular strategies of the dauer larva to survive extreme desiccation. **Cihan Erkut[1]**, Sider Penkov[1], Sebastian Boland[1], Andrej Vasilj[1], Hassan Khesbak[2], Daniela Vorkel[1], Bianca Habermann[3], Jean-Marc Verbavatz[1], Karim Fahmy[2], Andrej Shevchenko[1], Teymuras V Kurzchalia[1,4]. 1) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 2) Helmholtz-Zentrum Dresden-Rossendorf, Dresden, Germany; 3) Max Planck Institute for Biology of Ageing, Cologne, Germany; 4) Tbilisi Institute of Metabolic Genetics, Tbilisi, Georgia.

Terrestrial animals are almost always challenged by severe desiccation. However, many species have evolved ways to survive this by transiting into an ametabolic state known as anhydrobiosis (life without water). Although known for centuries, the molecular mechanisms underlying anhydrobiosis remained poorly understood because of the lack of a good genetic model. Recently, we showed that the *Caenorhabditis elegans* dauer is an anhydrobiote. It can survive losing almost its entire body water provided that it is first preconditioned at a mild desiccative environment. We showed that during this preparation, worms accumulate a large amount of the disaccharide trehalose. Trehalose-deficient mutants have a dramatically reduced desiccation tolerance because of extensive damage to plasma membranes and membrane-bound organelles. However, it is very unlikely that trehalose is the only factor involved in anhydrobiosis. In search for others, we surveyed the desiccation-induced changes in the transcriptome and proteome of the worm, which revealed that the desiccation response of *C. elegans* is focused and involves a small number of functional pathways. Mutants of genes in these pathways most of the time exhibited reduced desiccation tolerance. Some of these pathways have been implicated in drought resistance in plants and animals (e.g. ROS and xenobiotic detoxification, heat-shock response and intrinsically disordered protein expression) and some others have not been associated with anhydrobiosis before (e.g. fatty acid desaturation and polyamine biosynthesis). Our data also suggest that sensing the decrease of ambient humidity (hygrosensation) can be associated to the head neurons. A thorough understanding of the anhydrobiotic ability of the worm can shed light on the fundamental properties of metabolism as well as the material properties of the cell.

91. Heterochromatin organization through development: regulated anchorage by H3K9 methylation and a novel chromodomain protein. **Susan M. Gasser**, Benjamin Towbin, Adriana Gonzalez, Peter Zeller, Véronique Kalck. Mechanisms of Cancer, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.

Heterochromatin comes in several forms and becomes the dominant form of chromatin during terminal differentiation. At least one class of heterochromatin is positioned adjacent to the nuclear lamina. We have created a system in which we can track gene position in developing C. elegans by live fluorescence microscopy. We have found that in differentiated cells, developmentally regulated promoters are at the nuclear periphery when repressed, and shift inwards when active. In early embryonic cells gene positions are not fixed. Using an in vivo model of fluorescently tagged heterochromatin, we have screened for factors that are necessary for anchoring heterochromatin to the nuclear lamina. We find that peripheral anchoring is a direct consequence of sequential methylation reactions by two enzymes that modify histone H3 K9. Mono- and di-methylation of H3K9 mediates anchoring, while silencing of the array requires H3K9 trimethylation. A further screen has identified a novel chromodomain protein that mediates perinuclear anchorage by binding H3K9me, to link chromatin bearing this mark to the nuclear envelope. The physiological effects of disrupting the spatial organization of chromatin through loss of the anchoring machinery will be presented, as well as an RNAi screen for factors that are necessary for survival and differentiation in the absence of heterochromatin, but not in its presence. This reveals fundamental insights into the importance of heterochromatin in development.

92. Identification of small RNA pathway genes using patterns of phylogenetic conservation and divergence. **Yuval Tabach[1,2]**, Allison Billi[3], Gabriel Hayes[1,2], Martin Newman[1,2], Or Zuk[4], Harrison Gabel[1,2], Ravi Kamath[1,2], Brab Chapman[1], Susana Garcia[1,2], Mark Borowsky[1,2], John Kim[3], Gary Ruvkun[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, Massachusetts 02114, USA; 2) Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA; 3) Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109, USA; 4) Broad Institute.

Genetic and biochemical analyses of RNA interference (RNAi) and microRNA (miRNA) pathways have revealed proteins such as Argonaute and Dicer as essential cofactors that process and present small RNAs to their targets. Well-validated small RNA pathway cofactors such as these show distinctive patterns of conservation or divergence in particular animal, plant, fungal and protist species. We compared 86 divergent eukaryotic genome sequences to discern sets of proteins that show similar phylogenetic profiles with known small RNA cofactors. A large set of additional candidate small RNA cofactors have emerged from functional genomic screens for defects in miRNA- or short interfering RNA (siRNA)-mediated repression in Caenorhabditis elegans and Drosophila melanogaster, and from proteomic analyses of proteins co-purifying with validated small RNA pathway proteins. The phylogenetic profiles of many of these candidate small RNA pathway proteins are similar to those of known small RNA cofactor proteins. We used a Bayesian approach to integrate the phylogenetic profile analysis with predictions from diverse transcriptional coregulation and proteome interaction data sets to assign a probability for each protein for a role in a small RNA pathway. Testing high-confidence candidates from this analysis for defects in RNAi silencing, we found that about one-half of the predicted small RNA cofactors are required for RNAi silencing. Many of the newly identified small RNA pathway proteins are orthologues of proteins implicated in RNA splicing. In support of a deep connection between the mechanism of RNA splicing and small-RNA-mediated gene silencing, the presence of the Argonaute proteins and other small RNA components in the many species analysed strongly correlates with the number of introns in those species.

93. The CSR-1 22G-RNA pathway modulates histone H3 modifications associated with euchromatin. **Christopher J. Wedeles**, Julie M. Claycomb. Department of Molecular Genetics, University of Toronto, 1 Kings College Circle, Toronto ON. M5S 1A8.

CSR-1 (Chromosome Segregation and RNAi Deficient) is an Argonaute that localizes to the nucleus and associates with a subset of small RNAs, called 22G-RNAs. CSR-1 coupled small-RNAs are antisense to ~4200 germline expressed protein coding genes distributed along the length of each chromosome and target genomic loci which are adjacent to genomic regions enriched for the centromeric histone H3 variant, CENP-A. It was previously shown that CSR-1 associates with chromatin at its targeted genomic loci and loss of CSR-1 leads to the disorganization of a number of factors required for kinetochore function including CENP-A. Thus, we have hypothesized that the CSR-1 pathway is required for proper chromosome segregation and kinetochore assembly, however the mechanisms through which this occurs remain unclear. To deepen our understanding of the CSR-1 pathway in the nucleus, we are investigating the mechanisms through which CSR-1 influences chromatin at its targeted genomic loci in the germline. We have characterized the

composition of histone modifications at CSR-1 targeted genomic loci and show that reduced CSR-1 activity results in aberrant accumulation of particular histone H3 modifications at these loci. Although a recent report asserted that CSR-1 plays a role in promoting histone mRNA maturation, and that loss of CSR-1 leads to a depletion of histone proteins, we present evidence that that CSR-1 is also required for limiting the extent of particular histone modifications on a global scale. Previous data demonstrated that CSR-1 has the ability to target over 80% of all germline expressed transcripts in a small-RNA dependent manner, and we have obtained evidence supporting a model whereby germline transcription is sufficient to recruit CSR-1 to transcribed genomic loci. We are currently investigating how manipulating the recruitment of CSR-1 to transcribed genomic loci influences RNA Polymerase II activity and the distribution of histone modifications at these sites. Although most small RNA pathways that modulate chromatin have been implicated in the formation of heterochromatin, our results provide a novel role for the CSR-1 small-RNA pathway in regulating non-repressive histone modifications at its targeted loci.

94. Positive regulation of Pol II transcription by CSR-1 RNAi pathway in *C. elegans*. **Germano Cecere**[1], Ravi Sachidanandam[2], Sebastian Hoersh[3], Alla Grishok[1]. 1) Dept Biochem, Columbia Univ, New York, NY; 2) Dept Genetics and Genomic Sciences, Mount Sinai, New York, NY; 3) Koch Institute, MIT, Cambridge, MA.

Argonaute proteins and their small RNA co-factors are known to inhibit gene expression by a variety of mechanisms, which include inhibition of mRNA translation, mRNA or pre-mRNA degradation, and inhibition of transcription by promoting heterochromatin assembly. However, neither RNA-RNA interaction nor Argonaute-mediated cleavage excludes the possibility that small RNAs may have a positive effect on gene expression. There are two major endo-siRNA pathways in *C. elegans*: 1) The WAGO pathway, which silences pseudogenes, transposons, cryptic loci, and some protein-coding genes, and 2) The CSR-1 pathway, which mainly targets germline-enriched protein-coding genes (Gu et al., 2009). Interestingly, the Argonaute CSR-1 was shown to associate with chromatin in a siRNA-dependent manner suggesting its nuclear role (Claycomb et al., 2009). We have reported recently that the CSR-1 pathway positively regulates histone biogenesis and that depletion of core histone proteins in the CSR-1 pathway mutants significantly contributes to their lethality (Avgousti et al, 2012). Therefore, an intriguing possibility is that CSR-1 also positively regulates other target genes in the nucleus. In order to investigate the global impact of the CSR-1 pathway on transcription we used Global Run-On sequencing (GRO-seq) to map the precise position, amount, and orientation of the transcriptionally engaged Pol II in wild type and RNAi mutants. We used a loss-of-function mutant of Dicer-related helicase, *drh-3*, which is depleted of most endo-siRNAs and a partially rescued *csr-1(tm892)* strain. We found strikingly similar changes in Pol II transcription profiles in the RNAi mutants compared to wild type suggesting that CSR-1 affects Pol II transcription in a siRNA-dependent manner. Importantly, histone genes and most other CSR-1 target genes are positively regulated by CSR-1 at the transcriptional level in the germline. Therefore, endo-siRNAs may represent an important epigenetic mechanism that globally controls Pol II transcription during development.

95. Characterization of the AGO protein VSR-1 in small RNA-mediated gene silencing pathways in the worm. **Monica Z. Wu**, Julie M. Claycomb. Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Argonautes (AGOs) are the key effector components of RNA interference (RNAi) and related endogenous small RNA pathways. *C. elegans* possesses 26 AGO family proteins and although deletion mutant strains for each of the *C. elegans* AGOs have been generated, the functions of only a handful of these proteins are understood. The AGOs that have been characterized thus far have been shown to perform distinct functions. Therefore, further investigation of the remaining AGOs can lead to novel insights into small RNA mediated gene-silencing functions. We have been investigating the role that a relatively uncharacterized, but well-conserved AGO protein, which we have named VSR-1 (Versatile Small RNAs), plays in small RNA-mediated gene regulation in *C. elegans*. mRNA and protein expression analysis demonstrates that VSR-1 is expressed throughout development, but is enriched in later stages when the germline develops, implicating VSR-1 in germline small RNA functions. Immunolocalization studies show that VSR-1 localizes to embryonic P granules, consistent with localization patterns of other Argonautes, including WAGO-1 and CSR-1. VSR-1 also localizes to oocyte and embryonic chromatin, which has been confirmed through biochemical fractionation experiments, suggesting a role for this AGO in transcriptional regulation or other nuclear functions. To identify with which small RNAs VSR-1 interacts, we have cloned and Illumina sequenced small RNAs in both the *vsr-1* mutant background and from VSR-1 complexes. Consistent with VSR-1 playing a role in the biogenesis of particular small RNAs, the small RNAs enriched in VSR-1 complexes are depleted in the mutant. Our initial analyses indicate that VSR-1 associates with particular subsets of small RNAs spanning multiple classes. These small RNAs have been implicated in playing key roles in germline and embryonic development. These findings are exciting and point to a novel activity for VSR-1 in multiple small RNA pathways, as all other AGOs studied thus far have been shown to associate with one particular class of small RNAs in the worm. Our ongoing studies will reveal additional insights into the roles of VSR-1 in worm development.

96. Involvement of *C. elegans let-7-Family* developmental timing microRNAs in bacterial pathogen response. **Zhiji Ren**, Victor Ambros. Molecular Med, Univ Mass Medical School, Worcester, MA.

C. elegans let-7 family (*let-7-Fam*) microRNAs (*let-7*, *mir-48*, *mir-241* and *mir-84*) function semi-redundantly to regulate the developmental timing of stage-specific events in hypodermal seam cell lineages. We observed that upon infection of *C. elegans* larvae with human opportunistic pathogen *Pseudomonas aeruginosa* (*P. aeruginosa*), the developmental timing defects of certain *let-7-Fam* mutants are enhanced. This enhancement is mediated by the p38 MAPK innate immunity pathway, though interestingly the upstream TIR domain adaptor protein TIR-1 seems to be bypassed. The enhancement of *let-7-Fam* developmental timing defects by *P. aeruginosa* infection implies that the activity of one or more *let-7-Fam* microRNAs may be decreased in response to *P. aeruginosa*, perhaps to enhance the animal's antibacterial response. Consistent with this supposition, we observed that *let-7(mg279)* animals exhibit an improved survival against *P. aeruginosa* infection. Interestingly, *let-7(mg279)* animals are more resistant to *P. aeruginosa* even though they accumulate pathogen in their intestine more rapidly than wild type. *mir-48(0)*; *mir-84(0)* and *mir-48(0)*, *mir-241(0)* double mutants display a complex, two-phase survival curve on *P. aeruginosa*, suggesting possible positive and negative roles of these *let-7-Fam* microRNAs in pathogen resistance. Upon *P. aeruginosa* infection, we observed slight decreases in the levels of certain *let-7-Fam* microRNAs and an increase in LIN-28 protein level. Consistent with a possible role for *lin-28* in modulating pathogen response via *let-7*, we observed that *lin-28(lf)* animals exhibit an hypersensitivity to *P. aeruginosa*, and this hypersensitivity can be suppressed by *let-7(0)*. This suggests that post-transcriptional regulation by LIN-28, as well as transcriptional regulation by p38

MAPK responsive factors, may contribute to a down-regulation of *let-7-Fam* microRNA activity in response to *P. aeruginosa* infection. By exploring these dual functions of *let-7-Fam* microRNAs in pathogen response as well as developmental timing, we aim to achieve a better understanding of how these microRNAs are integrated into broader gene regulatory networks that confer robustness to developmental events against environmental stresses.

97. *In vivo* quantitative analysis of the heterochronic pathway reveals extensive target specificity of individual *let-7* miRNA family members. **Matyas Ecsedi**, Helge Grosshans. Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland.

During larval development, timing of cell proliferation and differentiation events in the hypodermis and vulva is accomplished by the heterochronic pathway, which comprises microRNAs (miRNAs) and their protein coding target genes. Despite extensive genetic characterization of the pathway, the in vivo spatio-temporal repression patterns created by miRNA activity have not been determined. As 3'UTRs of miRNA targets typically harbor binding sites for several distinct miRNAs, it is not clear how individual miRNAs, in particular members of a given miRNA family, contribute to target repression. Here, we have used target repression by the *let-7* and *lin-4* miRNA families as a paradigm to test miRNA target regulation in vivo. To this end, we have developed a quantitative imaging assay that examines the effects of endogenous miRNAs on single copy integrated reporter transgenes expressed in various tissues at defined, constitutively low (physiological) levels. By examining a number of 3'UTR reporters including *lin-41*, *daf-12*, *hbl-1*, and *lin-28*, we observe that distinct miRNA targets are regulated in highly specific manners regarding extent, timing, and tissue site of action. Systematic analysis of reporters in various mutants lacking *let-7* family members or the *lin-4* miRNA indicate that these repression patterns are explained on the one hand by co-regulation of individual targets by more than one miRNA and on the other hand by specificity among members of a miRNA family. In particular, we find that *let-7* and its sisters act in a strikingly non-redundant manner. This specificity is a function of target site sequence, specifically of base pairing in the non-seed region, which we demonstrate by reengineering a target of *let-7 proper* to be repressible by distinct family members. In sum, our findings provide novel insight into the regulatory relationships in the heterochronic pathway and reveal some unexpected new developmental functions of miRNAs, e.g. in the intestine and vulva. Our work further demonstrates how quantitative analysis of *C.elegans* development can be used to define the rules of miRNA target specificity *in vivo*.

98. MUT-14 and SMUT-1 are redundantly required for germline RNAi and endogenous siRNA production. **Carolyn M. Phillips**[1,2], Taiowa A. Montgomery[1,2], Peter C. Breen[1,2], Gary Ruvkun[1,2]. 1) Molecular Biology Dept, Massachusetts General Hospital, Boston, MA; 2) Genetics Dept, Harvard Medical School, Boston, MA.

Defects in *mutator*-class genes have active transposons, loss of endogenous siRNAs, and do not respond to exogenous dsRNA. We have characterized several factors in this pathway by cytological analysis and found that the *mutator* proteins localize to foci at the periphery of germline nuclei, near to, but not overlapping with P granules and nuclear pores. MUT-16, a Q/N-rich protein, is required for the integrity of the *Mutator* complex by cytology and immunoprecipitation. Currently, we are focusing on the role of another *mutator*-class gene, *mut-14*, and its paralog Y38A10A.6/*smut-1* (*Synthetic MUTator-1*), both of which are DEAD box helicases. The only available allele of *mut-14, pk738*, is a point mutation in the DEAD motif. Using this strain, we demonstrate that *mut-14* is required for the production of germline endogenous siRNAs (WAGO-class 22G and ERGO-class 26G) and is germline RNAi defective (Rde). However, we generated a deletion allele, *mut-14(mg464)*, and unlike *pk738*, *mg464* has only mild effects on endogenous siRNA levels and no Rde phenotype. In a subsequent RNAi screen for defects in endogenous siRNA formation and activity, we identified eight DEAD box helicases, including the *mut-14* paralog *smut-1*. Like the *mut-14* deletion, a *smut-1* deletion has only very mild effects on endogenous siRNA levels and no Rde phenotype, but a *mut-14 smut-1* double mutant resembles the *pk738*/DEAD mutant, suggesting that *pk738* may act dominantly, inhibiting *smut-1* function. Neither single mutant, nor the double mutant is required for somatic RNAi, suggesting a role for these DEAD box helicases specifically in the germline. Interestingly, *mut-14(pk738)* causes hyper-trimming and tailing of siRNAs, although both wild type MUT-14 and MUT-14(pk738) localize to Mutator foci and trimming and tailing is thought to occur in P granules. We are currently investigating a model where MUT-14 and SMUT-1 transport RNAs from P granules to *Mutator* foci to initiation siRNA amplification.

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99. The essential CHORD protein CHP-1 functions in small RNA pathways in *C. elegans*. **Wendy X. Cao**, Julie M. Claycomb. Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

Endogenous small RNA-mediated gene silencing pathways regulate gene expression throughout animal development. At the core of gene silencing activities are Argonaute proteins (AGOs), which are guided to target transcripts in a sequence specific manner by a small RNA (18-26 nucleotides), and catalyze gene silencing outcomes (i.e., transcript degradation, translational or transcriptional inhibition). AGO activity can be influenced by several factors, including interactions with accessory proteins, thus identifying new members of AGO complexes is of great interest. To identify factors physically associated with the essential AGO CSR-1 (Chromosome Segregation and RNAi Deficient), we performed a yeast two-hybrid assay and identified a highly conserved protein, known as CHP-1 (CHORD Protein). CHP-1 homologs possess CHORD domains (Cys and His Rich Domains) as well as a CS domain (CHORD/SGT1), and interacts with HSP-90 (Heat Shock Protein). CHORD proteins often act as HSP-90 co-chaperones in various species and in a variety of processes, from centrosome duplication to stress responses. In *C. elegans*, *chp-1* is essential, and is most highly expressed in the germline and embryos. Consistent with a role in small RNA pathways, we have found that *chp-1(tm2277)* mutants are germline RNAi deficient. Furthermore, Illumina sequencing of small RNA populations in *chp-1(tm2277)* mutants reveals a depletion of particular small RNAs. We have rescued *chp-1(tm2277)* with a transgene expressing 3XHA::CHP-1, and have characterized its subcellular localization throughout development. Immunoprecipitation of 3XHA::CHP-1 revealed that CHP-1 interacts with several AGO proteins, including CSR-1, as well as specific small RNAs. Because CHORD proteins can act as HSP-90 co-chaperones, and HSP-90 has been implicated in AGO/small RNA complex formation in other organisms, we are investigating the possibility that CHP-1 plays a role in the stability of AGO complexes in the worm. In sum, these studies enhance our understanding of the composition of AGO/small RNA complexes and are the first to demonstrate a role for this highly conserved and essential CHORD protein in small RNA pathways in any organism.

100. Periodic A/T rich DNA structures promote germline expression. **C. Frokjaer-Jensen**, M.W. Davis, E.M. Jorgensen. Biology, HHMI, University of Utah, Salt Lake City, UT.

Organisms face the challenge of distinguishing their own DNA from foreign DNA such as retrotransposons and DNA transposons. This is of particular concern in the germline, where any deleterious effect of transposition can affect all subsequent generations. Small RNAs (e.g. piRNAs) play an important role in identifying and silencing foreign DNA. Here we test a complementary model for silencing transposons in the germline proposed by Fire *et al.* (2006). In this model, stretches of mostly intronic DNA containing Periodic A/T Clusters (PATCs) identify endogenous genes and promote germline expression by preventing heterochromatic silencing. PATCs are enriched in genes expressed in the germline and residing on autosomal arms, which are strongly enriched for repressive chromatin marks. ***Do germ cells silence foreign DNA without PATCs by aggressive epigenetic silencing over large genomic regions?*** We have tested this model by inserting and monitoring germline-expressed transgenes at random genomic locations inside a Mos1 transposon. A transgene without PATCs (P*dpy-30*:GFP:H2B) is silenced at all locations outside the central region of autosomes. In contrast, a transgene with PATCs in the promoter (P*pie-1*:GFP:H2B) is expressed from a much broader region of the genome: autosome centers, some expression from the arms and a few from the tip of Chr. X. This position based silencing is reproducible; targeted insertions of a P*pie-1* transgene into seven MosSCI sites along Chr. V behave similarly. An endogenous gene (P*smu-1:smu-1*:GFP) with many PATCs is highly resistant to silencing and expressed from essentially all genomic locations, including the entire X chromosome. ***Does this type of silencing occur naturally and protect worms from foreign DNA?*** We show that a class of germline specific retrotransposons, *Cer1*, follow this pattern of silencing by identifying their genomic locations in natural *C. elegans* isolates. Silenced *Cer1* insertions are all integrated into the arms of autosomes or the X-chromosome, whereas most strains with an active *Cer1* have at least one copy in the center of an autosome. We think it is likely that PATCs promote germline expression and contribute to distinguishing endogenous genes from foreign DNA.

101. The period protein homolog LIN-42 negatively regulates microRNA biogenesis in *C. elegans*. **Priscilla M. Van Wynsberghe[1,2]**, Emily F. Finnegan[1], Thomas J. Stark[3], Evan P. Angelus[2], Kathryn Homan[2], Gene W. Yeo[3], Amy E. Pasquinelli[1]. 1) Division of Biology, UCSD, La Jolla, CA; 2) Biology Department, Colgate University, Hamilton, NY; 3) Dept. of Cellular and Molecular Medicine, UCSD, Sanford Consortium for Regenerative Medicine, La Jolla, CA.

MicroRNAs (miRNAs) are essential small RNAs that post-transcriptionally regulate development in *C. elegans* and other species. They are encoded in the genome and transcribed into primary (pri-) miRNAs that are capped and polyadenylated. Processing of a pri-miRNA by the RNase III enzyme Drosha produces the ~70 nt precursor (pre-) miRNA, which is further processed by a second RNase III enzyme, Dicer, in the cytoplasm to form the mature miRNA. In order to produce the appropriate amount of a particular miRNA in the correct location at the correct time, proper regulation of miRNA biogenesis is essential. Here we present our work that identifies the Period protein homolog LIN-42 as a new regulator of miRNA biogenesis in *C. elegans*. We mapped a spontaneous suppressor of the normally lethal *let-7(n2853)* allele to the C terminal region of *lin-42*. Mutations in this allele *(ap201)* or a second *lin-42* allele *(n1089)* caused increased mature let-7 miRNA levels at all time points throughout the 3rd and 4th larval stages (when mature let-7 miRNA is normally expressed). Levels of pri-let-7 and a let-7 reporter, which expresses GFP from the let-7 promoter, were also increased in *lin-42(n1089)* worms relative to wt. These results indicate that LIN-42 normally represses pri-let-7 transcription and thus the accumulation of let-7 miRNA. This inhibition of miRNA expression by LIN-42 is not specific to let-7, as pri- and mature levels of lin-4 and mir-35 are also increased in *lin-42* mutant worms and eggs, respectively. Furthermore, RNAseq analysis shows widespread increases of mature miRNAs in *lin-42* mutants in both egg and 4th larval stage worms. Thus, we propose that LIN-42 is a global regulator of miRNA biogenesis. Since LIN-42 is the homolog of *Drosophila* and mammalian Period proteins, our results raise the possibility that these proteins may share a conserved function in transcriptionally regulating miRNA biogenesis to ultimately control rhythmic processes.

102. Arginine methylation is required for piRNA mediated gene silencing in *Caenorhabditis elegans*. **Alexandra Sapetschnig**, Peter Sarkies, Eric Miska. Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, Cambs., United Kingdom.

The main Piwi protein in *C. elegans* is termed PRG-1. It associates with 21U-RNAs (the piRNAs of *C. elegans*) and is required for transposon silencing and normal germline development. We have recently shown that the piRNA pathway acts upstream of an endogenous siRNA pathway in *C. elegans*. The piRNA/Piwi complex serves as an initiator to trigger gene silencing via endo-siRNAs (22G-siRNAs). Production of the 22G-siRNAs requires a dicer-related helicase (DRH-3) and a class of proteins called Mutators (MUTs). Subsequent gene silencing is at least partly exerted by a nuclear germline-specific Argonaute protein termed HRDE-1. The mechanism by which the piRNA and the downstream endo-siRNA pathway interact and ensure loading of 22G-siRNAs into the correct Argonaute protein is not understood. Here, we show that HRDE-1 nuclear localisation depends on the biogenesis of 22G-siRNAs as it remains mostly cytoplasmic in *drh-3* and *mut-16* animals. These results indicate loading of 22G-siRNAs as a pre-requisite for nuclear localisation and function of HRDE-1. Surprisingly, *prg-1* mutant animals also show severely reduced nuclear localisation of HRDE-1 and reduced loading with 22G-siRNAs suggesting that the piRNA pathway might be the main input for this nuclear silencing pathway. Piwi proteins in several organisms have been shown to be methylated by protein arginine methyltransferases (PRMTs). This modification can be recognised by tudor domain proteins which have been implicated in the loading of piRNAs into Piwi proteins. Here, we show that *C. elegans* PRG-1 is methylated at several arginine residues in its N-terminus by PRMT-5. Animals carrying a deletion of *prmt-5* as well as several tudor gene mutants fail to silence piRNA targets indicating that arginine methylation is functionally required in the piRNA pathway. Furthermore, a *prg-1* transgene lacking the arginine methylation sites is not capable of rescuing the gene silencing defects of the *prg-1* mutant strain. We are currently analysing these arginine methylation-deficient strains for HRDE-1 nuclear localisation to assess how arginine methylation contributes to loading of HRDE-1 with 22G-siRNAs.

103. ALG-3/4 acts through the CSR-1 pathway to promote spermiogenic gene expression and to provide a paternally inherited memory of past gene expression. **Colin Conine[1]**, James Moresco[2], John Yates[2], Craig Mello[1,3]. 1) RNA therapeutics Institute, Univ Mass Med Sch, Worcester, MA; 2) Department of Chemical Physiology, The Scripps Research Institute; 3) Howard Hughes Medical Institute.

During each generation germline cells undergo truly dramatic alterations in gene expression and cellular morphology that are required to produce the male and female gametes, and do so while preserving both the genetic and epigenetic information required for pluripotency. In many animals the production of functional male gametes is particularly sensitive to temperature. We have been exploring the question of how small-RNA pathways contribute to the production of functional thermotolerant sperm in C. elegans. Here we show that ALG-3/4-associated 26G-RNAs act upstream of the

argonaute CSR-1. csr-1 mutant males are identical phenotypically to alg-3/4 males and like alg-3/4 mutants, exhibit temperature sensitive infertility correlated with reduced expression of spermiogenic mRNAs. CSR-1 protein associates with peripheral spermatogenic chromatin and also localizes prominently in mature spermatozoa. Interestingly, alg-3/4 and csr-1 heterozygous males are normally fertile at all temperatures, however heterozygous progeny produced after 3 to 5 generations of paternal homozygosity exhibit the same thermo-intolerant sterile phenotype observed for homozygous males. These findings are consistent with a role for the ALG-3/4, CSR-1 pathway in maintaining an epigenetic program for thermotolerant spermiogenesis. As expected CSR-1 engages small-RNAs targeting spermiogenic RNAs in males. However, we were surprised to find that CSR-1 also engages small RNAs antisense to female-specific germline mRNAs that are not expressed in males. Taken together our findings suggest that the ALG-3/4 CSR-1 pathway promotes theromtolerance by preserving robust spermiogenic gene expression during meiosis, and that CSR-1 functions epigenetically to transmit a memory of both male- and female-specific gene expression to successive generations.

104. *let-70*, an E2 ubiquitin-conjugating enzyme, promotes linker cell death in *C. elegans*. **Jennifer A. Zuckerman**, Yun Lu, Shai Shaham. Rockefeller University, New York, NY.

The *C. elegans* linker cell (LC) directs migration and development of the male gonad, dying in the L4-adult transition to allow *vas deferens*-cloaca fusion.We previously showed that the LC dies independently of apoptosis genes, displaying non-apoptotic features including nuclear membrane invagination, open chromatin, and organelle swelling.These features are conserved in dying cells in normal vertebrate development and in polyQ-expansion diseases. *let-70* encodes an E2 ubiquitin-conjugating enzyme we identified in an RNAi screen for LC death genes.*let-70*(RNAi) males are defective in LC migration and death and LC-specific RNAi using *rde-1* rescue blocks death but not migration. Thus, *let-70* functions in the LC to promote cell death and non-autonomously to control migration. *let-70* is transcriptionally induced in the LC as the cell begins to die. Similarly,we found that expression of a ubiquitin gene reporter, *ubq-1::gfp*,also increases, as does expression of PQN-41, a polyQ-repeat protein required for LC death (Blum et al, 2012).These results suggest that LC death is controlled by a transcriptional program. Inactivation of the MAPKK *sek-1* or the histone methyltransferase *set-16* blocks *let-70* expression and LC death (Blum et al, 2012). *nhr-67* RNAi induces early *let-70*::GFP expression in the LC; however, *nhr-67*(RNAi) blocks LC death and double RNAi against *nhr-67* and *let-70* synergistically increases cell survival.Thus, proper timing of expression and/or optimal levels of let-70 may be crucial for its cell death role.We screened for E3 ligases needed for LC death and found that RNAi against seven-in-absentia homolog *siah-1* or the RING-box *rbx-1* blocked LC death in 10% of animals. *siah-1*; *rbx-1* double mutants show nearly 30% LC survival, suggesting that they act in parallel. Furthermore, 2-hybrid studies show that LET-70 and SIAH-1 interact. We suggest, therefore, that *let-70* degrades proteins normally required to inhibit LC death. Supporting this notion, RNAi against proteasome regulatory components blocks LC death, as does RNAi against other SCF components.Our studies suggest a role for protein degradation in the control of a novel, conserved form of cell death with potential relevance to human neurodegeneration.

105. Translational Regulators GCN-1 and ABCF-3 Maternally Contribute to General Programmed Cell Death. **Takashi Hirose**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

In *C. elegans*, 131 somatic cells undergo apoptosis during wild-type hermaphrodite development. Extensive genetic screens have identified the BH3-only gene *egl-1*, the BCL-2 homolog *ced-9*, the APAF-1 homolog *ced-4* and the caspase gene *ced-3*, which together define an evolutionarily conserved cell-death execution pathway that drives most somatic cell deaths. Most screens for cell-death mutants have been performed by examining the F2 generation, so it is possible that maternal-effect genes involved in the cell-death execution pathway remain to be identified.

From a genetic screen for mutations that cause a defect in the death of the sister of the pharyngeal M4 motor neuron, we identified *ceh-32*, *ceh-34*, *eya-1*, *sptf-3* and *pig-1*, all of which promote cell-type specific apoptosis. We also identified *gcn-1* and *abcf-3* and showed that these genes promote apoptosis of most somatic cells. Maternal *gcn-1* and *abcf-3* are sufficient and partially required for the M4 sister to undergo apoptosis. These results suggest that maternally-contributed *gcn-1* and *abcf-3* function plays an important role in promoting apoptosis of possibly all somatic cells. GCN-1 and ABCF-3 proteins are highly conserved. The *S. cerevisiae* homologs of GCN-1 and ABCF-3 physically interact and are required for the phosphorylation of a conserved serine residue of eukaryotic initiation factor 2a (eIF2a), the phosphorylation of which causes a general inhibition of protein translation and the specific activation of translation of select mRNAs. We find that the *C. elegans* GCN-1 and ABCF-3 proteins interact *in vivo* and are required for the phosphorylation of eIF2a, suggesting a conserved function in translational regulation. To determine where GCN-1 and ABCF-3 function in the cell-death pathway, we performed genetic analyses of the interactions of *gcn-1* and *abcf-3* with known cell-death genes in the regulation of M4 sister cell death. GCN-1 and ABCF-3 act independently of *ced-9* and function in parallel to *ceh-34*, *sptf-3* and *pig-1*. We propose that GCN-1 and ABCF-3 act together to promote apoptosis generally through translational regulation in a pathway distinct from the known cell-death pathway.

106. The importance of multiple caspase downstream pathways to execution of cell death in *C. elegans*. **Akihisa Nakagawa**, Yu-Zen Chen, Ding Xue. Department of MCDB, University of Colorado, Boulder, CO.

Analysis of downstream pathways of important enzymatic biomolecules such as proteases and kinases, which have multiple *in vivo* substrates, has been a difficult challenge. Targets of these enzymes are difficult to identify through conventional genetic screens, because inactivating one of their downstream pathways usually causes a weak or non-detectable phenotype. Enzymatic targets are also difficult to identify through commonly used biochemical approaches that require stable protein interactions rarely seen between an enzyme and a substrate. Caspases, a family of cysteine proteases, play critical roles in apoptosis execution. However, the downstream pathways of caspases and their importance and contributions to cell death execution are poorly understood. We have identified five downstream pathways of the CED-3 caspase in *C. elegans* that promote different cell death execution events, including chromosome fragmentation, phosphatidylserine externalization, mitochondria elimination, and inactivation of the AKT survival pathway. Inactivation of each CED-3 downstream pathway causes a mild delay or non-detectable effect in apoptosis. Remarkably, simultaneous inactivation of all five CED-3 downstream pathways delays all embryonic cell deaths to late larval stages and blocks close to 50% of the cell deaths. Despite of the strong cell death defect in the quintuple mutant, CED-3 caspase is activated and active in cleaving CED-3 substrates, indicating that cell death is suppressed even after CED-3 is activated in the quintuple mutant. Moreover, most of the hermaphrodite-specific neurons (HSNs) in the quintuple mutant fail to die in response to a gain-of-function mutation in *egl-1* that causes ectopic death of HSNs and function properly to control egg laying of hermaphrodite animals. Our study

demonstrates that seemingly unimportant individual cell death execution events synergize to kill the cell and this finding may be generally applicable to downstream pathways of important enzymatic biomolecules.

107. A Redox Signaling Globin Regulates Germ Cell Apoptosis in *Caenorhabditis elegans*. **S. De Henau[1]**, L. Tilleman[2], M. Pauwels[3], A. Pesce[4], M. Nardini[5], M. Bolognesi[5], K. De Wael[3], L. Moens[2], S. Dewilde[2], B.P. Braeckman[1]. 1) Biol Dept, Ghent Univ, Belgium; 2) Biomedical Sciences Dept, Univ of Antwerp, Belgium; 3) Chemistry Dept, Univ of Antwerp, Belgium; 4) Physics Dept, Univ of Genova, Italy; 5) Biosciences Dept, Univ of Milano, Italy.

It has become clear that reactive oxygen species (ROS) can modulate signal transduction pathways, thereby influencing cellular functioning. A striking aspect hereby is that ROS can be purposely generated by enzymes. However, in the majority of reported redox-sensitive pathways, it is unclear what the enzymatic source is of ROS. We show that a globin of *C. elegans*, globin-12 (GLB-12), regulates multiple aspects in reproduction by redox signaling. *glb-12* RNAi causes severely reduced fecundity and multiple defects during oocyte development, including increased germline apoptosis levels. By focusing on the increase in germline apoptosis, we found that GLB-12 signals through the JNK and P38 MAPK pathways: abolishing these pathways eliminates the increase in germline apoptosis. Biochemical analysis of GLB-12 showed that, unlike the well studied hemo- and myoglobin, this globin cannot bind oxygen. Instead, we see that GLB-12 has a reduction potential sufficiently low to favor electron transfer from its heme iron to oxygen. In addition, the crystal structure of GLB-12 shows that this globin possesses unique properties that support a role in electron transfer. *In vitro*, GLB-12 can indeed actively convert oxygen to superoxide by electron transfer, thus creating a redox signal. *In vivo*, the unstable superoxide produced by GLB-12 could potentially be converted by superoxide dismutases (SODs) to the more stable hydrogen peroxide, which in turn may act as a biological messenger. Applying *glb-12* RNAi in mutants for the five *C. elegans sod* genes showed that fecundity is further reduced in the intracellular SOD-1 mutant and restored to almost normal levels in the extracellular SOD-4 mutant. We also observed such opposite effects of SOD-1 and SOD-4 on the antiapoptotic role of GLB-12, and on the inhibitory role of GLB-12 on the P38 pathway. SOD-1 and SOD-4 thus modulate the signal created by GLB-12, creating a redox signaling pathway that influences germline apoptosis levels.

108. A non-canonical role for the Caenorhabditis elegans dosage compensation complex in growth and metabolic regulation downstream of TOR complex 2. **Christopher M. Webster[1,2]**, Denzil Douglas[1,2], Alexander A. Soukas[1,2]. 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Harvard Medical School, Boston, MA.

The target of rapamycin complex 2 (TORC2) pathway is evolutionarily conserved and regulates cellular energetics, growth and proliferation. Loss of function of the essential TORC2 subunit, Rictor (rict-1), exhibits numerous pleiotropies in C. elegans such as decreased developmental rate, reduction of brood size, smaller body size, increased body fat mass, and a truncated lifespan. As major downstream effectors of TOR complex 2 belong to the AGC kinase family, we performed a rict-1 suppressor RNAi screen of genes encoding proteins that possess the phosphorylation sequence of AKT and SGK, the AGC-family kinases. Only RNAi to dpy-21 suppressed rict-1 slow developmental rate. The canonical role of DPY-21 is to function in the 10 protein dosage compensation complex (DCC) to downregulate expression of X-linked genes in hermaphroditic worms. However, we find that dpy-21 functions outside of its canonical role as RNAi is equally able to suppress TORC2 mutant developmental delay in rict-1 males and hermaphrodites. RNAi toward dpy-21 normalized several phenotypes in TORC2/rict-1 mutants including brood size and fat storage, but failed to restore normal body size and was highly detrimental to lifespan. Further dissection of the DCC via RNAi revealed that other complex members phenocopy the dpy-21 suppression of rict-1. Similarly, RNAi to the DCC effector set-1, which monomethylates histone 4 on lysine 20(H4K20) also had suppressive effects. TORC2/rict-1 animals were enriched for the H4K20me1 silencing epigenetic mark associated with DCC activity, and this enrichment was suppressed by knockdown of dpy-21. We demonstrate here DPY-21 is functioning epigenetically via SET-1. Loss of dpy-21 along with associated epigenetic silencing marks on chromatin results in antagonistic pleiotropy as evidenced by both enhancement and suppression of phenotypes associated with TORC2 mutation. Together the data suggest non-canonical, negative regulation of growth and reproduction by DPY-21/DCC downstream of TOR complex 2 in C. elegans.

109. WormGUIDES: an overview. Zhirong Bao[4], William Mohler[3], Javier Marquina[1], Hari Shroff[2], **Daniel A. Colon-Ramos[1]**. 1) Dept Cell Biol, Yale Sch Med, New Haven, CT; 2) Section on High Resolution Optical Imaging, National Institute of Biomedical Imaging and Bioengineering, National Institutes of Health, Bethesda, MD; 3) Genetics and Developmental Biology, UConn Health Center, Farmington, CT; 4) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

WormGUIDES (Global Understanding in Dynamic Embryonic Systems) is a novel resource that aims to create and share the first 4D atlas with single cell resolution of embryogenesis and neurodevelopment for any animal. The first goal of WormGUIDES is production of an interactive atlas of nuclear positions from zygote until hatching. A simple navigation program for computers or hand-held devices facilitates the use of WormGUIDES as a reference tool in cell identification, quantification of developmental processes and visualization of nascent patterns and symmetries in the embryo. This program and data will be demonstrated at the meeting. WormGUIDES will also incorporate a complementary atlas of neurodevelopmental processes—neurite outgrowth and synaptogenesis—that will facilitate a dynamic understanding of the connectome emerging throughout development. Building on the C. elegans community's open-sharing of systems-level knowledge and resources, WormGUIDES will further enhance the value of C. elegans as a model organism.

110. Using natural variation to decipher the complex genetic causes of *C. elegans* drug sensitivities. **Erik C. Andersen**, Tyler Shimko. Molecular Biosciences, Northwestern University, Evanston, IL.

C. elegans is an important model for a variety of biomedically relevant traits, including sensitivities to diverse compounds and pharmaceuticals. Sensitivities to these compounds vary in the human population, and we know little about the genetic causes of this variation. Using *C. elegans*, we hope to identify and characterize these variant genes exploiting the existing natural genetic and phenotypic variation of the species.

Quantitative geneticists use the correlations between genetic and phenotypic differences to identify the variants that control complex traits. Species-wide variation in genome-wide association studies or pairwise strain variation in linkage mapping studies is used to identify variant genes. Using these two approaches, one needs to score the phenotypes of a large number of independent strains. In *C. elegans*, we must phenotype over one hundred wild strains or nearly six hundred N2xCB4856 recombinant inbred lines in drug susceptibility assays. In order to score these large collections of independent strains for

drug susceptibility traits, we optimized highly reproducible and high-throughput growth, fecundity, and pharyngeal pumping assays to score 384 strains per day per researcher in the presence of any aqueous compound. These assays allow us to probe both the acute and chronic phenotypic effects of important drugs, like chemotherapeutics, pesticides, and anthelmintics.

To determine the efficacy of these approaches, we scored susceptibility to the most widely applied herbicide paraquat. In one week, we phenotyped growth and fecundity of 97 wild strains and 574 recombinant inbred lines between N2 and CB4856. These data implicated genomic intervals as small as 274 kb with 60 genes that control paraquat sensitivity. To verify these loci, we constructed nearly isogenic lines, in which a genomic region from one strain is isolated in another strain background. These results recapitulate the predicted effects, and we will discuss methods to characterize small phenotypic effects. Additionally, we will discuss recent progress towards scaling up these techniques to a larger set of compounds and pharmaceuticals.

111. Evolution of sperm activation in *Caenorhaditis* hermaphrodites. **Qing Wei**[1,2], Ronald E Ellis[2]. 1) GSBS, UMDNJ-SOM, Stratford, NJ; 2) Molecular Biology, UMDNJ-SOM, Stratford, NJ.

In *Caenorhabditis*, the *XX* animals of most species are female, and the *XO* animals are male. Because hermaphroditism evolved independently in three species, it provides a model for the origin of novel traits. Previous studies showed that this change required two steps: a mutation that caused *XX* spermatogenesis, and a separate mutation that allowed *XX* spermatids to activate and fertilize oocytes.

Sperm activation is controlled by redundant pathways in *C. elegans* males, but by only one pathway in *C. elegans* hermaphrodites. The *spe-8* pathway functions in both sexes, and contains *spe-12, spe-19, spe-27,* and *spe-29,* which act in response to an unknown signal. By contrast, the *try-5* protease pathway works only in males. Using both computer analyses and degenerate primers, we found that all *Caenorhabditis* species have orthologs of genes from both pathways. Thus, these redundant systems existed in the male/female ancestor of the genus.

RT-PCR confirmed that most of these genes are expressed in *C. briggsae* hermaphrodites, at appropriate times to regulate sperm activation. By creating null mutants with TALENs, we showed that *C. briggsae try-5* males and *spe-19* males are fertile, but that *try-5 spe-19* double mutants have inactive sperm. Thus, these pathways are redundant in *C. briggsae* males. By contrast, *try-5* hermaphrodites are fertile, whereas both *spe-8* and *spe-19* hermaphrodites are sterile. We conclude that the *spe-8* pathway was recruited to work in *C. briggsae* hermaphrodites. Finally, by studying *C. briggsae/ C. sp. 9* hybrids, we found that *C. briggsae* has a dominant factor promoting sperm activation; this factor might be the signal that acts on the *spe-8* group.

Did a developmental constraint force evolving hermaphrodites in *C. elegans* and *C. briggsae* to recruit the *spe-8* pathway for sperm activation? Preliminary studies with *C. sp. 11* show that neither *spe-19* nor *spe-27* are required for hermaphrodite sperm activation, so other regulatory solutions might also be possible.

112. Specificity of interaction between *Caenorhabditis* and their natural viruses. **Gautier BRESARD**[1,2,3], Marie-Anne FELIX[1,2,3]. 1) Institute of Biologie of the Ecole Normale Supérieure; 2) Centre National de la Recherche Scientifique UMR8197; 3) Institut National de la Santé et de la Recherche Médicale U1024, 46 rue d'Ulm, 75230 Paris Cedex 05, France.

Species involved in host-pathogen relationships exert selective pressures on each other. This co-evolution situation results in an arms race between host and pathogen, which may lead to specialisation of their interactions.

We recently found three related horizontally-transmitted RNA viruses that naturally infect *C. elegans* or *C. briggsae*, called Orsay, Santeuil and Le Blanc viruses (Félix et al. 2011, Franz et al. 2012). Here we study their specificity for *C. elegans* vs. *C. briggsae*, and at the intraspecific level in *C. briggsae*.

We first used viral filtrates to infect a set of *C. elegans* and *C. briggsae* isolates, and measured by RT-PCR the virus ability to replicate. We find that the Orsay virus can infect *C. elegans* but not *C. briggsae*, whereas Santeuil and Le Blanc viruses infect *C. briggsae*, but not *C. elegans*. Thus, each virus shows specificity toward one of these two *Caenorhabditis* species.

Given that *C. briggsae* can be infected by two viruses, we then measured viral replication after infection of *C. briggsae* isolates by either Santeuil or Le Blanc viruses, using RT-qPCR. We observed 1) wide variation among *C. briggsae* isolates; 2) correlation between the sensitivities to each virus; 3) an exception to the correlation. Schematically, *C. briggsae* isolates can be separated into two groups: sensitive isolates, in which the viruses replicate efficiently; and resistant ones, in which the viruses either disappear or are barely maintained. Strikingly, all sensitive strains belong to the temperate *C. briggsae* clade, raising the possibility that sensitivity is derived within this clade. The exception to the correlation in sensitivity is HK104, a temperate-clade isolate from Japan. HK104 is sensitive to the Santeuil virus, but resistant to Le Blanc. This result opens the possibility to study specificity of host-pathogen interactions through genetic analysis.

113. Influence of the Microbiome on *C. elegans* Growth in the Wild. **Buck S. Samuel**[1], Holli Rowedder[1], Christian Braendle[2], Marie-Anne Félix[3], Gary Ruvkun[1]. 1) Dept. of Molecular Biology, Mass. General Hospital, Boston, MA; 2) Institute of Dev. Biology and Cancer, CNRS, University of Nice Sophia-Antipolis, France; 3) Institute of Biology of the Ecole Normale Supérieure (IBENS), Paris, France.

Like us, *C. elegans* lives in a microbial world. In its natural habitats of rotting fruits and vegetation, these nematodes proliferate as they dine on an array of microbes. Interactions with microbes span a spectrum from constant confrontation (pathogens) to relative indifference (food) to perhaps even mutual benefit (symbionts). This study identifies these natural microbes and addresses whether microbiome composition influences proliferation of *C. elegans* in the wild.

To examine this question, we sequenced bacterial 16S (SSU) rDNA amplicons from habitats with wild *C. elegans* populations collected in France and Spain. Our results show that *C. elegans* encounters a broad array of bacteria in the wild—especially the divisions (phyla) of Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria. An abundance-weighted comparisons of phylogenetic differences (UniFrac) showed distinct clustering by habitat type, as rotting apples clustered separately from other habitats sequenced. Further, rotting apples clustered by large presence of proliferating or small non-proliferating (dauer) populations of worms. *C. elegans* appear to proliferate in apples with 'simpler' microbiomes (lower diversity, fewer species and Proteobacteria-rich). Specific alpha-proteobacteria were particularly enriched in apples with proliferating worms, while a number of genera were consistently found in apples with non-proliferating worms (e.g., *Pseudomonas*, several Bacteroidetes, etc.). Population size also correlated with apple rottenness, suggesting bacterial load is key to growth as well.

Similarly, Proteobacteria content does affect *C. elegans* (N2) growth rate in the lab, as worms grew faster on mixtures (and single isolates) with 80% Proteobacteria versus those with 40% Proteobacteria. Together, these studies define the microbial diet of *C. elegans* and implicate the natural microbiome as a key determinant of *C. elegans'* growth in the wild.

114. Evolution of *Caenorhabditis* Dosage Compensation. **Te-Wen Lo**, Caitlin Schartner, Barbara J. Meyer. HHMI/UC Berkeley.

Comparative studies have shown remarkable divergence in the conservation of developmental mechanisms. Strategies to determine sexual fate and to compensate X-chromosome dosage between sexes have evolved particularly rapidly: mammals, flies, and worms utilize different methods. Understanding such rapidly changing processes requires comparisons over shorter evolutionary time-scales, such as between *C. briggsae* and *C. elegans*. Comparison of sex determination and dosage compensation across nematode species using heritable, targeted mutagenesis protocols we developed has shown that key features of the dosage compensation complex (DCC) and the genetic pathway that coordinates sex determination and dosage compensation are conserved. Despite conservation of the DCC and its regulatory hierarchy, X-chromosome targeting mechanisms have diverged. The cis-acting DNA recruitment elements on X (rex) and their motifs are distinct. *C. elegans* rex sites ported to *C. briggsae* fail to bind the *C. briggsae* DCC. The reciprocal also holds: *C. briggsae* rex sites ported into *C. elegans* fail to bind the *C. elegans* DCC. Also, *C. briggsae* rex sites lack the X-enriched *C. elegans* DNA motifs pivotal for DCC recruitment. The divergence of DCC binding sites between *C. elegans* and *C. briggsae* prompted us to explore X targeting in *C. sp. 9*, which is closer to *C. briggsae* than to *C. elegans*. *C. sp. 9* proteins homologous to DCC subunits of *C. briggsae* and *C. elegans* co-localize on X chromosomes of *C. sp. 9* hermaphrodites. We established site-directed mutagenesis in *C. sp.9* using TALENs and recovered an XX-specific lethal mutation in the key component of the regulatory hierarchy that triggers assembly of the DCC onto X. Further TALEN knockouts and ChIP-seq experiments will determine the divergence in X-targeting mechanisms. Dosage compensation provides a unique opportunity to study the co-evolution of regulator proteins and their binding sites. The evolution of DCC binding sites followed a different pattern from that of binding sites for conserved regulatory proteins that control many unrelated cellular processes. For multi-functional proteins few significant changes have occurred in their DNA binding domains and cognate DNA binding motifs. In contrast, the DCC complexes, which lack the constraints of multiple functions, exhibit robust divergence in binding sites.

115. Sumoylated NHR-25/NR5A regulates cell fate during *C. elegans* vulval development. **Jordan D. Ward**[1], Nagagireesh Bojanala[2,3], Teresita Bernal[1], Kaveh Ashrafi[4], Masako Asahina[1,2,3,4], Keith Yamamoto[1]. 1) Department of Cellular and Molecular Pharmacology, UCSF, San Francisco, CA, USA; 2) Institute of Parasitology, Biology Centre ASCR, Ceske Budejovice, CZ; 3) University of South Bohemia, Ceske Budejovice,CZ; 4) Department of Physiology, UCSF, San Francisco, CA, USA.

Individual metazoan transcription factors (TFs) regulate distinct sets of genes depending on cell type and developmental or physiological context. The exquisite cell and tissue specificity of gene regulation by a given TF appear to be achieved combinatorially, within multifactor regulatory complexes with distinct compositions and activities. The precise mechanisms by which regulatory information from ligands, genomic sequence elements, co-factors, and post-translational modifications are integrated by TFs remain challenging questions. Here we examine how a single regulatory input (sumoylation) differentially modulates the activity of the conserved *C. elegans* nuclear hormone receptor, NHR-25, in different cell types. Through a combination of yeast two-hybrid analysis and in vitro biochemistry we identified the single *C. elegans* SUMO (SMO-1) as an NHR-25 interacting protein, and showed that NHR-25 is sumoylated on three lysines. Genetic studies revealed that loss of *smo-1* phenocopied NHR-25 overexpression, with respect to maintenance of the 3º cell fate in vulval precursor cells (VPCs) during development. Furthermore, *in vivo* overexpression using NHR-25 alleles that could not be sumoylated and SUMO-NHR-25 fusions indicated that NHR-25 sumoylation is critical for maintaining 3º cell fate. SUMO also regulated formation of an NHR-25 accumulation gradient in VPCs. Using an NHR-25::GFP translational reporter, we discovered that NHR-25 levels were uniform across VPCs at the beginning of development, but as cells began dividing a *smo-1*-dependent NHR-25 gradient formed with highest levels in 1º fated VPCs, lower levels in 2º fated VPCs, and the lowest levels in 3º fated VPCs. Our findings support a model where the ratio of unsumoylated to sumoylated NHR-25 regulates 3º cell fate determination and maintenance during vulval development. Supported by GAAV IAA500960906, MODBIOLIN 7FP-REGPOT-2012-2013-1, Terry Fox Foundation (#700046), CIHR (#234765) and the NIH (CA20535).

116. Vulva Precursor Cells dynamically regulate their sensitivity to the LIN-3/EGF morphogen gradient to control Notch ligand expression during vulva induction. **Jeroen S. van Zon**[1,3], Alexander van Oudenaarden[2,3]. 1) FOM Institute for Atomic and Molecular Physics, Amsterdam, Netherlands; 2) Hubrecht Institute, Utrecht, The Netherlands; 3) Massachusetts Institute of Technology, Cambridge, MA.

How equipotent cells reliably interpret morphogen gradients to establish precise spatial cell fate patterns is a general and unresolved question. During *C. elegans* vulva development, a spatial LIN-3/EGF gradient excreted from the anchor cell (AC) induces different cell fates in the Vulva Precursor cells (VPCs) in a distance-dependent manner: P6.p, closest to the AC, adopts the 1° fate, whereas the more distant neighbors P5.p and P7.p adopt the 2° fate. Notch signaling between VPCs is required to restrict 1° fate to a single VPC. Using the novel smFISH technique, we quantified Notch ligand expression in individual VPCs with single mRNA resolution during vulva induction. We found that upregulation of the Notch ligands *lag-2* and *apx-1* by LIN-3 followed a distinct temporal pattern: first, in the early L3 stage only *apx-1* was expressed, initially in several VPCs but then restricted exclusively to the prospective 1° cell, P6.p. During the late L3 stage both *lag-2* and *apx-1* expression in P6.p increased dramatically. Moreover, we found that in P6.p this temporal pattern of Notch ligand expression was highly robust to changes in LIN-3 dosage. We could reproduce these observations using a simple mathematical model with three parameters: the decay length of the LIN-3 gradient, the production rate of excreted LIN-3 and the sensitivity of the VPCs to the external LIN-3 signal. The model showed that the observed Notch ligand expression dynamics was driven not by changes in LIN-3 excretion, but instead by increased sensitivity of each VPC to external LIN-3, as we subsequently confirmed experimentally. In addition, the model indicated that the LIN-3 gradient itself narrowed significantly over the course of induction. Our results suggest that the induction of spatial gene expression patterns by morphogen gradients is determined not only by the shape of the gradient but also by the modulation of the morphogen-induced signal in the receiving cells.

117. Synapse location during growth depends on glia location. **Z. Shao**[1], S. Watanabe[2], R. Christensen[1], E. Jorgensen[2], D. Colón-Ramos[1]. 1) Program in Cellular Neuroscience, Neurodegeneration and Repair, Department of Cell Biology, Yale University School of Medicine; 2) Howard Hughes Medical

Institute, Department of Biology, University of Utah.

Synaptic contacts are largely established during embryogenesis and are then maintained during growth. How the nervous system maintains correct synaptic contacts and prevents formation of inappropriate ones during growth is not understood. To identify molecules involved in this process we conducted a forward genetic screen in *C. elegans* and identified *cima-1*. In *cima-1* mutants, synaptic contacts are correctly established during embryogenesis, but ectopic synapses emerge as the animal grows. *cima-1* encodes a novel solute carrier in the SLC17 family of transporters that includes Sialin, a protein that when mutated in humans results in neurological disorders. *cima-1* does not function in neurons but rather functions in the nearby epidermal cells to correctly position glia during growth. Our findings indicate that CIMA-1 antagonizes the FGF receptor/EGL-15, and does so most likely by inhibiting the FGF receptor/EGL-15's role in epidermal-glia adhesion rather than signaling. Our data suggest that epidermal-glia crosstalk, in this case mediated by a novel transporter and the FGF receptor/EGL-15, is vital to preserve embryonically-derived circuit architecture during organismal growth.

118. Attenuation of insulin signaling contributes to FSN-1-mediated regulation of synapse development. **Wesley L. Hung**[1], Christine Hwang[1,2], ShangBang Gao[1], Edward H Liao[1,3], Jyothsna Chitturi[1,2], Ying Wang[1], Hang Li[1], Christian Stigloher[4], Jean-Louis Bessereau[4], Mei Zhen[1,2,3]. 1) Dept Research, Samuel Lunenfeld Research Inst, Toronto, ON, Canada; 2) Institute of Medical Science, University of Toronto, Ontario, Canada; 3) Department of Molecular Genetics, University of Toronto, Ontario, Canada; 4) Institut de Biologie de l'École Normale Supérieure, Biology Department, INSERM, Paris, F-75005 France.

The conserved neuronal F-box protein FSN-1 regulates neuromuscular junction development by negatively regulating DLK-mediated MAPK signaling in the presynaptic terminal (Liao et al., 2004; Nakata et al., 20005; Wu et al., 2007). We show here that attenuation of insulin/IGF signaling also contributes to FSN-1-dependent synaptic development and function. Loss of *fsn-1* leads to aberrant synapse growth and a significant decrease in spontaneous vesicle release frequency from the neuromuscular junctions. These synaptic defects are partially and specifically rescued by decreasing insulin/IGF signaling activity in postsynaptic muscles, as well as by reducing the activity of EGL-3, a proprotein convertase that processes neuronal agonistic insulin/IGF ligands INS-4 and INS-6. FSN-1 interacts with, and potentiates the ubiquitination of EGL-3 in vitro, and reduces the level of EGL-3 in vivo. A constitutively activated MAPK, MKK-4(DD), can revert the suppression effect of *fsn-1* by *daf-2*, indicating that MAPK pathway may act genetically downstream of the insulin pathway. We propose that FSN-1 negatively regulates insulin/IGF signaling and MAPK pathways to coordinate synapse growth signals from both pre- and postsynaptic terminals. Liao, EH, Hung, W, Abrams, B, Zhen, M. (2004) Nature 430:345 Nakata, K, Abrams, B, Grill, B, Goncharov, A, Huang, X, Chisolm, A, Jin, Y (2005) Cell 120:407 Wu, C, Daniels, RW, DiAntonio, A (2007) Neural Dev. 2:16.

119. MicroRNA regulation of proteoglycan biosynthesis controls cell migration in *C. elegans*. Mikael Pedersen, Goda Snieckute, Konstantinos Kagias, Camilla Nehammer, Hinke Mulhaupt, John Couchman, **Roger Pocock**. BRIC, University of Copenhagen, Copenhagen, Denmark.

Proteoglycans are glycosylated proteins that control many aspects of biology. Appropriate proteoglycan glycosylation status is crucial to permit specific interactions with, and regulation of, growth factor receptors and adhesion molecules to enable faithful development of multiple tissues. However, the mechanisms that control proteoglycan biosynthesis are poorly understood. To study this phenomenon, we used the HSN neuronal developmental paradigm to dissect the requirement for regulation of proteoglycan biosynthesis. We found that HSN development is sensitive to disruption of both the chondroitin and heparan sulfate biosynthesis pathways.

Through analysis of the 3'UTRs of proteoglycan biosynthesis pathway components we identified a previously unappreciated role for the conserved *C. elegans* microRNA *mir-79*. We found that *mir-79* directly controls the expression of two proteins that are critical for the glycosylation of proteoglycans. Through this regulation, *mir-79* controls the balance of chondroitin and heparan sulfate substitution. Deletion of the *mir-79* locus in *C. elegans* causes specific HSN neurodevelopmental defects via dysregulation of proteoglycan biosynthesis in the hypodermis. We find that the resultant imbalance in glycosylation impinges on a LON-2/Glypican pathway and non-cell autonomously disrupts cell migratory capacity. Interestingly, we observe that the regulatory relationship between the *mir-79* and proteoglycan biosynthesis components is conserved in humans, suggesting that this is an ancient means of regulating proteoglycan architecture and developmental migration patterns.

120. Identification of a novel axon guidance regulator. **Nanna Torpe**, Roger Pocock. Biotech Research & Innovation Center, University of Copenhagen, Copenhagen, Denmark.

In the developing nervous system of both vertebrates and invertebrates the guidance of the axonal growth cones is regulated by multiple signalling mechanisms and interactions with the extracellular matrix (ECM). We identified a collagen prolyl 4-hydroxylase (P4H) a-subunit, DPY-18, as a novel protein required for axonal guidance in C. elegans. P4Hs are enzymes involved in the synthesis of collagen, however the role of these enzymes in neuronal development is unknown. We found that *dpy-18* is specifically required for axonal guidance decisions of the PVQ, PVP and HSN neurons. Tissue-specific rescue shows that *dpy-18* is needed in the hypodermis, muscle and nervous system, consistent with the expression pattern of *dpy-18*. We analysed *dpy-18* mutant animals by electron microscopy to determine whether defective axon guidance is caused by a structural change in the hypodermal ridge or the basement membrane. These data do not reveal any obvious structural changes and confirm that all axons (except the AVK axons) in the left axon bundle cross over to the right. We performed a forward genetic suppressor screen to investigate in which pathway *dpy-18* acts and two axon guidance suppressor mutants, encoding a cuticular collagen and an uncharacterized protein, were isolated from the screen. The two suppressor mutants suppress the *dpy-18* mutant HSN axon guidance defects from 98% to 61% and 36%, respectively. Both suppressor mutants have been confirmed by RNAi and transgenic rescue and we are currently working on elucidating the connection between the suppressor genes and the prolyl 4-hydroxylase, *dpy-18*.

121. Reversible dendrite arborization in dauers is regulated by KPC-1/furin. **Nathan Schroeder**[1], Rebecca Androwski[1], Alina Rashid[1], Harksun Lee[2], Junho Lee[2], Maureen Barr[1]. 1) Dept. of Genetics, Rutgers University, Piscataway, NJ; 2) Institute of Molecular Biology and Genetics, Seoul National University, Seoul, Korea.

Neuroplasticity in response to adverse environmental conditions can entail both hypertrophy and resorption of dendrites. How dendrite remodeling occurs in response to unfavorable environmental conditions is unclear. We discovered that the six IL2 sensory neurons undergo dendrite remodeling

during development of the stress-resistant dauer stage. Based on our findings we divide the IL2 neurons into two separate anatomical classes. The four IL2Q (quadrant) neurons undergo extensive dendritic arborization and a shift from bipolar to multipolar neurons during dauer formation. The two IL2Ls (lateral) extend only a single additional process during dauer formation. During dauer recovery, the IL2 arbor retracts, leaving behind remnant branches in post-dauer L4 and adult animals. We isolated a mutation in *kpc-1* (kex2/subtilisin-like proprotein convertase), from a forward genetic screen, which results in disorganized and truncated IL2Q arbors. In mammals, the KPC-1 homolog furin is responsible for the cleavage and activation of numerous proproteins associated with various pathologies including neurodegenerative diseases. While broadly expressed in *C. elegans*, *kpc-1* is upregulated in dauer IL2 neurons and acts cell autonomously in the regulation of dauer-specific arborization. The IL2s are required for nictation behavior. We found that *kpc-1* mutant dauers are defective for nictation. *kpc-1* is also required for multidendritic neuron morphology and behavior during non-dauer stages (See Rashid et al. abstract) suggesting that, similar to furin, KPC-1 plays multiple roles in *C. elegans*. We are currently searching for potential substrates of KPC-1 that affect dauer-specific IL2 remodeling (See Androwski et al. abstract). The *C. elegans* IL2 sensory neurons provide a paradigm to study stress-induced reversible neuroplasticity, and the role of environmental and developmental cues in this process. Our discovery of KPC-1 as required for dendrite morphogenesis provides insight into the role of proprotein convertases in nervous system development.

122. Axonal fusion in regenerating axons shares molecular components with the apoptotic cell recognition pathway. **Brent Neumann**[1], Sean Coakley[1], Hengwen Yang[2], Ding Xue[2], Massimo Hilliard[1]. 1) Queensland Brain Institute, The University of Queensland, St Lucia, Queensland, Australia; 2) Department of MCD Biology, University of Colorado, Boulder CO, USA.

Understanding the molecular mechanisms regulating axonal regeneration is essential for the development of effective therapies for nerve injuries. However, we have a very poor understanding of how target reconnection occurs. Previously, we and others have demonstrated that target reconnection in *C. elegans* severed mechanosensory neurons can occur through a process of axonal fusion, with the proximal regrowing fragment recognizing and re-establishing membrane and cytoplasmic continuity with its own separated distal fragment (Ghosh-Roy et al. *J Neurosci* 2010, Neumann et al. *Dev Dyn* 2011). We have now characterised the process of axonal fusion at the molecular level, uncovering a critical role for molecules previously shown to mediate the recognition of apoptotic cells by neighbouring phagocytes. We have discovered that the conserved apoptotic phosphatidylserine receptor, PSR-1, plays an important role in axonal fusion. In animals carrying mutations in the *psr-1* gene, the proximal axon regenerates and contacts the distal fragment, but is unable to fuse, as a result of which the distal fragment degenerates. PSR-1 has previously been shown to bind exposed phosphatidylserine (PS) on the surface of apoptotic cells, an “eat-me” signal necessary for recognition and engulfment by phagocytic cells. Furthermore, we have identified similar axonal fusion defects in animals lacking the secreted transthyretin-like protein TTR-52, which also binds PS, the phagocyte receptor CED-1 that TTR-52 directly interacts with, and the adaptor phosphotyrosine-binding protein CED-6, which acts downstream of CED-1 to transduce engulfment signals. Analyses of double and triple mutant strains reveal that all found genes likely act in the same genetic pathway. We propose that PSR-1, CED-1, and CED-6 all function cell-autonomously, while TTR-52 is expressed from the intestines, from where it binds and acts as a bridging molecule to mediate recognition between the regrowing axon and its distal fragment.

123. Inhibition of precocious DD motor neuron synapse formation by the single Ig domain protein, OIG-1. **Kelly L. Howell**, Oliver Hobert. Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY.

Synaptic remodeling is a key process in the neuronal development of many organisms; however, the transcription factors and molecular mechanisms that control the specificity and timing of rewiring are poorly understood. The DD GABAergic motor neurons of *C. elegans* undergo extensive remodeling during development. At the end of the L1 stage, synapses of DD motor neurons that initially innervate ventral muscle are removed and new synapses are formed to the dorsal muscle of the animal. The heterochronic gene and novel transcription factor *lin-14* functions to prevent precocious remodeling from occurring; in mutants lacking *lin-14*, DD motor neuron synapses remodel prematurely to the dorsal muscle of the animal before the end of the L1 stage. However, downstream targets of LIN-14 that function to block premature synapse formation have not yet been described. We have found that in mutants for *unc-30*, a homeobox gene required for GABAergic motor neuron differentiation, DD motor neurons also generate DD synapses in the dorsal cord prematurely, similar to the phenotype observed in *lin-14* mutants. How do *lin-14* and *unc-30* work to inhibit premature dorsal synapse formation? We have identified a presumptive downstream target of *lin-14* and *unc-30*, a single Ig domain-encoding gene, *oig-1*. *oig-1* is transiently expressed in the DD neurons and constitutively expressed in the VD neurons. In the absence of *oig-1*, DD synapses form prematurely before the end of the L1 stage, similar to the phenotype caused by absence of *lin-14* and *unc-30*. In both *lin-14* and *unc-30* mutants, expression of *oig-1* is decreased or eliminated. Neuron-type specific rescue experiments show that *oig-1* functions autonomously in the DD neurons. Our studies provide insights into how transcription factors and their downstream targets control synapse formation and remodeling.

124. GRDN-1/Girdin and SAX-7/L1CAM establish a glial guide for sensory dendrite extension. **Ian G McLachlan**, Maxwell G Heiman. Department of Genetics, Harvard Medical School and Children's Hospital Boston, Boston, MA.

The extension of dendrites to genetically programmed targets is a crucial step in neural circuit formation. To understand this process, we are investigating the development of the sensory anatomy in the head of *C. elegans*, where each neuron extends an unbranched dendrite to a distinct but stereotyped position at the nose tip. Previous work showed that amphid neurons extend dendrites by retrograde extension: the neurons are born at the nose, anchor dendrite tips there, and migrate away to stretch out dendrites behind them. The extracellular matrix component DYF-7 is required for anchoring, and its loss results in severely shortened amphid dendrites. To determine if this mechanism is shared among distinct neuron classes, we generated a panel of fluorescent markers to visualize most of the head sensory neurons, and analyzed dendrite lengths in *dyf-7* animals. We found that while all glial-ensheathed sensory neurons require *dyf-7* for dendrite extension, some non-ensheathed neurons—including the oxygen sensor URX—do not require *dyf-7*. We therefore performed a forward genetic screen for mutants that affect dendrite extension of URX, but not amphid, neurons. We identified eight alleles of two genes: *grdn-1*, a homolog of Girdin, a cytoskeletal adapter that localizes to cell-cell contacts in the nervous system; and *sax-7*, a homolog of the neuron-glia adhesion molecule L1CAM. Surprisingly, we found that GRDN-1 and SAX-7 do not act cell-autonomously in URX. Instead, *grdn-1* appears to act in glia. A 450 bp *grdn-1* promoter, sufficient for rescue when driving the *grdn-1* cDNA, drives expression in the glial cells of the outer

labial (OL) sense organs that fasciculate with URX. Expression of the *grdn-1* cDNA by other glial-specific promoters is also sufficient for rescue of the URX dendrite phenotype. Embryonic imaging suggests that the URX dendrite extends from a stationary cell body, unlike retrograde extension of the amphids. We hypothesize that GRDN-1 and SAX-7 act in OL glia to form an adhesive guide, along which the URX dendrite extends. These results thus identify a novel mechanism of glial-dependent dendrite extension, distinct from the *dyf-7*-mediated retrograde extension of amphid and other glial-ensheathed neurons.

125. Genes that function downstream of Notch define a novel mechanism for inhibiting axon regeneration. **Rachid El Bejjani**, Marc Hammarlund. Dept Gen, Yale Univ, New Haven, CT.

We have previously shown that Notch/lin-12 signaling functions cell autonomously in motor neurons to inhibit axonal regeneration. We now show that the conserved neuronal scaffolding protein Mint/lin-10 is a likely target of anti-regeneration Notch signaling. Like Notch, Mint/lin-10 functions cell-autonomously to inhibit axonal regeneration in GABA motor neurons. Further, Mint/lin-10 is necessary for Notch to inhibit regeneration: lin-10 null mutations fully suppress the decreased regeneration caused by lin-12/Notch gain of function alleles or by overexpression of the Notch Intracellular Domain (NICD). Finally, lin-10 contains LAG-1 binding consensus elements in its 5' UTR. Together, these data suggest that LIN-10 is a transcriptional target of Notch signaling during axonal regeneration. LIN-10 may inhibit regeneration by regulating trafficking of other signaling molecules. We find that in axotomized GABA neurons, LIN-10 GFP localizes to the proximal stump of the axotomized neurons (as well as at synapses and the Golgi). LIN-10-dependent inhibition of axonal regeneration requires retrograde transport, as inhibition of retrograde transport in a rab-6.2 null significantly increases regeneration and suppresses the effect of LIN-10 overexpression. Further, the increase in regeneration in rab-6.2 mutants is fully suppressed by mutations that affect clathrin-mediated endocytosis. We propose that LIN-10 and retrograde trafficking function in damaged axons to maintain membrane localization of anti-regeneration factors. We are currently testing candidate binding partners of MINT/LIN-10 to identify these unknown factors.

126. The LAD-2/L1CAM functions in EFN-4/ephrin-mediated axon guidance. Binyun Dong, Melinda Moseley-Alldredge, **Lihsia Chen**. Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN.

The ephrin-Eph signaling pathway plays important roles in many diverse developmental processes. In *C. elegans*, the ephrin-Eph signaling pathway functions in axon guidance and cell movements critical during embryogenesis and male tail morphogenesis, requiring the function of the sole Eph receptor, VAB-1, and one or more of the four GPI-linked ephrins, EFN-1, 2, 3, and 4. In this study, we present data that demonstrates a role for EFN-4 in axon guidance that requires the function for SUP-17/Kuzbanian ADAM metalloprotease, suggesting the importance of ectodomain shedding of EFN-4. Expression of an engineered secreted form of EFN-4 can rescue *efn-4(bx80)* null axon guidance defects, raising the possibility that EFN-4 can function as a diffusible guidance cue. Of the four ephrins, EFN-4 is thus far shown to have roles that are independent of VAB-1. Consistent with EFN-4 functioning via an alternative receptor, we provide evidence that EFN-4 functions in the same pathway as LAD-2, a transmembrane protein belonging to the L1 family of cell adhesion molecules (L1CAMs). We had previously uncovered a role for LAD-2 as a co-receptor for MAB-20/semaphorin in directing axon migration. Our preliminary results reveal LAD-2 and EFN-4 can form a biochemical complex. Taken together, these results suggest LAD-2 may act as a potential receptor or regulatory co-receptor in EFN-4-mediated axon guidance.

127. Brain-wide Ca^{2+}-imaging of neural activity in *Caenorhabditis elegans*. **T. Schroedel**[1], R. Prevedel[1,2], K. Aumayr[1], A. Vaziri[1,2], M. Zimmer[1]. 1) Research Institute of Molecular Pathology, Vienna, Austria; 2) Max F. Perutz Laboratories, Vienna, Austria.

The connectome of *C. elegans*, with its 302 neurons and 8000 synapses, has been known for more than 25 years. This anatomical wiring diagram has been an invaluable resource to the research community; however, this alone is not sufficient to predict all functional connections that lead to behavior. To make this possible, what is missing is a physiological map of the nervous system. In order to achieve this goal, we set out to develop the techniques required for brain-wide real-time fluorescence Ca^{2+}-imaging in *C. elegans*. Because most worm neurons have unique identities, such a technique must allow for both, single-cell resolution and reliable single-cell recognition. This is especially challenging given the dense packing of the worm's head ganglion. We generated transgenic worms expressing the Ca^{2+}-sensor GCaMP5K pan-neuronally, while allowing for high resolution imaging of single cells. Worms are immobilized in specially designed microfluidic devices, in which chemosensory stimuli can be reliably delivered. To achieve high-speed volumetric imaging we employ a two-photon light-sculpting microscope. This technique is based on ultra-fast lasers and enables imaging with 2-photon excitation in a wide-field configuration while maintaining a high axial confinement. In combination with a high-speed camera and piezo driver, three-dimensional Ca^{2+}-imaging of large volumes (70mm x 70mm x 30mm) can be performed with speeds up to 5 volumes per second. This enables us to simultaneously visualize Ca^{2+}-traces of up to one third of the entire nervous system of *C. elegans* with a high spatio-temporal resolution. First recordings reveal basal activity in at least one third of detectable neurons. In order to investigate how sensory information is processed at the level of the whole worm brain, we activated oxygen chemosensory neurons via the microfluidic device. Initial imaging experiments confirm robust activation. In order to analyze evoked activity in the rest of the brain, we are currently developing image-processing tools for automated identification of postsynaptic interneuron classes in *C. elegans*.

128. A database of C. elegans behavioral phenotypes. **Eviatar I. Yemini**[1,2], Laura J. Grundy[2], Tadas Jucikas[2], Andre E.X. Brown[2], William R. Schafer[2]. 1) Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, New York, NY, USA; 2) MRC Laboratory of Molecular Biology, University of Cambridge, Cambridge, UK.

Previous single and multi worm tracking experiments have produced summary statistics to phenotype small worm sets. We introduce a database of extensive and intensive single-worm phenotypes for over 300 strains of C. elegans with nervous system and locomotory defects as well as a reference of N2 variability composed of more than 1,200 young-adult hermaphrodites examined over the course of 3 years. The data is available online at **http://wormbehavior.mrc-lmb.cam.ac.uk** and includes a link to Worm Tracker 2.0 (our single-worm tracker used for data collection).

Our phenomic database provides multiple levels of representation, from high-level statistical strain summaries all the way down to detailed time-series measurements for over 10,000 single-worm experiments. Within our database are 76 mutants with no previously characterized phenotype, 15 genes with multiple allelic representation, and 13 double or triple mutant combinations (the majority of which are accompanied by single mutant representation as well). Annotated experimental videos are easily accessible alongside their data, with various degrees of processing, from the skeleton and outline

coordinates to the time series of extracted features, their histograms, and an in-depth view of collective strain statistics. For computational researchers, the database is a rich source of processed measures and raw data for developing new algorithms for segmentation, behavioral quantification, and bioinformatic approaches which link complex phenotypes with genetic perturbations. For neurogeneticists, the summary statistics and visualizations make it possible to identify behavioral phenotypes in mutants of interest.

Free Worm Tracker 2.0 software and simple plans to build its inexpensive hardware are available at **http://www.mrc-lmb.cam.ac.uk/wormtracker/**.

129. Cell-cell fusion, sculpting and mechanisms. **Benjamin Podbilewicz**. Technion - Israel Institute of Technology.

130. The midbody ring, not the midbody microtubules, dictates abscission patterning *in vivo*. **Rebecca A. Green**[1], Jonathan Mayers[2], Lindsay Lewellyn[1], Arshad Desai[1], Anjon Audhya[2], Karen Oegema[1]. 1) Dept Cell/Molec Med, 3071G, LICR - UCSD, La Jolla, CA; 2) University of Wisconsin, Madison.

During cytokinesis, the cell must transition from constriction phase into abscission phase, by patterning the abscission components at the appropriate spatiotemporal location. Two structural features within the intercellular bridge are poised to serve as guidance cues to promote this process - the midbody microtubules and the midbody ring. It has been widely anticipated that the midbody microtubules are required—since key abscission determinants localize to this structure. In this study, we find that the midbody microtubules are dispensable for abscission in vivo and provide the first detailed analysis of the steps that occure during abscission phase in an intact organism. We define three temporally distinct and assayable abscission steps *in vivo* in the *C. elegans* embryo: cytoplasmic isolation, membrane shedding onset and midbody/midbody ring release; ESCRT assemblies are required only for the final step. We find that midbody microtubules are not required for any step, and that key abscission determinants are recruited to the abscission site in the absence of midbody microtubules. Furthermore, we show that the midbody ring component, the septins, is important for cytoplasmic isolation and is essential for midbody release. These results suggest that the midbody ring orchestrates the constriction to abscission phase transition *in vivo*.

131. *In vivo* visualization of chromosome synapsis in *C. elegans*. **Ofer Rog**[1,3], Abby F. Dernburg[1,2,3]. 1) Department of Molecular and Cell Biology and California Institute for Quantitative Biosciences (QB3), University of California, Berkeley; 2) Lawrence Berkeley National Laboratory; 3) Howard Hughes Medical Institute.

Meiosis is the special cell division process that enables the production of haploid gametes. During meiotic prophase, chromosomes form linkages with their homologous partners to enable reductional segregation during the first meiotic division. Essential to this process is the pairwise alignment of homologous chromosomes along their entire lengths. In most eukaryotes this alignment is reinforced through synapsis, the assembly of a structurally conserved polymer called the synaptonemal complex (SC), which links homologous chromosomes. The SC promotes genetic exchanges (crossovers) between homologs, and likely regulates their number and location. Synapsis is a dynamic process, the details of which are difficult to infer from images of fixed cells or tissues. Our goal is to illuminate this process through analysis in living nematodes, using fluorescently tagged SC components and high-resolution time-lapse microscopy. Our observations have revealed that initiation of synapsis is a relatively infrequent event that is rate-limiting for completion of synapsis, consistent with evidence that initiation is subject to strict regulation. Initiation occurs at the “Pairing Centers” - regions near one end of each chromosome where initial pairing of homologous chromosomes is achieved. Once initiated, synapsis extends along the chromosome at a rate of ~160nm per minute. Individual chromosomes thus complete synapsis within 20-30 minutes of initiation. In *C. elegans*, synapsis is accompanied by rapid chromosome motions driven by dynein, which is coupled through the nuclear envelope to the Pairing Centers. Under conditions where this motion is severely abrogated, synapsis remains processive but is ~5-fold slower. This suggests that chromosome motion promotes homolog alignment, enabling synapsis to proceed more rapidly. Moreover, it reveals a novel function for the rapid chromosome motions that have been observed in diverse organisms during meiotic prophase.

132. The development of non-centrosomal MTOCs during epithelial polarization. **Jessica L. Feldman**, James R. Priess. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

The centrosome is the major microtubule organizing center (MTOC) in dividing cells. In many types of differentiated cells, however, MTOC function is reassigned to non-centrosomal sites. We are using C. elegans intestinal cells to analyze how MTOC function is reassigned to the apical surface of epithelial cells. After the terminal cell divisions, the centrosomes of intestinal cells move near the future apical membranes, and the post-mitotic centrosomes lose all, or most, of their associated microtubules. We show that microtubule-nucleating proteins such as g-tubulin that are centrosome components in dividing cells become localized to the apical membrane, which becomes highly enriched in microtubules. Our results suggest that centrosomes are critical to specify the apical membrane as the new MTOC. First, g-tubulin fails to accumulate apically in wild-type cells following laser ablation of the centrosome. Second, g-tubulin appears to redistribute directly from the migrating centrosome, forming a nascent MTOC at the lateral membrane before redistributing apically. Electron microscopy of embryos staged at the transition between lateral and apical g-tubulin show electron dense material associated with small clusters of microtubules at both lateral and apical sites. These data suggest that the reassignment of MTOC function from centrosomes to the apical membrane is associated with a physical hand-off of nucleators of microtubule assembly.

133. *C. elegans* meets single-molecule detection technologies; The embryonic cell polarity system is driven by state transition of PAR-2 protein molecules. **Yukinobu Arata**[1], Tetsuya Kobayashi[2], Michio Hiroshima[1,3], Chan-gi Pack[1], Tatsuo Shibata[4], Yasushi Sako[1]. 1) Cellular Informatics Laboratory, RIKEN; 2) Institute of Industrial Science, the University of Tokyo; 3) Laboratory for Cell Signaling Dynamics, Quantitative Biology Center (QBiC), RIKEN; 4) Laboratory for Physical Biology, RIKEN Center for Developmental Biology.

Genetics analyses have shown that asymmetric localization of PAR proteins is achieved by mutual inhibition and feedback controls. But how are the physical properties of the polarity proteins controlled *in vivo*? Recently, we succeeded in detecting single PAR-2 molecules in living embryos using total internal reflection fluorescence microscopy and fluorescence correlation spectroscopy. PAR-2 formed oligomers, and PAR-2’s high-degree oligomers accumulated asymmetrically in the posterior side of the embryo. The cortical PAR-2 localization is regulated by PKC-3-dependent phosphorylation. By comparing the dissociation rate constants from the cortex of low- and high-degree oligomers in hyper- and hypo-phosphorylated PAR-2 proteins, we found

that PKC-3-dependent phosphorylation promotes, but PAR-2 oligomerization negatively regulates PAR-2's dissociation. This dual regulation broadens the range of dissociation rate constants along the a-p axis. Unexpectedly, the association rate constant of PAR-2 proteins onto the cortex was larger in the posterior region, and thereby asymmetric along the a-p axis. This asymmetry was weakened in phospho-mimic PAR-2 proteins expressed in wild-type embryos normally polarized by endogenous proteins. This suggests that the association rate asymmetry requires dephosphorylation in the cytoplasm. A mathematical model, in which all parameters were determined by experimental measurements reproduced the asymmetric localization as a bi-stable system. The physico-chemical state transitions driven by phospho-dephosphorylation cycle and oligomerization produce asymmetric patterning of PAR-2 protein particles. Thus, quantitative measurements of protein dynamics using single-molecule detection technologies and mathematical modeling provide a bridge between molecular reactions and cellular pattern formation.

134. Analyses of C. elegans enhancer and promoter architectures reveals CpG island-like sequences and promoter activity of HOT regions. **R. Chen**[1], T. Down[1], E. Zeiser[1], P. Stempor[1], Q. Chen[2], T. Egelhofer[3], L. Hillier[4], T. Jeffers[2], J. Ahringer[1]. 1) Gurdon Inst, Univ of Cambridge; 2) Biology Dept, UNC at Chapel Hill; 3) Molecular, Cell and Developmental Biology Dept, UCSB; 4) Genetics Dept, Washington University.

RNA PolII transcription initiation sites are largely unknown in *C. elegans*. The initial 5' end of most protein-coding transcripts are removed by trans-splicing, and non-coding initiation sites have not been investigated. We identify 73,500 distinct clusters of initiation. Bidirectional transcription is frequent, with a peak of transcriptional pairing at 120 bp. We assign transcription initiation sites to 7691 protein-coding genes and find that they display features typical of eukaryotic promoters. Strikingly, the majority of initiation occurs in intergenic regions with enhancer-like chromatin signatures. Remarkably, productive transcription elongation across enhancers is predominantly in the same orientation as that of the nearest downstream gene. This oriented transcription at upstream enhancers could potentially deliver RNA Pol II to a downstream proximal promoter, or alternatively might function as a distal promoter. CG dinucleotides (CpG islands) are enriched in mammalian promoters. CpG density is thought to be irrelevant in invertebrates that lack DNA methylation such as *C. elegans*. We find that CpG enrichment at worm promoters shares features of mammalian CpG islands. CpG clusters are found at protein-coding promoters showing nucleosome depletion. In mammals, non-methylated CpGs are bound by Cfp1/CXXC1, which leads to H3K4me3 marking at promoters through recruitment of Set1. Interestingly, a worm Cfp-1 ortholog was reported to be required for global H3K4me3 levels. We found that the worm Cfp-1 is enriched at high H3K4me3 promoters containing high density of CpGs. Moreover, we find that highly occupied target (HOT) regions bound by multiple transcription factors are CpG-rich promoters in worm and human genomes, suggesting that the HOT regions may be caused by CpG-induced nucleosome depletion. Our results suggest that non-methylated CpG-dense sequence is a conserved genomic signal dictating an open chromatin state and marking by the H3K4me3 modification.

135. Neuropeptide Secreted from a Pacemaker Activates Neurons to Control a Rhythmic Behavior. **Han Wang**[1,2], Kelly Girskis[2], Tom Janssen[3], Jason P. Chan[2], Krishnakali Dasgupta[1,2], James A. Knowles[2], Liliane Schoofs[3], Derek Sieburth[2]. 1) Graduate Program in Genetic, Molecular and Cellular Biology, Keck School of Medicine, University of Southern California, Los Angeles, California 90033, USA; 2) Zilkha Neurogenetic Institute, Keck School of Medicine, University of Southern California, Los Angeles, California 90033, USA; 3) Functional Genomics and Proteomics Unit, Department of Biology, Katholieke Universiteit Leuven, Leuven, Belgium.

Rhythmic behaviors are driven by endogenous biological clocks in pacemakers, which must reliably transmit timing information to target tissues that execute rhythmic outputs. During the defecation motor program in *C. elegans*, calcium oscillations in the pacemaker (intestine), which occur about every 50 seconds, trigger rhythmic enteric muscle contractions through downstream GABAergic neurons that innervate enteric muscles. However, the identity of the timing signal released by the pacemaker and the mechanism underlying the delivery of timing information to the GABAergic neurons are unknown. Here we show that a neuropeptide-like protein (NLP-40) released by the pacemaker triggers a single rapid calcium transient in the GABAergic neurons during each defecation cycle. We find that mutants lacking *nlp-40* have normal pacemaker function, but lack enteric muscle contractions. NLP-40 undergoes calcium-dependent release that is mediated by the calcium sensor, SNT-2/synaptotagmin. We identify AEX-2, the G protein-coupled receptor on the GABAergic neurons, as the receptor for NLP-40. Functional calcium imaging reveals that NLP-40 and AEX-2/GPCR are both necessary for rhythmic activation of these neurons. Furthermore, acute application of synthetic NLP-40-derived peptide depolarizes the GABAergic neurons in vivo. Our results show that NLP-40 carries the timing information from the pacemaker via calcium-dependent release and delivers it to the GABAergic neurons by instructing their activation. Thus, we propose that rhythmic release of neuropeptides can deliver temporal information from pacemakers to downstream neurons to execute rhythmic behaviors.

136. Tasting Light: A *C. elegans* Pharyngeal Neuron Senses Hydrogen Peroxide Produced by Light. **Nikhil Bhatla**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA, USA.

Organisms use specific proteins to sense light: rhodopsin, phytochrome, xanthopsin, cryptochrome, phototropin and BLUF sensors. *C. elegans* uses a novel protein: the worm's avoidance of shortwave light requires LITE-1, a *Drosophila* gustatory GPCR homolog (Edwards..Miller 2008). We found that light inhibits pharyngeal pumping. This inhibition is partly independent of *lite-1*, indicating that a second light-sensing mechanism functions in the worm. Through laser ablation of pharyngeal neurons we found that loss of the I2 neuron increases the time it takes for light to inhibit pumping. Furthermore, light rapidly increases I2 calcium. I2 calcium increases even in *unc-13* mutants defective in synaptic signaling, suggesting that I2 might directly sense light. Two I2-expressed genes are required for both rapid inhibition of pumping and I2 calcium increase: *gur-3*, a *lite-1* paralog, and *prdx-2*, a peroxiredoxin. Since peroxiredoxins are antioxidants, we tested the effect of hydrogen peroxide (H_2O_2) on pumping. H_2O_2 odor elicits both avoidance and pumping inhibition. H_2O_2 odor and light act through the same mechanism to inhibit pumping, as both depend on *gur-3* and *prdx-2*. *lite-1* is required for H_2O_2 avoidance, and the *lite-1 gur-3* double mutant is completely defective in the pumping response to H_2O_2, just like with light. One possibility is that light produces H_2O_2. H_2O_2 can be detected by the generation of Prussian blue (Saito..Yoshida 2007). We found that shortwave light also generates Prussian blue in water, likely through the production of H_2O_2 as addition of catalase reduces pigment formation. Downstream of *gur-3* and *prdx-2*, *eat-4*, a vesicular glutamate transporter, functions in I2 to inhibit pumping, suggesting that I2 releases glutamate in response to light/H_2O_2. Two glutamate receptors, *avr-15* and *glc-2*, are required for the rapid inhibition of pumping. These receptors are expressed in pharyngeal muscle and function there to inhibit pumping in

response to light/H_2O_2. Overall, these results describe the molecular and cellular components of a light-sensing circuit. Sensing light via an H_2O_2 intermediary suggests that light-sensing in the worm might have evolved from a pre-existing H_2O_2 avoidance mechanism.

137. The connectome of the anterior nervous system of the *C. elegans* adult male. Travis A. Jarrell[1], Yi Wang[1], Adam E. Bloniarz[1], Steven J. Cook[1], Christopher A. Brittin[1], Kenneth Nguyen[1], Meng Xu[1], David H. Hall[2], **Scott W. Emmons[1,2]**. 1) Dept Gen, Albert Einstein Col Med, Bronx, NY; 2) D. Purpura Dept Neuroscience, Albert Einstein Col Med, Bronx, NY.

The innate behavioral repertoires of the two sexes of a species are guided by differing reproductive priorities. *C. elegans* male copulation is controlled by a neural network in the tail in which a majority of the neurons and muscles are specific to the male. But known differences in olfactory preferences and exploratory tendencies emanate from behaviors controlled by circuits in the head, where the complement of neurons is nearly identical in the two sexes. We determined connectivity in the anterior nervous system of the adult male from a 1,500 section-long thin section EM series extending from near the tip of the nose, through the nerve ring, and part way into the retrovesicular ganglion. This region contains the bulk of the synapses, excluding ventral cord nmj's. To make a comparison to the hermaphrodite, we re-reconstructed legacy Cambridge micrographs using our software, which allows us to score synaptic weights (see abstract by Cook et al). While our analysis is at an early stage, we can already see the essential result: in the adjacency matrices that display the connectivity, it is difficult to spot differences that appear greater than would be expected given the inherent variability of neuronal wiring. Known circuits in navigation and other responses are conserved. Thus behavioral differences likely emerge from differing circuit properties rather than differing connectivity. There are two possible exceptions: AIM synapses onto AIB and RIA synapses onto RIB in the male only. One set of male-specific synapses expected involves the male-specific head CEM sensory neurons, and the tail EF interneurons, which receive extensive input from the copulatory circuits in the tail and extend processes through the ventral nerve cord into the nerve ring. Both of these neuron classes have as their strongest targets the AVB command interneurons for forward locomotion. This suggests one of their functions may be to inhibit forward locomotion when a hermaphrodite is sensed or during copulation. They make additional connections to be further explored.

138. The degenerin family ion channel UNC-8 promotes activity-dependent remodeling of GABAergic synapses in *C. elegans*. **Tyne W Miller[1]**, Sarah C Petersen[1], Megan E Gornet[1], Ying Wang[2], Han Lu[2], Cristina Matthewman[2], Laura Bianchi[2], Janet E Richmond[3], Shohei Mitani[4], Sayaka Hori[4], David M Miller[1]. 1) Department of Cell & Developmental Biology, Vanderbilt University, Nashville, TN; 2) Department of Physiology & Biophysics, University of Miami, Miami, FL; 3) Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 4) Department of Physiology, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan.

Synaptic networks are extensively remodeled in the developing brain by mechanisms that require neural activity. Members of the DEG/ENaC (Degenerin/Epithelial sodium channel) family are known to modulate plasticity in the mammalian brain, but the molecular events that regulate this effect are poorly defined. Here we describe an activity-dependent mechanism in which the DEG/ENaC protein, UNC-8, promotes synaptic remodeling in *C. elegans*. GABAergic Dorsal D (DD) motor neurons reverse polarity by relocating synapses from ventral to dorsal muscles in the first larval stage. This pathway is blocked by the UNC-55/COUP-TF transcription factor in Ventral D (VD) GABAergic neurons, which ectopically remodel in an *unc-55* mutant. We exploited this mutant phenotype in a cell-specific profiling strategy to identify UNC-55-regulated transcripts. This approach revealed fifty UNC-55 targets that are required for GABA neuron remodeling, including UNC-8. We find that UNC-8 functions in DD neurons where it is localized near ventral DD synapses. Our results show that UNC-8 is required for removing ventral synapses in a mechanism that depends on GABA. A necessary role for neurotransmitter release is also suggested by our finding that a voltage-gated calcium channel subunit, UNC-2, promotes remodeling. *In vitro* reconstitution of an UNC-8 channel results in robust cation transport activity that is strongly inhibited by extracellular calcium. The negative effect of calcium on UNC-8 function is consistent with a model in which depletion of extracellular calcium by UNC-2 at active GABAergic synapses effectively relieves the calcium block and thereby induces UNC-8 activation. Our results support a model in which UNC-8 functions as an activity sensor in GABAergic DD motor neurons to trigger the deconstruction of ventral synapses, thus promoting the remodeling process.

139. Epidermal Growth Factor signaling mediates heat-induced quiescence in *C. elegans*. **Andrew Hill**, Cheryl Van Buskirk. California State University Northridge, Northridge, CA.

Despite widespread recognition of the importance of sleep, little is known about the molecular mechanisms that govern behavioral quiescence. We are using *C. elegans* to understand sleep behavior on a molecular basis. Gain-of-function studies have implicated the Epidermal Growth Factor (EGF) family of ligands as inducers of sleep-like states in *C. elegans*, *Drosophila*, and mammals, suggesting that EGF signaling may be part of a deeply conserved quiescence program. It has been shown that *C. elegans* exhibits sleep-like behavior during molting, and loss-of-function studies indicate that EGF signaling may play a minor or partially redundant role during these periods of lethargus. *C. elegans* also experiences states of behavioral quiescence in response to certain stressors such as heat. Here, we demonstrate the role of EGF signaling in heat-induced quiescence.

We and others have observed that in response to heat shock, *C. elegans* will cease pharyngeal pumping and locomotion, a transient effect that precedes the HSF-1-dependent transcriptional response to heat. We have found this behavior to be dependent on the thermosensory AFD neurons and independent of their synaptic targets, pointing to an endocrine signaling mechanism. Surprisingly, this behavioral quiescence is also dependent on known components of the EGF-mediated sleep pathway, including LIN-3/EGF, its receptor LET-23/EGFR and downstream phosphorylation target PLC-3, and the sleep-inducing ALA neuron. We propose that in response to heat, AFD neurons signal release of EGF from its membrane-bound precursor LIN-3. Soluble EGF then binds EGF receptors on the ALA neuron to induce quiescence.

Current efforts to characterize the *C. elegans* heat response are aimed at identifying the relevant neuropeptide(s) and protease(s) that bridge the gap between AFD thermosensation and EGF shedding. We are also testing whether the EGF pathway is engaged by other stressors. This unexpected connection between cellular stress and a conserved EGF-mediated quiescence program offers an exciting possible insight into the ancient function of sleep behavior.

140. Heterochronic Genes and Developmental Timing in *C. elegans*. **Victor Ambros**. Univ. of Massachusetts Medical School.

141. Feedback Control of Gene Expression Variability in the *Caenorhabditis elegans* Wnt pathway. **Ni Ji**[1], Teije Middelkoop[2], Remco Mentink[2], Hendrik Korswagen[2], Alexander van Oudenaarden[2,3]. 1) Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA; 2) Hubrecht Institute, KNAW, University Medical Center, Utrecht, Utrecht, the Netherlands; 3) Department of Physics and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Variability in gene expression contributes to phenotypic heterogeneity even in isogenic populations. Here, we use the stereotyped development of the *Caenorhabditis elegans* Q neuroblast to probe endogenous mechanisms that control gene expression variability. Posterior migration of the left Q neuroblast (QL) depends on a canonical Wnt signaling pathway which functions cell-autonomously to activate the expression of *mab-5/Hox*. To identify the mechanism that ensures robust transcriptional activation, we used single molecule Fluorescent *In Situ* Hybridization (smFISH) to quantify the transcript levels of *mab-5* and other genes of the Wnt pathway in QL in a series of wild type and Wnt signaling mutant strains. Interestingly, we found that mutants that perturb Wnt signaling frequently exhibited increased variability in *mab-5* expression. Unexpectedly, these mutants also perturbed the levels of Frizzled (*mig-1/Fz*, *lin-17/Fz* and *mom-5/Fz*) expression in QL, indicative of feedback regulations within the Wnt pathway. Combining computational network inference with quantitative gene expression profiling, we deduced a most probable network topology consisting of interlocking positive and negative feedback loops targeting the Frizzled receptors and *mab-5*. Interestingly, model analysis suggests that positive feedback may cooperate with negative feedback to reduce variability, while keeping the expression of the target gene (*mab-5*) at elevated levels. A minimal model of this signaling network predicts changes in gene expression variability across various mutants. Our results challenge the conventional view of the Wnt signaling pathway as a feedforward cascade and implicate gene regulatory network as an effective mechanism to ensure developmental robustness. In an ongoing effort, we attempt to elucidate the biochemical nature of these genetic interactions.

142. Integral nuclear pore components associate with Pol III-transcribed genes and are required for Pol III transcript processing in *C. elegans*. **Kohta Ikegami**[1,2], Jason Lieb[1,2]. 1) Department of Biology, University of North Carolina Chapel Hill, Chapel Hill, NC; 2) Carolina Center for Genome Sciences, University of North Carolina Chapel Hill, Chapel Hill, NC.

Nuclear pores associate with active protein-coding genes in yeast and have been implicated in transcriptional regulation. Here, we show that in addition to transcriptional regulation, key components of *C. elegans* nuclear pores are required for processing of small non-coding RNAs transcribed by RNA Polymerase III (Pol III). Chromatin immunoprecipitation of NPP-13 and NPP-3, two integral components of the nuclear pore, and importin-b IMB-1, provides strong evidence that this requirement is direct. All three proteins associate specifically with tRNA and small nucleolar RNA (snoRNA) genes undergoing Pol III transcription. These pore components bind immediately downstream of the Pol III pre-initiation complex, but are not required for its recruitment. Instead, NPP-13 are required for the cleavage of snoRNA precursor transcripts into mature snoRNAs, whereas processing of Pol II transcripts occurs normally. Our data suggest that integral nuclear pore proteins act to coordinate transcription and Pol III transcript processing in *C. elegans*.

143. The regulation of global histone acetylation during meiotic prophase in C.elegans. **Jinmin Gao**, Hyun-Min Kim, Andrew E. Elia, Stephen J. Elledge, Monica P. Colaiácovo. Harvard Medical School, Boston, MA.

Histone acetylation is well known for its role in transcriptional activation. Global changes in histone acetylation have been reported for mutants of enzymes involved in Acetyl-CoA metabolism in yeast. However, it is not known how global regulation of histone acetylation might be involved in specific cell differentiation processes. Here we report that CRA-1, a NatB domain-containing protein conserved from yeast to humans, promotes global histone acetylation in C. elegans. Acetyl-CoA regulator ACER-1 is identified as a binding-partner of CRA-1, and CRA-1 is required for maintaining a normal level of acetyl-CoA, through which it modulates levels of histone acetylation. Interestingly, CRA-1 expression in the germ cells is specifically inhibited before entrance into meiosis, and the meiosis-specific protein XND-1 is required for this inhibition. Our studies demonstrate that the proper expression pattern of CRA-1 contributes to a dynamic regulation of histone acetylation during meiotic prophase and affects the efficiency of programmed DNA double-strand break formation on the X chromosomes. Our findings reveal a novel pathway for the regulation of global histone acetylation and demonstrate the importance of dynamic regulation of histone acetylation during meiotic prophase.

144. Tissue integrity and laminopathic phenotypes correlate with subnuclear heterochromatin positioning. **A. Mattout**, SM. Gasser. FMI, Basel, Switzerland.

We have previously shown that wild-type lamin helps organize the subnuclear position of heterochromatin, and that a point mutation in lamin, Y59C, which in humans leads to Emery-Dreifuss muscular dystrophy, impairs the proper muscle-specific redistribution of a heterochromatic array away from the nuclear periphery. We also found that this muscle-specific misorganization of heterochromatin correlated with transcriptional defects and with defective locomotion and muscle integrity (Mattout et al, Curr Biol, 2011). It has remained unclear, however, whether the chromatin misorganization was a cause of the observed physiological defects, or a secondary effect due to an impaired gene expression. In order to clarify this, we took advantage of the cec-4 deletion mutant recently characterized in our laboratory, which specifically releases H3K9me-containing heterochromatin from the nuclear periphery. This release occurs in embryos and does not necessarily provoke transcriptional reactivation. We combined this mutation with expression of the lamin Y59C mutant, in a strain bearing a muscle-specific reporter array, which is heterochromatic due to its large size. We find that combining the cec-4 deletion with the Y59C lamin mutation restores the proper positioning of the muscle-specific heterochromatic array in developing muscle cells, and interestingly, also rescues the impaired locomotion phenotype of the Y59C lamin mutant. This result argues that the spatial reorganization of heterochromatin in the nucleus during differentiation can impact tissue integrity. Indeed, the failed tissue-specific release of heterochromatin in muscle, which stems from a dominant mutation in lamin, may indeed be responsible for the range of phenotypes correlated with lamin-related genetic diseases.

145. A Comprehensive Expression Map of Lysine Methyltransferases Reveals Germline-specific Function of *set-17*. **Christoph G. Engert**[1], Alexander van Oudenaarden[1,2], Bob Horvitz[1]. 1) Dept. Biology, MIT, Cambridge, MA; 2) Dept. Physics, MIT.

Posttranslational modification of histone tails is fundamental to controlling access to DNA. Lysine methyltransferases (KMTs) methylate lysine residues in histone tails to specify transcription or silencing. In humans, KMTs have been implicated in important biology, such as fertility, and disease, such as cancer. The SET domain is catalytically active in KMTs. The C. elegans genome encodes 38 putative KMTs, most of which have plausible mammalian orthologs. In C. elegans, four KMTs are required for viability and four KMTs have been implicated in germ cell fate specification; individual mutation or RNAi inactivation of the remaining KMTs revealed no other gross defects.[1] To analyze KMT function, we determined the endogenous mRNA expression profile of all KMTs in early L1 larvae using single molecule fluorescence in situ hybridization (smFISH). For each KMT, we created a smFISH probe-set of 48 DNA oligos. These probe-sets now constitute a comprehensive freely available resource to study endogenous KMT expression. In L1 larvae, most KMTs are expressed in a tissue-specific manner. Only ten KMTs are expressed broadly throughout the animal. Four KMTs display a muscle-specific expression pattern and four KMTs are expressed exclusively in the germline. Overall, 22 KMTs are expressed in the two primordial germ cells, Z2 and Z3. To investigate KMT function in the germline, we determined the brood size of all available mutants of germline-expressed KMTs. Four single KMT mutants show brood size defects: *met-1*, *met-2, set-17* and *set-32. set-17* is an uncharacterized broadly expressed KMT with a PR-type SET domain, the closest mammalian orthologs of which are PRDM9 and PRDM7. Loss of *set-17* causes a reduction in sperm number. A rescuing *set-17*::GFP single-copy transgene shows that SET-17 localizes in sparse foci (~5-8) to diplotene nuclei in both sperm and oocyte precursors. Based on these data, we propose that *set-17* functions in germ cell maturation or meiosis. The expression data and smFISH resource provide the basis for further specific investigation of the function of KMTs in C. elegans biology. 1 Andersen and Horvitz, Development, 134, 2991-9, 2007.

146. MRG-1 acts as an epigenome interpreter of Lys36 methylation on histone H3. **Teruaki Takasaki**[1,2], Thea Egelhofer[2], Andreas Rechtsteiner[2], Hiroshi Sakamoto[1], Susan Strome[2]. 1) Dept. of Biology, Graduate School of Science, Kobe University, Kobe, Japan; 2) MCD Biology, University of California Santa Cruz, CA.

We previously identified the autosome-associated protein MRG-1 as an essential maternal factor for proper germline development, yet its molecular function remains unclear (Takasaki et al., 2007). MRG-1 contains a CHROMO domain, which is found in numerous proteins that associate with methylated histones. In our search for the element(s) that recruits MRG-1 to autosomes, we found that oocyte and sperm chromosomes arrive in the zygote marked with methylated H3K36, and that MRG-1 accumulates predominantly on those methylated chromosomes. Genome-wide chromatin immunoprecipitation analysis in early embryos revealed that methylation of H3K36 is propagated on the body of germline-expressed genes by the histone methyltransferase MES-4 in an epigenetic manner (Rechtsteiner et al., 2010; Furuhashi et al., 2010). The distribution of MRG-1 exhibits a high correlation with the distributions of both MES-4 and H3K36me, and the accumulation of MRG-1 on germline-specific genes depends on MES-4-generated H3K36me. These findings suggest that MRG-1 recognizes germline-expressed genes using the epigenetic mark H3K36me generated by MES-4. Strikingly, we found that germline-specific loci exhibit an atypical H4K16 acetylation pattern, which is dependent on MES-4. By analogy to the *Drosophila* homolog of MRG-1, which acts in the dosage compensation complex (the MSL complex) to generate H4K16ac on the X chromosome, it is likely that MRG-1 contributes to the germline-gene-specific H4K16ac pattern. Supporting this idea, we identified MYS-2, which is a homolog of another component of the MSL complex, as an H4K16 acetyltransferase. Furthermore, we found that loss of MYS-2, like loss of MES-4 and MRG-1, suppresses ectopic expression of germline genes in somatic cells of *mep-1* mutant larvae. This suggests that H4K16 acetylation serves a pivotal role in germline development. Taken together, our results suggest that MRG-1 interprets the epigenetic H3K36me landmark and as part of an MSL-like complex generates H4K16ac specifically at germline-expressed genes.

147. Control of DNA accessibility by histone H2A variants revealed using *in vitro* analysis of *C. elegans* nucleosomes. **Ahmad N. Nabhan**[1], Francisco Guerrero[1], Geeta Narlikar[2], Diana Chu[1]. 1) SFSU, San Francisco, CA; 2) UCSF, San Francisco CA.

Though histone variants play vital roles in gene regulation in different tissues, mechanisms of how they do so remain a mystery. The *C. elegans* H2A variants HTAS-1 and HTZ-1 are an interesting case study because they localize to distinct transcriptional environments: HTZ-1 is incorporated in many cell types to regions poised for transcriptional activity while HTAS-1 is found only in sperm during a period of global repression and chromatin compaction. We hypothesize each variant is incorporated to regulate expression differentially by how they alter DNA accessibility. Variants can influence chromatin structure by altering parameters like reversible DNA unwrapping, which provides transient access to occluded DNA, and stability, which influences the turnover of histones. To assess these features, we reconstituted *C. elegans* nucleosomes *in vitro*. Canonical histones and the H2A variants HTZ-1 and HTAS-1 were cloned and expressed in bacteria, reconstituted into octamers, and combined with DNA to form nucleosomes. To determine H2A variant influence on DNA unwrapping, we analyzed restriction enzyme accessibility to show HTZ-1 increases reversible DNA unwrapping compared to H2A. This suggests that HTZ-1 maintains DNA in a more 'open' state for its role in transcription. On the other hand, HTAS-1 decreases the unwrapping rate, suggesting HTAS-1 induces a more 'closed' state, consistent with its incorporation during sperm-specific chromosome compaction. We also measured the effects of variants on nucleosome stability by monitoring FRET pairs on labeled nucleosomes under increasing salt concentrations. Surprisingly, HTZ-1 nucleosomes are more resistant to NaCl induced dissociation than H2A nucleosomes, suggesting HTZ-1 nucleosomes are more stable than those bearing H2A. From these results we suggest that more stable HTZ-1 nucleosomes are maintained at specific chromatin sites to modulate DNA unwrapping to influence transcription. FRET studies measuring the role of HTAS-1 in nucleosome stability are underway. Overall, our *in vitro* studies show that H2A variants in *C. elegans* influence stability and DNA accessibility independently, presenting a potential mechanism for their observed roles in gene regulation *in vivo*.

148. Epigenetic reprograming during germ line development. B. Hargitai, I. Kalchhauser, **S. Gutnik**, R. Ciosk. Friedrich Miescher Institute, Basel, Switzerland.

Cell fate reprograming is critical for development. A profound reprograming takes place during germ cell development, which is why germ cells, and the various pluripotent cell lines derived from them, have been invaluable for dissecting the mechanisms controlling pluripotency. One conserved mechanism for restricting pluripotency is the formation of facultative heterochromatin by the Polycomb Repressive Complex 2 (PRC2). In mammals, subsets of PRC2-

repressed genes become activated at different times during germ cell development, and thus, the PRC2-mediated repression of specific targets can be reversed. However, the mechanisms regulating both the reactivation and the specificity for particular targets remain unresolved. Similarly to mammals, the C. elegans PRC2 (consisting of the MES-2, -3, and -6 proteins) is required during germ cell development. Using this model to understand epigenetic reprograming, we find that the reactivation of a subset of PRC2-repressed genes is mediated by the Notch signaling pathway. GLP-1^{Notch} signaling appears to directly relay information to chromatin, as the putative Notch-response elements in the promoter region of Notch-regulated genes are required for the reactivation. Because a number of the identified Notch-activated genes impact self-renewal and/or differentiation in the germ line, we propose that Notch signaling affects these cell fate events, at least in part, by inducing epigenetic remodeling.

149. Spatial Control of Gene Expression in the *C. elegans* Intestine. **Aidan Dineen**, Jim McGhee. University of Calgary, Alberta, Canada.

Our goal is to understand the molecular mechanisms that control transcription in the *C. elegans* intestine. The mature intestine is composed of 20 cells, arranged in nine rings along the A/P axis of the animal. All intestinal cells are derived from a single progenitor (E), specified by the GATA transcription factors END-1 and END-3. END-1/END-3 activate expression of the GATA factor ELT-2, which drives expression of most terminally differentiated genes in the organ. Some intestinal genes are expressed only in a subset of cells; for example, *pho-1* is expressed only in the posterior 14 cells. Such observations lead to the question: how does gene expression in the intestine become spatially patterned? ELT-2 is expressed uniformly in all intestinal cells, suggesting that patterned genes could be regulated by a combination of ELT-2 with some other trans acting factor(s). Previous studies have shown that the Wnt/b-catenin/POP-1 asymmetry pathway is necessary to repress *pho-1* expression in the anterior six cells and specify the anterior fate of these cells. Currently, it is unclear if this pathway patterns *pho-1* expression at the level of cell fate or by direct action on the *pho-1* promoter. Our evidence suggests that the heterochronic gene *lin-14* also functions to repress anterior *pho-1* expression. LIN-14 is expressed in the intestine from late embryogenesis until the end of L1 and is required for the binucleation of the posterior 14 cells at the end of this stage. We observe *pho-1* reporter expression initially in all intestinal cells in early/mid stage L1 larvae, although expression is weaker in int-II and IX and occasionally in int-I. Expression of the *pho-1* reporter in the anterior six cells decreases by late L1 and later developmental stages suggesting a correlation with the patterned binucleation event. RNAi mediated knockdown of *lin-14* function results in increased *pho-1* reporter expression in int-I and II. Analysis of the *pho-1* promoter identifies three putative LIN-14 binding sites suggesting that this regulation may be by direct repression of transcription. In summary, our results suggest a novel role for the heterochronic gene *lin-14* in spatial patterning of gene expression in the intestine.

150. Defining regulatory pathway coupling cell division timing and cell fate differentiation in *C. elegans* by automated lineaging . **Vincy Wing Sze Ho[1]**, Ming-Kin Wong[1], Xiaomeng An[1], Jiaofang Shao[1], Kan He[1], Dongying Xie[1], Jinyue Liao[2], Long Chen[3], Xiaotai Huang[3], Leanne Chan[3], King Chow[2], Hong Yan[3], Zhongying Zhao[1]. 1) Department of Biology, Hong Kong Baptist University, Hong Kong; 2) Division of Life Science, the Hong Kong University of Science and Technology, Hong Kong; 3) City University of Hong Kong, Hong Kong.

Coordination of division pace among different cells is essential for proper formation of various tissues and organs during metazoan development. Failure in the coordination frequently leads to tumorous growth or abnormal cell death. How cell division paces are regulated to accommodate cell fate differentiation remains poorly understood. *C. elegans* embryogenesis provides a unique opportunity to address the issue due to its invariant development. To identify the regulatory proteins coupling cell division and fate determination, we performed a high-content screening of defects in embryonic cell lineage and cell fate determination for around 400 *C. elegans* genes using automated lineaging. We prioritized the gene list based on their conservation and potential defects during embryogenesis after inactivation. We inactivated each of the 400 genes through RNAi by injection and imaged three embryos per gene followed by lineaging analysis. The injected strain carries both a lineaging and a tissue marker, allowing us to simultaneously trace cell divisions and cell fate differentiation for every minute of embryogenesis. We curated embryonic cell lineage up to 350 cell stage with two replicates per gene. Preliminary analysis of cell division and/or expression phenotypes have not only confirmed function of genes with known function, but also helped assign functions to uncharacterized genes. Systematic analysis of defects in cell division and cell fate differentiation allows us to assemble regulatory pathway coupling the two biological processes. Our dataset provide an information-rich resource for further prediction of gene functions in *C. elegans* embryogenesis which likely have direct relevance to human biology. Methods for inference of gene network coupling cell division and cell fate determination based on our accumulated data will be presented.

151. Linking dosage compensation complex assembly to X chromosome gene regulation. **Bayly Wheeler[1,2]**, Christian Frøkjær-Jensen[2,3,4], Erik Jorgensen[2,3], Barbara J. Meyer[1,2]. 1) Department of Molecular and Cell Biology, University of California, Berkeley 94720; 2) Howard Hughes Medical Institute; 3) Department of Biology, University of Utah, Salt Lake City, UT 84112-0840; 4) Department of Biomedical Science, University of Copenhagen, Denmark.

Dosage compensation (DC) is an essential process required to balance levels of gene expression between the two X chromosomes of females and the single X of males. As DC controls gene expression across the entire X, it serves as an exemplary system to understand mechanisms of long-range gene regulation. In *C. elegans,* DC is achieved by reducing gene expression from both hermaphrodite X chromosomes by half. DC is enacted by the DC complex (DCC), a condensin complex that is recruited to both hermaphrodite X chromosomes through DNA recruitment elements called *rex* sites. How these *rex* sites recruit the DCC, facilitate spreading to neighboring territories, and repress gene expression remains unknown. To understand the relationship between DCC binding and function, we used RNA-seq to identify dosage-compensated genes with high confidence and resolution. Consistent with previous analyses, we find that DCC-regulated genes are interspersed with genes that are not regulated by the DCC. DCC binding within a gene is not predictive of whether that gene will be dosage compensated. Since the DCC acts at a distance to control gene expression, we sought to identify the factors that confer DC status. We have monitored the expression of single-copy gfp transgenes integrated onto autosomes and onto X at different distances from endogenous and engineered *rex* sites in wild-type and DCC-defective worms. We found that the DCC represses transgene expression at six ectopic sites on X but not on autosomes. Thus, DC controls the expression of introduced and endogenous genes. Furthermore, we show that promoter sequence is not sufficient to determine DC status. We are conducting experiments to address the role of local regulatory elements and chromosome domain structure, as determined by chromosome conformation capture, in dictating DC status. This work will illuminate how regulatory elements on the *C. elegans* X chromosome achieve appropriate patterns of gene expression chromosome-wide.

152. Silencing of Germline-Expressed Genes by DNA Elimination in Somatic Cells and a Mechanism for Selective DNA Segregation. **Richard E Davis**[1], Jianbin Wang[1], Makedonka Mitreva[2], Matthew Beriman[3], Alicia Thorne[1], Vincent Magrini[2], Stella Kratzer[1], Maggie Balas[1], Georgios Koutsovoulos[4], Sujai Kumar[4], Mark Blaxter[4]. 1) Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO, US; 2) The Genome Institute, Washington University School of Medicine, St. Louis, MO, US; 3) The Wellcome Trust Sanger Institute, Genome Campus, Hinxton, UK; 4) Institute of Evolutionary Biology, University of Edinburgh, UK.

Chromatin diminution is the programmed elimination of specific DNA sequences during development. It occurs in diverse species, but the function(s) of diminution and the specificity of sequence loss remain largely unknown. Diminution in the nematode *Ascaris suum* occurs during early embryonic cleavages and leads to the loss of germline genome sequences and the formation of a distinct genome in somatic cells. We found that ~43 Mb (~13%) of genome sequence is eliminated in *A. suum* somatic cells, including ~12.7 Mb of unique sequence. The eliminated sequences and location of the DNA breaks are the same in all somatic lineages from a single individual, and between different individuals. At least 685 genes are eliminated. These genes are preferentially expressed in the germline and during early embryogenesis. Soma-specific elimination provides a unique mechanism of gene repression and differentiation between germline and soma. We found no temporal or any other correlation of small RNAs with diminution. Preliminary data suggest that a possible mechanism of differential segregation of DNA following the breaks may be due to differential deposition of CenpA (as well as other histone marks) on retained vs eliminated DNA sequences. For comparison, we have also sequenced the germline and somatic genomes of a second nematode with a single large haploid chromosome that undergoes DNA elimination, *Parascaris univalens*. These data will be discussed. Overall, our studies suggest that diminution is a unique mechanism of germline gene regulation that specifically silences genes involved in gametogenesis and early embryogenesis through their elimination and that this process contributes to the soma-germline differentiation.

153. Exploring *C. elegans* heterochromatin through the HP1 homolog HPL-2. **Jacob M. Garrigues**, Susan Strome. MCD Biology, UC Santa Cruz, Santa Cruz, CA.

Formation of heterochromatin is important for organization of the genome and regulation of gene expression. Heterochromatin is typically concentrated at pericentric regions, where it plays important roles in helping to define centromeres. The holocentric nature of *C. elegans* chromosomes raised interesting questions about the distribution of worm heterochromatin. Some hallmarks of heterochromatin include the enrichment of Heterochromatin Protein 1 (HP1) and histone H3 methylated at Lysine 9 (H3K9me), which HP1 can directly bind. To infer the distribution of heterochromatin in worms, we performed chromatin immunoprecipitation followed by microarray analysis (ChIP-chip) of the worm HP1 homolog HPL-2, and compared its distribution to H3K9me. We observed that HPL-2, like H3K9me, is enriched on autosomal “arms” and depleted from the X chromosome. Consistent with the autosomal arms being worm heterochromatin, they contain more repetitive DNA elements compared to chromosome interiors. In agreement with previous work showing worm HP1 proteins to be dispensable for centromere formation (Yuen et al. Current Biology 21: 1800-1807, 2011), the distribution of HPL-2 lacks a clear relationship to that of HCP-3, a centromeric histone H3 variant. Previous studies in other systems have shown that the distribution of HP1 highly correlates with both H3K9me2 and H3K9me3. Interestingly, while correlating highly with H3K9me2, HPL-2 correlates poorly with H3K9me3. To investigate the dependence of HPL-2 association on H3K9me, we performed ChIP-chip of HPL-2 in *met-2 set-25* double mutants, which were shown by mass spectrometry to lack H3K9me (Towbin et al. Cell 150: 934-947, 2012). Surprisingly, the distribution of HPL-2 in *met-2 set-25* mutants resembles that in wild type. This finding suggests that HPL-2 can localize to and persist on chromatin independently of H3K9me. Consistent with the H3K9me independence of HPL-2, we also observed that *hpl-2* mutants display significantly stronger germline phenotypes (transgene desilencing and sterility) than *met-2 set-25* double mutants. Taken together, these results suggest that HPL-2 has functions independent of H3K9me, and that H3K9me may not be an essential component of worm heterochromatin.

154. Aurora A is essential for the organization of the female meiotic spindle in late anaphase. **Eisuke Sumiyoshi**, Yuma Fukata, Asako Sugimoto. Graduate School of Life Sciences, Tohoku University, Sendai, Miyagi, Japan.

In animal mitotic cells, spindles are formed mainly from microtubules assembled at centrosomes, with an additional contribution of microtubules assembled around condensed chromosomes. In contrast, female meiotic spindles are assembled independently of centrosomes, because centrosomes are eliminated during oogenesis. In *C. elegans*, the formation of female meiotic spindle is initiated inside the nucleus of mature oocytes, and then the spindle undergoes dynamic structural changes during meiosis progression. In late anaphase, the meiotic spindle is re-organized and microtubules are formed between chromosomes to segregate them. It has been reported that the gamma-tubulin complex, the major microtubule nucleator during mitosis, is dispensable for the formation of female meiotic spindles in *C. elegans*.

We previously reported that, in mitosis of *C. elegans* zygotes, the assembly of microtubules around condensed chromosomes requires the kinase-inactive form of Aurora A (AIR-1) but not gamma-tubulin (TBG-1). Here, we examined the role of AIR-1 in the assembly of microtubules composing female meiotic spindles. Immunostaining revealed that the AIR-1 protein localized to meiotic spindle microtubules and cytoplasmic microtubules in zygotes undergoing meiotic divisions, whereas the localization of phosphorylated (kinase-active) AIR-1 is limited to microtubules between chromosomes in meiotic anaphase. *air-1(RNAi)* did not inhibit the initial stage of meiotic spindle formation, but in late anaphase the reorganization of microtubules between chromosomes was severely affected, resulting in chromosome segregation defects. In addition, *air-1(RNAi)* significantly reduced the cytoplasmic microtubules at late anaphase of female meiosis. Live-imaging and photo-bleaching experiments revealed that cytoplasmic microtubules flowed into the meiotic spindle in which microtubules were rapidly turned over. Taken together, we propose that cytoplasmic microtubules stabilized and/or formed dependently on AIR-1 may contribute to the microtubule reorganization of the female meiotic spindle in late anaphase.

155. Formation of the nuclear envelope as a distinct subdomain of the endoplasmic reticulum requires spatial regulation of Lipin activation. **Shirin Bahmanyar**[1], Ronald Biggs[1], Jon Audhya[3], Arshad Desai[1], Jack Dixon[2], Thomas Mullert-Reichert[4], Karen Oegema[1]. 1) Ludwig Institute for Cancer Research, San Deigo, CA; 2) University of California, San Diego, San Deigo, CA; 3) University of Wisconsin-Madison Medical School, Madison, Wisconsin; 4) Dresden University of Technology, Dresden, Germany.

The nuclear envelope is a subdomain of the endoplasmic reticulum (ER) that forms a double membrane sheet surrounding chromatin. In mitosis, the

nuclear envelope retracts into the ER to promote spindle assembly. While the nuclear envelope and ER are within a contiguous membrane system, they execute distinct functions. Here, we use the first division of the C. elegans embryo to determine how the nuclear envelope forms as a functionally distinct subdomain of the ER. We show that localized activation of the phosphatidic acid phosphatase lipin alters flux within the de novo phospholipid synthesis pathway to distinguish the phospholipid content in the nuclear envelope from the ER. Lipin lies at a branch point for production of structural phospholipids versus phosphatidylinositol (PI). Activation of lipin by the protein phosphatase CNEP-1 specifically at the nuclear envelope excludes synthesis of PI in the nuclear envelope by biasing flux towards structural phospholipids. Biochemical analysis of phospholipids in cnep-1 mutant worms shows an increase in PI levels, whereas all other phospholipids remain unchanged. Control of lipin by CNEP-1 prevents the ER from forming excess sheet-like structures when a nuclear envelope is present. In the absence of CNEP-1, excess sheet like structure encase the nuclear envelope and perturb nuclear envelope breakdown (NEBD) in mitosis. Reducing PI synthesis in a CNEP-1 mutant suppresses excess ER sheets and NEBD defects. These results show that phospholipid synthesis must be spatially regulated within the ER and nuclear envelope to partition the ER into distinct subdomains.

156. Homolog pairing and feedback control during meiosis are mediated through CHK-2 phosphorylation of pairing center proteins. **Y. Kim**[1,2], A. F. Dernburg[1,2]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

During meiotic prophase, each chromosome must pair and undergo recombination with its homolog. In C. elegans, homolog pairing is mediated by Pairing Centers (PCs), special regions near one end of each chromosome. CHK-2, a homolog of the mammalian checkpoint kinase Chk2, is essential for homolog pairing, timely synapsis, and recombination. Despite its central role in meiotic prophase, direct substrates of CHK-2 have not yet been identified. Here we demonstrate that CHK-2 is recruited to PCs upon meiotic entry and phosphorylates a family of zinc-finger proteins, HIM-8, ZIM-1, ZIM-2 and ZIM-3, which are required for PC function. Phosphorylation by CHK-2 creates binding sites for the Polo-like kinase PLK-2, which mediates homolog pairing and synapsis. A phospho-specific antibody reveals that PC proteins are modified by CHK-2 at the onset of meiotic prophase, and remain phosphorylated until mid-pachytene, which coincides with the timing of double-strand breaks and crossover recombination. In mutants that disrupt crossover formation, CHK-2-dependent phosphorylation of the PC proteins is prolonged, consistent with other recent evidence for feedback regulation of crossover formation. Interestingly, the zone of the germline with phosphorylated HIM-8 and ZIMs is not extended in mutants that disrupt axial element structure, despite an absence of crossovers. This work illuminates the molecular basis for checkpoint mechanisms that act during meiosis to ensure faithful chromosome inheritance from parents to progeny.

157. Intestinal pathogens hijack the host apical recycling pathway for fecal-oral transmission. **Suzy Szumowski**, Emily Troemel. Biological Sciences, University California San Diego, San Diego, CA.

Exiting from host cells is a critical step for the spread of intracellular pathogens, however this process is poorly understood *in vivo*. Many intracellular pathogens utilize fecal-oral transmission, and these pathogens must exit from the apical side of intestinal cells into the lumen, in order to reach the intestinal tract and be defecated out and consumed by new hosts. Because of the challenges of investigating intestinal cell exit *in vivo*, it is unknown how microbial pathogens directionally exit from polarized cells. We are using *C. elegans* to show how its natural intracellular pathogen *Nematocida parisii* exits from polarized intestinal cells *in vivo* for fecal transmission. *N. parisii* spores exit non-lytically from the intestine on the apical side, with thousands of spores shed per hour into the lumen for defecation. Here we show that *N. parisii* spores enter the vesicle trafficking system of the host intestinal cell and escape via exocytosis. With a genetic screen we found that *N. parisii* hijacks the apical recycling pathway in the intestine in order to exit specifically into the lumen. Spores become coated with the apically polarized small GTPase RAB-11 and then fuse with the apical plasma membrane in a process that requires core components of the exocyst. These findings demonstrate how intestinal pathogens can find the correct way out of their hosts *in vivo*, in order to complete a fecal-oral life cycle.

158. Essential function for the exocyst complex in seamless tube formation. **Stephen Armenti**, Emily Chan, Jeremy Nance. NYU School of Medicine, New York, NY.

Cell polarization is an essential developmental event needed for asymmetric cell division, cell specialization, and morphogenesis. The PAR proteins PAR-3, PAR-6 and PKC-3 polarize many different cell types by establishing a spatially restricted signaling domain. Recent studies have suggested that the PAR proteins are closely linked to regulators of intracellular trafficking. For example, loss of the PAR proteins or members of the exocyst complex, which targets vesicles to discrete regions of the cell surface, prevents single lumen formation in MDCK three-dimensional culture. However, the functional relationship between PAR proteins and the exocyst is poorly understood. In particular, it is unclear which complex functions upstream, or whether the complexes function together, as cell polarity is established.

We characterized the expression and subcellular localization of several components of the exocyst complex. Strikingly, exocyst components SEC-5, SEC-8, and SEC-15 develop cortical asymmetries in polarized cells, including the zygote, early embryonic cells, and epithelial cells, and in each cell type, exocyst components co-localize with PAR-6 and PKC-3. By analyzing exocyst and PAR protein localization in *par* mutant embryos, and in embryos lacking SEC-3, SEC-5, SEC-8 and exocyst regulator RAL-1, we show that the exocyst complex localizes downstream of the PAR proteins and is a likely polarity effector. Although loss of exocyst function did not affect cell polarity, we identified a cell autonomous function for the exocyst in elongation and morphogenesis of the excretory canal. The excretory canal forms an intracellular apical lumen, and lumenogenesis requires fusion of subapically enriched vesicles with the apical surface. We show that the PAR proteins and exocyst complex both localize subapically within the excretory canal, and we demonstrate genetic interactions between exocyst components and genes required for canal lumenogenesis. We propose that the exocyst complex functions downstream of the PAR proteins to elaborate polarity, and may promote the fusion of apical vesicles needed for excretory canal lumenogenesis.

159. A context-specific role for Syndecan/SDN-1 in Wnt-dependent spindle orientation. **Katsufumi Dejima**, Sukryool Kang, Andrew Chisholm. University of California San Diego, La Jolla, CA.

Mitotic spindle orientation determines the cell division axis and is important for animal development. External cues such as Wnts can provide directional information and rotate the mitotic spindle in vivo. However, how extracellular cues drive spindle rotation are regulated during mitosis is poorly

understood. In early *C. elegans* embryos, the Wnt (MOM-2) and MES-1/SRC-1 signaling pathways act partly redundantly to control rotation of the mitotic spindles of the ABar and EMS blastomeres. We have found that the heparan sulfate proteoglycan (HSPG) syndecan/SDN-1 is required for spindle orientation specifically in ABar. Using immunostaining against heparan sulfate (3G10) we found that SDN-1 is the major HSPG expressed in the early embryo. We followed cell lineages in HS synthesis and core protein mutants using semiautomated nuclear tracking. We found that loss of HS biosynthesis (*rib-1*, *rib-2*, *hst-1*) or of *sdn-1* core protein genes resulted in impaired rotation of the ABar spindle. Epistasis analysis indicates that SDN-1 acts in the Wnt pathway in parallel to the SRC-1 pathway. Overexpression of SDN-1 results in increased rotation of the ABar spindle, dependent on DSH-2 and MIG-5, suggesting that SDN-1 can instructively regulate Wnt-spindle signaling. ABar spindle orientation is dependent on Wnt expressed by the C blastomere. We find that functional SDN-1::GFP transiently accumulates on the ABar surface as it contacts the C blastomere, before mitosis, and is bi-directionally internalized during or after anaphase. HSPGs such as syndecan can play a variety of roles in Wnt signaling and have been implicated in negative regulation of EGL-20 signaling in later development. Our data suggests SDN-1 plays a positive role in the MOM-2 Wnt signal, reminiscent of the role of Syndecan-4 in Wnt/PCP signaling. Interestingly, SDN-1 seems to be dispensable for EMS spindle rotation. The EMS spindle rotates towards a pre-existing cell contact with P2, whereas ABar forms a novel contact site with C prior to rotation. As SDN-1 is not required for the formation of the ABar-C contact, we hypothesize that SDN-1 is specifically involved in relaying or amplifying Wnt signaling in the specific context of a newly formed cell contact site.

160. Cell signalling and membrane trafficking - an unbreakable relationship. **Zita Balklava**, Navin Rathnakumar. School of Life and Health Sciences, Aston University, Birmingham, United Kingdom.

Membrane trafficking pathways target various molecules to their specific destinations within the cell as well as in and out of the cell, and thus are essential for fundamental aspects of eukaryotic life. Since the discovery of RNAi/siRNA, several genome-wide screens have been performed in order to identify novel regulators of membrane trafficking and many have found signalling molecules among the regulators of membrane transport, thus strengthening the emerging paradigm that membrane transport and signal transduction are tightly coupled in the cell. However, to what extent membrane trafficking is subjected to regulation by the cellular signalling machinery remains unclear. Recently, we performed a genome-wide screen in C.elegans to identify novel membrane trafficking regulators, and the worm Fibroblast Growth Factor Receptor (FGFR) encoded by egl-15 was the only growth factor receptor found as a candidate regulator of membrane transport in the screen. Strikingly, several of the EGL-15 downstream effectors were also identified as positives in the screen, thus making the FGFR and FGFR-mediated signalling pathway an excellent candidate to exploit in order to understand how membrane transport regulation is accomplished through signal transduction. In this study we used the previously described YP170-GFP trafficking assay to investigate all FGFR activated downstream signalling pathways for their potential role in the regulation of membrane transport. We show that FGFR signalling through PLCg and MAPK pathways disrupt proper trafficking of YP170-GFP. To pinpoint which membrane transport step is regulated by FGFR mediated signalling we further analysed the expression pattern of a number of GFP-tagged intracellular membrane markers in the intestine of WT and FGFR mutant worms by fluorescence microscopy. The results suggest that FGFR controls the early steps of endocytosis through signalling via PLCg pathway.

161. *ttm-1* encodes CDF transporters that excrete zinc from intestinal cells of *C. elegans* and act in a parallel negative feedback circuit that promotes homeostasis. Hyun Cheol Roh[1], Sara Collier[1], Krupa Deshmukh[1], James Guthrie[2], J. David Robertson[2], **Kerry Kornfeld[1]**. 1) Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110, USA; 2) Research Reactor Center and Department of Chemistry, University of Missouri, Columbia, MO 65211, USA.

Zinc is an essential metal involved in a wide range of biological processes, and aberrant zinc metabolism is implicated in human diseases. The gastrointestinal tract of animals is a critical site of zinc metabolism that is responsible for dietary zinc uptake and distribution to the body. However, the role of the gastrointestinal tract in zinc excretion remains unclear. Zinc transporters are key regulators of zinc metabolism that mediate the movement of zinc ions across membranes. We identified a comprehensive list of 14 predicted Cation Diffusion Facilitator (CDF) family zinc transporters in *C. elegans* and demonstrated that zinc is excreted from intestinal cells by one of these CDF proteins, TTM-1B. The *ttm-1* locus encodes two transcripts, *ttm-1a* and *ttm-1b*, that use different transcription start sites. *ttm-1b* expression was induced by high levels of zinc specifically in intestinal cells, whereas *ttm-1a* was not induced by zinc. TTM-1B was localized to the apical plasma membrane of intestinal cells, and analyses of loss-of-function mutant animals indicated that TTM-1B promotes zinc excretion into the intestinal lumen. Zinc excretion mediated by TTM-1B contributes to zinc detoxification. These observations indicate that *ttm-1* is a component of a negative feedback circuit, since high levels of cytoplasmic zinc increase *ttm-1b* transcript levels and TTM-1B protein functions to reduce the level of cytoplasmic zinc. We showed that TTM-1 isoforms function in tandem with CDF-2, which is also induced by high levels of cytoplasmic zinc and reduces cytoplasmic zinc levels by sequestering zinc in lysosome-related organelles. These findings define a parallel negative feedback circuit that promotes zinc homeostasis and advance the understanding of the physiological roles of the gastrointestinal tract in zinc metabolism in animals.

162. The SYS-1/b-catenin regulatory machinery controls multiple functions of APR-1/APC during seam cell division. **Austin T. Baldwin**, Bryan T. Phillips. Biology, University of Iowa, Iowa City, IA.

The tumor suppressor Adenomatous Polyposis Coli (APC) is involved in diverse cellular processes in stem cells ranging from gene regulation through Wnt/b-catenin signaling to chromosome stability via interaction with microtubules. However, the mechanism by which these disparate functions of APC are differentially controlled in vivo is not well understood. The C. elegans APC homolog, APR-1, stabilizes microtubules during asymmetric cell division to drive nuclear export of the b-catenin WRM-1 as part of the Wnt/b-catenin Asymmetry (WbA) pathway (Sugioka et al 2011). WbA function also depends on a second b-catenin, SYS-1, which directly interacts with POP-1/TCF to modulate gene expression. Here we show that APR-1 regulates SYS-1 levels during asymmetric seam cell division in addition to its previously described role in stabilizing microtubules. APR-1 loss of function also results in decreased nuclear retention of SYS-1 in the unsignaled daughter after division, indicating there may be another role of APR-1 in regulating SYS-1 outside of stability. We show that SYS-1 levels are regulated by KIN-19/CKIa downstream of APR-1. We also demonstrate that KIN-19 regulates APR-1 localization such that loss of KIN-19 results in disruption of WRM-1 localization. Finally, we show that APR-1 cortical localization is controlled by PRY 1/Axin, but that PRY-1 is dispensable for negative regulation of SYS-1 levels. Since PRY-1 has been previously demonstrated to be unnecessary for WRM-1 nuclear export

(Mizumoto & Sawa 2007), these results inform a model whereby Wnt signaling differentially regulates the function of two functionally distinct pools of APC. One pool of APR-1, controlled by KIN-19 and PRY-1, negatively regulates SYS-1 levels in the unsignaled daughter. The other pool, independent of KIN-19 and PRY-1, regulates WRM-1 nuclear export through asymmetric microtubule stability. Together, these results inform the mechanism of SYS-1 regulation during asymmetric division while also providing new insights into the function and regulation of the b-catenin destruction complex.

163. CEH-20/Pbx and UNC-62/Meis function upstream of *rnt-1*/Runx to regulate asymmetric divisions of the *C. elegans* stem-like seam cells. **Samantha L. Hughes**, Charles Brabin, Alison Woollard. Dept Biochem, Oxford University, United Kingdom.

Seam cells divide asymmetrically, typically producing an anterior daughter that differentiates and a posterior daughter that proliferates further, although there are deviations from this pattern. The asymmetric outcome of these divisions is controlled by the Wnt/b-catenin asymmetry (WBA) pathway, with the b-catenin WRM-1 being enriched at the anterior cortex and posterior daughter nuclei at division. In addition, *rnt-1* (the homologue of the mammalian cancer-associated Runx genes) is thought to act in parallel to the WBA pathway, being preferentially expressed in posterior daughters where it promotes the proliferative fate. We isolated the interacting CEH-20/Pbx and UNC-62/Meis transcription factors during a genome-wide RNAi screen for novel regulators of seam cell number. *ceh-20* and *unc-62* mutants display seam cell hyperplasia caused by the symmetrisation of normally asymmetric seam cell divisions towards the proliferative stem-like fate. Although WRM-1 localisation is perturbed in *ceh-20(RNAi)* animals, the hyperplasia is not dependent on WRM-1, suggesting that CEH-20/UNC-62 function downstream of or in parallel to Wnt signalling. Interestingly, in wild type animals, we observed that WRM-1 is always enriched at the anterior cortex and posterior nucleus during all seam cell divisions, even the symmetrical divisions occurring at L2 and in anterior L1 divisions where polarity is reversed. Thus, WRM-1 (and POP-1) asymmetry is somehow over-ridden in seam cell divisions that do not follow the canonical pattern. The *ceh-20/unc-62* hyperplasia is completely suppressed in *rnt-1* mutants, suggesting that CEH-20 and UNC-62 function upstream of *rnt-1* to limit proliferative potential to the appropriate daughter cell. Our data suggest that CEH-20/UNC-62 normally down-regulate *rnt-1* in anterior daughters that are destined to exit from the cell cycle and terminally differentiate. Furthermore, we find that CEH-20 is asymmetrically localised in seam daughters following an asymmetric division, being predominantly restricted to anterior nuclei. Thus, we have identified *ceh-20* and *unc-62* as crucial regulators of seam cell development, acting via *rnt-1* to regulate the balance between proliferation and differentiation.

164. Regulation of maternal Wnt mRNA translation in *C. elegans* embryos reveals mechanistic parallels between 3' UTRs and transcription enhancers. **Marieke Oldenbroek**[1], Scott Robertson[1], Tugba Guven-Ozkan[2], Caroline Spike[3], David Greenstein[3], Rueyling Lin[1]. 1) UT Southwestern Medical Center, Dallas, TX; 2) Scripps Research Institute, Jupiter, FL; 3) University of Minnesota, Minneapolis, MN.

The importance of 3' UTRs and RNA-binding proteins in regulating expression of maternally-supplied mRNAs is well established. Restricted spatiotemporal translation of maternal mRNAs is critical for correct cell fate specification in early *C. elegans* embryos. Genetic screens for maternal genes essential for early blastomere fate specification identified many RNA-binding proteins. These proteins are translated in oocytes and localize to one or a few blastomeres in a spatially and temporally dynamic fashion unique for each protein and each blastomere. We characterized the translational regulation of maternally-supplied *mom-2* mRNA, which encodes a Wnt ligand essential for two cell-cell interactions in early embryos. We show that the *mom-2* 3' UTR confers localization information for both the mRNA and protein. A GFP reporter that contains the *mom-2* 3' UTR is translationally repressed in oocytes and early embryos and correctly translated in known Wnt signaling cells. Nine maternally-supplied RNA-binding proteins bind to the *mom-2* 3' UTR in vitro and regulate *mom-2* translation in vivo. OMA-1 and OMA-2 are IFET-1-dependent translational repressors in oocytes. The other seven RNA-binding proteins restrict the translation of *mom-2* to a subset of early blastomeres. PIE-1, MEX-1, and POS-1 promote, whereas SPN-4, MEX-5/6, and MEX-3 repress *mom-2* translation. In vitro competition RNA-binding experiments reveal a hierarchy of binding to the *mom-2* 3' UTR among these RNA-binding proteins. MOM-2 expression in specific blastomeres results when repression is relieved by a positive regulator. The net translational readout for *mom-2* mRNA is controlled by the combination of competitive binding between positive and negative regulators, along with the distinct spatiotemporal localization patterns of these regulators. We propose that 3' UTRs of maternally-supplied mRNAs function like translational enhancers in C. elegans embryos, analogous in many ways to transcriptional enhancers.

165. Life at the edge of robustness: Partial guts suggest that endoderm specification is not all-or-none. **Morris F. Maduro**[1], Francisco Carranza[2], Farhad Ghamsari[1], Gurjot Walia[1], Gina Broitman-Maduro[1]. 1) Dept Biol, Univ California, Riverside, Riverside, CA; 2) MarcUstar, Univ California, Riverside, Riverside, CA.

The C. elegans intestine is derived from the embryonic blastomere E. The paradigm for E specification is that maternal SKN-1- and POP-1-dependent input cause activation of the E-specific gut specification factors end-1 and end-3. After reaching a threshold of expression, these activate elt-2, which maintains its expression by autoregulation and drives the commitment to gut differentiation. Prior work suggests that gut specification may not be all-or-none, and that some of the descendants of E are capable of adopting a gut fate independently of others. We have created strains in which E is specified by single-copy transgene forms of end-1 and/or end-3 that are mutated for binding sites for the MED-1,2 GATA factors, themselves direct targets of SKN-1. In such strains, different embryos make variable numbers of apparently normal-sized gut cells, suggesting that specification has become subject to stochastic variations among embryos, and that commitment to a gut fate can occur later in the E lineage. Counting of embryonic elt-2 transcripts by single-molecule FISH suggests that activation of elt-2 is more graded in these strains, as opposed to an all-or-none mode as was previously reported for SKN-1-depleted embryos (Raj et al., 2010). As these effects are confined to the E lineage due to the nature of the strains constructed, we are able to evaluate adults derived from embryos in which functional guts were made. We find that such adults store lipids at significantly higher levels and display other variable pleiotropic phenotypes, suggestive of primary defects in gut function. Together, these results build a picture in which specification of gut is not an all-or-none event, and that in animals that do make an intestine, the endoderm differentiation network is not fully self-correcting for partially compromised specification. We will present these results, including the outcome of an attempt to computationally model the stochasticity of elt-2 activation.

166. Identification of SEL-10/Fbw7 substrates regulated in cell fate patterning events via a conserved phosphodegron motif. **Claire de la Cova**, Iva Greenwald. HHMI and Dept Biochem, Columbia Univ, New York, NY.

The conserved E3 ubiquitin ligase SEL-10, the ortholog of the human Fbw7 tumor suppressor, specifically binds phosphorylated protein substrates that contain a high-affinity site called a Cdc4-phosphodegron (CPD) and targets them for ubiquitin-mediated protein degradation. As many known substrates of Fbw7 are oncoproteins, the identification of new substrates may offer insight into cancer biology as well as proteome regulation. However, global proteomic strategies have not been efficient at identifying new substrates. We therefore devised a simple computational approach based on the presence of an evolutionarily conserved CPD to identify candidates that could be validated through functional studies in *C. elegans*. Initially, we focused on LIN-45, the *C. elegans* Raf ortholog and component of the Ras-Raf-ERK signal transduction pathway underlying vulval induction. We found that LIN-45 protein stability is patterned during Vulval Precursor Cell fate specification such that LIN-45 protein is down-regulated in P6.p, where Ras-Raf-ERK signaling is active. Our analysis indicated that SEL-10 directly targets LIN-45 protein for down-regulation via a CPD that is conserved in human Braf, and the downstream kinase MPK-1/ERK appears to mediate phosphorylation of the LIN-45 CPD as part of a negative feedback loop that ensures a high Ras-Raf-ERK activity threshold for vulval induction. We have recently obtained additional genetic evidence that supports this model and suggests that SEL-10-mediated protein degradation is one of several negative feedback mechanisms that are functionally redundant for preventing ectopic induction. Furthermore, we have been evaluating the generality of the CPD-conservation approach by examining other potential SEL-10 substrates for increased activity or the stabilization of fluorescently tagged candidate proteins in the absence of *sel-10*. Our results suggest that this approach will be a powerful adjunct to proteomics-based methods generally, and effective for identifying signaling pathways modulated by SEL-10 in other developmental contexts in *C. elegans*.

167. A Genome-Wide RNAi Screen of Caenorhabditis elegans Identifies Translational Machinery Genes Involved in Fat Regulation. **Elizabeth Pino**[1,2], Christopher Carr[3], Alexander Soukas[1,2]. 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA; 2) Department of Medicine, Harvard Medical School, Boston, MA; 3) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

Many pathways regulating energy homeostasis are conserved between humans and *C. elegans*, making it an ideal model for the integrated study of fat regulatory processes. Previous methods used to study fat mass in *C. elegans* have proved to be indicative of a separate biology, leading our laboratory to develop and optimize a new, validated, fat staining technique that truly highlights lipid droplets. The method is rapid, compatible with fluorescent microscopy, and quantitative, making it ideal for small and large-scale studies of fat mass. Using this new methodology, we performed the first bona fide genome-wide screen for the detection of fat regulatory genes. We identified 122 gene inactivations that lead to decreased fat, and 391 gene inactivations that lead to increased fat. These include several expected genes such as *sbp-1*, *pod-2*, and *lpd* (lipid depleted) genes, as well as many novel lipid regulators. We analyzed genetic interactions between each individual 513 RNAi gene inactivations across several different genetic mutants. Our screen identified a group of genes encoding components of translational machinery, specifically amino-acyl tRNA synthetases, that when inactivated lead to 2-4 fold increases in fat mass in the worm. These RNAi-mediated increases in fat are independent of *daf-16* and other members of the insulin-signaling pathway, and are not a product of germline stem cell deletion. In addition, we found that animals with these gene inactivations fail to predictably lose lipid mass upon exercise and starvation, but instead continue to increase fat stores. We hypothesize that this gain in fat mass is part of an ancient stress response pathway to down regulation of translation, implicating amino-acyl tRNA synthetases in non-canonical metabolic signaling roles. We anticipate that these and many other newly identified fat regulatory genes in the worm may be universal regulators of fat storage and potential targets for obesity and related diseases.

168. Identification of a novel *C. elegans* protein that detects bacteria. **Darym Alden**, Jonathan Dworkin. Columbia University, New York, NY.

Bacteria serve as both a nutrient source and as a pathogen of *C. elegans*. However, how bacteria are detected is not understood. Here, we present evidence that LMD-1, a previously uncharacterized *C. elegans* membrane protein, mediates this recognition, and an *lmd-1* deletion results in constitutive dauer production. LMD-1 homologs are present in all metazoans including humans, suggesting that this mechanism of recognition is phylogenetically conserved.

LMD-1 contains a single transmembrane domain and an extracellular domain consisting of a LysM domain, a motif found in bacterial and plant proteins that binds peptidoglycan, the primary component of the bacterial cell wall. We find that LMD-1 is expressed in the pharyngeal gland cells whose processes are exposed to the worm's pharyngeal lumen. Furthermore, *lmd-1(tm5735)* worms that harbor a deletion that removes the LysM domain have a daf-c phenotype, suggesting that this mutation impairs the ability of worms to accurately assess the presence of bacteria.

We found that RNAi against *daf-16*, the downstream transcription factor of the insulin/IGF-I signaling pathway, suppresses this phenotype. In addition, a transcriptional reporter for DAF-28 which codes for the only known nutrient-regulated insulin homolog in *C. elegans* is down-regulated in the neurons of *lmd-1* larvae. The involvement of *lmd-1* in insulin signaling suggests a general role for bacterial recognition in nutrient signaling.

169. Nuclear Receptor NHR-8 Regulates Cholesterol, Bile Acid and Fat Metabolism, and Modulates Reproduction and Lifespan. **Daniel Magner**[1], Joshua Wollam[1], Yidong Shen[1], Caroline Hoppe[1], Dongling Li[1], Christian Latza[1], Veerle Rottiers[2], Harald Hutter[3], Adam Antebi[1,2]. 1) Max-Planck Institute for Biology of Ageing, Cologne, Germany; 2) Huffington Center on Aging, Baylor College of Medicine, Houston, TX, USA; 3) Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada.

Nuclear receptors are hormone-gated transcription factors that couple metabolic and environmental signals to transcriptional outputs. We show that the *C. elegans* nuclear receptor NHR-8, a conserved homologue of mammalian Liver-X and Vitamin-D receptors, regulates the choice between reproductive development and arrest at the long-lived dauer diapause stage in response to available cholesterol. NHR-8 promotes production of dafachronic acids (DA), the bile acid ligands of the DAF-12 nuclear receptor, which govern the dauer decision. Loss of *nhr-8* results in misregulation of endogenous cholesterol levels and related metabolites, including reduced levels of both 7-dehydrocholesterol and DA. These phenotypes arise in part from reduced expression of the *daf-36* Rieske oxygenase, which carries out the first step in DA biosynthesis, converting dietary cholesterol to 7-dehydrocholesterol. Transcriptome analysis reveals that *nhr-8* regulates genes enriched in fatty-acid metabolism, oxidation-reduction, proteolysis, defense response, and determinants of lifespan. In particular, expression of the D^9- and D^{12}-fatty acid desaturases (*fat-5*, *fat-7*, and *fat-2*) is downregulated in *nhr-8* mutants, resulting in reduced polyunsaturated fatty acid (PUFA) production. Cholesterol supplementation rescues various *nhr-8* phenotypes, including developmental arrest, unsaturated fatty acid deficiency, reduced fertility, and shortened lifespan, suggesting that *nhr-8* alters cholesterol deposition or transport. *nhr-8* also interacts with *daf-16/FOXO* to regulate steady-state cholesterol levels, and is lethal when mutated in combination with insulin signaling mutants that

promote excess growth, revealing a novel connection between insulin signaling and sterol metabolism. Similar to its mammalian counterpart LXR, our data define NHR-8 as an important regulator of cholesterol, bile acid, and fatty acid homeostasis, which ultimately modulates *C. elegans* reproduction, development, and lifespan.

170. The Tumor Suppressor Rb Critically Regulates Starvation-induced Stress Response in *C. elegans*. **Mingxue Cui**, Max Cohen, Cindy Teng, Min Han. HHMI, MCD Biology, University of Colorado at Boulder, Boulder, CO.

During evolution, organisms have developed complex mechanisms to adapt to food-deprived environments. Understanding the regulation of the starvation response is also closely related to the treatment of cancers, as indicated by the recent promotion of starvation-based cancer therapy. Newly hatched *Caenorhabditis elegans* respond to food deprivation by halting development and promoting long-term survival (L1 diapause), thereby providing an excellent model to study the starvation response. Through a genetic search, we have discovered that the tumor suppressor Rb homolog, *lin-35*, critically promotes survival during L1 diapause and likely does so by regulating the expression of genes in both insulin-IGF-1 signaling (IIS)-dependent and -independent pathways, mainly in neurons and the intestine. Global gene expression analyses suggested that *lin-35/Rb* maintains the "starvation-induced transcriptome" and represses the "re-feeding induced transcriptome", including the repression of many pathogen/toxin/oxidative stress-inducible and metabolic genes, as well as the activation of many other stress-resistant genes, mitochondrial respiratory chain genes, and potential IIS receptor antagonists. Notably, the majority of genes dysregulated in starved L1 *lin-35/Rb(-)* animals were not found to be dysregulated in fed conditions. Our tissue/cell specific expression of the wild type Rb gene indicated that the neuron and intestine are major sites for its role in promoting L1 starvation survival. Additionally, Rb's function in the L1 starvation response is largely independent of its well-known role in regulating the expression of cell division cycle regulators and *lin-35/Rb(-)* animals display no prominent defects in survival rates upon exposure to pathogen and osmotic stress. Together, these findings identify Rb as a critical regulator of the starvation response and may implicate mammalian tumor suppressor function in nutrient/starvation responses and the validity of fasting-coordinated cancer therapy.

171. A novel ascaroside controls the parasitic life cycle of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Jaime H. Noguez[1], Joshawna K. Nunnery[1], Elizabeth S. Connor[2], Yue Zhou[1], Todd A. Ciche[3], Justin R. Ragains[2], **Rebecca A. Butcher[1]**. 1) Dept. of Chemistry, University of Florida, Gainesville, FL; 2) Dept. of Chemistry, Louisiana State University, Baton Rouge, LA; 3) Dept. of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI.

Entomopathogenic nematodes survive in the soil as stress-resistant infective juveniles (IJs) that seek out and infect insect hosts. Upon sensing internal host cues, the IJs regurgitate bacterial pathogens from their gut that ultimately kill the host. Inside the host, the nematode develops into a reproductive adult and multiplies until unknown cues trigger the accumulation of IJs. Here, we show that the entomopathogenic nematode *Heterorhabditis bacteriophora* uses a pheromone to control IJ development. The pheromone, which likely increases in concentration at higher nematode densities, prevents IJ recovery to the J4 stage, allowing IJs to amass late in the infection process. Using activity-guided fractionation and NMR spectroscopy-based structure elucidation, we identify the chemical structure of the pheromone. The pheromone is structurally related to the dauer pheromone ascarosides that the free-living nematode *Caenorhabditis elegans* uses to control its development. However, none of the *C. elegans* ascarosides are effective in *H. bacteriophora*, suggesting that there is a high degree of species specificity. Our report is the first to show that ascarosides are important regulators of development in a parasitic nematode species.

172. A Conserved SREBP/Transketolase Regulatory Circuit Governing Lipid Homeostasis in Metazoans. **V. Rottiers[1,2]**, P. Mulligan[1,2], A. K. Walker[3], J. L. Watts[4], A. C. Hart[5], A. M. Näär[1,2]. 1) Cancer Ctr, Massachusetts Gen Hosp, Charlestown, MA; 2) Dept of Cell Biology, Harvard Medical School, Boston, MA; 3) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; 4) School of Molecular Biosciences, Washington State University, WA; 5) Department of Neuroscience, Brown University, Providence RI.

Obesity, a major cause of morbidity in the developed world, is characterized by excessive fat accumulation. Fat/lipid homeostasis is regulated by the conserved SREBP transcription factors. Reduction of *C. elegans* SREBP homolog *sbp-1* results in clear, sterile, lethal and slow growth phenotypes that are rescued by addition of oleic acid (OA). OA is a precursor for triacylglycerides (TAGs), the major constituent of adipose fat, and is produced by the SBP-1 regulated stearoyl-CoA-desaturases (SCDs) *fat-6/fat-7*. Based on the central role for OA and SCDs in SBP-1 function we performed two screens for novel lipid regulators. In the first screen, we assessed 1,311 RNAi clones with "*sbp-1*-like" phenotypes for dietary rescue by OA. The second, genome-wide, RNAi screen identified genes that altered *fat-7::gfp* expression. We focused our analysis on *tkt-1*/transketolase, a hit in both screens. Depletion of *tkt-1* strongly reduced fat storage, confirming its important role in lipid homeostasis. Transketolase is part of the Pentose Phosphate Pathway (PPP) and necessary for the production of NADPH, a crucial cofactor for lipid synthesis. SREBP activates transcription of NADPH generating enzymes, and we found that SBP-1 regulates several PPP genes in addition to *tkt-1*, including G6PDH (*gspd-1*), PGD (T25B9.9) and cytosolic IDH (*idh-1*). Mammalian SREBP also activated TKT through direct promoter binding, indicating that TKT/*tkt-1* is a novel SREBP/SBP-1 target. Interestingly, inhibition of TKT or modifying other PPP components altered SREBP levels, indicating that TKT regulates SREBP in a feed-forward fashion. Our results suggest that a conserved SREBP and TKT regulatory circuit couples fatty acid production and PPP activity to allow tight control of lipid homeostasis. Specific TKT inhibitors may potentially serve as novel obesity therapeutics.

173. C.elegans community behavior affects the dynamics of pathogen avoidance. **Andrzej Nowojewski**, Erel Levine. Department of Physics, FAS Center for Systems Biology, Harvard University, Cambridge MA 02138, USA.

Pathogen avoidance is a known behavior in which animals move away from pathogen-rich compounds. In C. elegans, pathogen avoidance is recognized for example in slow-killing assays, where worms exposed to Pseudomonas aeruginosa PA14 reverse their initial attraction within several hours and avoid the bacterial lawn. While genetic and neuronal factors involved in this behavior have been characterized, little is known about the population dynamics aspect of avoidance.

We built an automatic acquisition system capable of imaging 48 assay plates at several minute intervals. An image processing workflow allows us to

extract the locations of animals and monitor bacterial spreading over a few days. We used this setup to investigate quantitative aspects of nematode behavior during slow killing experiments.

We found that the dynamics of pathogen avoidance is strongly dependent on the number of animals on the plate and on the size of the lawn. Our results suggest that avoidance signal is conveyed at least partially through a chemical or morphological modifications to the bacterial lawn by infected worms. Training assays, where animals were pre-exposed to the pathogen, revealed that learning could change the response of animals to this signal. We present a phenomenological, quantitative model of this behavior of the animals and its relation to their survivability.

We suggest that this experimental set up and a rigorous mathematical treatment will open new possibilities for systematic study of the population-level aspects of this model host-pathogen interaction.

174. Triggering antifungal innate immunity. **Olivier Zugasti**[1], Barbara Squiban[1], Jerome Belougne[1], Leo Kurz[2], Neelanjan Bose[3], Frank Schroeder[3], Nathalie Pujol[1], Jonathan Ewbank[1]. 1) CIML, Marseille, France; 2) IBDM, Marseille, France; 3) Cornell University, Ithaca, NY.

Infection of worms by the fungus *D. coniospora* leads to the up-regulation of genes such as *nlp-29*, one of a cluster of six paralogous *nlp* antimicrobial peptide genes, via a PMK-1/p38 MAP kinase pathway. The worm genome, however, lacks orthologs of the receptors known to be important for pathogen recognition in other species. How *D. coniospora* triggers an immune response is an open question.

For epidermal defenses, the p38 signaling cassette acts downstream of the Ga protein GPA-12, implicating a G-protein coupled receptor (GPCR) in the immune response. By RNAi, we knocked down individually three-quarters of the worm's >1,500 GPCR genes. Quantification with the COPAS Biosort of the effect of each RNAi clone on the expression of a *nlp-29*p::*gfp* reporter following infection allowed us to identify *dcar-1* (dihydrocaffeic acid receptor) as a candidate innate immune receptor gene.

dcar-1 mutants exhibit an almost complete block of *nlp* genes induction following infection and a heightened susceptibility to infection compared to wild-type worms. We found that *dcar-1* is expressed in the major epidermal syncytium, hyp7, site of expression of the infection-inducible *nlp* genes. Restoring *dcar-1* expression in the epidermis rescued *nlp* gene expression and the mutant's resistance to infection. DCAR-1 has been previously shown to have a high affinity for dihydrocaffeic acid (DHCA) a derivative of DOPA. We found that unlike DOPA, direct addition of DHCA or oxidized DOPA to worms triggers the expression of *nlp* genes but could not demonstrate the production *in vivo* of DHCA. This suggests that DHCA might not be the natural ligand of DCAR-1. Our current hypothesis is that a derivative of DOPA is rapidly produced upon infection and triggers an immune response via DCAR-1. We are currently applying genetic and biochemical techniques to identify the putative DCAR-1 ligand.

Thanks to Christophe Melon, and Reina Aoki and Yoshio Goshima for reagents.

175. The *mir-58* family of microRNAs regulates the tissue-specific expression of PMK-2 p38 MAPK that functions in host defense. **Daniel J. Pagano**, Elena R. Kingston, Dennis H. Kim. Department of Biology, MIT, Cambridge, MA.

Host defense in *C. elegans* requires a conserved TIR-1-NSY-1-SEK-1-p38 mitogen-activated protein kinase (MAPK) cascade. The TIR-1-NSY-1-SEK-1 module has dual tissue-specific roles in response to pathogen; in the intestine this module regulates the expression of immune effector genes, whereas in the sensory nervous system this module regulates serotonin-dependent aversive behavior. The p38 MAPK PMK-1 functions downstream of this module to regulate the response to pathogen in the intestine. We performed a forward genetic screen for suppressors of the enhanced susceptibly to pathogen conferred by *pmk-1* loss-of-function and identified an allele of *pmk-2*, *qd171*. The *pmk-2* gene is paralogous to *pmk-1* and encodes a p38 MAPK. The *pmk-1* and *pmk-2* genes are clustered together in an operon, although their expression patterns differ. We observe nearly ubiquitous expression of *pmk-1*, whereas *pmk-2* is expressed specifically in the nervous system. The *qd171* allele of *pmk-2* contains an insertion/deletion in the 3' UTR that allows for expression of *pmk-2* in the intestine where it can compensate for loss of *pmk-1* function. We found highly conserved microRNA seed match sites for the *mir-58* family of microRNAs in the 3' UTR of *pmk-2* that are absent in the *qd171* mutant. We determined the *mir-58* family of microRNAs acts to inhibit expression of *pmk-2* in the intestine and establishes tissue-specificity of p38 MAPKs that are co-transcribed. We next isolated a loss-of-function allele of *pmk-2*, *qd279*, and defined a physiological role for PMK-2 in regulating serotonin-dependent behaviors of host defense. These data demonstrate that the *mir-58* family of microRNAs governs the tissue-specific expression of p38 MAPKs that act through distinct modes to provide host defense against pathogen.

176. Role of Manganese Homeostasis in Aging and Disease: Implications for Parkinson's Disease. **Suzanne Angeli**[1], Kathryn Page[2], David Killilea[3], Gordon Lithgow[1], Julie Andersen[1]. 1) Buck Institute for Research on Aging, Novato, CA; 2) University of California, Berkeley, Berkeley, CA; 3) Children's Hospital Oakland Research Institute, Oakland, CA.

Parkinson's disease (PD) is a debilitating motor and cognitive neurodegenerative disorder for which there is no cure. Currently, there is no clear understanding of what causes PD, although age, genetic susceptibility, and environment are all implicated. Environmental exposure to the heavy metal manganese (Mn) is a leading risk factor for developing PD. Further, exposure to high levels of Mn is associated with the syndrome known as manganism, which has pathological features that resemble PD. Thus, understanding the molecular cascades of Mn toxicity could shed light on the pathogenesis of PD. We have developed a model of manganism in *C. elegans* that exhibits mitochondria impairment, widespread metallomic dysfunction, and shortened lifespan. Further, Mn exacerbates neuronal degeneration in aged animals expressing a-synuclein, the toxic protein associated with PD. Mn also increases the pathology in models of Alzheimer's diseases and Huntington's disease. To determine how cellular regulation of Mn may impact an organism's susceptibility to disease, we screened over 60 putative metal transporters and identified several novel genes that modulate endogenous Mn levels in *C. elegans*. We find that knockdown of certain putative transporters can extend lifespan and protect animals from pathologies associated with PD and other diseases of protein misfolding. Conversely, upregulation of endogenous levels of Mn appears to exacerbate disease pathologies. These findings suggest that relatively small changes in metal homeostasis can have a significant impact on the health of an organism. Moreover, these findings underscore the importance of metal homeostasis in aging and age-related diseases and open new avenues to understand the complex, synergistic interactions among genes, environment, and aging that may lead to disease onset.

177. HIF-1, DAF-16, and ZIP-2 coordinate the *C. elegans* defense against *P. aeruginosa* pathogenesis. **Natalia Kirienko**[1,2], Daniel Kirienko[1,2], Jonah Larkins-Ford[1], Gary Ruvkun[1,2], Fred Ausubel[1,2]. 1) Molecular Biol, Mass General Hospital, Boston, MA; 2) Genetics, Harvard Medical School Boston, MA.

Pseudomonas aeruginosa is a serious risk to human health. Despite the identification of virulence factors, understanding of disease determinants and effective treatments remain incomplete. As *C. elegans* shares many human innate immune pathways, it is an invaluable model host. We used a liquid-based *C. elegans-P. aeruginosa* PA14 model to identify a mechanism for host killing. Unlike other models, colonization and quorum-sensing pathways were dispensable for killing. Instead pyoverdin, a secreted iron-scavenging polypeptide, induced a lethal hypoxic crisis in the host. Furthermore, we showed that loss of the hypoxia-inducing factor HIF-1 exacerbated PA14 pathogenesis. Previous work showed that *P. aeruginosa* infection on solid media triggered a panel of immune response genes dependent upon the p38 MAP kinase PMK-1. Unexpectedly, *C. elegans* exposed to PA14 in liquid did not show their upregulation. Moreover, RNAi knockdown of multiple components of the PMK-1 signaling cascade resulted in increased survival in liquid, whereas the same knockdowns exhibit enhanced susceptibility on solid media. In addition, targets of the SKN-1 detoxifying pathway were upregulated during plate-based infections, whereas DAF-16 targets and hypoxic response genes were activated in liquid. By comparing our microarray results with previous data from other pathogens, we identified a set of 121 genes that comprise a unique stress signature for exposure to pyoverdin and PA14 in liquid. A significant overlap between these genes and hypoxia response genes was observed. Bioinformatic analysis identified a nucleotide motif highly enriched in these genes' promoters that may confer resistance to exposure. We are currently investigating the role of this promoter element in the innate immune response to PA14. In several murine models, pyoverdin production has been shown to be indispensable for pathogenesis, but mechanisms for its toxicity are still unclear. Our work both proposes a mechanism and characterizes the host's response to the damage, and should provide a useful foundation for further studies in mammalian models.

178. A new role of DCR-1/DICER in C. elegans innate immunity against the highly virulent bacterium Bacillus thuringiensis DB27. **Igor Iatsenko**, Amit Sinha, Christian Rödelsperger, Ralf J. Sommer. Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Tuebingen, Germany.

Bacillus thuringiensis (BT) produces toxins that target invertebrates including Caenorhabditis elegans. Known C. elegans defense mechanisms against BT are largely limited to the Cry5B toxin and are not universal. Virulence of Bacillus strains is often highly specific, such that the BT strain DB27 represents one of the most virulent pathogens of C. elegans, but shows no virulence to another nematode model Pristionchus pacificus. To uncover the underlying mechanisms of differential response of the two nematodes to BT DB27 and to reveal C. elegans defense mechanisms against this pathogen we conducted a genetic screen for C. elegans mutants resistant to BT DB27. We identified a BT DB27 resistant C. elegans mutant that is identical to nasp-1, the C. elegans homolog of the nuclear-autoantigenic-sperm protein. Further characterization and gene expression analysis indicate a substantial overlap between genes down regulated in nasp-1 and targets of C. elegans dcr-1/Dicer, suggesting that dcr-1 is repressed in nasp-1 mutants. Indeed, quantitative PCR confirmed down-regulation of dcr-1 in nasp-1 mutants. Consistent with this, nasp-1 exhibits RNAi deficiency and reduced longevity similar to dcr-1 mutant. Building on these surprising findings we further explored a potential role of dcr-1 in C. elegans innate immunity. Indeed, we show that dcr-1 mutant alleles deficient in miRNA processing, but not those deficient only in RNAi, are resistant to BT DB27. Furthermore, dcr-1 overexpression rescues nasp-1 resistance, suggesting that repression of dcr-1 function determines nasp-1 resistance. We identified the collagen col-92 as one of the downstream effectors of nasp-1. Knockdown of col-92 results in hyper-susceptibility of wild-type worms, whereas col-92(RNAi) in a nasp-1 mutant background abrogates resistance indicating that this collagen plays an important role in resistance to DB27. These results uncover a previously unknown role of DCR-1/Dicer in C. elegans antibacterial immunity that is largely associated with miRNA processing.

179. A candidate host receptor exploited by microsporidia for intestinal infection in *C. elegans*. **Robert J Luallen**, Malina Bakowski, Emily Troemel. Biological Sciences, UCSD, La Jolla, CA.

The initial interactions between a host and pathogen often occur at the receptor level, with pathogens exploiting host receptors to initiate infection and hosts using receptors to detect pathogens. While several signal transduction pathways have been discovered to promote host defense in *C. elegans*, little is known about host receptors that interact with microbes. To study early host/pathogen interactions, we use a natural pathogen of *C. elegans*, a species of microsporidia called *Nematocida parisii*. *N. parisii* is an obligate intracellular pathogen of the intestine and offers an opportunity to study interactions between a natural host/pathogen pair. To search for host factors important for infection, we conducted a targeted RNAi screen for *C. elegans* genes that inhibited *N. parisii* infection, using nematode larval arrest as a readout for infection. A hit from this screen was a transmembrane protein containing leucine-rich repeats (LRRs), a domain found in numerous pathogen receptors, such as TLRs. RNAi against this gene, *naom-1*, caused resistance to *N. parisii* infection, leading to less larval arrest and lower pathogen load. This suggests that NAOM-1 is exploited by *N. parisii* to facilitate infection. Using transgenic *C. elegans* strains, we determined that the putative *naom-1* promoter is active in the intestine, where *N. parisii* exclusively infects. Also, intestinal-specific RNAi of *naom-1* inhibited larval arrest, suggesting that *naom-1* expression in the intestine is necessary for exploitation by *N. parisii*. Since the LRR domain of some proteins can interact with pathogen-derived molecules, we expressed recombinant NAOM-1 and found it can bind to *N. parisii* spores in an LRR-dependent manner. Furthermore, this recombinant NAOM-1 showed specificity to *N. parisii*, as the protein interacted less efficiently with spores from other microsporidia species that infect humans and grasshoppers. Altogether, the data suggests that the transmembrane LRR protein NAOM-1 may be a host receptor hijacked by *N. parisii* to facilitate infection in *C. elegans*. As NAOM-1 has a putative human ortholog, dissecting its mechanism of action in early infection may inform us about intestinal microsporidian infection in humans.

180. The EBAX-type Cullin-RING E3 ligase and Hsp90 guard the protein quality of the SAX-3/Robo receptor in developing neurons. **Zhiping Wang**[1], Yanli Hou[2], Xing Guo[3], Monique van der Voet[4], Jack Dixon[3,5], Mike Boxem[4], Yishi Jin[1,5]. 1) Division of Biological Sciences, UC San Diego, La Jolla, CA 92093; 2) Department of MCD Biology, UC Santa Cruz, CA 95064; 3) Department of Pharmacology, UC San Diego, La Jolla, CA 92093; 4) Department of Biology, Utrecht University, Utrecht, The Netherlands; 5) Howard Hughes Medical Institute.

Developing neurons require robust protein synthesis and strict protein quality control (PQC) to meet the requirement of neuronal wiring. Although PQC is generally perceived as important for the development of the nervous system, the underlying mechanisms of neuronal PQC have remained poorly understood. Here, we report that *C. elegans* EBAX-1 (Elongin BC-Binding AXon regulator), a conserved BC-box protein, regulates axon guidance through

PQC of the SAX-3/Robo receptor. EBAX-1 is expressed widely in mid-late embryos and highly enriched in the embryonic nervous system. Mass spectrometry and yeast-two hybrid screen have revealed that EBAX-1 is a conserved substrate-recognition subunit in the Elongin BC- and cullin-2 containing cullin-RING E3 ligase complex (CRL) and interacts with the cytosolic molecular chaperone DAF-21/Hsp90. The EBAX-type CRL and DAF-21/Hsp90 regulate SAX-3-mediated axon pathfinding in a collaborative manner. Biochemical and imaging assays indicate that EBAX-1 specifically recognizes misfolded SAX-3 receptors and promotes their degradation *in vitro* and *in vivo*. Together, our findings demonstrate a "triage" PQC mechanism mediated by the EBAX-type CRL and DAF-21/Hsp90 for controlling the accuracy of neuronal wiring. We propose that as a substrate recognition subunit specifically for aberrant proteins, EBAX-1 recruits DAF-21/Hsp90 to facilitate the refolding of misfolded SAX-3, while permanently damaged SAX-3 proteins are removed by protein degradation mediated by the EBAX-1-containing CRL. Importantly, we demonstrate that vertebrate EBAX also shows substrate preference towards aberrant human Robo3 proteins implicated in the horizontal gaze palsy with progressive scoliosis disorder (HGPPS). These data support that the PQC mechanism involving EBAX-1 reported here is likely conserved in mammals.

181. Feeding state, NPR-1 and circuit dynamics regulate chemoreceptor expression. **Matt Gruner**[1], Rebecca Hintz[1], Samuel Chung[2], Chris Gabel[2], Alexander van der Linden[1]. 1) Department of Biology, University of Nevada, Reno, NV 89557-0314; 2) Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA 02118.

Feeding state either hunger or satiation can dramatically alter an animal's olfactory response to odors. Dynamic changes in the expression of chemoreceptor (CR) genes may underlie some of these changes in olfactory behavior. We have shown for the first time that CR gene expression in the olfactory neuron type, ADL, in C. elegans is modulated by feeding state. We found that the candidate chemoreceptor, srh-234, in ADL is expressed when animals are fed, but not when animals are starved. Using subcellular laser surgery and genetic analysis, we showed that ADL likely functions cell-autonomously as a food sensor to regulate srh-234 expression. However, when animals are fed inedible food that produces the same odor profile as edible food, the expression of srh-234 was reduced. These results suggest that both food signals and internal nutritional signals regulate srh-234 expression in ADL. What are the molecular and neural mechanisms underlying this plasticity in srh-234 expression? Enhancing ADL output by expressing pkc-1(gf) exclusively in ADL enhances the expression of srh-234, whereas blocking ADL output by expressing tetanus toxin in ADL does not significantly affect srh-234 expression. Thus, peptide signals secreted from ADL may modulate srh-234 expression. We show that neuropeptide Y receptor, npr-1, signaling in RMG interneurons connecting to ADL via gap junctions regulate srh-234 gene expression in ADL. Modulation of CR gene expression is thought to be defined primarily by external inputs and not by circuit inputs. Our results reveal for the first time that, in addition to the cell-autonomous function of ADL, feedback modulation from RMG regulates CR gene expression in ADL. We propose an intriguing model by which feeding state- and NPR-1-dependent regulation of CR genes alter sensory perception in ADL. We expect that our findings will not only yield insights into the mechanisms underlying CR expression in sensory neurons in other organisms, but also provide information regarding the basic mechanisms underlying olfactory plasticity.

182. Sensory responses to graded stimuli: a role in bidirectional chemotaxis? **Michael Hendricks**, Luo Linjiao, Aravi Samuel, Yun Zhang. Harvard Univ, Cambridge, MA.

C. elegans can migrate up or down salt gradients toward a remembered cultivation concentration. Both positive and negative chemotaxis are driven by biased random walks of opposite sign, and the chloride-sensing neuron ASER is required for chemotaxis in both directions. We are interested in how the same neural circuit can mediate opposite behaviors. Responses of ASER, measured with genetically-encoded calcium indicators, have been previously examined in response to large, abrupt step changes in salt concentration. However, during chemotaxis assays, crawling animals experience smooth changes in salt concentration. To better match behavioral conditions, we exposed semi-restrained worms to quasi-linear graded changes in salt concentration that match what is experienced by animals migrating on plates (~50 mM/s). ASER shows sustained activity in response to decreasing salt gradients, however the temporal dynamics of these responses differ depending on whether the animals are being tested above or below their cultivation concentration. ASER exhibits activity during increases in salt concentration only above its cultivation concentration. Thus, ASER responses can represent both the direction of the temporal salt gradient and whether it is "toward" or "away" from a remembered set point. We hypothesize that downstream interneurons differentially integrate or respond to the various activity patterns in ASER.

183. Neuropeptide signaling remodels chemosensory circuit composition. **Sarah Leinwand**[1,2], Sreekanth Chalasani[1,2]. 1) Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA; 2) University of California, San Diego, Neuroscience Graduate Program, La Jolla, CA.

Sensory neural circuits detect dynamic changes in the environment and drive behavior. In particular, chemosensory neural circuits have the challenge of representing a potentially limitless set of novel smell and taste stimuli. Neural circuits may achieve this by switching their path of information flow between alternative circuit configurations[1,2]; however, the mechanisms underlying circuit remodeling are poorly understood. We combine genetics, *in vivo* calcium imaging, and behavioral analysis in *C. elegans* to understand how a neural circuit driven by the ASE sensory neuron represents specific changes in salt stimuli to drive appropriate behavioral responses.

We define a novel, sensory context-dependent and neuropeptide-regulated switch in the composition of a *C. elegans* salt sensory circuit. The primary salt detectors, ASE sensory neurons, use a specific peptide-processing enzyme (the proprotein convertase, BLI-4), which previously had no known function in the nervous system, to release insulin neuropeptides in response to large but not small changes in external stimuli. The insulin neuropeptides signal through the tyrosine kinase receptor, *daf-2*, and PI3-Kinase, *age-1*, to functionally transform the AWC olfactory sensory neuron into an interneuron in the salt neural circuit. Consistent with these results, animals with disrupted ASE-AWC neuropeptide signaling show a specific deficit in high, but not low, salt driven behaviors, suggesting that peptidergic signaling potentiates the normal response to high salt. This novel, peptide-regulated high salt circuit configuration may be critical in reinforcing salt appetite to maintain ion homeostasis. Our results show that sensory context and peptidergic signaling select the active routes of information flow from alternative neural circuit configurations, which may be a general mechanism for encoding dynamic environments and driving appropriate behaviors. [1]Anderson, C. & Van Essen, D. PNAS (1987). [2]Weimann, J.M. & Marder, E. Curr Biol (1994).

184. Thermal memory and behavioral regulation revealed by calcium imaging of the cultured neurons and neural circuits. **Kyogo Kobayashi**, Ikue Mori. Div of Bio Sci, Nagoya Univ, Nagoya, Japan.

Many organisms including human are capable of choosing an appropriate behavioral strategy based on the past experience. Unveiling the mechanisms underlying behavioral plasticity is a fundamental question in neuroscience. *Caenorhabditis elegans* shows a memory-based behavior called thermotaxis: the animals migrate to the cultivation temperature on a temperature gradient after cultivation at a certain temperature with food (Hedgecock and Russell 1975). Previous studies have revealed a neural circuit that regulates thermotaxis in which the thermosensory neuron AFD plays a prominent role (Kimata *et al*., 2012). *In vivo* calcium imaging showed that the AFD neuron increases intracellular calcium concentration by responding to warming around the cultivation temperature. This suggests that the AFD neuron itself remembers cultivation temperature and implicates a possibility that AFD neuron functions as not only a thermo-sensing neuron but also a thermal memory cell (Kimura *et al*., 2004). Here, we established *in vitro* calcium imaging system for primary cultures of *C. elegans* neurons, aiming to analyze the AFD neurons in an isolated state from the neural circuit. Cultured AFD neuron responded to temperature stimuli in a culture-temperature depending manner, suggesting that AFD neuron remembers the temperature cell-autonomously. To gain the detailed insight into the molecular mechanism underlying thermotaxis, we also performed *in vivo* calcium imaging of AFD neuron and downstream interneurons AIY and RIA in several mutants. The loss of CREB homolog CRH-1 and CaMKI/IV homolog CMK-1 in AFD neuron changed responding temperature of AFD to lower and broader temperature than that of wild type, respectively, and consequently perturbed AIY activity. In contrast, the loss of CaMKII homolog UNC-43 in AFD neuron did not affect AFD activity, but diminished calcium dynamics in AIY neuron. These results suggest that CRH-1 and CMK-1 act in the memory machinery in AFD and UNC-43 acts in the AFD-AIY synapses.

185. Functional memory loss: Msi-1 is an inhibitor of memory. Nils Hadziselimovic[1], Fabian Peter[1], Petra Hieber[1], Vanja Vukojevic[1], Philippe Demougin[1], Andreas Papassotiropoulos[1,2], **Attila Stetak[1,2]**. 1) Division of Molecular Neuroscience, University of Basel, Switzerland; 2) University Psychiatric Clinics, University of Basel, Switzerland.

In order to maintain a highly flexible nervous system, not only learning and generation of memory but forgetting is also essential to eliminate unnecessary memories to allow adaptation to a constantly changing environment. We recently performed a targeted candidate-gene based screen and we found that the RNA-binding protein musashi (*msi-1*) mediates memory loss. MSI-1 is expressed in neurons and tissue specific rescue demonstrates that MSI-1 function is exclusively necessary in AVA neuron. Among the previously identified MSI mRNA binding partners in vertebrate cells, ACTR2 is one subunit of the Arp2/3 protein complex serving as a nucleation core for the branching of the actin cytoskeleton. Using immunoprecipitation and subsequent RT-qPCR, we found that mRNAs of the *arx-1*, *-2* and *-3* subunits of the Arp2/3 complex associate with MSI-1 *in vivo*. We also show that the protein levels of ARX-1, ARX-2 and ARX-3 are down-regulated upon associative learning that is mediated by the translational inhibitory activity of MSI-1. Using genetic epistasis, we establish a link between MSI-1 and the levels and activity of the Arp2/3 protein complex. We show that activation of the Arp2/3 protein complex through expression of a constitutive active form of *wsp-1* in AVA interneuron can block memory loss similar to deletion of the msi-1 gene. The role of msi-1 in memory loss is also reflected in msi-1 mutants by the strong and persistent consolidation of GLR-1 containing synaptic size increase induced by associative learning. Finally, we demonstrate that GLR-1 signaling regulates both actin capping through the activation of adducin (*add-1*) and inhibition of the Arp2/3 complex mediated actin branching by *msi-1* regulated translational repression and both mechanisms act in concert to establish the proper memory. Thus, our results demonstrate that MSI-1 induces memory loss and represent a novel mechanism of memory regulation linking translational repression to regulation of the structure and complexity of the actin cytoskeleton in neurons.

186. Betaine acts on a ligand-gated ion channel in C. elegans. **Aude S Peden[1]**, Patrick Mac[1], You-Jun Fei[2], Cecilia Castro[3,4], Guoliang Jiang[2], Kenneth J Murfitt[3,5], Eric A Miska[3,5], Julian L Griffin[3,4,6], Vadivel Ganapathy[2], Erik M Jorgensen[1]. 1) Howard Hughes Medical Institute, Department of Biology, University of Utah, Salt Lake City, UT 84112, USA; 2) Departments of Biochemistry and Molecular Biology, Georgia Health Sciences University, Augusta, GA USA 30912, USA; 3) Department of Biochemistry, University of Cambridge, Tennis Court Rd, Cambridge CB2 1GA, UK; 4) Cambridge Systems Biology Centre, University of Cambridge, 80 Tennis Court Road, Cambridge, CB2 1GA, UK; 5) Wellcome Trust Cancer Research UK Gurdon Institute, University of Cambridge, The Henry Wellcome Building of Cancer and Developmental Biology, Tennis Court Rd, Cambridge CB2 1QN, UK; 6) The Medical Research Council Human Nutrition Research, Elsie Widdowson Laboratory, Fulborn Road, Cambridge, CB1 9NL, UK.

Prior to the advent of synthetic nematocides, natural products such as seaweed were used to reduce nematode infestations. The nematocidal agent in seaweed is postulated to be betaine, a small amino acid that acts as an osmolyte in most organisms. The molecular mechanism of its toxicity in nematodes is not known. Here, we demonstrate that betaine acts on ACR-23, a nematode-specific ligand-gated ion channel of the cys-loop family in the nematode C. elegans. ACR-23 is negatively regulated by the activity of the betaine transporter SNF-3 and phospholipase Cb (EGL-8). Mutating the betaine transporter (snf-3) in egl-8 background causes the animals to be hypercontracted and paralyzed, due to excessive betaine in the extracellular space. These behavioral defects can be suppressed by mutations in acr-23. The ACR-23 receptor is directly activated by betaine, but not other transmitters such as acetylcholine or choline. ACR-23 is expressed in the nervous system and is required for normal locomotion rate. ACR-23 was previously identified as the only target for a highly specific novel class of anthelmintic drugs, the amino acetonitrile derivatives (AADs). The presence of this novel receptor explains the sensitivity of nematodes to betaine as well as to AADs. Thus, ancient and modern anthelmintics act on the same target -a betaine-activated ion channel that is only found in nematodes.

187. Novel function of the polarity gene PAR-1 in control of activity at the NMJ. **Clara L. Essmann[1]**, Emma Hiley[1], Zhitao Hu[2], Joshua Kaplan[2], Stephen Nurrish[1]. 1) MRC LMCB, Univ College, London, United Kingdom; 2) Massachusetts General Hospital Department of Molecular Biology, Boston, MD.

PAR-1 is a serine/threonine kinase that acts among other PAR proteins to establish cell polarity during the first cell division. PAR proteins, and their orthologs, are highly conserved in metazoans where they are known to form and maintain cell polarity in polarized cells like neurons. Defects in PAR function have been implicated in diseases like Alzheimers disease, however, until now, a role for PAR-1 in C. elegans neuronal function has not been reported. Here we describe a new mutation par-1(nz90) that alters the expression levels of a subset of par-1 isoforms including a new isoform par-1l. Unlike previous par-1 mutants, par-1(nz90) had no obvious affect on embryonic development and homozygous animals were fertile. Expression of par-1l

measured by qPCR was reduced 50% in par-1(nz90). A MOS transposon insertion 5' of the par-1l start ATG was lethal suggesting that par-1l is essential and the true null phenotype of par-1 is lethality. par-1(nz90) mutant animals were strongly resistant to aldicarb suggesting reduced levels of acetylcholine(ACh) release. This was confirmed using electrophysiological recordings of the muscle, which showed a strong decrease in evoked ACh currents. We visualized the expression pattern of this new isoform par-1l and found it expressed in the GABA-ergic but not the cholinergic motorneurons. par-1l, when expressed under a GABA-specific promotor(p.unc25) rescued the aldicarb phenotype of this mutant. Interestingly, rescue did not require PAR-1 kinase activity suggesting that PAR-1 might acts as a scaffold, a novel property of PAR-1. Quantification of presynaptic markers showed a small but significant increase in presynaptic release sites in the GABA-neurons of par-1(nz90) mutants. We speculate that GABA-neurons are forming extra synapses onto the cholinergic motorneurons and cause their inhibition. In support of our model the double mutant par-1(nz90);unc-25 had the same sensitivity to aldicarb as the GABA-synthesis mutant(unc25) alone. We are currently trying to identify the GABA receptor that mediates the resistance to aldicarb in the par-1(nz90) mutants.

188. Neuroligin organizes C. elegans GABAergic NMJs. **Géraldine Maro**[1], Shangbang Gao[2], Michael Liu[1], Mei Zhen[2], Kang Shen[1]. 1) Howard Hughes Medical Institute, Department of Biology, Stanford University, Stanford, California, USA; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON M5G1X5, Canada.

The trans-synaptic adhesion complex neurexin-neuroligin plays key roles in synapse maturation and excitatory/inhibitory balance. Multiple neuroligins have been hypothesized to function both redundantly, and distinctly to influence synaptic connectivity or validate specific types of chemical synapses. The C. elegans genome contains a single neuroligin and neurexin encoding genes, and therefore represents a simplified genetic system to address the role of these molecules in the developing and mature nervous system. We found that at the C. elegans NMJs, NLG-1 localizes specifically at inhibitory GABAergic postsynaptic terminals. Moreover, postsynaptic NLG-1 is required for clustering of the GABAA receptor UNC-49, and nlg-1 mutants show a decrease in both the frequency and amplitude of spontaneous inhibitory postsynaptic events. Interestingly, nrx-1 null mutants do not share GABAergic NMJ morphological defects. We propose that NLG-1 is recruited to GABAergic postsynaptic terminals through a trans-synaptic interaction that is largely independent of NRX-1.

189. Synaptic engineering: an ionic switch of behavior. **Jennifer K Pirri**, Diego Rayes, Mark J Alkema. Neurobiology, UMass Medical School, Worcester, MA.

The unraveling of the human brain connectivity map is considered by many as an essential step in the understanding how the brain controls behavior and how brain malfunction underlies behavioral disorders. Although the behavioral output of neural networks depends on a delicate balance between excitatory and inhibitory synaptic connections, the neural connectivity map does not carry information about the sign of synaptic connections.. Is it possible to reverse the behavioral output of a neural circuit by changing the sign of a synapse? Does the sign of a synapse provide constraints to the development, the specification, and behavioral output of a connectome? Here, we address these questions using the neuronal circuit that mediates the C. elegans escape response. In the escape response a single pair of tyraminergic neurons coordinate the suppression of head movements with backward locomotion through the activation of an inhibitory postsynaptic receptor, the tryamine-gated chloride channel LGC-55. LGC-55 is a member of the Cys-loop ligand gated ion channel (LGIC) family, whose ion selectivity is determined by the M2 domain. Amino acid substitution of the M2 domain LGC-55 allowed us to change the selectivity from Cl^- to Na^+ and K^+. We generated transgenic animals that express the excitatory LGC-55 cation channel under control of its endogenous promoter in an LGC-55 anion mutant background. A fluorescently tagged LGC-55 cation channel localizes opposite to tyramine release sites indistinguishable from the wild-type LGC-55 anion channel. Exogenous tyramine induces neck muscle relaxation and backward locomotion in the wild-type LGC-55 anion animals, but induces neck muscle contraction and forward locomotion in transgenic animals that express the LGC-55 cation. Similarly, touch or optogentically induced release of endogenous tyramine triggers opposite behavioral responses in animals that express the LGC-55 cation vs LGC-55 anion channel. Our data show that changing the nature of a synapse within a neural circuit can reverse its behavioral output and indicate that the C. elegans connectome is established independent of the nature of synaptic activity or behavioral output.

190. Multiple independent calcium pools in a nociceptive neuron in *C. elegans*. **Jeffrey Zahratka**, Richard Komuniecki, Paul Williams, Bruce Bamber. Biological Sciences, University of Toledo, Toledo, OH.

To navigate constantly changing environments, animals integrate sensory inputs and internal state information to formulate proper locomotory commands. Monoamines and neuropeptides encode internal state information, modulating sensorimotor circuits at several levels, including the sensory neurons themselves. We study aversive behaviors in *C. elegans* to better understand how neuromodulators affect circuit function, in terms of the physiological states of single identifiable neurons. *C. elegans* reacts to the noxious odorant 1-octanol by reversing locomotory direction, to move away from the odor source. Worms off food respond to diluted 1-octanol in 10s, but in the presence of food, response time shortens to 5s. Endogenous 5-HT and neuropeptides are key signaling intermediates in this food-dependent modulation, relying in part on a 5-HT receptor (SER-5) in the ASHs (the pair of nociceptive neurons that sense 1-octanol). Using the GCaMP3 Ca^{++} indicator, we show that a saturating concentration of 1-octanol dissolved in buffer (~2 mM) elicits robust excitation of ASHs when applied to the nose. At least 3 distinct Ca^{++} pools exist in ASHs: distal dendrite, proximal dendrite/soma, and axon. Proximal dendrite/soma Ca^{++} responses are sensitive to Nemadipine-A, a blocker of L-type Ca^{++} channels. Distal dendrite responses, and more importantly, axonal responses are resistant to Nemadipine-A. This finding suggests that somal Ca^{++} influx is unnecessary for signal propagation from one end of the cell to the other, consistent with isopotentiality within *C. elegans* sensory neurons. What then is the role of somal Ca^{++} influx? Rather than amplifying or relaying chemosensory signals to axons, we hypothesize it is intermediate in sensory modulation, and in support, monoamine signals regulating aversive behavior also regulate ASH somal Ca^{++} signals. We are currently testing whether the different Ca^{++} pools within ASHs are the result of regionalized expression of Ca^{++} channels, characterizing the monoamine and neuropeptide signaling cascades affecting ASH somal Ca^{++}, and elucidating the roles for ASH somal Ca^{++} by direct measurement of ASH synaptic release onto postsynaptic targets and behavioral assays.

191. Two Minds of a Worm: Comparison of the L4 and Adult Hermaphrodite Connectomes. **S. Cook**[1], C. Brittin[1], T. Jarrell[1], D. Hall[2], S. Emmons[1,2]. 1) Departments of Genetics; 2) Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

The functional output of a nervous system is governed by the properties and connectivity of its individual neurons and the circuitry they form. How

neurons wire together during development remains a fundamentally unanswered neurobiological question. We have improved the description of nervous system connectivity of the C. elegans hermaphrodite by performing a complete reconstruction of four of the five serial section EM series used by White et al., 1986. Our new data (see wormwiring.org), include spatial coordinates for neurons, synapses, and NMJs as well as a measurement of synaptic weights. Our adult data (N2U) include a significantly greater number of chemical synapses (presynaptic densities) and gap junctions than in previous reconstructions. These newly scored synapses create 1199 new chemical edges (connected neuron pairs) for a total of 3287, and 234 new gap junction edges for a total of 1034, mostly small, in the graph of connectivity. Comparison of our newly annotated L4 series (JSH) to N2U (an "old" adult) reveals significant ongoing synaptogenesis between these two time points. The adult animal contains twice as many chemical synapses in the nerve ring region (5939 vs 2784) yet a similar number of gap junctions (766 vs 727). Of the 3155 synapses added between L4 and late adulthood, the majority are small, but half create new edges. Conversely, the L4 harbors 609 chemical edges not present in the adult. These could be inter-worm differences, but may also suggest some synaptic pruning during development. Comparison of L4 and adult connectomes using graph theoretic techniques reveals nearly identical network characteristics, including clustering coefficient, path length, and similarity in connectivity between homologous L/R neurons. Partitioning the graph according to the amount of connectivity between neurons reveals biologically relevant communities associated with known circuits involving pheromone sensation, thermosensation, head mechanosensation and locomotion, touch, and motor neuron output. However, some differences between the L4 and adult affecting the connectivity of AIA, AIB, and command interneurons suggest possibly significant differences in navigation that can be tested.

192. Gene Regulatory Networks. **Marian Walhout**. Univ. of Massachusetts Medical School.

193. A Transdifferentiating Cell Requires Dynamic Histone Modifying Activities. **Steven Zuryn**, Arnaud Ahier, Marie Charlotte Morin, Sophie Jarriault. Development and Stem Cells, IGBMC, Strasbourg, France.

Postmitotic cellular identity is generally a stable feature of multicellular organisms. However, there are naturally occurring instances whereby cells can transdifferentiate into other cell types with different functions. Documented examples are rare, but intriguing, as they necessitate a highly dynamic and wide change in the gene expression program from one cell-type to another. A key question in the field is whether the complete direct conversion of one cell into a different functional cell involves epigenomic changes, especially as the state of the epigenome has been suggested to represent a barrier to induced reprogramming. Here, through the use of a forward genetic screen in *C. elegans*, we found that the robustness of a highly precise transdifferentiation event whereby a postmitotic hindgut epithelial cell (called Y) changes into a motoneuron (called PDA) requires the sequential and overlapping activities of a histone H3 K27 demethylase (HDM) and a H3 K4 methyltransferase (HMT). Perturbation of their catalytic activities, as well as artificial modulation of histone methylation states within the Y cell decreased the efficiency of successful transdifferentiation events. We uncovered that the different steps of the conversion process have different requirements for the HMT and HDM activities. The HMT is required early to permit the loss of hindgut epithelial identity; a role performed in association with a *C. elegans* Nanog and Oct4 Deacetylase complex, a known pluripotentcy complex in mammalian cells. Next, the HDM and HMT act together to promote re-differentiation into a motoneuron via induction of PDA specific genes. The exquisite timing of this step-wise epigenetic reprogramming is correlated with dynamic protein levels of the HDM during conversion. Ectopic presence of the HDM at inappropriate times negatively impacted on proper transdifferentiation, suggesting that a critical balance between H3 K4 methylation and H3 K27 demethylation activities at specific steps of conversion are required. Together, our data suggests that step-wise epigenomic reprogramming - mediated by histone H3 K27 demethylating and H3 K4 methylating enzymes - permits robust and precise conversion of postmitotic cell identity.

194. Holocentromeres are dispersed point centromeres localized at transcription factor hotspots. **Florian A. Steiner**[1,2], Steven Henikoff[1,2]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA.

Centromeres are universally marked by the conserved variant histone cenH3 (also called HCP-3 or CENP-A), but vary greatly in size and sequence composition. They range from 'point' centromeres with a single cenH3-containing nucleosome to 'regional' centromeres embedded in tandemly repeated sequences to holocentromeres that extend along the length of entire chromosomes. However, the precise distribution of centromeric nucleosomes in regional and holocentromeres has remained elusive. We used high-resolution mapping of cenH3-associated DNA to show that *Caenorhabditids elegans* holocentromeres are organized as dispersed but discretely localized point centromeres, each forming a single cenH3-containing nucleosome. These centromeric sites co-localize with kinetochore components, and their occupancy is dependent on the cenH3 loading machinery. The sites coincide with non-specific binding sites for multiple transcription factors ('HOT' sites), which become occupied when cenH3 is lost, suggesting that HOT site factors act as centromere 'placeholders'. Our results provide the first insight into the precise organization of centromeric nucleosomes outside of budding yeast. We show that the point centromere is the basic unit of holocentromeres and provide a basis for understanding how centromeric chromatin is maintained.

195. A gene-activating pathway mediated by small RNAs (RNAa) protects self-transcripts from epigenetic silencing in C. elegans. **Meetu Seth**[1], Masaki Shirayama[1,2], Weifeng Gu[1], Takao Ishidate[1,2], Darryl Conte Jr[1], Craig C. Mello[1,2]. 1) RNA Therapeutics Institute, University of Massachusetts Medical School, Worcester, MA; 2) Howard Hughes Medical Institute.

Plants and animals employ a sophisticated array of mechanisms to detect and silence pathogenic nucleic acids. For example, the well-studied, sequence-specific silencing mechanism, RNA interference (RNAi), detects pathogenic activity by scanning for double-stranded (dsRNA), a hallmark of RNA-dependent RNA replication. However, many pathogens do not produce dsRNA, and it is clear that organisms can utilize additional strategies to detect and silence foreign gene expression. Recent studies on transgene silencing in C. elegans have uncovered an RNA surveillance system mediated by the Piwi Argonaute, PRG-1, and its genomically-encoded piRNA cofactors. Remarkably this system does not appear to recognize a structural or molecular feature of foreign sequences, but rather identifies foreign RNA by comparing the sequence information itself to a memory of previous gene expression. Interestingly, our findings also suggest that under some circumstances foreign sequences can be adopted (or "licensed", see Johnson and Spence (2011) Science, 333:1311) as "self." One phenomenological manifestation of licensing is the ability of an "adopted" transgene to send a sequence-specific anti-silencing signal that can convert a homologous epigenetically silent allele to an active state. We now have evidence that this transactivation mechanism is mediated by the CSR-1 Argonaute. We find that CSR-1 activity is required parentally (both maternally and paternally) and that heterozygosity for a csr-1 deletion (in either

parent) abolishes transactivation. The cloning of CSR-1-associated small RNA reveals that transactivation correlates with the accumulation of CSR-1-22G-RNAs targeting the foreign (GFP) part of the transgene. We also provide evidence that a licensing signal builds up over multiple generations, such that expressed GFP transgenes become more resistant to silencing over time. Our findings are consistent with a model in which Argonaute systems function together to recognize foreign sequences by constantly comparing de novo RNA expression with a memory of RNA expression from previous generations.

196. X-Chromosome Restructuring Imposed by the Dosage Compensation Complex and Its Relationship to Nuclear Pores. **Qian Bian**, Emily Crane, Satoru Uzawa, Barbara J. Meyer. HHMI and U.C.Berkeley.

In C. elegans, dosage compensation equalizes X-chromosome gene expression between males (XO) and hermaphrodites (XX) by reducing transcription from both hermaphrodite X chromosomes by half. Dosage compensation is implemented by a dosage compensation complex (DCC) that contains 5 subunits homologous to canonical subunits of condensin, a complex that restructures mitotic and meiotic chromosomes in preparation for their segregation. The similarity between the DCC and condensin suggests the DCC may modulate X-linked gene expression by remodeling X-chromosome structure. To test this hypothesis, we first examined the relative nuclear location of DCC binding sites using 3D FISH. We found that pairs of rex sites (DCC recruitment sites on X) between 500 kb and 8 Mbp apart colocalize at a higher frequency in wild-type XX embryos and XO mutant embryos with a DCC-bound X than wild-type XO embryos lacking DCC binding on X. Both 5C and Hi-C experiments confirmed the DCC-dependent interactions between rex sites and revealed significant differences in X-chromosome architecture between wild-type embryos and DCC-deficient embryos. We also examined the 3D location of non-DCC binding sites on X using FISH. We found that several pairs of non-DCC binding sites 500 kbp apart are in physical proximity more frequently when the DCC is bound to X. This result suggests that regions of X distal to DCC binding sites become more compacted upon DCC binding, likely a result of direct interactions mediated by DCC binding at distal rex sites. We also found that distant rex sites preferentially co-localize at the nuclear periphery in embryos with DCC binding to X, but not in wild-type XO embryos. Using 3D structured illumination microscopy, we performed sub-diffraction imaging of the nuclear periphery in embryos and observed that the pairs of rex sites in close proximity are associated with nuclear pore complexes (NPC) and not nuclear lamin. Furthermore, we show that the DCC also associates with NPCs at the nuclear periphery. Our results show that the DCC imposes a structure on X and imply that nuclear pores may assist in restructuring X chromosomes through physical interactions with the DCC.

197. A Developmental Time Course of Transcription and Subsequent Computational Bayesian Unification Reveals Multiple Global Waves of Gene Regulation. **Max E. Boeck**[1], Chau Huynh[2], Lou Gevirtzman[2], Daniel Mace[2], LaDeana Hillier[2], Owen Thompson[2], Pnina Strasbourger[2], Guilin Wang[3], Valerie Reinke[3], Robert Waterston[2]. 1) Biology, University of North Carolina, Chapel Hill, NC; 2) Genome Sciences, University of Washington, Seattle, WA; 3) Genetics, Yale University, New Have, CT.

We sequentially harvested populations of highly synchronous embryos every 30 minutes across development followed by RNA-seq. By combining multiple independent time courses using a novel Bayesian unification method we mapped transcriptional output to specific developmental timepoints. This revealed a transcriptional program of high temporal resolution and with digital expression measurements of whole genes, exons, splice junctions, splice leaders and ncRNAs. We found the previously known systemic down regulation of genes prior to the onset of zygotic transcription; we also uncovered two subsequent waves of regulation post zygotic transcription. The second wave is a downregulation of genes just after gastrulation while the third is a large, genome-wide upregulation and downregulation of genes prior to hatching. These third wave of upregulated genes are enriched for GO terms associated with larval development, indicating a global onset of larval cell fate. The concurrent downregulated genes are enriched for GO terms associated with nucleotide biosynthesis, indicating a systemic down regulation of DNA replication genes following the terminal embryonic cell division. We also found more than 1000 instances of differential exon and splice usage during embryonic development. Within operons we found a trend for high correlation of gene expression, but also a number of operon genes with lower correlation. By mining existing modENCODE chromatin data we found those operons with lower correlations are enriched for activating histone marks upstream of internal transcription start sites and show strong evidence for more ordered nucleosomes. These operons with low correlations then are likely the result of independent, internal promoters. These trends were particularly strong for the second gene within the operon. By developing this time series we hope to provide a resource for the worm community and an example of how to combine multiple modENCODE datasets.

198. Epigenetic control of terminal neuronal differentiation in *Caenorhabditis elegans*. **Chaogu Zheng**, Siavash Karimzadegan, Martin Chalfie. Department of Biological Science, Columbia University, New York, NY.

Although epigenetic control of the cell fate choice in embryonic stem cells is well established, little is known about the importance of epigenetic regulation during the terminal differentiation of neurons. We found that the subtype diversification of VC neurons requires H3K9 trimethylation, which restrained the expression of functionally important transcription factor UNC-4 in the vulval subtype of VC neurons. Using a rapidly degraded form of GFP, We found that only the vulval VC neurons (VC4 and VC5) but not the nonvulval subtype (VC1-3 and VC6) expressed *unc-4*. Mutations in H3K9 methyltransferases (MET-2/SETDB1 and MET-1) and H3K9me3-binding proteins (CEC-3/Chromodomain protein, HPL-2/HP1, LIN-13/HP1-interacting protein, and LIN-65/MBT domain-containing protein), as well as a Q/N-rich domain-containing protein PQE-1, caused ectopic expression of *unc-4* in all six VC neurons, suggesting that H3K9 trimethylation was essential for silencing *unc-4* expression. We then found that *unc-4* expression in VC neurons depended on signal from the vulval tissue, since Muv mutants showed ectopic expression of *unc-4* in extra VC neurons flanking the pseudo vulvae. EGF signal secreted from the vulF cells triggered *unc-4* expression in the vulval VC neurons through the EGFR signaling, because either ablation of vulF cells or mutations in the EGFR/RAS/RAF/MAPK pathway eliminated *unc-4* expression in VC4 and VC5 neurons. Epigenetic silencing of *unc-4* was established in all six VC neurons prior to the differentiation cue. EGF signal from the developing vulva induced derepression of *unc-4* gene in the proximal VC4 and VC5 neurons, but the signal could not reach the distant nonvulval VC cells, which kept *unc-4* silenced. Downstream of the EGFR signaling in vulval VC neurons, the transcription factor LIN-11 and H3K9-specific histone demethylases removed the suppressive histone marks and derepressed *unc-4* transcription. Behaviorally, expression of UNC-4 in all the VC neurons caused an imbalance in the egg-laying circuit and resulted in egg-laying defects. Thus, epigenetic mechanisms by establishing subtype-specific gene expression regulate the terminal neuronal differentiation needed for optimal activity of a neural circuit.

199. MNR-1/menorin, a novel skin-derived cue, controls arborization of sensory dendrites in *C. elegans*. **Y. Salzberg[1]**, C.A. Diaz-Balzac[1], N. Ramirez[1], M. Attreed[1], E. Tecle[1], Z. Kaprielian[2,3], H. Buelow[1,3]. 1) Department of genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Pathology, Albert Einstein College of Medicine, Bronx, NY; 3) Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Sensory dendrites depend on cues from their environment to pattern their growth and direct them towards their correct target tissues. Yet, little is known about dendrite-substrate interactions during dendrite morphogenesis. Here we describe MNR-1/menorin (*W01F3.1*), which is part of the completely unstudied, conserved Fam151 family of proteins, and is expressed in the hypodermis to control the elaboration of the 'menorah'-like dendrites of the mechanosensory PVD and FLP neurons in *C. elegans*. We provide biochemical and genetic evidence that MNR-1 acts as a contact-dependent or short-range cue, in concert with the neural cell adhesion molecule SAX-7/L1CAM in the hypodermis, and through the neuronal transmembrane receptor DMA-1 on sensory dendrites. Our data describes a previously unknown pathway that provides spatial information from the skin substrate to non-autonomously pattern sensory dendrite development.

200. Reduced Insulin/IGF1 signaling restores germ cell immortality to *Caenorhabditis elegans prg-1* Piwi mutants. **Matt A. Simon[1,2,3]**, Peter Sarkies[4], Kohta Ikegami[3], Leonard Goldstein[4], Aisa Sakaguchi[1], Eric Miska[4], Shawn Ahmed[1,2,3]. 1) Department of Genetics, UNC, Chapel Hill, NC; 2) Curriculum in Genetics and Molecular Biology, UNC, Chapel Hill, NC; 3) Department of Biology, UNC, Chapel Hill, NC; 4) The Gurdon Institute, University of Cambridge, Cambridge, UK.

Germ cells can maintain themselves indefinitely over many generations, effectively free of proliferative damage. Pathways that promote germ cell immortality could be relevant to forms of stress that accumulate to cause aging as human somatic cells proliferate. Deficiency for the *C. elegans* Piwi orthologue *prg-1* has been previously reported to result in sterility at high temperature (1, 2, 3). We found that outcrossed *prg-1* strains display germ cell mortality, in which lineages display normal fertility for many generations but ultimately become completely sterile. Although PRG-1 can repress the Tc3 transposon, our data suggest that transposition is unlikely to cause sterility. Instead, several lines of evidence suggest that *prg-1* mutants may become sterile as a consequence of a heritable form of stress. For example, the transgenerational lifespan of *prg-1* mutants (number of generations to sterility) was doubled by brief periods of starvation at high temperature, a condition that promotes formation of stress-resistant dauer larvae. In addition, reduced levels of *daf-2*-mediated insulin/IGF1 signaling, which promotes dauer formation and somatic longevity, rescued the progressive sterility phenotype of *prg-1* mutants, and even restored fertility to sterile *prg-1* mutants. Desilencing of repetitive segments of the genome occurred in late-generation *prg-1* mutants but not in late-generation *prg-1; daf-2* mutants. Further, reduced insulin/IGF1 signaling restored levels of 22G siRNAs that target repetitive loci in *prg-1* mutants. Thus, desilencing of repetitive loci could be relevant to germ cell mortality of *prg-1* mutants. We propose that the Piwi/PRG-1 small RNA silencing pathway represses a heritable epigenetic stress that can be transmitted via gametes, that can be ameliorated by insulin/IGF1 signaling, and that could be relevant to proliferative aging of somatic cells. 1. Batista, P.J. *et al*. (2008). 2. Das, P.P. *et al*. (2008). 3. Wang, G. & Reinke, V. (2008).

201. A deletion polymorphism in the *C. elegans* RIG-I homolog disables antiviral siRNA formation and immunity. **Jeremie Le Pen[1,2]**, Alyson Ashe[1,2], Peter Sarkies[1,2], Tony Bélicard[1,2], Amy Cording[1,2], Nicolas J. Lehrbach[1,2], Marie-Anne Félix[1,2], Eric A. Miska[1,2]. 1) Wellcome Trust Cancer Research UK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK; 2) Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK.

RNA interference is an important effector of antiviral immunity in plants and animals. Yet, how Dicer can sense the foreign genomes of RNA viruses is still poorly understood. We address this question using the nematode *Caenorhabditis elegans* and its natural pathogen, the RNA virus Orsay. Genomic studies on *C. elegans* wild isolates permitted us to identify a common 159 base-pair deletion in the *drh-1* gene as a major determinant of viral sensitivity. We show that DRH-1, a known Dicer interactor in *C. elegans* and the homologue of the human RIG-I receptor, is required for the generation of viral-derived siRNAs (viRNAs) by Dicer. It is well established in humans that RIG-I can sense foreign nucleic acids and trigger an interferon-based innate immunity response upon infection by RNA viruses such as *Influenza*. Our work with nematodes demonstrates that the conserved RNA binding domain of RIG-I has an ancient role in viral recognition outside of mammals. We therefore propose that RIG-I/DRH-1 acts as modular viral receptor that can couple viral sensing to different effector pathways including RNAi and interferon responses.

202. Death by worm-star: a new way of killing *C. elegans*, with implications for ether lipid metabolism. **Jonathan Hodgkin[1]**, Marie-Anne Félix[2], Laura C. Clark[1], Delia M. O'Rourke[1], Dave Stroud[1], Maria J. Gravato-Nobre[1]. 1) Dept Biochem, Univ Oxford, Oxford, United Kingdom; 2) IBENS, Paris, France.

A bacterial strain known as Verde1, belonging to the coryneform genus Leucobacter, has been found to have a remarkable ability to aggregate *C. elegans* and other rhabditid nematodes by means of tail adhesion. The adhesion leads to the formation of "worm-stars" containing tens to hundreds of worms stuck together by their tails. Star formation only occurs when worms are swimming in liquid but it is rapid (less than 5 minutes), efficient (a few thousand bacteria suffice), largely irreversible and ultimately lethal. Worms trapped in the stars are immobilized and die within 12-48 hours, with concomitant growth of bacteria. This host-killing strategy provides a bacterial equivalent to the nematode-trapping strategies used by some soil fungi. Surprisingly, Verde1 is not lethal to wildtype *C. elegans* when worms are grown on bacterial lawns, despite adhering to the cuticle and inducing a hypodermal damage response. However, it is completely lethal on lawns to many Srf and Bus mutants that have altered surface glycosylation and associated resistance to complementary related pathogens (*Microbacterium nematophilum, Leucobacter* Verde2). This lethality allows efficient selection of Verde1-resistant mutants, which so far define 9 complementation groups. Most of these mutants exhibit reduced surface adhesion by Verde1 and fail to form worm-stars. All Verde1-resistant mutants have generally increased cuticle permeability and fragility. Amongst these are many alleles of *agmo-1*, which encodes the only enzyme able to cleave the ether bond in alkyl glycerols; this suggests that ether lipid derivatives may be important in the worm's outermost surface layers, which provide both the major external permeability barrier, and also targets for pathogenic adhesion and attack.

203A. The novel regulators of RNT-1 stabilization in stress response. **Soungyub Ahn[1]**, Kiho Lee[1], Peter Swoboda[2], Junho Lee[1,3]. 1) School of Biological Sciences, Seoul National University, Seoul, Seoul, Korea; 2) Department of Biosciences and Nutrition, Karolinska Institute, Stockholm, Sweden; 3) World Class University, Department of Biophysics and Chemical Biology, Seoul National University, Seoul, Korea.

Abrupt changes in environment is sometimes dangerous for survival of living organisms. Thus, proper response to environmental signal gives an advantage to living organisms. RUNX transcription factor family proteins have essential roles in development and carcinogenesis. We previously reported that RNT-1, the sole homolog of RUNX protein family in *Caenorhabditis elegans*, is degraded by ubiquitin-proteasome system in normal conditions and that the stabilization of RNT-1 protein is induced by various stress responses in the intestine. The p38 MAPK pathway, a well-known pathway for stress response, mediates the increase of RNT-1 stability. But the mechanism by which RNT-1 protein is degraded in normal conditions has been still unknown except that the ubiquitin-proteasome system is involved. To answer this question, we are trying to find novel regulators involved in this RNT-1 stabilization by using forward genetic analysis. Now, we are isolating and characterizing RNT-1 stabilized mutants in normal conditions.

204B. Trade-offs and bet-hedging in reproductive performance during heat stress. **Erin Zucker Aprison**, Ilya Ruvinsky. Ecology and Evolution, Institute for Genomics and Systems Biology, University of Chicago, Chicago, IL 60637.

Organisms have to protect their reproductive capacity from harsh environmental extremes. We tracked changes in the *C. elegans* reproductive system during chronic heat stress to uncover the strategies used by worms to deal with high temperatures of unknown duration. *C. elegans* can recover from prolonged heat stress and resume laying eggs. The likelihood of producing viable offspring after recovery falls precipitously after exposure to temperatures greater than 28°C but takes a curious upturn at 31°C, and then continues to fall at higher temperatures. We show that most of the loss of fecundity at high temperature is due to sperm damage. We can explain the spike in the likelihood of producing viable progeny after exposure to 31°C because at that temperature worms do not begin to ovulate. Absent ovulation, which is necessary for spermatid activation, all spermatids remain as spermatids, a cell type that appears to be more heat tolerant than mature sperm. In addition, failure to ovulate mitigates damage to the reproductive system. Consistent with this idea, we show that mutants that continue to ovulate at high temperature are less likely to recover progeny, while chemical treatments suppressing ovulation increase the likelihood of recovering progeny. We interpret these dynamic responses as an adaptation to life in variable and unpredictable conditions. The reproductive system of *C. elegans* is exquisitely tuned, such that when temperatures rise above those consistent with larval viability, worms stop ovulating and shut down the reproductive system. This provides a fail-safe response that protects the system from damage by heat stress and preserves its capacity for a time when conditions improve. We show, however, that there is a cost associated with this strategy - it takes considerable time to recover and produce offspring. At temperatures approaching the fail-safe temperature, worms must make a decision to continue reproducing or shut down. Our results suggest that depending on the duration of heat stress either strategy may be advantageous. Heterogeneity that we observed in response to harsher conditions may be a form of bet hedging to ensure the continuation of the population in fluctuating environments.

205C. Protective effect of *Paullinia cupana*, the Guarana, on *Caenorhabditis elegans* under oxidative stress. **Leticia P Arantes**, Marina Machado, Daniele Zamberlan, Cintia Tassi, João da Rocha, Felix Soares. Chemistry Dept,UFSM, Santa Maria, RS, Brazil.

Paullinia cupana (Guarana) is a plant originally from Brazil with growing consumption in many countries. It has several reported functions, including antioxidant activity *in vitro*, and popularly has been linked to a delay in the aging process. However, few results from *in vivo* studies exist. Therefore, we used the well established model organism *Caenorhabditis elegans* to investigate the effects of chronic treatment with guarana fruit extract on oxidative stress *in vivo*. After synchronization, L1-larva stage wild type (WT) worms were transferred to agar plates seeded with *E. coli* OP50 and guarana extract in the concentrations of 100, 500 and 1000 mg/ml.To assessment of oxidative resistance, L4 WT treated worms were exposed to juglone 125 mM for 1 hour in M9 buffer and scored for viability in a microscope. In order to measure reactive species, L4 WT and TK22 (mev-1) worms treated with extract were exposed to CM-H_2DCFDA 20 mM for 2 hours. Fluorescence was detected by a multiplate reader and images were taken in a fluorescent microscope. Expression of *sod-3* was quantified after treatment through the fluorescence of L4 worms of CF1553 (*sod-3*::GFP) strain, under normal conditions and after exposure to juglone 50 mM, using a multiplate reader. Images were also taken. Our results showed that guarana extract protected worms against juglone-induced oxidative damage, increasing survival in 26% at 100 mg/ml and 60% at 500 mg/ml. The treatment with extract did not reduce the levels of reactive species under normal conditions but reduced the levels generated by TK22 strain, a respiratory chain defective mutant, with an overproduction of superoxide. The expression of *sod-3* was not altered under normal conditions neither after juglone exposure. As aging and mortality are closely related to external stress, the effects of guarana extract demonstrated on *C. elegans* under oxidative stress are very important. More studies are needed to investigate the mechanisms of antioxidant protection and the effects on aging. Keywords: Antioxidants, Guarana, Oxidative stress, *Paullinia cupana* .

206A. Evaluation of the activity of *Luehea divaricata* Mart. leaf extract against different pro-oxidants in *Caenorhabditis elegans* . **Leticia P Arantes**[1], Dirleise Colle[1], Marina Machado[1], Cintia Tassi[1], Daniele Zamberlan[1], Ritiel da Cruz[2], João da Rocha[1], Melânia Manfron[2], Margareth Athayde[2], Felix Soares[1]. 1) Chemistry Dept, UFSM, Santa Maria, RS, Brazil; 2) Industrial Pharmacy Dept, UFSM, Santa Maria, RS, Brazil.

Oxidative stress has been implicated as a major cause of many clinical abnormalities. Thus, different research groups have been interested for new antioxidant substances, especially natural. Since there are only few studies about the pharmacological effects of *Luehea divaricata* Mart., we investigated the antioxidant potential of its leaf extract against different pro-oxidants *in vivo* by use of *Caenorhabditis elegans*. To assessment of oxidative damage, the concentrations of juglone, sodium nitroprusside (SNP), H_2O_2 and Fe^{2+} that killed ±50% of L4-larva stage wild type worms in 1 hour were determinated. To evaluate the antioxidant potential, L4-nematodes were pretreated with extract (100-800 mg/ml) in M9 buffer for 1 hour, exposed to the determinated concentrations of pro-oxidants and scored for viability using a microscope. In order to analyse effects of the extract, worms were observed 24 hours after exposure to 800 mg/ml for developmental and some behavioral parameters. Rutin was identified in the extract after HPLC analyses and was used for comparison in all assays in same concentrations of the extract. The extract increased worm survival (±23%) in adverse conditions generated by juglone (100 mM) and Fe^{2+} (200 mM) but did not protect against the effects caused by SNP (50 mM) and H_2O_2 (200 mM). *L. divaricata* did not alter larval development, body bends and defecation cycle length, but increased pharyngeal pumping rate. It can be due to the reported effect of flavonoids as acetylcholinesterase inhibitors. Results were similar to rutin in all assays. Together, these results contribute to the increase of knowledge about plant extracts and its compounds that can be used as medicines to prevent and treat health disorders associated with oxidative stress, including Alzheimer's disease. Keywords: Antioxidants; Natural products; *Luehea divaricata* .

207B. Screening of antioxidant organochalcogen compounds in Caenorhabditis elegans: focus on modulation of DAF-16/FOXO pathway. **Daiana S Avila**[1], Suzi Wollenhaupt[1], Ana Thalita Soares[1], Willian Salgueiro[1], Diego Avila[2]. 1) Universidade Federal do Pampa- UNIPAMPA, Uruguaiana; 2) Universidade Federal de Pelotas- UFPel.

The nematode Caenorhabditis elegans has emerged as an important tool in pharmacology and toxicology because of its invariant and fully described developmental program, well-characterized genome, ease of maintenance and genetic manipulation, short and prolific life cycle, and small body size. This model has shown to be useful in the evaluation of the biological activity and the mechanisms of action of new synthetic compounds such as organoselenium and organotellurium compounds. Most of these compounds depict antioxidant potential in other models, however little is known regarding their mechanisms. Antioxidant response can be modulated by intracellular signaling, such as FOXO pathway, which is DAF-16 pathway in worms. This work was based on the hypothesis that depending on the chemical structure of the compounds, they would modulate the insulin-like pathway DAF-16 and consequently depict higher antioxidant potential against pro-oxidants. N2, TJ356 and CF1553 strains were handled and maintained at 20°C on E. coli OP50/ NGM plates. The lethal dose 50% (LD50) of the compounds was determined with doses ranging from 0.1 to 2250 mM. Synchronized L1 worms were treated with the compounds for 30 min. For stress- resistance assays, worms were pre-treated for 30 min with compounds and then exposed for 30 min to the prooxidant paraquat. We determined the LD_{50} using a sigmoidal dose-reponse curve and for the resistance assays we used one-way ANOVA to compare groups. Our results showed that xylofuranosides and some quinolines containing Se or Te have very low toxicity. We have found that Te-containing compounds can modulate DAF-16 pathway at lower concentrations, with consequent increase in SOD-3::GFP expression. Consequently, these compounds presented higher antioxidant potential at sublethal concentrations in wild type worms, as observed by protection against paraquat. We also observed that quinolines with a donator group have higher antioxidant activity. Furthermore, these studies show that C. elegans can be contribute to the rational drug synthesis field.

208C. Role of amino-acid pool size in aging-related muscle atrophy. **Meenakshisundaram Balasubramaniam**[1,3], Ramani Alla[2,3], Robert J Shmookler Reis[2,3], Srinivas Ayyadevara[2,3]. 1) Department of Bioinformatics, University of Arkansas at Little Rock, Little Rock, AR, & UAMS; 2) Department of Geriatrics, University of Arkansas for Medical sciences, Little Rock, AR; 3) Central Arkansas Veterans Healthcare System, Little Rock, AR.

Skeletal muscle mass declines to varying degrees with age and this is a significant contributor to frailty, loss of mobility and increased mortality. Reduced muscle mass can result from a decrease in muscle synthesis and/or an increase in degradation, as a consequence of trauma, aging and many age-onset disorders. A previous NMR study reported that a long-lived mutant strain has elevated pool levels of several amino acids, in particular the branched-chain amino acids leucine, isoleucine, valine, phenylalanine, tyrosine and tryptophan. To learn whether specific amino acid pools are generally associated with improved muscle retention or lifespan across diverse longevity mutants, we have determined the free aminoacid levels in a panel of C.elegans longevity mutants.In our initial assay we found increased pools of several branched-chain amino acids in the long-lived mutant strains. Remarkably, dietary supplementation of these aminoacids reduced age-dependent sarcopenia and moderately extended life span for a wild-type strain. Based on these results in a model system, we predict that prolonged amino acid supplementation may enhance muscle mass, strength and function in older persons by increasing muscle growth and quality.

209A. Microfluidic devices for electrical measures of pharyngeal health. **Stephen A. Banse**[1,2], John. H. Willis[2], Kristin J. Robinson[1], Janis C. Weeks[1], Patrick C. Phillips[2], Shawn R. Lockery[1]. 1) Institute of Neuroscience, University of Oregon, Eugene, OR; 2) Institute of Ecology and Evolution, University of Oregon, Eugene, OR.

The conventional method of health span screening in *C. elegans* currently faces three critical barriers: the absence of rigorously standardized culture conditions, the difficulty of performing longitudinal studies on individuals, and the challenge of high-throughput quantification of feeding behavior, one of the most reliable measures of health and a predictor of longevity in the worm. Whereas new microfluidic technologies are being developed to provide controlled growth chambers that minimize the first two challenges, the technology to automate measurements of feeding behavior is lagging behind. In *C. elegans* aging research, pump rate (0-5 Hz) is currently recorded and analyzed manually by direct observation of slow-motion videos of single worms while feeding on agar plates. It currently takes 5 hours to record and analyze 10 minutes of video, a 30:1 ratio that has become a bottleneck in *C. elegans* aging research, particularly in health-span screens requiring large data sets.

We have devised an alternative approach using a recently developed microfluidic device for recording the electrical activity of the pharynx - an electropharyngeogram (EPG). These recordings can be made on eight or more worms at once, and analyzed computationally to extract pump frequencies, as well as higher-order pump features not visible in videos. Consistent with traditional measures, EPGs reveal generalized decay in pharyngeal pumping with age, with effects revealing themselves as early as 5 days. Additionally, we observe that interventions that slow aging have clear EPG phenotypes. We believe that compared to traditional video analysis, this approach will be more amenable to automation, less subjective, and provide a more information-rich readout of pharyngeal health. Additionally, this technology presents the possibility of combining EPG with other microfluidic devices to create arrays of sealed, individually addressable culture chambers that permit longitudinal assays of feeding behavior and other established measures of *C. elegans* health in tens, and ultimately hundreds, of worms at a time.

210B. Persistent effects of starvation on multiple life history traits. Moses Sandrof, Meghan Jobson, James Jordan, **Ryan Baugh**. Dept Biol, Duke Univ, Durham, NC.

Starvation during early human development can increase adult disease risk, but it is thought that epigenetic effects of starvation could be adaptive if famine persists. We wanted to know if C. elegans has a potentially adaptive epigenetic response to starvation. To address this question, we characterized the response to early larval starvation during L1 arrest. We found a variety of phenotypic effects following recovery from extended L1 arrest. Remarkably, some of these effects persist for multiple generations. Growth and development are delayed after extended starvation, producing smaller adults, and fertility is reduced, but heat resistance increases. Starvation causes a striking amount of phenotypic variation among isogenic individuals, and those that develop slowest are least fertile but most heat resistant. But do these observations reflect an adaptive response to starvation, perhaps involving an epigenetic fitness trade-off, or are they part of a pathological response? L1 arrest increases heat resistance within hours, and resistance persists but

declines through larval development, persumably due to chaperone dilution. Slower growing animals retain resistance longer, suggesting that the primary effect of extended starvation is actually slow growth. Slow growth appears to result from impaired feeding - animals subjected to extended starvation have lower rates of pumping throughout development, they exhibit a grinding defect as evidenced by the presence of whole bacteria in the gut, and individuals with the lowest pumping rates at the L1 stage tend to grow slowest. It was also recently shown that tissue damage is evident in the gonad after extended L1 arrest, resulting in reduced fertility upon recovery. These observations suggest that the effects of extended starvation we have characterized are pathological in nature, stemming from irreversible damage incurred during starvation. However, the manner by which these effects are transmitted to progeny remains an open and interesting question under investigation.

211C. RER-1 - Finding New Roles For An Old Protein. **Kunal Baxi**[1], Ata Ghavidel[2], Troy Harkness[2], Carlos Carvalho[1]. 1) Dept of Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 2) Dept of Anatomy and Cell Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Protein homeostasis is essential for cell survival. In eukaryotes, the ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP) are the two major protein degradation pathways. Accumulation of misfolded/unfolded proteins in the cell elicits a cascade of events collectively termed the Unfolded Protein Response (UPR). UPR activation leads to attenuation of global transcription as well as upregulation of autophagy. Defects in UPS and ALP have been implicated in a variety of neurodegenerative conditions like Parkinson's and Alzheimer's wherein insoluble a-synuclein and b-amyloid protein aggregates accumulate in the brain. Both ALP and UPS activity deteriorates progressively during aging and this is thought to be a major contributing factor in many neurodegenerative conditions. We undertook a genetic screen in yeast to search for mutants with increased lifespan. One such mutation inactivated a gene called rer-1 that encodes a conserved Golgi protein RER-1 involved in Golgi-ER retrotransport. Our data shows that rer-1 knockdown via RNAi in C. elegans leads to an increase in both cellular and reproductive lifespan. In addition, ¬rer-1 mutants show increased UPR stress and autophagy. C. elegans rer-1 mutants are able to alleviate a-synuclein accumulation in body wall muscles and inactivation of lgg-1, a key component of autophagy, is sufficient to reverse this alleviation, presumably via upregulation of UPR. We are currently trying to elucidate downstream effectors of this process and our results suggest a role of intestinal/germline signaling in this regulation.

212A. 7-ketocholesterol acts through steroid receptor DAF-12 to regulate dauer formation and longevity. **Ben Becker**, Adam Antebi. Molecular Genetics of Ageing, MPI for Biology of Ageing, Cologne, Germany.

In favorable environmental conditions C. elegans develops rapidly through four larval stages to adulthood, termed reproductive development. In unfavorable environmental conditions including high temperature, food scarcity, high population density or sterol depletion, C. elegans enters the stress-resistant long-lived dauer stage. During reproductive development, activation of the insulin/IGF-1-like signaling (IIS) pathway results in inhibition of DAF-16/FOXO, thereby stimulating production of bile-acid like steroids called the dafachronic acids (DA). DAs are endogenous ligands of the nuclear hormone receptor DAF-12, a key determinant of dauer formation, and promote reproductive development and short life. Conversely, when these pathways are downregulated, unliganded DAF-12 promotes dauer formation and long life. Aside from DA, relatively little is known about other endogenous signaling molecules that modulate the dauer pathways. To identify novel signaling molecules we performed a dauer enhancer/suppressor screen in the Insulin receptor mutant *daf-2(e1368)* background. We fed animals small molecules metabolites and nutrients, including representative sugars, amino acids, fatty acids, and sterols, and identified modulators of dauer formation in all four compound groups. One of the small molecules we identified was 7-ketocholesterol (7KC). 7KC synergistically enhanced dauer formation of *daf-2(e1368)* (up to 98%) and increased median life-span of N2 wild-type (20%). Interestingly both phenotypes were DAF-16/FOXO independent but strongly dependent on DAF-12. Consistently we found the DAF-12 targets, *mir-84*, *mir-48* and *mir-241*, to be modestly downregulated in 7KC fed animals. Preliminary experiments showed that 7KC suppressed DA dependent DAF-12 activation in cell culture assays. We are currently elucidating the transcriptional output of 7KC treatment. These studies suggest that 7KC either directly or indirectly modulates DAF-12 activity towards longevity enhancing mechanisms. Conceivably similar small molecules could modulate nuclear receptor activity and life span in higher animals.

213B. Identifying the role of *apl-1* through an RNAi Screen and Mosaic Analysis. **Maisam T. Begum**[1], Pei Zhao[2], Chris Li[1,2]. 1) Biology, City College of New York, New York, NY; 2) Graduate Center, City University of New York.

Over 30 million people worldwide are affected by Alzheimer's Disease (AD). One of the pathological characteristics of AD is the presence of senile plaques in patients' brains. The major component of the plaques is the beta-amyloid peptide, which is a cleavage product of the amyloid precursor protein (APP). Mammals have several functionally redundant APP related proteins and the family has an essential function, making study of one individual protein difficult in mammals. *C. elegans* has only one APP-related protein, APL-1. *apl-1* knockouts are not viable and have several other morphological defects. Expression of *apl-1* in neurons, however, is sufficient to rescue the *apl-1* lethality. To identify proteins in the APL-1 pathway, a genome wide RNAi screen is being conducted to identify suppressors of the *apl-1* lethality. Since *apl-1* deletions mutants are not viable, heterozygous *apl-1(yn10)* mutants with flanking genetic markers are being screened to identify viable homozygous *apl-1* mutants. More than half of the C. elegans' genome has been screened, but no suppressors have been identified thus far. We will continue with the RNAi screen to find possible suppressors. Although expression of *apl-1* in neurons is sufficient to rescue the *apl-1* lethality, the specific neurons that require *apl-1* activity are unknown. We are using homozygous *apl-1(yn10)* mutants that are viable because they carry an extrachromosomal array containing the *apl-1* genomic region and a SUR-5::GFP marker to perform mosaic analysis to identify which neurons require *apl-1* activity. We have discovered that animals always require *apl-1* expression in the ventral cord neurons for viability. We are still identifying other neurons in which *apl-1* activity is essential for viability.

214C. Modulation of HIF-1 activity and its effect on stress tolerance and longevity in C. elegans. **P Bharill**, F Fabretti, H Gharbi, B Schermer, T Benzing, R Mueller. Department 2 of Internal Medicine and Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany; Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases, University of Cologne, Cologne, Germany.

While stabilization of HIF-1a can lead to tumor formation in mammals, activation of its worm orthologue HIF-1 extends lifespan. HIF-1a plays a central role in the cellular response to varying oxygen levels. While in worms, hypoxia leads to longevity and increased stress resistance by activation of HIF-1, in

mammals ischemic or hypoxic preconditioning can be exploited to protect organ integrity from a row of damaging stimuli. These findings hold the promise to provide us with novel approaches to also prevent organ failure in the clinical setting of patients being exposed to toxic therapies or ischemic damage. For being able to exploit these findings in an optimal way a better understanding of the underlying molecular mechanisms will be of utmost importance. Due to its simple maintenance and easy genetic amenability C. elegans is the perfect model to study the molecular basics behind the increase in cellular stress resistance underlying the effects of preconditioning. Using gene expression profiling coupled to differential stress testing after activation of hypoxia signaling we are trying to unravel the key players involved. Doing so will not only extend our understanding of the hypoxia pathway in longevity and cellular stress resistance but may also yield a putative list of targets that can be tested in mouse models of organ preconditioning.

215A. The Mechanistic Basis of Neuroendocrine Control of Longevity. **Konstantinos Boulias**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA.

A fundamental question in the field of aging is how different tissues communicate with each other to coordinate the rate of aging of the entire organism. Recent work has underscored the importance of the interplay between the nervous system and the intestine in the regulation of *C. elegans* lifespan by the gonad-dependent and the mitochondrial respiratory signaling pathways (Boulias and Horvitz, 2012; Durieux et al., 2011). Which specific neurons participate in this interaction, which neuroendocrine molecules are secreted, which downstream signaling effectors are used and whether these components are shared among the different longevity pathways (Insulin/IGF-1, gonad-dependent, mitochondrial, etc.), are questions that remain to be addressed. When the germline of *C. elegans* is removed, either by laser microsurgery or by mutations that block germ-cell proliferation, animals live up to 60% longer than controls. This lifespan extension requires the activities of the FOXO family transcription factor DAF-16 and of the steroid hormone receptor DAF-12. Our previous studies identified the microRNA *mir-71* as a novel component of a DAF-16-dependent pathway by which the germ cells regulate lifespan (Boulias and Horvitz, 2012). We showed that *mir-71* acts in neurons to promote the localization and activity of DAF-16 in the intestine, suggesting that signaling among the gonad, the nervous system and the intestine coordinates organismal lifespan. We are now seeking to identify the neuronal networks, signaling molecules (neurotransmitters and/or neuropeptides) and downstream effectors (ion channels, G protein-coupled receptors, etc.) that function to transduce the effects of germ cells on lifespan. To this end, we have started a systematic analysis of neural synaptic function mutants for defects in their lifespan response to germ cell loss. Our preliminary results suggest that *mir-71*-mediated lifespan extension in animals lacking germ cells depends on acetylcholine signaling, G protein signaling and synaptic release via dense core vesicles. We are currently testing whether specific neuromodulators function to promote germline-mediated longevity by regulating the localization and activity of DAF-16.

216B. Proteomics approach to identify potential targets of tyrosol, an olive oil phenol that stimulates longevity and stress resistance in Caenorhabditis elegans. **Ana Cañuelo**, J. Peragón, P. Pacheco. University of Jaen, Jaen, Jaen, Spain.

Tyrosol is one of the most representative phenols of olive oil and olive fruit that induce significant lifespan extension and resistance to thermal and oxidative stress in *Caenorhabditis elegans*. To further understand the cellular mechanisms underlying the action of tyrosol, we have used 2-DE coupled with MS to identify proteins differentially expressed in nematodes grown in a medium containing 250 mM tyrosol. After the comparison of the protein profiles from 250 mM tyrosol and from control, 28 protein spots were found to be altered in abundance (^{3}2-fold). Analysis by MALDI-TOF/TOF and peptide mass fingerprinting (PMF) allowed the unambiguous identification of 23 spots, corresponding to 18 different proteins. These proteins were: vitellogenin-5 (VIT-5), vitellogenin-2 (VIT-2), gex interacting protein 7 (GEI-7), selenocysteine-specific translation elongation factor (EEFSEC), elongation factor thermo unstable (EF-Tu), elongation factor 2 (EFT-2), acyl CoA dehydrogenase (ACDH-3), sorbitol dehydrogenase 1 (SODH-1), adenosylhomocysteinase (K02F2.2), ras related nuclear protein (RAN), chorionic somatomammotropin hormone precursor (CSHP); cytokeratin 9 (CK9), heat shock protein 4 (HSP-4), heat shock protein 90 kDa (T05E11.3), outer membrane protein F chain A (OMPF-A), vacuolar H ATPase 12 (VHA-12), vacuolar H ATPase 13 (VHA-13) and immunoglobulin heavy chain constant alpha1 membrane bound isoform 1 (IGHCA1M). The changes found in the protein-expression levels of these proteins explain how cell functions such as vitellogenesis and embryonic morphogenesis, regulation of protein synthesis, fatty acids, sorbitol and amino acids metabolism, transport of proteins into nucleus, hormone, cytoskeleton, heat shock proteins, and membrane proteins are among the main mechanisms probably implied in the increase of longevity induced by tyrosol in this species.

217C. Mutations in the Translation Initiation Factor Subunit *eIF-3.k* Suppress the Stress Sensitivity of *xbp-1* Mutants. **Douglas Cattie**, Kirthi Reddy, Claire Richardson, Dennis Kim. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

The Unfolded Protein Response (UPR) is a conserved homeostatic mechanism that functions to balance the folding capacity of the endoplasmic reticulum (ER) with the flux of unfolded protein into the compartment. We are interested in the physiological challenges that necessitate UPR function in *C. elegans*. Previously, we demonstrated a specific requirement for the *ire-1/xbp-1* branch of the UPR in mediating the secretory load that results from innate immune activation, as mutants deficient in *xbp*-1 die during larval development when infected with the human opportunistic pathogen *Pseudomonas aeruginosa*. In order to better understand the physiological and pathological role of the UPR we conducted a forward genetic screen to identify mutations that would facilitate larval development of *xbp-1* mutant worms developing on *P. aeruginosa*. From a screen of approximately 10,000 haploid genomes, we isolated 24 mutants and have further characterized three complementation groups. One suppressor mutation of *xbp-1* larval lethality on *P. aeruginosa* was identified in the translation initiation factor subunit *eIF-3.k*. Mutants deficient in *eIF-3.k* also exhibited enhanced resistance to tunicamycin treatment and elevated temperatures, indicating that the resilience of these mutants is not limited to pathogen-induced proteotoxic stress. RNAi knockdown of several other *eIF-3* subunits in the *xbp-1* mutant background similarly restored successful larval development when infected with pathogenic bacteria. Mutations that result in attenuated translation likely contribute to survival in response to ER proteotoxic stress much in the way PEK-1 phosphorylation of eIF2a functions in the UPR by limiting the influx of unfolded protein into the ER. We are currently investigating whether ER stress tolerance is restricted to knockdown of *eIF-3* complex components or whether attenuating translation by other genetic mechanisms also confers these phenotypes.

218A. Biological behavior of carbon nanoparticles in *Caenorhabditis elegans*. **Yun Jeong Cha**[1], Shin Sik Choi[1,2]. 1) Department of Energy and Biotechnology; 2) Department of Food and Nutrition, Myongji University, Yongin 449-728, South Korea.

Although a number of researches on biological applications of nanomaterials have been investigated for the last ten years, the genetic mechanism

behind a biological behavior of nanoparticles has not been fully understood yet. In this study, we illuminated a genetic interaction between carbon nanoparticles and physiological toxicity in *Caenorhabditis elegans*. Water stable suspension of fullerene (nC_{60}) and hydroxyllated form ($C_{60}(OH)_{19-24}$) reduced survival ratio in adulthood of *C. elegans* resulting in diminished body growth and brood size in wildtype N2. In order to elucidate toxic mechanism of nanoparticles, the genes required for lower viability were discovered using mutations in apoptosis-, membrane permeability-, and stress-related regulators.

219B. Novel Kinases that interact with the Insulin-IGF-1-like signaling pathway to control longevity. **Manish Chamoli**, Awadhesh Pandit, Mukund Sudharsan, Arnab Mukhopadhyay. Molecular Aging Lab, National Institute of Immunology, New Delhi, India.

Extensive research over the past decade has identified the Insulin-IGF-1-like signaling (IIS) axis, predominantly a kinase cascade, as a major regulator of aging across the animal kingdom. In *C. elegans*, the IIS pathway regulates dauer diapause, metabolism, stress/pathogen resistance as well as longevity. In order to identify additional kinases that may interact or cross-talk with this pathway and regulate life span, we developed a high throughput 96-well liquid RNAi screening protocol using dauer formation as the quantifiable output. We prepared a kinase mini-library that contained RNAi clones for around 400 serine-threonine kinases and screened for ones that affect dauer formation in *daf-2(e1370)*. We identified several novel as well as previously studied modulators of the IIS pathway. Here we report characterization of two such kinases that enhanced dauer formation of an IIS pathway mutant. Although both the kinases possess activity *in vitro* and increased dauer formation of *daf-2(e1370)*, their mechanisms of action are completely different. One of the kinases works downstream of the IIS pathway in a manner similar to DAF-16, the FOXO homolog in worms and its knockdown suppresses the long life span of *daf-2(e1370)*. The other kinase is a novel signaling intermediate in the dietary restriction pathway and its knockdown increases life span of wild-type worms by ~60% while synergizing with IIS pathway mutants. We will discuss the identity and possible functions of these genes.

220C. Characterization of a gene whose expression correlates with the food type-dependent effects on lifespan. Wolfgang Maier[1,2], **Rashmi Chandra**[3], Joy Alcedo[1,3]. 1) Friedrich Miescher Institute, Basel, Switzerland; 2) Institute for Biology 3, Albert-Ludwig University, Freiburg, Germany; 3) Department of Biological Sciences, Wayne State University, Detroit, MI, USA.

The lifespan of wild-type *C. elegans* is affected by different types of bacterial food sources through a mechanism that is distinct from food level restriction [1]. Because the mechanism that underlies the food type-dependent effects on lifespan remains unknown, we used microarray analyses to identify genes that are involved in this process. Through this approach, we have identified a set of 26 genes whose expression changes are correlated with longevity on different *E. coli* strains [1]. Strikingly, this set is enriched for "pathogen-response" genes. However, unlike their expression on different *E. coli* strains, these genes do not exhibit a similar effect in response to pathogenic bacteria [2]. Indeed, in some cases, the same gene could be up-regulated on one pathogen, but strongly down-regulated on another pathogen. Hence, the regulation of these genes does not necessarily reflect food toxicity, but a more complex modulation of gene expression in response to different food types.

To gain a better understanding of how these genes might affect lifespan in a food type-dependent manner, we are characterizing one of these genes, the acyl coA dehydrogenase *acdh-1*, which has also been recently identified as a dietary sensor [3]. Thus, we are determining the *acdh-1* mutant phenotypes in correlation with the gene's spatial and temporal expression patterns on different food sources. Since we have also found that sensory neurons and neuropeptide signaling are involved in the food-type effects on lifespan [1], we are assessing whether sensory inputs and neuropeptides affect *acdh-1* function on different food types. Together, these studies could yield insight into the mechanisms underlying the food-type effects on lifespan and physiology.

Refs: [1] Maier et al., 2010. *Plos Biol* 8, e1000376; [2] Shapira et al., 2006. *PNAS* 103, 14086-14091; [3] MacNeil et al., 2013. *Cell* 153, 240-252.

221A. *daf-16/FoxO* isoform-specific mutants reveal differential contributions to longevity in the contexts of reduced DAF-2 insulin-like signaling and germline ablation. **Albert Chen**[1], Chunfang Guo[1], Kathleen Dumas[1], Travis Williams[1], Sawako Yoshina[2], Shohei Mitani[2], Patrick Hu[1]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Physiology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.

FoxO transcription factors promote longevity in invertebrates and may influence age-related disease in mammals. In *C. elegans*, the sole FoxO ortholog DAF-16 is required for lifespan extension in the contexts of reduced DAF-2 insulin-like signaling and germline ablation. The *daf-16/FoxO* locus encodes multiple protein isoforms that have distinct N-terminal amino acid sequences while sharing C-terminal sequences. Although the central role of DAF-16/FoxO in life span control by DAF-2 and the germline is well established, the relative contribution of distinct DAF-16/FoxO isoforms to longevity in these contexts is not known. Here, we characterize the first isoform-specific mutant alleles of *daf-16/FoxO*. Two independent *daf-16a* mutations and one *daf-16f* mutation fully suppress dauer arrest of Class 1 *daf-2(e1368)* animals, while partially suppressing dauer arrest of Class 2 *daf-2(e1370)* animals. Both *daf-16a* mutations partially suppress life span extension to the same extent in both *daf-2* alleles. In contrast, *daf-16f* mutation fully suppresses life span extension in *daf-2(e1368)* but has no effect on *daf-2(e1370)* longevity. In *glp-1(e2141)* animals lacking a germline, both *daf-16a* mutations and the *daf-16f* mutation partially suppress longevity to the same extent. Our data suggests that DAF-16A plays a modest but consistent role in all three backgrounds, whereas DAF-16F contributes differentially to longevity in distinct contexts. Our work on multiple DAF-16/FoxO isoforms should inform research on aging in mammals, where multiple FoxO proteins modulate age-related disease phenotypes associated with Type 2 diabetes, cardiovascular disease, osteoporosis, and cancer.

222B. Effects of *C. elegans sgk-1* mutations on life span, stress resistance, and DAF-16/FoxO regulation. **Albert Chen**[1], Chunfang Guo[1], Kathleen Dumas[1], Kaveh Ashrafi[2], Patrick Hu[1]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Physiology, University of California, San Francisco, CA.

The AGC family serine-threonine kinases Akt and Sgk are similar in primary amino acid sequence and substrate specificity *in vitro*, and both kinases are thought to directly phosphorylate and inhibit FoxO transcription factors. In the nematode *Caenorhabditis elegans*, AKT-1 and SGK-1 are thought to act in concert to limit life span by phosphorylating and inhibiting the nuclear translocation of the FoxO transcription factor DAF-16. Here we show that AKT-1 and SGK-1 act in opposition to control C. elegans life span. In contrast to *akt-1* null mutations, which prolong life in a *daf-16/FoxO*-dependent manner and

confer stress resistance, *sgk-1* null mutations shorten life span and enhance sensitivity to ultraviolet radiation and oxidative stress. Accordingly, an *sgk-1* gain-of-function mutation increases life span in a DAF-16/FoxO-dependent manner. Therefore, SGK-1 promotes longevity. Intriguingly, although *sgk-1* null mutants are short-lived, they are thermotolerant, suggesting that they are not short-lived due to general frailty secondary to developmental defects caused by lack of SGK-1 activity. Whereas AKT-1 inhibits DAF-16/FoxO target gene expression by promoting the sequestration of DAF-16/FoxO in the cytoplasm, SGK-1 does not significantly influence DAF-16/FoxO subcellular localization and does not regulate the expression of DAF- 16/FoxO target genes. Thus, in spite of their similar *in vitro* substrate specificities, Akt and Sgk influence longevity through distinct mechanisms *in vivo*. Our findings highlight the need for re-evaluation of current paradigms of FoxO regulation by Sgk.

223C. Sestrin confers the regulations of muscle aging and lifespan in *Caenorhabditis elegans*. Ya-Luen Yang[1], Kah-Sin Loh[2], Bang-Yu Liou[2], I-Hua Chu[3], Cheng-Ju Kuo[1,2], Huan-Da Chen[1,2], **Chang-Shi Chen[1,2]**. 1) Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan, Taiwan; 2) Department of Biochemistry and Molecular Biology, National Cheng Kung University, Tainan, Taiwan, Taiwan; 3) College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Aging is associated with the progressive decline in body function and physiology that is shared by all multicellular organisms. The free radical theory argues that the accumulation of damage caused by reactive oxygen species (ROS) over the time is the cause of aging. Sestrins are evolutionarily conserved in metazoans and are required for ROS clearance. However, whether sestrins *per se* regulate longevity in multicellular organisms is still unclear. Here, we report that SESN-1, the only Sestrin ortholog in *Caenorhabditis elegans*, is a positive regulator of lifespan. *C. elegans sesn-1* mutants exhibit shorter lifespans, hypersensitivity to oxidative stress, and premature aging in muscle. When *sesn-1* gene was overexpressed and rescued, the lifespan of *sesn-1* mutant worms could be extended. These findings imply that SESN-1 is required to protect against general life stressors, is important in regulation of lifespan and healthspan late in life, and might play a key role in muscle integrity.

224A. The Effect of Hydrolysable Tannins from Eucalyptus Leaves on C. elegans Lifespan. **Y. chen[1,2]**, B. Onken[2], H. Chen[1], Q. Huang[2], S. Xiao[1], M. Driscoll[2], Y. Cao[1]. 1) College of Food Science, South China Agricultural University, Guangzhou 510640, China; 2) Rutgers, The State University of New Jersey, U.S.A.

To identify potential pharmacological compounds that delay the progression of age-related degenerative changes and illness, we monitored the effects of six hydrolysable tannins with high antioxidant activities isolated from Eucalyptus leaves (Oenothein B (OEB), 1,2,3,4,6-penta-O-galloyl-b-D-glucose (PGG), Tellimagrandin I (T1), Tellimagrandin II(T2), Pedunculagin (Ped) and Gemin D (GD)) on C. elegans lifespan at four different concentrations. We found that of the six, OEB and PGG extended lifespan in a dose-dependent manner and increased median lifespan by up to 22%. OEB significantly prolonged youthful locomotory ability. We also found that the survival curves of T1-treated animals at all tested concentrations were significantly increased, although median lifespan was not significantly improved. OEB, PGG and T1 did not significantly affect the age-associated physiological functions of reproduction, pharyngeal pumping rate, or age pigment accumulation. Animals treated with 40 mM Ped significantly extended median lifespan by 11%, but we did not observe any significant benefits at other Ped concentrations. T2 and GD did not cause significant lifespan extension at any tested concentration. To further determine how OEB and PGG prolong C. elegans lifespan, we investigated the genetic requirements for these benefits. Strikingly, we found that lifespan extension with OEB and PGG treatment was driven by the insulin signaling, dietary restriction, and mitochondrial function pathways, indicating that these compounds target multiple longevity mechanisms to promote lifespan. Together, our results demonstrate broad-based aging benefits with these botanical compounds, which may be exploited in novel therapies to promote healthy aging.

225B. Nutritional Control of Insulin-Like Peptide Expression during L1 Arrest and Recovery. **Yutao Chen**, Ryan Baugh. Biology Dept, Duke University, Durham, NC.

Animals must coordinate development with fluctuating nutrient availability. Nutrient availability governs post-embryonic development in *C. elegans*: larvae that hatch in the absence of food do not initiate post-embryonic development but enter “L1 arrest” (or “L1 diapause”) and can survive starvation for weeks. Insulin-like signaling is a key regulator of L1 arrest and recovery. However, the *C.elegans* genome encodes 40 putative insulin-like peptides (ILPs), and there is evidence that they can function to promote development (“agonists”) or arrest (“antagonists”). We used the nCounter platform to measure high-resolution mRNA expression dynamics for all 40 ILPs in response to feeding and fasting in recently hatched L1 larvae. Expression of 23 of the ILPs is significantly affected by nutrient availability. We classified several ILPs as candidate agonists or antagonists based on up-regulation in response to feeding or fasting, respectively. 10 candidate agonists (daf-28, ins-3, ins-4, ins-5, ins-6, ins-7, ins-9, ins-26, ins-33 and ins-35) were selected for further analysis. Phenotypic analysis of single or several multiple deletion mutants has not revealed detectable effects on L1 growth or development. Destabilized YFP reporter gene analysis shows that ten putative agonists are expressed in the intestine and various neurons. 6 out of 10 are expressed in the chemosensory neuron ASI and interneuron PVT. ASI is known to regulate dauer formation and lifespan but PVT has not been shown to be directly involved in feeding or stress response. Quantitative image analysis confirms nutritional control of transcription and reveals the intestine as the primary site of transcriptional regulation. Up-regulation in response to feeding in ASI is also evident in 3 candidates. We are currently testing if intestinal expression is regulated autonomously by ingestion of food or non-autonomously by neuronal signals. In summary, our expression analysis reveals the dynamics and sites of ILP expression in response to nutrient availability, providing insight into how post-embryonic development is governed by insulin-like signaling.

226C. Transcriptional profiling reveals a principle role for *wdr-23* in regulating SKN-1 and potential interactions with molting and the cuticle. Lanlan Tang, Andrew Deonarine, Chi K. Leung, **Keith P. Choe**. Department of Biology and Genetics Institute, University of Florida, Gainesville, FL.

The transcription factor SKN-1 regulates responses to oxidants and electrophiles. We previously identified WDR-23 as a repressor of SKN-1. Loss of *wdr-23* causes accumulation of SKN-1, induction of detoxification genes, resistance to oxidants and electrophiles, and increased longevity. Loss of *wdr-23* also slows growth and reduces brood size suggesting that SKN-1 activation may have important consequences. Here, we identify over 800 genes upregulated in *wdr-23(tm1817)* worms, the majority of which are suppressed by *skn-1(RNAi)*. We also demonstrate that stress resistance, longevity, and reproduction phenotypes of *wdr-23(tm1817)* are completely suppressed by a *skn-1* deletion allele suggesting that the primary function of WDR-23 is to tightly regulate SKN-1. We conducted Gene Set Enrichment Analysis for 26 previously published gene sets regulated by a variety of stress and longevity pathways. Gene

sets previously reported to be *skn-1* dependent during stress were among the most strongly enriched for upregulation in our *skn-1* dependent *wdr-23* expression data set indicating that loss of *wdr-23* mimics transcriptional changes induced by SKN-1 during stress. Surprisingly, the gene set with the third greatest enrichment for upregulation in our data set was upregulation in *dpy-10(e128)*. DPY-10 is a cuticle collagen and *dpy-10(e128)* alters cuticle structure. Interestingly, many of the overlapping genes the *wdr-23* and *dpy-10* data sets were previously shown to be required for molting. We have confirmed upregulation of a subset of these molting genes in *wdr-23(tm1817)* worms and preliminary experiments suggest that *wdr-23* is required for normal cuticle function. In addition to disrupting cuticle formation, *dpy-10(e128)* was previously reported to constitutively activate osmotic stress responses; several detoxification genes are also upregulated in *dpy-10(e128)* worms. Taken together, these data suggest functional interactions between the cuticle and the xenobiotic responsive SKN-1/WDR-23 pathway. This work is funded by NSF grant IOS-1120130.

227A. Peroxide Stress Response and Ferritin Synthesis Regulation by the REF-1 Family Member HLH-29. **H.T. Chou**, T.K. Quach, C.M. Johnson. Dept of Biology, GSU, Atlanta, GA.

In *Caenorhabditis elegans*, the six members of the REF-1 family have been identified as functional homologs of the Hairy/Enhancer of Split (HES) proteins. These transcription factors act in both Notch dependent and Notch-independent pathways to regulate embryonic events during development; however, their post-embryonic functions are not well defined. Gene expression microarray analysis from previous reports of our laboratory suggest HLH-29 targets in L4/young adult stage animals are genes needed for the regulation of growth, lifespan, oxidative stress response, fatty acid metabolism, and ferritin biosynthesis. Here we characterize HLH-29 regulation on the ferritin gene *ftn-1* expression via promoter sequences upstream the iron-dependent element, the recognition site of the hypoxia inducible factor, HIF-1. Additionally, *hlh-29* mutants are more resistant to peroxide stress than wild-type animals and *ftn-1*(RNAi) animals, even in the presence of excess iron. Finally we show that HLH-29 acts parallel to DAF-16 but upstream of the microphthalmia transcription factor ortholog, HLH-30, to regulate *ftn-1* expression under normal growth conditions.

228B. Down regulation of miR-124 in both Werner syndrome DNA helicase mutant mice and mutant *Caenorhabditis elegans wrn-1* reveals the importance of this microRNA in accelerated aging. **A. Dallaire**[1], C. Garand[1], E. R. Paquet[1], S. J. Mitchell[2,3], R. De Cabo[2], M. J. Simard[1], M. Lebel[1]. 1) Laval University Cancer Research Centre, Quebec City, Quebec, Canada; 2) Laboratory of Experimental Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, USA; 3) Sydney Medical School, University of Sydney, Sydney NSW, Australia.

Small non-coding microRNAs are believed to be involved in the mechanism of aging but nothing is known on the impact of microRNAs in the progeroid disorder Werner syndrome (WS). WS is a premature aging disorder caused by mutations in a RecQ-like DNA helicase. Mice lacking the helicase domain of the WRN ortholog exhibit many phenotypic features of WS, including a pro-oxidant status and a shorter mean life span. *Caenorhabditis elegans* (*C. elegans*) with a nonfunctional *wrn-1* DNA helicase also exhibit a shorter life span. Thus, both models are relevant to study the expression of microRNAs involved in WS. In this study, we show that miR-124 expression is lost in the liver of Wrn helicase mutant mice. Interestingly, the expression of this conserved *miR-124* in whole *wrn-1* mutant worms is also significantly reduced. The loss of *mir-124* in *C. elegans* increases reactive oxygen species formation and accumulation of the aging marker lipofuscin, reduces whole body ATP levels and results in a reduction in life span. Finally, supplementation of vitamin C normalizes the median life span of *wrn-1* and *mir-124* mutant worms. These results suggest that biological pathways involving WRN and miR-124 are conserved in the aging process across different species.

229C. Activation of the hexosamine pathway improves ER protein quality control and slows aging. **Martin S. Denzel**, Nadia J. Storm, Adam Antebi. Max Planck Inst f Biol of Ageing, Cologne, Germany.

Aging is the progressive loss of cellular homeostasis - including protein quality control. The endoplasmic reticulum (ER) is the site of protein synthesis for all secreted and membrane proteins. ER quality control depends on N-glycosylation of nascent peptide chains and on chaperones as well as degradation mechanisms such as ER associated degradation (ERAD) and autophagy. ER function is critical for normal *C. elegans* lifespan and is implicated as an output of longevity pathways. We hypothesized that novel mutants with improved ER protein quality control might show improved cellular homeostasis and longevity. After EMS mutagenesis, we selected for developmental resistance to the N-glycosylation inhibitor tunicamycin (TM), and analyzed lifespans of TM resistant mutants. We found that gain-of-function (gof) mutations in the key enzyme of the hexosamine pathway (HP), glucosamine-fructose 6-phosphate aminotransferase (*gfat-1*, F07A11.2), result in ER stress resistance and lifespan extension. The HP provides UDP-N-acetylglucosamine (UDP-GlcNAc) that is required in the first step of N-glycan synthesis. Notably, feeding wild type *C. elegans* with GlcNAc resulted in TM resistance and extended lifespan in a dose dependent manner. We tested the activity of the unfolded protein response (UPR), but could detect no activation of UPR target genes, even during ER stress. To address the link between N-glycosylation and lifespan extension we used an aggregation-prone point mutant of SRP-2 as a fluorescent sensor of ER protein quality control. *gfat-1* gof significantly reduced SRP-2 aggregation, which was dependent on ERAD and on autophagy. We detected induced autophagosome formation in *gfat-1* gof mutants and further found that the lifespan extension required autophagy. We next tested cytosolic protein aggregation using polyglutamine expansion and detected a reduction of polyQ toxicity that was likewise autophagy-dependent. This could be mimicked by GlcNAc supplementation. Together, we demonstrate a genetic or pharmacological intervention that links N-glycoslyation and ER protein quality control with autophagy and lifespan extension. This makes the HP a potential target for the treatment of age-related proteotoxic diseases.

230A. Proteostasis in the aging model *Caenorhabditis elegans*. **I. Dhondt**[1], G. Depuydt[1], H. Cai[1], J. Staal[2], A. Borghi[2], L. Verstrepen[2], L. Baten[1], R. Beyaert[2], B.P. Braeckman[1]. 1) Department of Biology, Ghent University, Proeftuinstraat 86 builing N1, B-9000 Ghent, Belgium; 2) Department for Molecular Biomedical Research - VIB, Department of Biomedical Molecular Biology - Ghent University, Technologiepark 927, Ghent B-9052, Belgium.

Protein turnover is considered as an important mechanism to avoid the accumulation of molecular damage, a major hallmark of aging [1]. A first proteostasis system we study is mitochondrial homeostasis which appears to be maintained by mitophagy [2]. To test whether mitochondrial turnover is an important determinant of longevity, we optimized a new technique which involves a mitochondrially targeted photoswitchable protein DENDRA2 to study mitochondrial autophagy in long-lived *daf 2(e1370)III* mutants. Our results suggest that the turnover of photoconverted DENDRA2 is reduced in *daf-2(e1370)III* mutants compared to control worms. However, the mechanism responsible for the degradation of converted DENDRA2 in our transgene

worms is not yet known. Besides mitophagy, the mitochondrial proteases may be important in mitochondrial quality control. A second system we investigate is protein aggregation clearance. MALT1 (mucosa-associated lymphoid tissue), a human paracaspase, is known as a positive regulator of the inflammatory transcription factor NF-kB in lymphocytes [3]. Recently, loss of MALT1 in mouse fibroblasts and neuronal cells was found to result in the accumulation of damaged mitochondria and protein aggregates, suggesting an unexpected function of MALT1 in autophagosomal degradation. We currently investigate the role of F22D3.6 (MALT1 homolog) in protein aggregate clearance in *C. elegans*. First results show that neuronal-specific RNAi inactivation of F22D3.6 caused a subtle lifespan shortening. We are exploring the effect of F22D3.6 knockdown on protein aggregates by the use of FRAP experiments in transgene worms in which fluorescently tagged aggregation-prone polyglutamine (polyQ) proteins are expressed throughout the nervous system. Preliminary results suggest a conserved role of the MALT1 homolog F22D3.6 in protein aggregation clearance. [1] Levine RL , Stadtman ER (2001) [2] Lemasters JJ (2005) [3] Staal J, Bekaert T , Beyaert R (2011).

231B. Pharmacological enhancers of physical activity and their impact on metabolic functions and longevity in C. elegans. **Sharon Epstein**, Matthew McGee, Pankaj Kapahi. Buck Institute, Novato, CA.

In humans increased physical activity is associated with lower mortality and exercise can delay/prevent many age-related diseases. Recently, our lab has also demonstrated that the protective effects of dietary restriction maybe mediated through an increase in physical activity. However the molecular and physiological mechanisms through which exercise improves health remains poorly understood and its elucidation would have ground-breaking consequences for treatment of a wide range of age-related disorders including diabetes, obesity, cancer and cardiovascular disease, as well as how we view aging itself. To try to mimic the effect of exercise in worms, we performed a screen for enhancers of physical activity using a Natural Compound Library composed of natural molecules primarily from plants and also from bacteria, fungus, and animal sources. Using a WMicrotracker we tested the worms on three different conditions: at L4 larval stage in the presence of food, at L4/adults while starved and adults that had food added back after a long period of starvation. Of the 640 drugs tested in the initial screen we found 37 compounds that increased physical activity by at least 2 fold in one of the conditions. Re-testing of these candidates showed that at least 9 drugs that have the capacity to enhance movement. The main hits fall into two categories: sterols and flavonoids. We are currently investigating how these compounds activate metabolic pathways to affect movement and longevity. We are also testing how these metabolic pathways are influencing activity-related pathways such as mTOR, dietary restriction, TGF-beta and stress response.

232C. An underlying dauer-independent DAF-2 longevity program implicates collagen homeostasis in longevity. **Collin Ewald**[1], Jess Landis[2], Jess Porter Abate[1], Coleen Murphy[2], T. Keith Blackwell[1]. 1) Joslin Diabetes Center, Harvard Medical School, Department of Genetics, Boston, MA; 2) Lewis-Sigler Institute of Integrative Genomics, Princeton University, Department of Molecular Biology, Princeton, NJ.

Reductions in insulin/IGF-1 signaling (IIS) increase lifespan in diverse species. In *C. elegans* this longevity extension requires the transcription factor DAF-16/FoxO, which is inhibited by IIS. DAF-16 and IIS also regulate dauer development, and some mutations in the insulin/IGF-1 receptor DAF-2 are associated with temperature-dependent dauer-like phenotypes during adulthood. Accordingly, it has remained unclear whether the effects of IIS on longevity and the dauer program can be fully separated from each other. Here we examined how reduced DAF-2 activity extends lifespan under conditions that do not predispose to dauer (low temperature, *daf-2* RNAi). Lifespan extension then required the stress defense transcription factor SKN-1/Nrf, which is inhibited by IIS but had previously seemed to play a comparatively minor role in IIS-associated longevity. Our new data identify an underlying DAF-2/IIS longevity program that can be distinguished from the dauer pathway by its requirement for both DAF-16/FoxO and SKN-1/Nrf. We performed expression profiling to identify genes that are regulated by SKN-1/Nrf when DAF-2 activity is reduced. Surprisingly, collagens were the most overrepresented category among SKN-1/Nrf-upregulated genes. Collagens are well known as cuticular proteins, but in humans collagens make up 1/3 of the total protein and are critical in many organs. Their function declines during aging, but it is not understood how this influences longevity. In *C. elegans*, expression of most collagens decreases with age. We found that reduced IIS and other conditions that promote longevity oppose this decline by increasing collagen expression in aging adults, and that adulthood knockdown of collagens decreased lifespan in long-lived *daf-2* or *eat-2* (calorie restricted) but not wild-type animals. Enhancement of collagen function thus seems to be important in multiple longevity pathways, suggesting that a decline in collagen homeostasis contributes to aging.

233A. Adaptation to hydrogen sulfide induces a reversible developmental plasticity in *C. elegans*. **Emily Fawcett**, Dana Miller. Dept. Biochemistry, University of Washington, Seattle, WA.

Developmental plasticity, a phenomenon where early environmental conditions dictate adult phenotypes, predicts future environmental conditions to increase the likelihood of survival. However, there may be negative consequences if the future environment is different than predicted. Understanding the molecular underpinnings of plastic phenotypes, and how they may be reversed, may provide insight into how individuals respond differently to changes in the environment. We discovered that transient exposure to low levels of the gas hydrogen sulfide (H_2S) during embryogenesis allows for survival of otherwise lethal H_2S concentrations as adults in *C. elegans*. We have found that memory of adaptation to H_2S requires the conserved SWI/SNF chromatin-remodeling complex, suggesting that the response to H_2S stimulates epigenetic adaptations with long-lasting consequences. Remarkably, we have also demonstrated that H_2S memory is reversible, suggesting that these epigenetic changes are malleable. The memory of H_2S adaptation is erased by brief periods of fasting, but not by exposure to other environmental stresses. These results suggest that there is a specific interaction between the fasting response and the memory of adaptation to H_2S. Consistent with this model, we show that the insulin/IGF1-like signaling (IIS) pathway modulates the persistence of H_2S memory in fasting. Intriguingly, IIS is also required for the dauer developmental decision, another example of developmental plasticity in *C. elegans*. We found that well-fed animals with loss-of-function mutations in the FOXO transcription factor DAF-16 maintained H_2S memory. However, adaptation to H_2S was lost even faster in *daf-16* mutant animals after fasting than wild-type controls. In contrast, animals with constitutively active DAF-16 are insensitive to fasting - they retain the memory to H_2S significantly better than wild-type. Our results demonstrate that the cellular response to nutrient availability through the insulin-like signaling pathway is required for the persistence of H_2S memory upon subsequent nutritional stress. We are currently working to understand the molecular underpinnings that integrate IIS signaling with the epigenetic memory of adaptation to H_2S.

234B. Examining neuromuscular deficits and oxidative damage after exposure to common anthropogenic chemicals in *Caenorhabditis elegans*. **Denise B. Flaherty**, Christopher W. Dukes, Seth V. Malhotra, Ashlin L. Niedzwiecki, Daniel C. Kovarik, Julia M. Billington. Biology, Eckerd College, St. Petersburg, FL.

In the state of Florida between 2003 and 2006, almost 20 million pounds of pesticides were used on 40 different crop types per year. Between 2007 and 2009, that number jumped to over 40 million pounds on only 14 crop types per year. While the Federal Environmental Protection Agency (EPA) does set maximum tolerated residue levels per crop for each pesticide, it does not take into account possible combinations of pesticides being applied to the same crops. With this drastic increase in pesticide use, further research needs to be conducted to ensure the safety of pesticide use even at low "residual" concentration levels when combinations are applied. Using motility assays and GFP expression in the *hsp-16.2::GFP* strain,this study looks to determine possible neuromuscular and oxidative stress effects after exposure to low concentrations of three common pesticides used on various crops: chlorpyrifos, carbaryl, and indoxacarb. Results from this study conclude that individually, effects from these pesticides are minor at low concentrations in relation to both neuromuscular damage and oxidative stress. However, combinations of chlorpyrifos and carbaryl or chlorpyrifos and indoxacarb can have detrimental effects on motility even when concentration levels are below the EPA's maximum tolerated residue levels and when no oxidative stress is observed.

235C. Lysosomal lipolysis promotes longevity through a lipid-responsive nuclear hormone receptor signaling pathway. **Andrew Folick**[1], Holly Doebbler[1], Yong Yu[1], Meng Wang[1,2]. 1) Huffington Center on Aging, Baylor College of Medicine, Houston, TX; 2) Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX.

Lipids are known for roles in long-term storage of energy and cellular architecture, but they also act as signaling molecules involved in gene expression and signaling transduction. Although fat storage and metabolism has been associated with metabolic health and aging, the role that lipid signaling plays in the regulation of the aging process has not been elucidated. Previous work identified *lipl-4*, a homolog of human lysosomal acid lipase, as a novel regulator of longevity in C. elegans, and showed that constitutive expression of this lipase in intestinal fat storage tissue decreases fat storage and increases both mean and maximum lifespan. We hypothesized that lipolysis may generate key lipid messengers to modulate lifespan-regulating signaling processes. Through metabolomic profiling, we found *lipl-4* over-expression (*lipl-4 Tg*) increases abundance of oleoylethanolamide, a N-acyl ethanolamide known to function as an endogenous agonist of PPARa in mammals. Both the *lipl-4 Tg* and feeding of oleoylethanolamide are sufficient to induce the expression of the fatty acid binding protein *lbp-8*. This induction is dependent on the nuclear hormone receptors NHR-49 and NHR-80 and the mediator subunit MDT-15. Interestingly, *lbp-8* is not only required for the lifespan extension, but also sufficient to promote longevity. Accordingly, *nhr-49*, *nhr-80* and *mdt-15* are also required for the lifespan extension phenotype of the *lipl-4 Tg*. These results suggest that *lipl-4*-mediated lipolysis extends worms lifespan by activating an oleoylethanolamide-NHR-49/NHR-80 transcriptional signaling pathway. The *lipl-4* over-expression also induces expression of the acyl-CoA synthase *acs-2*, and *acs-2* is required for the lifespan extension phenotype suggesting that this signaling pathway may promote longevity in part by increasing mitochondria fatty-acid oxidation. In summary, we have identified a new pathway by which lipid metabolism regulates lifespan, and identified a potential longevity-promoting lipid metabolite.

236A. Genes that Affect Glucose-Fed *C. elegans* Exposed to Oxygen-Deprivation. **Anastacia Garcia**, Pamela Padilla. University of North Texas, Denton, TX.

A dramatic increase in the consumption of sugar is correlated with an increase in obesity and type 2 diabetes. Individuals with these conditions have a 2-4 fold increased risk of death from oxygen-deprivation related disease such as myocardial infarction and stroke. A question of interest is to address whether individuals with altered metabolism, due to dietary-induced obesity or hyperglycemia, are more susceptible to oxygen deprivation and why. We are using *C. elegans* to investigate how sugars influence anoxia response and survival and are taking a genetic approach to identify alleles that modulate this response in glucose-fed animals. Wild-type hermaphrodites survive short-term (24hr) anoxia exposure. However, animals fed glucose or fructose supplemented diets have a dramatic reduction in survival in response to the same treatment, suggesting that a homeostatic balance of carbohydrates is important for anoxia survival. Wild-type, 1-day old hermaphrodites exposed to long-term (72hr) anoxia do not survive, while *daf-2(e1370)* and *glp-1(e2141)* mutants do. Correlated with long-term anoxia resistance is an increase in intestinal carbohydrate stores. Animals supplemented with the type 2 diabetes drug Metformin also survive long-term anoxia, further establishing a link between metabolic homeostasis and anoxia survival. Yet, not every genetic mutation that confers long-term anoxia resistance enhances glucose-fed anoxia survival. While *daf-2(e1370)* mutants display a glucose-fed anoxia resistant phenotype, *glp-1(e2141)* mutants do not. Suppression analysis of the *daf-2(e1370)* glucose-fed anoxia resistance phenotype will identify genes important for combating the dual stress of hyperglycemia and oxygen-deprivation. Additionally we conducted a forward genetic screen to identify novel genes that confer a glucose-fed anoxia resistant (GFAR) phenotype, and are in the process of confirming phenotypes. This work will provide a greater understanding of the associations between diet, genotype and oxygen deprivation responses and has potential to compliment epidemiological and genetic association studies to provide insight into complex diseases like type 2 diabetes and ischemic events.

237B. Role of Autophagy in Long-lived *C. elegans* Subjected to Dietary Restriction. **Sara Gelino**[1,2], Jessica Chang[2], Malene Hansen[2]. 1) Graduate Program of Biomedical Sciences; 2) Developement and Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA.

Multiple conserved pathways and processes can modulate lifespan, including dietary restriction (DR). While the underlying mechanism for how DR promotes longevity remains poorly understood, we and others have recently shown a direct role for the cellular recycling process autophagy, in lifespan extension induced by DR. Specifically, autophagy is induced in response to DR, and autophagy genes are required for DR to extend lifespan in *C. elegans* (Hansen et al., 2008). However, these studies did not address how autophagy may prolong organismal lifespan in response to DR at the cellular or molecular level. To address the cellular role of autophagy in DR, we are investigating autophagy in different tissues of dietary-restricted *C. elegans*. First, we are using different markers to systematically monitor the autophagy process in all major tissues. Second, we are utilizing tissue-specific RNA interference models to inactivate autophagy and functionally evaluate the role of autophagy in select tissues. Knowledge of where in the animal that autophagy is occurring and may have a rejuvenating effect will shed light on the underlying mechanism by which autophagy promotes longevity. Our preliminary studies suggest that DR induces autophagy in multiple tissues and depletion of autophagy genes in single tissues, including the intestine, is

sufficient to abrogate DR-mediated lifespan extension. These data indicate that DR broadly induces autophagy in multiple tissues of *C. elegans*, and several tissues engage autophagy in a lifespan-promoting fashion in response to DR. Future work will investigate if autophagy induced by DR is similarly engaged in all tissues. We propose that the autophagic turnover of tissue-specific material, the nature of which is still to be identified, may prolong the youthfulness of a cell, tissue, and organism.

238C. Quasi-programmed yolk synthesis contributes to *C. elegans* aging. Yila de la Guardia, Eleanor Tyler, Alex Benedetto, **David Gems**. Institute of Healthy Ageing, University College London, London, United Kingdom.

Discovering the mechanisms of aging in *C. elegans* could help to understand human aging. An influential paradigm in biogerontology views aging as the result of stochastic molecular damage, protected against by somatic maintenance processes. However, the role of damage accumulation as a primary cause of aging has proved difficult to establish. It is therefore important to test alternative theories [1]. We have been exploring the application to *C. elegans* of the new hyperfunction theory of aging [2]. This suggests that aging is caused not by stochastic molecular damage, but by the non-adaptive running on in later life of developmental and reproductive programmes. Such quasi-programmes give rise to hyperfunction, i.e. functional excess, leading via dysplasia (including hypertrophy and hyperplasia, and atrophy) to age-related pathologies that cause death. A survey of previous studies of pathology in elderly worms reveals that they show many pathological changes consistent with hyperfunction [3]. These include oocyte hypertrophy to form tumour-like masses, proximal gonad disintegration, cuticular hypertrophy and neurite outgrowth. A clear example of quasi-programmed hyperfunction contributing to aging is yolk accumulation. Oocytes are provisioned by yolk synthesized in the intestine. After hermaphrodite sperm depletion, intestinal yolk synthesis continues, resulting in yolk accumulation in the body cavity [4]. We find that accumulation of the yolk proteins YP170, YP115 and YP88 continues through much of adulthood, to very high levels - i.e. yolk synthesis is never switched off. *vit-6(RNAi)* blocked accumulation of YP115 and YP88 and increased lifespan, implying that yolk accumulation contributes to mortality. Overall our findings suggest that the hyperfunction theory is a plausible alternative to the molecular damage theory to explain aging in *C. elegans*. To what extent the hyperfunction paradigm should replace the damage/maintenance paradigm remains an interesting open question. 1. Gems, Partridge, *Ann. Rev. Physiol*. **75**: 621 (2013). 2. Blagosklonny, *Cell Cycle* **7**: 3344 (2008). 3. Gems, de la Guardia, Antiox. *Redox Signal.* Sep 24. [Epub ahead of print] (2012). 4. Herndon et al., *Nature* **419**: 808 (2002).

239A. Characterizing Adult Reproductive Diapause Longevity. **B. Gerisch**, D. Magner, A. Antebi. Max Planck Institute for Biology of Ageing. Cologne, Germany.

One of the major environmental factors impacting life span is diet. When *C. elegans* animals are starved late in larval development, they arrest growth and enter the adult reproductive diapause (ARD) (Angelo and Van Gilst 2009) Animals in ARD are long lived and can survive beyond 60 days without food. Remarkably upon return to food, a fraction of worms rejuvenate their tissues and recover to give progeny, revealing extraordinary means to maintain reproductive competence and survival under starvation. However, relatively little is known about the mechanisms and the pathways involved. To better characterize ARD, we sought to identify environmental and genetic modulators of the process. The principle inducer of ARD is food deprivation. Other environmental factors, such as high temperature, high dauer pheromone concentrations, or population density (up to 500 worms) have no effect. Worms in ARD have adult characteristics, yet bear the size of L3/L4 worms, suggesting modulation of growth pathways. Moreover, pumping and movement are dramatically reduced in these animals. To understand the effect of starvation on life span, we developed demography experiments in which we followed defined cohorts throughout ARD. We found that N2 wild type grown at 20°C lives 2.5 times longer than under ad libitum food conditions. Moreover, ARD longevity is largely independent of other longevity pathways including gonadal signaling (*glp-1(e2141)*), insulin signaling (*daf-2*/IGF and *daf-16*/FOXO) and dafachronic acid signaling (*daf-9*/CYPP450 *daf-12*/NHR double mutant), since life span is still significantly enhanced in these backgrounds. The nuclear receptor NHR-49 has been previously implicated in fat metabolism and entry into ARD. Surprisingly, *nhr-49* mutants are long lived under ARD conditions, but short lived in ample food conditions, suggesting that NHR-49 modulates life span in response to diet. Metabolic and transcriptional profiling should reveal further insights into the underlying mechanisms and regulation of ARD.

240B. The Dual Roles of TCER-1/TCERG1 in Balancing Reproductive Fitness and Longevity. Francis RG Amrit[1], Arshi Arora[2], Takis Benos[2], **Arjumand Ghazi[1]**. 1) Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Department of Computational and Systems Biology, University of Pittsburgh, Pittsburgh, PA.

The debilitating effects of advacing age on fertility are well-known, but recent studies have shown that reproductive status reciprocally alters aging. We describe a role for TCER-1, the worm homolog of human transcription elongation factor, TCERG1, in establishing the balance between reproductive efficiency and somatic maintenance based on the procreative status of the animal. In *C. elegans*, removal of germline-stem cells (GSCs) extends lifespan. TCER-1/TCERG1 promotes longevity specifically upon GSC loss, likely by influencing the activity of the conserved longevity determinant, DAF-16/FOXO. We used massively parallel RNA-Sequencing to map the contribution of TCER-1/TCERG1 and DAF-16/FOXO to the transcriptional profile of GSC(-) worms. We found that these proteins share about a third of their transcriptomes and jointly up-regulate a spectrum of genes that alter lipid metabolism and xenobiotic-stress resistance. These targets improve cellular homeostasis and are essential for the longevity of the sterile adult. Strikingly, TCER-1/TCERG1 represses more genes than it activates in GSC(-) worms, and these genes are highly enriched for reproductive roles. We found that *tcer-1* mutants have reduced fecundity under normal conditions, including diminished brood size, poor egg viability and premature unfertilized-oocyte production. *tcer-1* is expressed in the germline and its mutant phenotypes can be attributed to both defective sperm and eggs. Accordingly, many genes repressed by TCER-1/TCERG1 in GSC(-) worms are important for reproduction and reducing their functions impairs fertility. Our results reveal that TCER-1/TCERG1 plays a dynamic function depending on the reproductive status of the worm. In fertile adults, it promotes reproductive fitness. Upon GSC loss, it represses the expression of reproduction genes, and (in combination with DAF-16/FOXO) elevates the expression of pro-longevity genes. This molecular dexterity of TCER-1/TCERG1 facilitates communication between the germline and the soma, and orchestrates the balance between reproductive vigor and somatic preservation.

241C. Genetics of praziquantel resistance in *C. elegans*. **Rajarshi Ghosh**, Anya Levinson, Conrad Tenenbaum, Leonid Kruglyak. Lewis Sigler Institute for Integrative Genomics, Department of Ecology and Evolutionary biology, Princeton University, Princeton, NJ.

The anthelmintic praziquantel (PRZ) is widely used for treating parasitic flatworm infections in human. It is the most-used drug for treating schistosomasis, a debilitating fluke-borne disease. Despite this prevalence, the target of PRZ is still unknown. Dependence on a single drug with unknown target, for a disease affecting 240 million people worldwide, makes the problem of emergence of drug resistance a special concern.

The target and mechanism of action of PRZ has been elusive perhaps because it is thought that nematodes are unaffected by this drug. Indeed we found that the laboratory strain N2 is resistant to PRZ relative to other wild isolates of *C. elegans*. However, we observed that, as in flatworms, PRZ induces spastic paralysis in several nematode species and wild isolates of *C. elegans*. To identify the genetic targets of PRZ, we mutagenized N2 and Hawaiian (CB4856) strains and selected mutants resistant to high dose of PRZ. We isolated nine and two mutants in the N2 and CB4856 background respectively. Through whole genome sequencing, complementation tests, RNAi and transgenic rescue we identified the first genes contributing to PRZ resistance in *C.elegans*. Interestingly different genes in the N2 and CB4856 background conferred PRZ resistance. Tissue specific knockdown experiments and epistatsis analysis suggested that PRZ disrupts osmoregulation in *C. elegans*.

We also found that CB4856 animals were significantly more sensitive to PRZ than N2 animals. To identify genetic basis of individual differences in responses to PRZ, we took a quantitative trait loci mapping approach and identified a significant locus on chromosome IV. Currently we are using transgenic rescue to fine map the QTL and testing the interactions between the QTL and the genes identified through mutagenesis. Using classical and quantitative genetic approaches and by studying wild isolates beyond the laboratory strain, we uncovered novel insights into mechanism of action of PRZ .

242A. The Mediator subunit MDT-15 is required for the oxidative stress response. **Grace Y.S. Goh**, Kulveer S. Parhar, Ada W.L. Kwong, Marcus A. Wong, Stefan Taubert. Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, British Columbia, Canada.

Reactive oxygen species (ROS) can damage cellular components, yet they are also required for a multitude of physiological processes. Thus, ROS levels must be tightly controlled. One critical level of control is through transcriptional regulation of ROS detoxification genes. Here we identify MDT-15, a subunit of the Mediator complex, as a key player in ROS detoxification. MDT-15 acts as a transcriptional coregulator and is required to express many detoxification genes; as such, it is required for worms to survive on both the metalloid sodium arsenite and the organic peroxide t-BOOH. This requirement is independent of MDT-15's previously characterized role in lipid metabolism, as knockdown or mutation of the fatty acid desaturase *fat-6*, a known target of MDT-15, did not reduce survival on t-BOOH, and supplementation with polyunsaturated fatty acids did not rescue the hypersensitive phenotype of *mdt-15* mutants. The conserved transcription factor SKN-1/Nrf is required for arsenite-dependent gene inductions, and we find evidence for functional and physical interaction between MDT-15 and SKN-1. Specifically, we show that in a yeast two-hybrid system, MDT-15 physically interacts with SKN-1 via a previously uncharacterized region of MDT-15. MDT-15 is also required for the induction of SKN-1 target genes, making it a novel transcriptional coregulator of SKN-1 in the context of oxidative stress. Furthermore, others have shown that the t-BOOH response is largely SKN-1 independent, suggesting that other transcription factors must cooperate with MDT-15 in this context. Our preliminary data suggest that Nuclear Hormone Receptors may cooperate with MDT-15 to regulate the response to t-BOOH. In sum, we propose that MDT-15 is broadly required for the transcriptional response to oxidative stress by acting in concert with multiple transcription factors.

243B. Proteotoxicity Models of Transthyretin Amyloid Disease in *C. elegans*. **E. Greiner[1]**, J. Paulsson[1,2], S. Choi[1], S. Wolff[1,2], D. Ong[1], A. Dillin[2], S. Encalada[1,3], J. Kelly[1]. 1) Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; 2) Howard Hughes Medical Institute, Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 3) Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA.

The transthyretin (TTR) amyloid diseases are the most common autosomal-dominantly inherited systemic amyloidoses, wherein TTR aggregation leads to peripheral and autonomic nervous system degeneration and/or heart dysfunction. These diseases include Familial Amyloid Polyneuropathy (FAP), Familial Amyloid Cardiomyopathy (FAC), Senile Systemic Amyloidosis (SSA), and the rare central-nervous-system-selective amyloidosis (CNSA). The tetrameric TTR protein is primarily secreted by the liver, and undergoes dissociation and monomer denaturation enabling extracellular TTR aggregation in the heart, peripheral, and/or autonomic nervous system. However, the mechanism by which this putative cell-non-autonomous process results in TTR proteotoxicity remains unscrutinized experimentally in an animal model system. To test the hypothesis that TTR proteotoxicity occurs in a cell-non-autonomous manner, we have generated *C. elegans* transgenic lines carrying human wild-type and various TTR mutations involved in TTR diseases expressed under the body wall muscle-specific promoter *unc-54p*. In humans, aggregated WT TTR results in SSA, aggregation of V30M and L55P mutations lead to FAP, and D18G and A25T mutations result in CNSA. Our results show that depending on the mutation, TTR is secreted differentially from the muscle, it aggregates, and results in a shortened lifespan and in age-related paralysis. These phenotypes are the direct result of TTR proteotoxicity, as these phenotypes are rescued upon TTR RNAi treatment. Using the TTR variants, we find a strong inverse correlation between onset of paralysis and energetic stability of the protein. TTR variants differ in the degree to which they exhibit extracellular TTR localization, and we find evidence for the formation of both extracellular and intracellular aggregates. Furthermore, we will discuss the potential to reduce TTR proteotoxicity in our TTR *C. elegans* models, by treatment of worms with a potent TTR kinetic stabilizer.

244C. The neuroglobin GLB-5 regulates C. elegans responses to hypoxic exposure. **E. Gross[1]**, Z. Soltesz[2], V. Zelmanovich[1], M. de-Bono[2]. 1) The Hebrew University of Jerusalem, Jerusalem, Israel; 2) MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, U.K.

Fine-tuned O_2 metabolism is essential for most animals, requiring animals to monitor and adapt to changes in O_2 levels. We previously identified a polymorphic neuroglobin, GLB-5, that acts in O_2-sensing neurons and enables *C. elegans* to respond to small changes in O_2 concentration (Persson et al, 2009). Here we show that GLB-5 is essential for fast behavioural recovery after exposure to hypoxia. Whereas *glb-5(Haw)* animals recovered from hypoxia within minutes, *glb-5(Bri)* recovered slowly, over four hours. By combining genetics, biochemistry, and Calcium imaging we provide evidence that *glb-5* enables fast recovery of O_2-sensing neurons after prolonged hypoxic exposure. We designed mutagenesis screens to explore how GLB-5 regulates recovery from hypoxia. One mutant we identified disrupts a conserved chaperone that regulates the activity of the O_2 sensing neurons. The chaperone regulates the spatial organization of soluble guanylate cyclases in these neurons, altering the way they interact with the neuroglobin to control the O_2 response

properties of these neurons. Persson A, Gross E, Laurent P, Busch KE, Bretes H, de Bono M (2009) Natural variation in a neural globin tunes oxygen sensing in wild Caenorhabditis elegans. Nature 458: 1030-1033.

245A. SIR-2.1, an HDAC, is required to maintain male mating ability during aging of C. elegans. **Xiaoyan Guo**[1], Luis Rene Garcia[1,2]. 1) Department of Biology, Texas A&M Universtiy, College Station, TX; 2) HHMI.

C. elegans males display a significant deterioration of mating behavior during 'early aging' prior to the structural dysfunction of neuromuscular circuitry. The mating deterioration is correlated with an increase of the excitability in the male mating circuitry(1). Here, we showed that the mating behavior of males with sir-2.1 null mutation declines even prematurely compared to that of wild-type males, and the mating circuitry of sir-2.1(ok434) is more excitable than that of wild type males. Through Ca2+ imaging in mating males, we demonstrated that the hyper excitation of sex muscles during mating blocks the process of sperm transferring in 2-day-old sir-2.1(ok434) males, hence leading to the failure of mating. Furthermore, we illustrated that sir-2.1(ok434) males generate more reactive oxygen species (ROS) possibly due to enhanced catabolism in the mating circuits and/or reduced ability to scavenge ROS because of reduced expression of ROS-scavenger genes such as superoxide dismutase sod-1and glutathione transferase gsto-1. Meanwhile, we found that artificially increasing ROS stress by feeding males with paraquat elevates the excitability of the mating circuitry and reduces the mating potency. In addition, reducing ROS by feeding males with N-Acetyl Cysteine (NAC), an antioxidant reagent, lowers the excitability of the mating circuitry and improves mating. Taken together, we conclude that SIR-2.1, a histone deacetylase (HDAC) regulates the physiological state of the mating circuitry possibly through regulation of the oxidative stress. Reference: 1.Guo X, Navetta A, Gualberto DG, Garcia LR. Behavioral decay in aging male C. elegans correlates with increased cell excitability. Neurobiol Aging. 2012.

246B. Bacterial nitric oxide extends C. elegans lifespan. **Ivan Gusarov**[1], Laurent Gautier[1], Olga Smolentseva[1], Ilya Shamovsky[1], Svetlana Eremina[2], Alexander Mironov[2], Evgeny Nudler[1]. 1) Biochemistry and Molecular Pharmacology, NYU School of Medicine, New York, NY; 2) State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow, Russia.

Organisms ranging from bacteria to human synthesize NO by a family of enzymes known as NO-synthases (NOS). Small, freely diffusible and short-lived NO possesses the properties of an ideal signaling molecule. It is involved in numerous physiological and pathological processes in mammals. The vital role NO in vasodilation, inhibition of platelet aggregation, neuronal transmission, and cytotoxic activity has been well documented. Surprisingly the round worm Caenorhabditis elegans lacks its own NOS. However, in its natural environment C. elegans feeds on bacteria that possess functional NOS. Here we demonstrate that Bacillus subtilis-derived NO increases C. elegans longevity and stress resistance. NO upregulate the expression of 65 genes that function under the dual control of HSF-1 and DAF-16 transcription factors. Our work provides an example of interspecies signaling by a small molecule and illustrates the life-long value of commensal bacteria to their host.

247C. Temperature-dependent effects of *C. elegans* N-acylethanolamine biosynthetic enzymes. **Neale Harrison**[1], Ifedayo Victor Ogungbe[1], Pedro Reis-Rodrigues[1], Thomas Gallagher[2], Young-Jai You[2], Matthew S. Gill[1]. 1) Metabloism & Aging, The Scripps Research Institute Florida, Jupiter, FL; 2) Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA 23298.

We have previously identified several N-acylethanolamines (NAEs) in *C. elegans* and found that they are important for development and influence adult lifespan. In mammals, the activity of NAEs is regulated by the relative activity of the biosynthetic enzyme N-acyl phosphatidylethanolamine (NAPE-PLD) and the hydrolytic enzyme fatty acid amide hydrolase (FAAH). Here we focus on the two *C. elegans* NAPE-PLD homologs, *nape-1* and *nape-2*, and characterize their roles in NAE biosynthesis as well as their effects on life history traits. Although these enzymes have strong sequence similarity, and are expressed in overlapping tissues, they are also expressed in discrete areas suggesting the possibility of divergent functions. We find that they are both capable of liberating NAEs from N-acyl phosphatidylethanolamine substrates *in vitro* and over-expression of each enzyme increases NAE levels *in vivo*. Surprisingly, this effect is temperature-dependent, with *nape-1* being most effective at 25°C and *nape-2* at 15°C. These alterations in NAE levels are reflected in temperature-dependent differences in phenotypes. *nape-1* over-expressers have growth delay and shortened lifespan at 25°C, but not at 15°C. In contrast, *nape-2* over-expression results in a significant fraction of L1 arrest at 15°C but extends adult lifespan in those animals that do not arrest. *nape-1* over-expressers show decreased satiety quiescence in both the unfasted state and during refeeding after fasting, but *nape-2* over-expression has no apparent effect on satiety quiescence. Interestingly, when the nape over-expressers are crossed into a *faah-1* deletion mutant, the growth phenotype of *nape-1* is exacerbated, as expected, but the L1 arrest associated with *nape-2* is fully rescued. In summary, these results provide evidence for a conservation of NAE metabolism between *C. elegans* and mammals, and indicate that *nape-1* and *nape-2* have different roles in mediating temperature-dependent effects of NAEs.

248A. The E3 Ligase LIN-23/bTRCP Influences SKN-1/NRF2 Activity and Reduces Toxic Proteins in *daf-2* Mutants. **Kyle Holden**[1], Emmanuel Schrieber[2], Mani Balasubramani[2], Arjumand Ghazi[1]. 1) Dept. of Pediatrics, University of Pittsburgh, School of Medicine, Pittsburgh, PA; 2) University of Pittsburgh, GPCL, Pittsburgh, PA.

Protein homeostasis is vital to an organism's ability to respond to environmental stress and to cope with the changing internal landscape associated with aging. Critical to this homeostasis are proteasomal E3 Ligases that control the ubiquitination of substrates. LIN-23, an F-Box Adaptor protein of the SCF E3 complex, is known to regulate cell-cycle progression during development as well as determination of adult lifespan in *daf-2* mutants. LIN-23 is homologous to human bTRCP that regulates the transcription factor NRF-2. The worm homolog of NRF-2, SKN-1, is a key mediator of oxidative stress response and is essential for the longevity of *daf-2* mutants. We provide evidence that LIN-23/bTRCP influences SKN-1/NRF2. LIN-23/bTRCP is required for the oxidative stress resistance of *daf-2* mutants, and reduction of LIN-23 results in decreased expression of SKN-1/NRF2 target genes that help combat oxidative stress. Paradoxically, this reduced SKN-1/NRF2 activity is accompanied with increased intestinal nuclear localization, suggesting a complex regulatory relationship. To map the proteome governed by LIN-23, we used iTRAQ followed by LC-MS/MS and identified 223 proteins that are consistently stabilized following *lin-23* inactivation in *daf-2* mutants. We found this group to be enriched for the canonical {DSG(x)nG} as well as a non-canonical LIN-23/bTRCP phosphodegron motif {(DDG(x)nG)}. These proteins are down-regulated in long-lived worms, and reduction of their function increases the lifespan of wild-

type animals. The levels of 96 proteins were lowered following LIN-23 inactivation, including known targets of SKN-1/NRF2. We tested the functional relevance of these longevity-promoting genes in *daf-2* mutants. Our studies show that LIN-23/bTRCP and SKN-1/NRF2 share an evolutionarily conserved relationship that determines stress-resistance and lifespan in worms, and that LIN-23/bTRCP performs degradative as well as non-degradative functions to influence aging. The LIN-23/bTRCP substrates we identified can provide valuable information about aging as well as other LIN-23/bTRCP-mediated processes.

249B. Profiling of genotoxic stress response of *C. elegans* and investigation of protective effects of selected natural compounds. **S. Honnen**[1], C. Henninger[1], C. Büchter[1], Y. Chovolou[1], P. Proksch[2], G. Fritz[1]. 1) Toxicology, Heinrich Heine University, Duesseldorf, Germany; 2) Pharmaceutical Biology and Biotechnology, Heinrich Heine University Duesseldorf, Germany.

Due to its short generation time, well-characterized developmental timing, transparency as well as easy genetic accessibility, the nematode *C. elegans* has become a well-established model system to study both DNA damage repair and effects of natural compounds *in vivo*. As in other multicellular organisms, an evolutionary conserved set of DNA repair and apoptosis pathways can be activated after ionizing radiation (IR) to maintain genome stability (e.g. *hus-1*(hHUS1), *atm-1*(hATM) and *rad-51*(hRAD51)). Various natural compounds have been shown to protect against genotoxic insults in cell-culture systems or, rarely investigated, in living animals, often because of their antioxidant capacities. Our aim is to systematically analyze dose-response relations between IR and selected functional read-outs in *C. elegans*, including development, fertility, viability as well as life span and specific gene expression profiles. These findings will provide the basis for studying genoprotective effects of selected natural compounds with low antioxidant capacity. By now we have established an optimized liquid culture system for easy application of natural compounds in a reproducible manner and low volume. Dose-response analyzes were performed to investigate radiosensitivity of *C. elegans* regarding fertility and viability. Using this setup we were able to discover genoprotective effects from compounds with high antioxidant capacity like chlorogenic acid and myricetin. Interestingly, aaptamine, which has only low antioxidant capacity, is even more potent. Now we aim to identify more natural compounds which distinctively influence the IR-induced gene expression profile and have beneficial effects with respect to the aforementioned functional readouts under genotoxic stress. Currently, we focus on marine sponge constituents like the alkaloid aaptamine, which enhances viability of embryos after acute irradiation (120 Gray) by more than 100%.

250C. A Novel Link between Ubiquitin-dependent Proteolysis and Mitochondrial Metabolism. **Thorsten Hoppe**, Alexandra Segref. University of Cologne, Institute for Genetics, Cologne, Germany.

The ubiquitin/proteasome system (UPS) is pivotal for the elimination of damaged or regulatory proteins, and plays a crucial role in development and tissue functionality. Substrate ubiquitylation is mediated by an enzymatic cascade that involves ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin protein ligases (E3). Chains of four to six ubiquitin moieties linked via K48 of ubiquitin usually promote degradation of modified substrates by the 26S proteasome. Using green fluorescent protein (GFP)-based model substrates, we have established an in vivo degradation assay that allows rapid quantification of ubiquitin-dependent proteolysis in Caenorhabditis elegans. We used this in vivo degradation assay to delineate the physiological relevance of ubiquitin-mediated protein turnover in multicellular organisms. Beside E2 and E3 enzymes or regulators of the 26S proteasome, our main goal was to identify proteolytic factors with novel mechanistic and tissue specific functions. Therefore, we performed forward genetic screens for mutants that are defective in the degradation of GFP-based ubiquitin fusion proteins. Surprisingly, we identified mitochondrial mutants that strongly stabilize different model substrates. The corresponding genes encode the acetyl-CoA synthetase ACS-19 and the isovaleryl-CoA dehydrogenase IVD-1, which are both involved in acetyl-CoA metabolism. Substrate stabilization can be suppressed by adding the antioxidant N-acetyl cysteine (NAC), suggesting enhanced oxidative and/or metabolic stress. Additional data indicate that general defects in mitochondrial respiration interfere with the UPS. Recessive mutations in the human homolog of IVD-1 cause errors of leucine metabolism and accumulate isovaleryl-CoA derivatives linked to morbidity and mortality of the patients. Intriguingly, a related model substrate is also stabilized in patient cell lines lacking IVD-1, supporting the idea that defects in ubiquitin-mediated proteolysis might be fundamental to human mitochondrial pathology. In summary, our work offers intriguingly new mechanistic insights how ubiquitin-dependent proteolysis is fine-tuned to maintain the cellular and organismal physiology.

251A. The *Caenorhabditis elegans* homolog of co-chaperone p23/prostaglandin E synthase-3 regulates lifespan in response to temperature. **M. Horikawa**, A. Antebi. Max Planck Inst., Biol. Ageing, Cologne, Germany.

Poikilotherms face the challenge of adapting normal physiological functions over a wide range of ambient temperatures. In *C. elegans*, increasing temperature from 15-25°C increases metabolic rate. Longevity is inversely correlated with temperature, suggesting that increased metabolic rates could limit lifespan. Recent studies reveal that thermal effects on various physiologic processes, including lifespan, are regulated and not simply a consequence of altered metabolism. Dauer formation is a process intimately connected to thermal sensing, and is induced by elevated temperatures. How animals sense temperature and coordinate the various processes however is poorly understood. We have studied the role of *daf-41/ZC395.10*, encoding the *co-chaperone p23/prostaglandin E synthase-3* homolog, in thermal control of dauer formation and longevity. *Daf-41* mutants constitutively enter dauer at 27°C. Daf-c phenotypes are suppressed by Daf-d mutants of neurosensory (*daf-10, osm-1, osm-3*), insulin/IGF (*daf-16/FOXO*), and steroidal (*daf-12/FXR*) signaling. These patterns of epistasis suggest that *daf-41* works early in dauer signaling at the level of neurosensory processing. Accordingly, *daf-41* is highly expressed in neuronal cells, and mutants exhibit defects in chemotaxis to benzaldehyde and isoamylalcohol. *HSP90* and *p23* are known to physically and functionally interact in mammals, suggesting these molecules could work together to regulate dauer formation. Surprisingly, genetic interactions suggest they do not work in a simple linear pathway, as *daf-21* mutants enhance *daf-41* dauer formation at 25°C but suppress it at 27°C. *Daf-41* mutants also exhibit curious temperature dependent effects on lifespan. At 20°C, mutants have normal lifespans, but are unexpectedly longlived at 25°C and shortlived at 15°C. *daf-41(+)* overexpression abolished lifespan extension of *daf-41* null mutants at 25°C, but not at 20°C. Finally *daf-41* longevity is suppressed by *daf-16/FOXO* at 25°C, but not further shortened at 15°C. Thus, *daf-41* might have a novel role in dauer formation and temperature-dependent lifespan adaptation via insulin/IGF signaling.

252B. Translational Effect of Hydrogen Sulfide and a Novel Role for HIF-1. **Joe Horsman**, Dana Miller. Dept. Biochemistry, University of Washington, Seattle, wa.

The conserved transcription factor hypoxia inducible factor-1 (*hif-1*), responsible for responding to low concentrations of oxygen (hypoxia), is critical in the initial response to hydrogen sulfide (H_2S). Hypoxia induces a dramatic decrease in translation. It has been shown that H_2S can affect translation in specific mammalian tissues, suggesting mechanistic overlap. By studying translation in H_2S we hope to uncover the mechanisms by which H_2S elicits phenotypic changes in *C. elegans*. We have shown that exposure to low levels of H_2S do not change global levels of translation in *C. elegans*. However, *hif-1* is required to maintain translation in H_2S. The role of *hif-1* in modulating translation rates suggests a new and unique role for this key transcription factor. We hypothesize *C. elegans* actively maintains translation in H_2S. While *hif-1* is necessary for both survival and to maintain translation, our data suggests these two phenomena can be uncoupled. Preliminary results show that *hif-1* can affect translation in a cell non-autonomous manner, as rescuing *hif-1* only in the neurons is able to dramatically affect how the organism adapts to H_2S. Our findings are a preliminary look at the the molecular mechanisms by which H_2S affects *C. elegans* and suggests a novel *hif-1* function in modulating translation rates.

253C. SAMS-1: A protein that senses nutrient levels and mediates the longevity response to dietary restriction in the intestine. Tsui-Ting Ching[1,3], Alex Kramer[2], Alisha Paal[1], Linda Zhang[1], **Ao-Lin Hsu[1,2]**. 1) Department of Internal Med, Division of Geriatric and Palliative Med, University of Michigan, Ann Arbor, MI, USA; 2) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA; 3) Institute of Biopharmaceutical Sciences, National Yang-Ming University, Taipei, Taiwan.

Dietary restriction (DR) results in a robust increase in lifespan while maintaining the physiology of much younger animals in a wide range of species. Here, we examined the role of *sams-1* in mediating the anti-aging effect of DR in *C. elegans*. *sams-1* has previously been identified as a DR-responsive gene in *C. elegans*, as its expression level is significantly reduced in DR animals. Moreover, reduction in the expression of *sams-1* has been shown to extend lifespan. However, the molecular mechanisms by which *sams-1* influences longevity remain unknown. *sams-1* encodes a S-adenosyl methionine (SAM) synthetase that catalyzes the biosynthesis of the universal methyl group donor S-adenosyl methionine (SAM). In this study, we found that SAMS-1 functions primarily in the intestine to influence longevity, and that dietary supplementation of SAM significantly reversed the lifespan phenotypes of *sams-1* mutants. We also found that over-expression of *sams-1* completely eliminates the longevity effects of DR. Most intriguingly, we found that SAMS-1 undergoes a rapid subcellular re-localization in the intestine, followed by a reduction in overall protein level in response to nutrient changes or TOR knockdown. These new findings strongly suggest that SAMS-1 may act downstream of TOR signaling to mediate the anti-aging effect of DR. To identify the downstream effectors that mediate the longevity effects of *sams-1* mutants, we examined the effects of various methyltransferases on lifespan. Our preliminary results suggest that SAMS-1 might influence longevity by modulating RNA maturation, histone methylation, and phospholipid synthesis.

254A. The role of *C. elegans* BRAP-2 in the SKN-1 mediated oxygen radical detoxification response. **Queenie Hu[1]**, Lesley MacNeil[2], Marian Walhout[2], Terrance J. Kubiseski[1]. 1) Department of Biology, York University, Toronto, Ontario, Canada; 2) Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA.

To prevent cellular damage caused by reactive oxygen species (ROS), eukaryotes have developed conserved defense mechanisms to protect themselves from these stressors. The *C. elegans* BRCA1 associated protein 2, BRAP-2, has been identified to have a role in responding to ROS. A deletion mutant of *brap-2* (EEED8.16) is sensitive to oxidizing conditions, resulting in developmental L1 larval arrest or lethality. However, the molecular mechanism of how BRAP-2 relieves stress is poorly understood. Thus, it is important to understand the precise signaling pathway in which BRAP-2 regulates stress. We have found that *brap-2* mutants display an enhanced *gst-4* expression in the intestine and hypodermis, and this increase is dependent on the transcription factor SKN-1/Nrf2. Although the exact mechanism of BRAP-2/SKN-1 regulation requires further investigation, our results indicate that BRAP-2 physically interacts with both LET-60/Ras and KSR-1/KSR-2, suggesting that MAPK is the pathway involved in the regulation of SKN-1 by BRAP-2.

We have used a genetic approach in order to identify novel genes that function in the BRAP-2/SKN-1 detoxification pathway. Using a transcription factor specific library, an RNAi screen was used to identify factors required for the enhanced *gst-4::gfp* expression in the *brap-2* strain. We have identified twenty suppressors that potentially represent either a novel transcription factor or co-activator of SKN-1 to promote its biological effect. Our results indicate that *elt-3* is a potential candidate involved in the SKN-1/Nrf2 signaling pathway. We found that ELT-3/GATA is required for enhanced *gst-4* expression in the *brap-2* mutant, through binding to SKN-1. Furthermore, no lifespan extension was observed in overexpressing SKN-1 worms when we knock down *elt-3*, indicating ELT-3 is crucial to co-activate SKN-1 in promoting longevity. Taken together, these results suggest a model where BRAP-2 acts as negative regulator of KSR and MAPK activity on SKN-1/ELT-3 dependent *gst-4* expression.

255B. Nanoparticle size, shape, coat, and charge alter the bioactivity of nanosilver in *C. elegans*. **Piper R. Hunt[1]**, Steven J. Oldenburg[2], Nicholas Olejnik[1], Robert L. Sprando[1]. 1) Center for Food Safety and Applied Nutrition, FDA, Laurel, MD; 2) nanoComposix, Inc., San Diego, CA.

The antimicrobial activity of nanosilver has made it the largest and fastest growing class of nanomaterial used in consumer products. Studies on the effects of nanosilver exposure in mammals are limited, and new methods for rapid safety evaluation of nanomaterials are urgently required. Additionally, Congress has mandated that alternative test methods which replace mammals be developed and validated. To address this mandate, several government agencies are collaborating on an initiative informally known as Tox21 to assess the predictive capacity of economically efficient model systems for toxicity testing. Previously, we have demonstrated correlations between chemical toxicity ranking in *C. elegans* and rat. We have also shown that larval growth and DNA damage responses to 10 nm silver spheres in *C. elegans* cultured in axenic liquid media correlate with data from rat and mammalian cell cultures. We are now assessing the effects of nanosilver size, shape, coat, and charge on bioactivity in *C. elegans* using TEM-spectral analysis, larval growth assays, and a novel method to measure fertility using COPAS microfluidics technology and GFP transgenics. Nanosilver species used in this study include spheres with diameters ranging from 10 to 110 nm, coated in citrate, PVP, PEG, or BPEI (a highly aminated, positively charged organic moiety), and hexagonal disks called nanoplates which can be “tuned” to resonate at specific frequencies by altering their size. Preliminary results indicate that 1) nanosilver spheres and plates in the 100 nm range have minimal or no toxicity to *C. elegans* at concentrations of up to 100 mg/mL, 2) toxicity increases with decreasing size and with positively charged coats, and 3) all forms of nanosilver studied to date are considerably less toxic than equimolar ionic silver. Limited published data

from fish and rodents indicate that the physiochemical properties of nanosilver similarly effect toxicity profiles in vertebrates, providing further evidence that rapid and inexpensive *C. elegans* screening assays may have potential for predicting toxic response in mammals.

256C. Analysis of *miro-1*. **Takao Inoue**, Yanqing Shen, Natarie Pei Wen Low, Thilo Hagen, Li Fang Ng, Jan Gruber. Biochemistry, National University of Singapore, Singapore.

miro-1 encodes the *C. elegans* homolog of mitochondrial rho (Miro), a mitochondrial outer membrane protein involved in mitochondrial movement. We found that a mutant carrying a deletion in *miro-1* (*tm1966*) exhibits extended life span. In addition, *miro-1(tm1966)* mutants have altered distribution of mitochondria in intestinal cells and reduced amount of mitochondria in body wall muscles. Consistently, the mitochondrial DNA copy number is reduced in *miro-1(tm1966)* mutants. Interestingly, we found no difference in the oxygen consumption of *miro-1(tm1966)* mutant compared to the wild-type. We are carrying out further analysis to determine how the function of *miro-1* relates to *C. elegans* life span.

257A. AMPK regulates protein homeostasis in response to hypoxia and nutrient deprivation. **Nicole Iranon**[1], Dana Miller[2]. 1) Molecular and Cellular Biology, University of Washington, Seattle, WA; 2) Department of Biochemistry, University of Washington, Seattle, WA.

In order to survive in changing environmental conditions, organisms must be able to successfully sense and integrate diverse environmental signals to respond appropriately. We are interested in how the energy sensor AMP-activated protein kinase (AMPK) integrates environmental cues regarding oxygen and nutrient availability to regulate protein homeostasis. Protein homeostasis (proteostasis) involves the coordination of protein synthesis, folding, degradation, and quality control in order to maintain a functional proteome. Maintaining proteostasis is required for the successful completion of development, healthy aging, and the ability to resist stressful environmental conditions. We have found that specific concentrations of low oxygen (hypoxia) cause a disruption of protein homeostasis in C. elegans, as measured by increased aggregation of polyglutamine proteins. Here, we show that nutritional cues regulate the effect of hypoxia on proteostasis. Animals that are fasted develop dramatically fewer protein aggregates compared to their fed counterparts when exposed to hypoxia. The effects of hypoxia and nutrient deprivation on protein aggregation are mediated through AMPK, a cellular nutrient sensor that coordinates energy homeostasis. Both hypoxia and a lack of nutrients can activate AMPK. We discovered that, when fed, AMPK mutant animals do not display increased protein aggregation in hypoxia. Moreover, fasting does not protect against hypoxia-induced aggregation in these mutant animals. We previously found that AMPK activity is required for developmental progression in hypoxia. Taken together, our results suggest that AMPK synthesizes input from various environmental cues in order to coordinate energy-consuming processes including proteostasis and development, which maximizes survival in a changing environment.

258B. Genes that influence longevity, dauer formation and pathogen responses downstream of sensory neurons in *C. elegans*. **Dae-Eun Jeong**[1], Marta M. Gaglia[2], Eun-A Ryu[1], Dongyeop Lee[1], Cynthia Kenyon[2], Seung-Jae Lee[1,2]. 1) Department of Life Sciences/I-BIO/WCU ITCE, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Korea; 2) Neuroscience Graduate program and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94158, USA.

Sensory neurons of *C. elegans* affect the decision between arrest in the dauer stage and reproductive growth during larval development, and modulate the lifespan of the animals in adulthood. However, the molecular mechanisms by which sensory neurons influence these physiological processes are incompletely understood. Here we determined how different genes act downstream of sensory neurons to influence three physiological outputs - longevity, dauer formation and pathogen responses. Through genome-wide microarray analysis, we identified transcripts whose levels were influenced by mutations in the intraflagellar transport protein *daf-10*, which perturb morphology and function of many ciliated sensory neurons in *C. elegans*. First, among the genes with increased expression levels in *daf-10* mutant animals, we identified a novel longevity factor, *mct-1/2* (monocarboxylate transporter 1/2). *mct-1/2* is predicted to transport small metabolites or hormones in the body, and we found that *mct-1/2* was expressed in the pharyngeal tissue. Importantly, we demonstrated that *mct-1/2* was required for the longevity of *daf-10* mutants and was sufficient for increasing lifespan. Second, we found that a subset of genes whose levels were altered by *daf-10* mutations are transcriptional targets of the DAF-12/nuclear hormone receptor. We showed that DAF-12/NHR specifically influenced the high-temperature-induced dauer-formation phenotype of *daf-10* mutants, but did not affect their extended lifespan. Third, we found that several pathogen-responsive genes were repressed in *daf-10* mutant animals. We found that *daf-10* sensory mutants exhibited altered susceptibility to and behavioral avoidance of the bacterial pathogen, *P. aeruginosa* (PA14). Taken together, sensory input seems to influence the expression of diverse downstream genes that modulate basic biological processes in *C. elegans*.

259C. *nhr-176* regulates *cyp-35d1* to control hydroxylation-dependent metabolism of thiabendazole in *C. elegans*. **Laura M. Jones**[1], Anthony Flemming[2], Peter E. Urwin[1]. 1) School of Biology, University of Leeds, Leeds, West Yorkshire, UK; 2) Syngenta, Jealotts Hill Int Res Ctr, Bracknell, Berkshire, UK.

Knowledge of how anthelmintics are metabolised and excreted is essential for understanding the factors that determine their efficacy, spectrum of activity and mechanisms of resistance. Thiabendazole was the first benzimidazole to be commercially available and remains one of the most important anthelmintic drugs for medical use. We have characterised how *Caenorhabditis elegans* metabolises thiabendazole and shown that members of the cytochrome P450s (CYPs) are highly induced by thiabendazole in *C. elegans*. The most strongly induced CYP (encoded by *cyp-35d1*) is regulated by *nhr-176* and this nuclear hormone receptor mediates resistance to thiabendazole. HPLC analysis showed that nematodes which had been exposed to thiabendazole contained more of the compound when *nhr-176* was knocked down. This correlated with a lower concentration of hydroxylated thiabendazole i.e. the metabolised compound, being detected in the culture medium. This suggests that the down-stream target of *nhr-176*, CYP-35D1 catalyses hydroxylation of thiabendazole prior to its excretion from *C. elegans*. Finally, we show that recombinantly-expressed NHR-176 binds thiabendazole.

260A. Modulation of the Ubiquitin-Proteasome System and C. elegans Longevity by Neuroendocrine and Growth Factor Signaling Pathways. **Kishore K Joshi**, Tejash Shah, Christopher Rongo. Department of Genetics, Waksman Institute, Rutgers University, Piscataway, New Jersey, 08854-8020.

Protein homeostasis is a key facet of longevity, and failure in protein quality control is linked to both age-associated decline and neurodegenerative

disorders. One key regulator of protein homeostasis is the Ubiquitin-Proteasome System (UPS), which acts globally to degrade damaged and oxidized proteins. Multiple hormonal and growth factor signaling pathways are implicated in regulating longevity, but the precise interaction between these signaling pathways, global UPS activity, protein homeostasis, and aging is not well understood. We recently demonstrated that components of the Ubiquitin Fusion Degradation (UFD) pathway promote longevity in C. elegans by mediating the polyubiquitination and degradation of damaged and unfolded proteins. We also showed that growth factor signaling modulates the level of global UPS activity at different points during adult maturation and aging, thereby promoting longevity and healthspan. Using an in vivo reporter for UPS activity, we have now performed an RNAi screen (followed by validation with true genetic mutants) for additional regulators of global UPS and identified a dopamine (DA) receptor as a regulator of UPS activity in maturing adults. Here we analyze the detailed molecular interactions between DA signaling and UPS activation in maturing adults by targeting genes involved in DA synthesis, release, reception, and signal transduction. We find that DA is used as an extracellular neurohormone to adjust UPS activity in specific tissues and at specific points in development and aging. Molecules involved in this DA-mediated regulation of the UPS are thus potential therapeutic targets for minimizing age-associated decline and neurological disorders.

261B. Role of mir-35-41 family in embryonic development and hypoxia. **Konstantinos Kagias**, Roger Pocock. BRIC, Copenhagen, Denmark.

miRNAs are small molecules that negatively regulate gene expression at the post-transcriptional level. miRNAs that can target the same transcript(s) are grouped into the same family. In C. elegans most miRNA knockout mutants have no obvious developmental defects under normal breeding conditions even when they lack multiple members of the same family. However, little is known on whether these mutants can survive and develop under stress. The mir-35 worm-specific family has been reported to be crucial for early embryonic development and we found that it is also involved in the embryonic response to hypoxia. Despite systematic efforts in the past to find downstream effectors of the mir-35 family, the mode of action of this miRNA family in embryogenesis remains unknown. We conducted whole RNA sequencing and microarrays to identify miRNAs that are regulated by hypoxia. We then screened through mutants of such miRNAs and found 7 sensitive and 3 resistant to 0,5% oxygen, one of which is the mir-35-41 knockout mutant. This mutant exhibits ~50% embryonic lethality under normoxic conditions (21% oxygen), however this lethality increases to almost 100% when embryos develop in hypoxia (0,5% oxygen). At present, we are trying to gain further insights into this regulation, as well as to investigate the previously unknown role of this miRNA family in embryonic hypoxia response. It is becoming clear that miRNAs play an important role during normal development. miRNAs are also emerging as stress regulators. Here we present evidence that mir-35-41 family has a crucial role in both normal development and environmental stress response (hypoxia). In addition, we have identified a potential direct target of mir-35 miRNA family that is conserved in humans. Thus, non-conserved miRNAs can target conserved gene products to regulate important biological events such as embryogenesis and embryonic stress resistance.

262C. LEA genes in Antarctic nematode, *Panagrolaimus davidi*. **Hiroshi Kagoshima**[1,2], Yuji Kohara[2]. 1) Transdisciplinary Research Integration Center, Tokyo, Japan; 2) National Institute of Genetics, Mishima, Shizuoka, Japan.

In Antarctica, terrestrial animals live in one of the most extreme environments on Earth. They normally can be active and grow only during the brief summer when sufficient heat is available in their microhabitats for liquid water to be present from melting snow or other sources. However, they have to withstand freezing and desiccation stresses for long periods over winter, and periodically (and often unpredictably) at other times of the year. We would like to know how the Antarctic animals adapt to the extreme environment, using Antarctic nematode *Panagrolaimus davidi* as a model organism. We analyzed total 50,000 ESTs prepared from *P. davidi* cultivated at 20°C (good condition for growth) and at 4°C (cold-stressed condition). We found several candidate genes for environmental tolerances in the cDNA library. In particular, we picked up LEA genes for further analysis. The LEA gene (named after "Late Embryo Abundant") was originally found in wheat seed. This gene has been isolated from plants, bacteria and animals, which have environmental tolerances. The LEA genes share characteristic 11 amino acids repeat (LEA repeat), which adopt amphipathic α-helix during desiccation condition, and may function in retention of water and protection of proteins/membrane. *P. davidi* had more than 10 LEA genes and some of them had highly diverged LEA repeats, suggesting they may have different functions from that of typical LEA genes. We are analyzing *P. davidi* LEA genes *in vitro* by recombinant LEA proteins and *in vivo* by transgenic *C. elegans* carrying *P. davidi* LEA genes.

263A. 20-hydroxyecdysone prevents age-associated decline in *C. elegans* . **Shaunak Kamat**, Shrutika Yeola, Monica Driscoll. Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ.

Disruption of cellular homeostasis is the underlying cause of a variety of disorders associated with ageing across species. Maintenance of cellular homeostasis is thus key to healthy ageing, and a major focus of therapeutic drugs. Plant extracts have long been used across diverse cultures for therapeutic purposes and to improve vitality and health. The isolation, identification and bioactive properties of compounds derived from plant extracts is thus a growing focus in the field of ageing research. Here we report on the effects of 20-hydroxyecdysone, a plant-derived polyhydroxylated sterol on ageing and healthspan in *C. elegans*. *C. elegans* fed 500uM 20-hydroxyecdysone over a period of 7 days showed significant improvement in locomotory performance among the bottom 25% of animals tested, while we noted no increase in median or maximum values. The lower quartile animals also showed significantly lower morbidity and increased AGE pigment levels as compared to controls. We observed that the effects of 20-hydroxyecdysone are dependent on *daf-2* and *hsf-1*, and partially independent of *daf-16*. Furthermore, we found that 20-hydroxyecdysone acts independently of *skn-1*. We found no significant increase in mitochondrial biogenesis in 20-hydroxyecdysone-treated animals. Our results suggest a role for 20-hydroxyecdysone in improving health of the weakest segment of the population via the glucose metabolism pathway.

264B. Quest for life-lengthening signals within gonadal longevity. **Oezlem Karalay**[1], Shuhei Nakamura[1], Adam Antebi[1,2]. 1) Max Planck Institute for Biology of Ageing, Gleueler Str. 50a, D-50931, Cologne, Germany; 2) Department of Molecular and Cellular Biology, Huffington Center on Aging, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA.

The reproductive system of an animal is important for procreation but also has an impact on longevity. In *Caenorhabditis elegans*, removal of the germline during early development results in lifespan extension. This longevity is dependent on the presence of somatic gonad, which is a source of life-lengthening signals (so called 'gonadal longevity'). Although several molecular mechanisms are known to be involved in gonadal longevity, the signals

coming from the somatic gonad are not yet well defined. Evidence suggests that one such signal is a steroid hormone, called dafachronic acid, which works through the steroid receptor DAF-12 to promote gonadal longevity. DAF-12 in turn upregulates the *let-7*-related microRNAs, *mir-84* and *mir-241*, to facilitate DAF-16/FOXO nuclear localization and activity. However, the mechanisms activating the hormone signaling pathway are unknown. To identify novel signaling pathways that regulate hormonal signaling within gonadal longevity, we conducted RNAi-based suppressor screens. We screened through transcription factors, kinases and phosphatases, and have identified about 30 evolutionarily conserved factors, loss of which shorten the lifespan of germlineless animals. Subsequent gene expression analysis suggested that knockdown of a handful of candidate genes also abolish the activation of hormonal signaling in germlineless animals. Ongoing demography and further gene expression analyses will identify new candidates that are involved in the regulation of hormonal signaling mechanisms upon the loss of germline. In addition, to understand the timing of events, candidates will be analyzed for their interaction with the other known key players of germline longevity. This will not only allow us to define a temporal network but also reveal how signaling factors involved in different processes such as hormone production, lipolysis, and autophagy cooperate to guarantee homeostasis within gonadal longevity.

265C. The HIF-1 Pathway and organismal senescence. **Jordan Kardos**, Sudhir Nayak. The College of New Jersey Biology Dept., 2000 Pennington Road, Ewing, NJ 08628-0718.

Several highly conserved pathways regulate senescence on both cellular and organismal levels across organisms. Hypoxia Inducible Factor-1 (HIF-1) is a transcriptional activator that functions as the cellular and physiological regulator of oxygen homeostasis under hypoxic conditions. HIF-1 has been shown to regulate a variety of important cellular processes in *C. elegans* including increased lifespan under hypoxic conditions. This has indicated a possible regulatory role of the HIF-1 pathway in organismal senescence. We tested the ability of $CoCl_2$, a hypoxia mimicking molecule, to up regulate both HIF-1 expression and HIF-1 activity in *C. elegans*. We found that HIF-1 expression and activity were increased with $CoCl_2$ exposure and resulted in a relative increase in both lifespan and brood size. Our data suggest that the relative increase in lifespan is indicative of a protective effect for post-mitotic ageing, while the relative increase in brood size is indicative of a protective effect for mitotic divisions.

266A. *C. elegans* EXO-3 contributes to progeny production by repairing AP site in gonad. **Yuichi Kato**, Takahito Moriwaki, Masafumi Funakoshi, Qiu-Mei Zhang-Akiyama. Kyoto University, Kyoto, Japan.

DNA damage occurs continuously in living cells even under normal conditions, and the apurinic/apyrimidinic (AP) site is an example of such DNA damage. Since it inhibits DNA replication and transcription, an unrepaired AP site has deleterious consequences such as mutation and cell death. AP endonuclease is a DNA repair enzyme that recognizes the AP site and cleaves the sugar phosphate backbone. This is followed by DNA resynthesis by DNA polymerase and DNA ligase to complete repair of the AP site. It is believed that AP site repair is indispensable, especially in germ cells. In the mammalian testis, previous studies reported that the expression of AP endonuclease was higher while the AP site level was lower compared to some somatic tissues. Also of note, knockout of AP endonuclease resulted in embryonic lethality. In spite of these indications of the relationship between AP site repair and its consequences in multicellular organisms, direct evidence has yet to be shown. To understand in detail how AP site repair is involved in the reproduction of multicellular organisms, we used *C. elegans* as the simplest model, and focused on EXO-3, which is one of the AP endonucleases in *C. elegans*. We firstly found that the *exo-3* gene is mainly expressed in the gonads of both hermaphrodites and males. Secondly, to determine the level of the AP site in the gonad, we measured the AP site level in the *glp-4* mutant, which does not develop gonads at restrictive temperature. The AP site level per total DNA was increased in the *glp-4* mutant, and this indicates that the AP site level in the gonad is suppressed compared to other tissues. Thirdly, we analyzed the *exo-3* mutant, which completely lacks AP endonuclease activity derived from EXO-3. The mutant was viable under normal conditions, but also exhibited some abnormality in reproduction, such as a reduced self-brood size. Taken together, we have shown a negative correlation between the expression of AP endonulcease and level of the AP site in the gonad. We also found that germ cells are preferentially protected from harmful effects of the AP site by a higher expression of AP endonuclease in the gonad, and this suggests the importance of AP site repair in reproduction.

267B. TAF-4 is Required for the Life Extension of *isp-1*, *clk-1* and *tpk-1* Mit Mutants. **Maruf H. Khan**[1], Lauren Temmer[2], Melissa Ligon[1], Bryce Hufnal[2], Robert Farber II[1], Andrew Dillow[1], Erynn Kahlig[1], Amanda Rodriguez[1], Shane L. Rea[1]. 1) Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center, San Antonio, TX; 2) Institute for Behavioral Genetics, University of Colorado at Boulder, Boulder, CO.

Impairment of the mitochondrial electron transport chain (ETC) in the nematode *Caenorhabditis elegans* can lead to life extension. Genetic and RNAi-induced mutants of *C. elegans* that have disrupted ETC function and which are long-lived are collectively called Mit (Mitochondrial) mutants. In order to understand why Mit mutats are long-lived, we hypothesized that defective mitochondrial function activated a transcriptionally-encoded response. Using an RNAi screening approach we have now identified 15 transcription factors that, when reduced-in-function, reproducibly and differentially alter the development, stress responsiveness, and/or fertility of *isp-1(qm150)* Mit mutants relative to wild type worms. Of these transcription factors seven, AHA-1, CEH-18, HIF-1, JUN-1, NHR-27, NHR-49 and the CREB-1 interacting protein TAF-4, were required for *isp-1* life extension. When we tested the involvement of these same transcription factors in the life extension of two other Mit mutants, namely *clk-1(qm30)* and *tpk-1(qm162)*, only TAF-4 and HIF-1 were consistently required. Our findings suggest that the Mit mutant phenotype is under the control of multiple transcriptional responses, and that CREB-1, TAF-4 and HIF-1 may be part of a signalling axis that specifies Mit mutant life extension.

268C. Fat, reproduction and longevity phenotypes associated with altered protein synthesis capacity. **Akshat Khanna**[2], Sean P. Curran[1,2,3]. 1) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; 2) Dornsife College of Letters, Arts, and Sciences, Department of Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 3) Keck School of Medicine, Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.

Mutations in the protein synthesis machinery have been shown to increase the lifespan of *C. elegans*. In addition, lipid homeostasis and reproductive capacity can have profound effects on the health and lifespan of an organism. In addition to increased longevity, post-developmental RNAi depletion of cellular protein synthesis components leads to decreased fecundity and increased lipid stores. An inverse relationship between lipid homeostasis and

reproduction has been observed in multiple longevity mutants. To better understand the coordination of lipid homeostasis, reproduction, and lifespan, we screened regulators of cellular protein synthesis that altered brood size and fat storage antagonistically. We identified one RNAi clone that targets an evolutionarily conserved regulator of global protein synthesis, which results in decreased intestinal lipid stores and increased progeny production, but a normal lifespan. This indicates that the lipid homeostasis and reproduction phenotypes can be uncoupled from longevity. We contrast our genetic and RNAi suppression studies with strains overexpressing regulators of global protein synthesis, and discuss our characterization of the fat, reproduction, and longevity phenotypes associated with modulating these components. A better understanding of how altering global protein synthesis leads to changes in lipid metabolism, reproduction, and lifespan will help us understand how these complex phenotypes are regulated and mechanistically linked.

269A. Gene expression studies to evaluate the size related toxicity of ZnO nanoparticles on the soil nematode, *Caenorhabditis elegans*. **Priyanka Khare**, Madhavi Sonane, K.C Gupta, Aruna Satish. ENVIRONMENT AND NANOMATERIAL TOXICOLOGY DEPARTMENT, CSIR-INDIAN INSTITUTE OF TOXICOLOGY RESEARCH(IITR), POST BOX NO-80, MAHATMA GANDHI ROAD, LUCKNOW -226001, UTTAR PRADESH, India.

Zinc oxide (ZnO) nano-particles (NPs) are extensively used in cosmetics, sunscreens, food products, paints, drugs, ground water remediation and water treatment because of their unique physico-chemical properties. The enormous usage of NPs demands risk assessments on health and environment. In this regard the safety assessment of different sizes of ZnO NPs was examined in the soil nematode *Caenorhabditis elegans. C. elegans* were maintained on nematode growth medium with *E.coli* OP50 as food source. 50nm and 100nm ZnO NPs (SIGMA) along with bulk was tested in the present study. The NPs were characterized in the exposure medium using TEM and Zeta-sizer. Dose response in L4 stage worms was estimated for all the particles, followed by oxidative stress (ROS and MTT assay) at sub-lethal concentrations. Physiological end-points such as growth, behavior and reproduction were also analyzed. Quantitative PCR for genes involved in stress response pathway followed by biochemical assay and/or transgenic GFP strain screening was also carried out to investigate relationship between toxicity and size of ZnO NPs. Our studies revealed that lethality on exposure to ZnO NPs was both dose as well as size dependent. Further in comparison to bulk the ZnO NPs showed (1) more prominent adverse effects on physiology of nematodes, (2) increase in ROS levels, reduced glutathione peroxidase and reduced glutathione level, (3) enhanced lipid peroxidation, protein carbonylation and DNA damage, (4) suppression of Insulin/IGF- like signaling pathway and activation of stress response pathway through up regulation of genes which are controlled by DAF-16/HSF-1/SKN-1transcription factors and (5) reduced acetylcholine enzyme level suggesting hampered neuro-transmission. Therefore we conclude that ZnO NPs have size-related eco-toxic potentials in *C. elegans*. Further our study also provide evidenced that ZnO NPs are genotoxic and disrupts various physiological processes at very low concentration (0.23ug/mL) in *C. elegans*.

270B. Characterization of changes in metal composition in C. elegans with age. **Ida M. Klang[1,2]**, David Killilea[3], Tracy Barhydt[1], Peter Swoboda[2], Daniel Edgar[1], Gordon Lithgow[1]. 1) Buck Inst Age Res, Novato, CA; 2) Karolinska Institute Center for Biosciences at NOVUM Department of Biosciences and Nutrition Hälsovägen 7 S-141 83 Huddinge Sweden; 3) CHORI, 5700 Martin Luther King Jr Way, Oakland, CA.

A loss of homeostasis in various systems is one of the major complications with human aging. A loss of metal homeostasis (metallostasis) is characteristic of a number of age-related neurodegenerative diseases. Surprisingly little is known about the relationship between metal regulation and aging processes. We have found that aging of C. elegans is associated with alteration in the metallomic profile, with large segmental increases in metals with known physiological functions. Manipulations of these metals can modulate lifespan and affects various models of protein misfolding. We are investigating if known aging pathways modulate the metal profile. Overall our results suggests that levels and ratios of specific metal abundance could be associated with healthy aging and longevity.

271C. Natural genetic polymorphisms in *set-15* and a casein kinase 1a homolog shape the mortality schedule of worms. **Gunnar Kleemann[1,2]**, Hua-Jay Cherng[1,2], Alina Yang[1,2], Thomas Kuhlman[3], Joshua Bloom[2], Edward Cox[1], Leonid Kruglyak[2], Coleen Murphy[1,2]. 1) Lewis-Sigler Institute, Princeton University, Princeton, NJ; 2) Dept Molecular Bioloy, Princeton Univ, Princeton, NJ; 3) Dept of Physics, University of Illinois, Urbana-Champaign, Ill.

Large-scale loss-of-function *C. elegans* screens have demonstrated the profound flexibility of the aging schedule and identified major, conserved longevity pathways. A new challenge is to understand how longevity pathways work in the context of normal physiology, which will give us insight into the natural function of pathways controlling aging schedules and the genetic basis of naturally occurring disease states, and should lead to mechanistic insights that will allow us better control over disease and aging processes. In order to identify stable genetic polymorphisms in longevity pathways, we performed Quantitative Trait Loci (QTL) analysis using our rapid lifespan profiling platform (Chronos) to estimate lifespans across a panel of Bristol (N2) X Hawaiian (CB4856) Recombinant Inbred Lines (RILs) (Rockman *et al*, 2009). We identified a region on the left arm of Chromosome IV associated with variation in longevity. This region was further refined using a panel of Near Isogenic Lines (NILs) that contain short genomic fragments from the Hawaiian strain on the genetic background of the Bristol strain. Finally, using transgenic lines that rescue the longevity defect in our short-lived NILs, we identified two polymorphic genes, *set-15* and a close homolog to human casein kinase 1a, which shape the mortality schedule of *C. elegans*. Since *set-15* is a histone methyl transferase homolog, the polymorphism in *set-15* may influence longevity by regulating epigenetic modification of the genome. This is particularly interesting since the polymorphisms in the longevity-linked region have been linked to gene expression differences (expressionQTLs; eQTLs) across the genome. The regulation of these eQTLs provides a potential mechanism by which natural allelic variation can lead to large changes in gene expression and subsequently, longevity. Such natural variation likely contributes to differences in longevity observed in human populations, as well.

272A. Molecular aging driven by Wnt signaling in *C. elegans*. **B. Koenders- van Sintanneland**, M. Lezzerini, Y. Budovskaya. University of Amsterdam, Amsterdam, Netherlands.

We are using a system biology approach to reveal the molecular basis for aging in nematodes *C. elegans* by characterizing gene expression differences between young and old animals and then determining at a molecular level how these changes contribute to aging. We used DNA microarrays to profile gene expression changes associated with aging. This analysis revealed that gene expression differences between young and old animals are under control of a relatively simple gene regulatory network that involves the *elt-3, elt-5*, and *elt-6* GATA transcription factors. Expression of *elt-5* and *elt-6* increases in old age, leading to decreased expression of *elt-3*, thus causing changes in the expression of the many downstream target genes. Understanding upstream

control of the *elt-3/elt-5/elt-6* pathway will provide key insights into the causes of the molecular differences between young and old worms. In *C. elegans*, Wnt/Wingless signaling pathway activates *elt-5* and *elt-6* expression during development. Therefore, we hypothesised that the Wnt signaling pathway continues to function past development and has detrimental effects on longevity. To test this hypothesis, we began a mini screen where we compared the life span of 11 different Wnt signaling mutants to that of wild type worms. Then, we measured the expression level of the *elt-3*, *elt-5,* and *elt-6* GATA transcription factors in each worm. We discovered that mutations in either *cwn-2*/Wnt or *mom-2*/Wnt increase both median and maximum life span by ~20-40%, whereas, mutations in *egl-20*/Wnt and *lin-44*/Wnt decrease both median and maximal life span by~15-40%. This suggests that Wnt signaling acts to limit life span in *C.elegans*. In addition, expression of both *elt-5* and *elt-6* GATA was significantly decreased in all Wnt signaling ligands mutants, whereas the same Wnt signaling mutations led to higher expression of *elt-3* compared to control animals. This suggests that *elt-3*, *elt-5*, and *elt-6* GATA transcriptional factors are under control of the Wnt signaling during aging. In this study we are planning to assess how much of normal aging can be accounted for by changes in Wnt signaling using high-throughput sequencing technologies.

273B. Age-related degeneration of the egg-laying system promotes matricidal hatching in *Caenorhabditis elegans*. Christopher L. Pickett, **Kerry Kornfeld**. Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110.

The identification and characterization of age-related degenerative changes is a critical goal because it can elucidate mechanisms of aging biology and contribute to understanding interventions that promote longevity. We have documented a novel, age-related degenerative change in *C. elegans* hermaphrodites, an important model system for the genetic analysis of longevity. Matricidal hatching—intra-uterine hatching of progeny that causes maternal death—displayed an age-related increase in frequency and affected ~70% of mated, wild-type hermaphrodites. The timing and incidence of matricidal hatching were largely independent of the levels of early and total progeny production and the duration of male exposure. Thus, matricidal hatching appears to reflect intrinsic age-related degeneration of the egg-laying system rather than use-dependent damage accumulation. Consistent with this model, mutations that extend longevity by causing dietary restriction significantly delayed matricidal hatching, indicating age-related degeneration of the egg-laying system is controlled by nutrient availability. To identify the underlying tissue defect, we analyzed serotonin signaling that triggers vulval muscle contractions. Mated hermaphrodites displayed an age-related decline in the ability to lay eggs in response to exogenous serotonin, indicating that vulval muscles and/or a further downstream function that is necessary for egg-laying degenerate in an age-related manner. By characterizing a new, age-related degenerative event displayed by *C. elegans* hermaphrodites, these studies contribute to understanding a frequent cause of death in mated hermaphrodites and establish a model of age-related reproductive complications that may be relevant to the birthing process in other animals such as humans.

274C. Regulation of the ER Stress Response by HPL-2 and other chromatin associated proteins in *Caenorhabditis elegans* . **Lucie Kozlowski**, Steve Garvis, Cécile Bedet, Francesca Palladino. LBMC, ENS LYON, Lyon, France.

Cellular adaptation to environmental changes and stress relies on a wide range of regulatory mechanisms which are tightly controlled at several levels, including transcription. Chromatin structure and chromatin binding proteins are important factors contributing to the transcriptional response to stress. However, it remains largely unknown to what extent specific chromatin factors influence these distinct responses in a developmental context. One of the best characterized stress response pathways is the unfolded protein response (UPR), which is activated by accumulation of misfolded proteins in the endoplasmic reticulum (ER). Here, we show that *Caenorhabditis elegans* HPL-2, the homologue of the HP1 chromatin associated protein, is required for the ER stress response. Inactivation of HPL-2 results in enhanced resistance to ER stress dependent on the XBP-1 branch of the UPR and the closely related process of autophagy. Increased resistance to ER stress in animals lacking HPL-2 is associated with increased basal levels of XBP-1 activation and ER chaperones under physiological conditions. Using tissue specific rescue experiments, we find that HPL-2 plays antagonistic roles in intestinal and neuronal cells to influence the ER stress response. We further show that chemical inhibition of histone deacetylase activity mimics the HPL-2 loss of function phenotype, and that increasing or decreasing histone H3 lysine 4 methylation (H3K4me) has antagonistic effects on animal survival in response to ER stress. Altogether our results point to an important function for specific chromatin factors and chromatin modifications in maintaining ER homeostasis in a developmental context.

275A. Age-dependent neuronal changes are the result of multiple intrinsic and extrinsic factors. **Anagha Kulkarni**, Claire Bénard. Neurobiology, UMass Medical School, Worcester, MA.

While the age-dependency of neurodegenerative diseases is well-known, the mechanisms by which aging triggers them are largely unknown. Our research aims to decipher the molecular mechanisms that protect the nervous system throughout life. To establish the progression of age-related changes in the nervous system and elucidate mechanisms of neuronal protection from aging, we undertook a quantitative analysis of changes across neuronal classes in wild-type worms at different ages. We find that old worms display disorganization of ganglia, defasciculation and displacement of axon bundles, and appearance of novel neurites. Our results are largely in agreement with others (1,2,3) for mechanosensory, cholinergic and dopaminergic neurons. To broaden our understanding of the effects of aging on neurons in different organismal contexts, we expanded our analysis to include other neuronal classes and structures. Age-related changes vary for different neurons, indicating differential susceptibility of neurons to the stresses of aging likely due to multiple layers of regulation.

To determine if age-related neuronal changes are regulated by lifespan determination pathways, we examined the degree of defasciculation, branching and displacement in several neuronal classes in the long-lived mutants clk-1, daf-2 and eat-2, as well as in the short-lived mutant daf-16. We find that extending lifespan does not simply delay age-dependent neuronal changes. Our analysis reveals that even for the same neuronal class the appearance of age-dependent neuronal changes is delayed in some long-lived mutants, while accelerated or unchanged in other long-lived mutants. Similarly, the occurrence of age-dependent changes is accelerated in some but not all neurons in short-lived mutants. These results, together with other manipulations we have carried out, indicate that a combination of intrinsic and extrinsic factors impinges upon age-dependent neuronal changes. Taken together, our results suggest that multiple factors, including the class of neurons, their structural context, and lifespan regulators, impact on the state of neurons during aging. 1.Pan et al, 2011 2.Tank et al, 2011 3.Toth et al, 2012.

276B. An ACE inhibitor extends *Caenorhabditis elegans* life span. **Sandeep Kumar**, Nicholas Deitrich, Kerry Koenfeld. Development Biology, Washington University School of Medicine, St. Louis, MO.

Aging is characterized by progressive, degenerative changes in many organ systems. Age-related degeneration of somatic tissues is a major contributor to disability and death. Treatments that delay age-related degeneration of humans are desirable, but no drugs that delay normal human aging are available. To identify drugs that delay age-related degeneration, we used the C. *elegans* model system to screen for FDA-approved drugs that can extend the adult lifespan of worms. We showed that captopril, an angiotensin converting enzyme (ACE) inhibitor used to treat high blood pressure, extended mean life span significantly. Captopril inhibits the enzyme ACE in humans, and the worm homolog of ACE is encoded by the *acn-1* gene. We discovered that reducing the activity of *acn-1* extended the mean life span of worms. *acn-1* has previously been shown to play a role in larval molting. Combining treatment with captopril and reducing the activity of *acn-1* did not have an additive effect on life span extension, indicating that these interventions may affect the same pathway. Captopril treatment and *acn-1* RNAi can further extend the life span of many long-lived mutants, including mutants with defects in caloric intake, mitochondrial function, and insulin/IGF-1 signaling. However, captopril treatment and *acn-1* RNAi did not extend the life span of *daf-16* mutants. Based on these findings, we hypothesize that captopril extends life span by inhibition of the *acn-1* gene, and this life span extension requires DAF-16, the target of the insulin/IGF-1 signaling pathway. The ACE pathway has been implicated in life span regulation in rodents, suggesting this may be an evolutionarily conserved system of life span regulation.

277C. Integrin-linked kinase regulates longevity and thermo-tolerance via HSF-1 in *C. elegans*. **C. Kumsta[1]**, A. Davis[1], T.-T. Ching[2], M. Nishimura[1], C.-C. Chu[1], S. Gelino[1], B. Ong[1], R. Bodmer[1], A.-L. Hsu[2], M. Hansen[1]. 1) Sanford-Burnham Medical Research Institute, La Jolla, California, USA; 2) University of Michigan, Ann Arbor, Michigan, USA.

The integrin-signaling complex (ISC) transmit cues from the extracellular matrix to the cytoskeleton and is essential for many cellular processes including cell adhesion. A central signaling component of the ISC is highly conserved Integrin-linked kinase (ILK). We previously identified components of the ISC, including PAT-4/ILK, as new longevity determinants in *C. elegans* through a genome-wide RNAi screen. ISC components also modulate aging in *Drosophila*, and thus the ISC may constitute a conserved longevity paradigm. However, the underlying mechanism by which the ISC modulates aging remains unknown. To investigate the longevity role of the ISC, we have examined how ILK affects lifespan. We find that ILK inhibition also promotes thermo-tolerance in both worms and flies, suggesting that stress resistance may be a conserved cellular mechanism underlying the long lifespan observed in animals with reduced ISC. In *C. elegans*, both thermo-tolerance as well as lifespan extension upon ILK inhibition is mediated by the transcription factor HSF-1, a major regulator of stress responses and proteostasis. Specifically, we observe that the reduction of ILK leads to an increase in HSF-1 levels and activity, at least partially inducing the heat-shock response (HSR). In *C. elegans*, the HSR is regulated both cell-autonomously as well as systemically through signaling via specific thermo-sensory neurons (AFD/AIY). Notably, ILK is expressed in muscle, somatic gonad, and in neurons different from AFD/AIY. We find that AFD/AIY neurons are required for the thermo-tolerance and increased longevity in animals with reduced ILK levels, implicating that ILK-mediated signals from specific somatic tissues are processed in AFD/AIY neurons to mediate organismal effects. In conclusion, we show that genetic manipulations of the ISC component ILK lead to the activation of HSF-1, and that this activation requires thermo-sensory neurons for the induction of thermo-tolerance and longevity. This describes a novel trigger for HSF-1 activation that via complex, inter-tissue signaling extends lifespan in *C. elegans*.

278A. The Impact of Age and Lipid Stores: An Analysis of Oxygen Deprivation Response in *C. elegans*. **M.L. Ladage[1]**, J.M. Goy[2], V. Shulaev[1], P.A. Padilla[1]. 1) University of North Texas, Denton, TX; 2) Harding University, Searcy, AR.

Human health issues prevalent in the aging population and associated with disruption of oxygen and metabolic homeostasis (heart disease, stroke, and type 2 diabetes) are also associated with obesity. Further, oxygen homeostasis and delivery are negatively affected by obesity in humans. The presence of large adipose tissue likely impacts oxygen delivery, yet a question of interest is whether cellular lipid profiles influence oxygen homeostasis. We are using *C. elegans* to understand the impact of oxygen deprivation on aging animals and examining alteration of cellular phenotypes and metabolic profiles by oxygen deprivation exposure relative to diet and genotype. We analyzed long-term anoxia survival relative to age and determined that young gravid adults are very sensitive to anoxia, adults 3-5 days of age are less sensitive and as the animals age anoxia sensitivity increases. Additionally, wild-type males become increasingly sensitive to long-term anoxia as they age. The anoxia sensitivity phenotype is significantly decreased in *daf-2* and *glp-1* mutants and analysis of the *daf-2;glp-1* double mutant suggests that the insulin-signaling pathway and notch-signaling pathway act synergistically to confer anoxia resistance. Suppression analysis of anoxia resistance in *glp-1(e2141)* animals indicates that the KRIT1 homologue *kri-1* is required for anoxia survival as the animal ages. Microscopy analysis indicates that the *kri-1* mutation suppresses morphological phenotypes associated with the *glp-1(e2141)* animal. We are using cell biological approaches (BODIPY) to examine lipid profiles in animals sensitive and resistant to anoxia exposure. To further dissect the mechanisms by which wild-type and mutant animals survive anoxia exposure, we have taken a metabolomics approach. Lipids were extracted from animals on the first day of adulthood and analyzed by LC-MS. Metabolomic profiles will be presented for worms of various genotypes and environmental conditions. This work is significant for determining the impact of metabolomic and physiological profiles, influenced by genotype and age, on surviving the stress of oxygen deprivation.

279B. SIR-2.1 regulation by the NAD+ Salvage Pathway and Environmental Stress. **Stephanie E. Lange**, Wendy Hanna-Rose. BMB, Penn State Univ, University Park, PA.

Sirtuins are an important class of lysine deacetylases that have been implicated in aging and stress response. The enzymatic activity of SIR-2.1 consumes NAD+ and is inhibited by its product, nicotinamide (NAM). Thus, NAD+ and NAM levels are hypothesized to regulate SIR-2.1 in vivo. The first enzyme in the NAD+ salvage biosynthesis pathway (PNC-1) impacts both NAD+ and NAM levels. In S. cerevisiae, Pnc1p indeed regulates Sir2. (1) We hypothesize that this regulation by PNC-1 is likewise present in C. elegans. Superoxide dismutase 3 (sod-3) is an oxidative stress response gene that is upregulated by SIR-2.1 in a DAF-16 dependent manner. (2) If PNC-1 regulates SIR-2.1, it should be reflected by the downstream modulation of sod-3 expression. To test this, we examined Psod-3::gfp expression in combination with mutants and overexpression constructs of sir-2.1 and pnc-1 loss-of-function. We found that PNC-1

regulates SIR-2.1 in C. elegans. We furthermore assayed the relative impact of changes to NAD+ and NAM to this regulation, as well as the role of this regulation in response to environmental stress. 1) Anderson et al. 2003. Nature 423(6936):181-5 2) Berdichevsky et al. 2006. Cell 125(6):1165-77.

280C. DNA damage leads to replicative aging but extends lifespan of long lived mutant animals. Jessica M. Lindvall[2], Karen Thijssen[1], Andrea E Karambelas[1], Daniel Cupac[1], Øyvind Fensgård[2], Gert Jansen[1], Jan H.J. Hoeijmakers[1], Hilde Nilsen[2], Wim Vermeulen[1], **Hannes Lans[1]**. 1) Department of Cell Biology and Genetics, Erasmus MC, Rotterdam, The Netherlands; 2) The Biotechnology Centre, University of Oslo, Oslo, Norway.

Nucleotide Excision Repair (NER) is a versatile DNA repair pathway that removes a wide variety of helix-distorting DNA lesions, including those induced by UV irradiation. Two subpathways in NER can be discerned, one which detects DNA damage anywhere in the genome, called global genome NER (GG-NER), and one which detects damage in the transcribed strand during transcription, called transcription-coupled NER (TC-NER). Following detection, the damaged DNA strand is excised and the resulting gap is repaired by novel DNA synthesis. In humans, NER-deficiency is associated with severe clinical disorders characterized by cancer predisposition and/or pleiotropic developmental defects and accelerated aging of which the pathogenesis is only partially understood.

We use *C. elegans* to understand the *in vivo* impact of DNA damage and the biological relevance of NER in a multicellular, developing organism. Previously, we showed that UV-exposed germ cells mainly depend on functional GG-NER, while postembryonic cells predominantly depend on TC-NER. Further analysis of development and aging associated with NER deficiency shows that, contrary to expectation, DNA damage accumulation does not decrease adult lifespan of post-mitotic tissue. Surprisingly, NER deficiency even further extends life-span of long-lived *daf-2* mutants, through an adaptive activation of stress signaling. Contrary, NER deficiency leads to a striking decrease in replicative lifespan and transgenerational aging of proliferating cells. DNA damage accumulation induces severe, stochastic impairment of development and growth, which is most pronounced when additional DNA repair pathways besides NER are impaired. These results show that different DNA lesions contribute to replicative aging and suggest that also in patients there might be a direct link between symptoms and the level of DNA repair deficiency.

281A. Dissecting Ageing-Related Pathways by Studying Protein Changes after Calorie Restriction. **Mark Larance**, Ehsan Pourkarimi, Anton Gartner, Angus Lamond. Centre for GRE, University of Dundee, Dundee, United Kingdom.

Calorie restriction has been identified in many organisms to mediate increased life-span. Many pathways are involved in ageing including insulin/IGF-1 signalling, AMPK, autophagy, and TOR. In this study, we have used state-of-the-art quantitative proteome analysis of *C. elegans*, combined with follow-up genetics studies to identify the protein-level mechanisms that mediate the response to calorie restriction. We have recently established a method to enable stable isotope labelling with amino acids in cell culture (SILAC) for quantitative proteomic analysis in *C. elegans*. We have used advanced chromatography to fractionate *C. elegans* peptide digests to enhance sensitivity. To date, we have quantified the proteome-wide response in nematodes to short term calorie restriction. After characterising >4000 proteins across three biological replicates, we first identified those proteins changing significantly after calorie restriction. As a harsh threshold we required that proteins changed in abundance by more than two-fold and had a p-value <0.05. These cut-offs yielded 341 proteins significantly increased and 127 significantly decreased after calorie restriction for 16 h. We have also quantified many post-translational modifications detected in our unbiased dataset, including some that change significantly in response to calorie restriction. Gene ontology enrichment analysis based on biological processes showed that the up-regulated proteins were associated with nucleosome assembly, ageing, the response to heat, and macromolecular complex assembly. Conversely, the down-regulated proteins were associated with the biological processes of fatty acid metabolism, amino acid biosynthesis, cell division, and the positive regulation of growth. Of the 341 up-regulated proteins, 30 of the genes encoding these proteins have previously been shown to bind the transcription factor DAF-16, which plays a critical role in the increased longevity caused by calorie restriction. The mechanisms that regulate the remaining proteins remain to be elucidated. These datasets provide a comprehensive overview of the networks that mediate the response to calorie restriction and which may provide the associated increased longevity.

282B. Mutations in ribosomal S6 kinase lengthen lifespan through increasing the activity of heat shock transcription factor 1. Keunhee Seo[1], Eunseok Choi[1], Dongyeop Lee[1], Dae-Eun Jeong[1], Sung Key Jang[1,2], **Seung-Jae Lee[1,3,4]**. 1) Life sciences; 2) WCU IBB; 3) WCU ITCE; 4) IBIO, Pohang University of Science and Technology, Pohang, South Korea.

It has been established that aging is subject to regulation by classical signaling pathways, including insulin/IGF-1 signaling (IIS) and target of rapamycin (TOR) signaling. The TOR signaling pathway has emerged as a major regulator of aging, and one of the well-characterized effectors of TOR signaling is ribosomal protein S6 kinase whose inhibition increases the lifespan of yeast, *C. elegans*, *Drosophila* and mice. Here, we show that inhibition of S6 kinase (*rsks-1*) exerts lifespan-extending effects on *C. elegans* via increasing the activity of the heat shock transcription factor 1 (HSF-1), a crucial longevity transcription factor known to act downstream of the IIS pathway. We initially noticed that *hsf-1* genetically interacted with *rsks-1* through a genome-wide RNAi screen. We then found that *hsf-1* was required for the extended life span of *rsks-1* mutants. In addition, inhibition of *hsf-1* suppressed the longevity caused by the reduction of other components in the TOR signaling pathway, including raptor (*daf-15*), Rag GTPase (*ragc-1*) and ribosomal protein subunits (*rps-6* and *rps-15*). Moreover, we found that *hsf-1* was required for the enhanced resistance of *rsks-1* mutants against oxidative stress, protein aggregation, and pathogenic *P. aeruginosa* (PA14). Since HSF-1 is a longevity transcription factor crucial for the induction of various chaperone genes, we examined whether *rsks-1* mutations increased the mRNA levels of several HSF-1 target genes. Among them, *hsp-16* and *hsp-70* were induced by *rsks-1* mutations, and *hsp-16* was at least partially required for the longevity of *rsks-1* mutants. We then asked whether HSF-1 acts as a downstream longevity factor for both IIS and TOR signaling. We found that the enhanced longevity of *rsks-1; daf-2* double mutants was mediated by HSF-1. Together, our findings suggest that HSF-1 functions as a hub transcription factor that integrates longevity signals from IIS and TOR/S6K pathways.

283C. Investigating the role of the *wrn-1* helicase in the nematode worm, *C. elegans*. **Hayley Lees**, Alison Woollard, Lynne Cox. Biochemistry, University of Oxford, Oxford, Oxfordshire, United Kingdom.

In order to ameliorate the deleterious consequences of old age, the biology of human ageing must be understood. The study of human progeroid disorders which recapitulate many of the features of normal ageing have helped to contribute to our understanding of normal human ageing. Werner

syndrome is a canonical progeroid disorder, caused by mutation of the *WRN* gene. *WRN* encodes both RecQ helicase and exonuclease activities, and is known to participate in DNA replication, repair, recombination and telomere maintenance. In addition, although many interacting partners have been identified, the exact molecular functions of the *WRN* gene remain largely unknown. In order to dissect the roles of WRN helicase in ageing, we use mutants of the WRN homologue and interacting partners in the nematode worm, *C. elegans*.

Reduction of function by RNA interference of the *C. elegans* WRN homologue *wrn-1* leads to ageing phenotypes and shortened lifespan [1]. We have shown that mutation of *wrn-1* leads to genomic instability: interestingly, this phenotype is enhanced in a mutant *cep-1* background (the *C. elegans* p53 homologue). Notably, lifespan also shows significant modulation, while brood size remains unchanged from that of either single mutant.

Therefore we suggest that *cep-1* status has a significant effect upon the role of *wrn-1* helicase in longevity and germline maintenance in worms.

References 1.Lee, SJ; Yook, JS; Han, SM; Koo, HS. A Werner syndrome protein homolog affects C. elegans development, growth rate, life span and sensitivity to DNA damage by acting at a DNA damage checkpoint. Development, 2004. 131(11): p. 2565-2575.

284A. Development of small molecule inhibitors of SKN-1 dependent detoxification genes identified in a screen of ~364,000 compounds. **Chi K. Leung**[1], Satyamaheshwar Peddibhotlal[3], Patrick Maloney[3], Paul M. Hershberger[3], Michelle Bousquet[2], Hendrik Luesch[2], Siobhan Malany[3], Keith P. Choe[1]. 1) University of Florida, Department of Biology and Genetics Institute, Gainesville, FL; 2) University of Florida, Department of Medicinal Chemistry,, Gainesville, FL; 3) Sanford-Burnham Center for Chemical Genomics at Sanford-Burnham Medical Research Institute, Orlando, FL.

Multidrug resistance is a growing problem in parasitic nematodes that is poorly understood. SKN-1 regulates numerous detoxification genes and mediates resistance to toxins. Pharmacological compounds that target SKN-1 would provide tools for studying drug resistance and have the potential to increase the efficacy of anthelmintics. Using a high-throughput fluorescence assay for transcriptional activity of *gst-4*, a gene tightly regulated by SKN-1, we screened the NIH Molecular Libraries Small Molecule Repository of ~364,000 compounds in 1536-well plates. We identified 1795 hits that reduced induction of *Pgst-4::GFP* by the electrophile acrylamide. Re-testing 1381 hits at multiple doses confirmed 364 (26%) and identified 128 compounds with IC50 doses of less than 10 mM; 125 of these compounds did not inhibit induction of *Phsp-16.2::GFP* by heat-shock suggesting that they do not generally interfere with transcription or expression of transgenes. Two chemical scaffolds were selected for medicinal chemistry and structure-activity relationship studies. Analogs of both scaffolds inhibit induction of *Pgst-4::GFP* and *Pgst-30::GFP* (a second detoxification gene reporter) by acrylamide and juglone (a natural electrophile and redox cycler) at ~1 mM without inhibiting *Phsp-16.2::GFP* or causing general toxicity. Importantly, these same analogs also strongly inhibit *Pgst-4::GFP* in worms with a *skn-1* gain-of-function allele suggesting that they may act within the SKN-1 pathway. Ongoing work includes testing for inhibition of the homologous Nrf2 pathway, confirmation of effects on endogenous genes, defining mode-of-action and physiological effects, and testing in a range of parasitic species. This work is funded by NIH grant R21NS067678-01.

285B. A dual role of the Wnt signaling pathway during aging in *Caenorhabditis elegans*. **M. Lezzerini**, Y. Budovskaya. Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, The Netherlands.

Wnt signaling is a major highly conserved developmental pathway that guides many important events during embryonic and larval development. In adulthood, misregulation of Wnt signaling has been implicated in tumorigenesis and various age-related diseases. These effects are accomplished through highly complicated cell-to-cell interactions mediated by Wnt secreted proteins. Despite that its role during development is well studied, very little is known about the possible role of Wnt signaling in natural aging. In this study, for the first time *Caenorhabditis elegans* serves as a model system to determine the role of Wnt ligands in aging. *C. elegans* has 5 Wnt proteins, *mom-2*, *egl-20*, *lin-44*, *cwn-1*, and *cwn-2*. We show that all five Wnt ligands are expressed and active past the development stages. Through the use of hypomorphic mutants and RNAi manipulations, we show that *mom-2*/Wnt plays a major detrimental role in longevity, whereas the function of *lin-44*/Wnt is beneficial for long life. Although the identification of important targets is currently under study, interestingly, we found that longevity variations deriving from Wnt signaling manipulations do not involve cellular or oxidative stress pathways in response to heat shock temperature and Paraquat exposure. Our results suggest that Wnt signaling regulates aging-intrinsic genetic pathways, opening a new research direction on the role of Wnt signaling in aging and age-related disease.

286C. Characterization of novel mutants with dysfunctional mitochondrial stress pathways. **Jacqueline Lo**[2], Sean P. Curran[1,2,3]. 1) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; 2) Dornsife College of Letters, Arts, and Sciences, Department of Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 3) Keck School of Medicine, Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.

Often referred to as the powerhouse of the cell, mitochondria are essential for many developmental, physiological, and metabolic events in eukaryotes. An exceptionally important topic in mitochondrial biology is the mitochondrial stress response that regulates mitochondria homeostasis. It is likely that there are signaling pathways that relay messages from the mitochondria to the nucleus in response to specific stress conditions. We have completed two EMS mutagenesis screens in *C. elegans* to look for novel regulators of mitochondrial stress pathways. The first looks for negative regulators of mitochondria stress response using *hsp-6p::gfp* as a reporter system. These mutants are characterized by increased *hsp-6p::gfp* expression and the YOuthful mitochondria for Death Avoidance phenotype. This screen identified two recessive alleles *lax205* and *lax208* and a dominant allele *lax212* (LGIII). The second screen looks for positive regulators by searching for suppression and enhancement of the signal in the strain with *isp-1; hsp-6p::gfp*, which has a constitutive activation of the reporter. These mutants are characterized by a Variable Adaptation to Diminished Respiration phenotype. Here we report the characterization of the stress, reproduction, lipid profile, and lifespan phenotypes associated with the mutants with altered mitochondrial stress response. Once the genes that are mutated have been identified they can be ordered into relevant genetic pathways that influence lifespan and cellular physiology responses that depend upon proper mitochondrial function. Together, these two approaches will further our understanding of the essential role of mitochondria in cellular homeostasis by identifying new players and mechanisms of regulation.

287A. New roles for *wdr-23* in organismal survival and stress adaptation. **Jacqueline Lo[2]**, Akshat Khanna[2], Elaine Roh[1,2], Megan Bernstein[2], Sean P. Curran[1,2,3]. 1) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; 2) Dornsife College of Letters, Arts, and Sciences, Department of Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 3) Keck School of Medicine, Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.

SKN-1/Nrf (NF-E2 related factor) plays multiple essential roles in organismal development and maintaining cellular homeostasis. In *C. elegans* WDR-23 negatively regulates SKN-1 by facilitating delivery to the proteasome for degradation. Post-developmental inactivation of *wdr-23* by RNAi leads to increased longevity while early inactivation results in developmental pleiotropies. In a genetic screen to isolate mutants with constitutive SKN-1 activation we isolated a complementation group that consists of eight novel recessive alleles of*wdr-23* (*lax101, lax123, lax124, lax126, lax129, lax134, lax211*, and *lax213*). These mutations differentially alter normal physiology, growth, development, stress adaptation, and lifespan. To better define the antagonistically pleiotropic roles played by *wdr-23* we will compare and contrast the developmental and adult-aging phenotypes associated with each of these mutants. Our findings will provide mechanistic insight into an evolutionarily conserved axis of SKN-1/Nrf2 activation that adds further dimensions to the complexity of this essential pathway.

288B. Exploring the health benefits of phytoestrogens in C. elegans. Jessica M. Ochoa, Vanessa Parada, Gabriela Gutierrez, Breann De Santiago, Erika Perez, Peaches Ulrich, Emanuel Zamora, **Sylvia A. Lopez-Vetrone**. Biology, Whittier College, Whittier, CA.

The mitochondrial free radical theory of aging suggests that accumulation of highly reactive oxygen species (ROS), or free radicals within cells leads to the oxidative damage of DNA, lipids, and proteins, and in turn is responsible for cellular aging. Estrogen signaling has been shown to increase the production of antioxidants, and is correlated with a reduction in ROS and oxidative damage. Equally, phytoestrogens, plant-derived estrogen-like molecules that can bind the estrogen receptor, have also been shown to increase the production of antioxidants and decrease ROS, suggesting that their consumption may hold many health benefits for humans. The nematode Caenorhabditis elegans (C. elegans) is widely used to study the effects of oxidative stress and aging, and also express estrogen-like receptors that can bind estrogen. Therefore, in this study we investigated the potential health benefits of phytoestrogen administration to C. elegans, and if these benefits might be mediated through the DAF-2 Insulin-like pathway. Briefly, C. elegans strains (N2 bristol wild type, AGE-1, AKT-1, and DAF-16) were fed OP50 E. coli with or without the addition of phytoestrogen(s) (daidzien, genistein, or daidzein and genistein in conjunction) and maintained at 22°C. Assessments on fertility, metabolism, longevity, oxidative stress, and innate immunity were then conducted. The results of these experiments demonstrate that phytoestrogen adminstration (both alone or in combination) to C. elegans correlate with a statistically significant increase in lifespan and their ability to withstand both temporary and acute oxidative stress exposure when compared to their control treated groups. Likewise, the innate immunity challenge assays revealed a statistical increase in bacterial resistance. Our findings extended across all strains studied, suggesting that the physiological effects might be mediated through the DAF-2 Insulin like pathway. While we believe these results are very exciting, our future efforts will focus on conducting biochemical assays to confirm our results.

289C. Investigating the Toxic Effect of Biosensor Nanoparticles Using the Caenorhabditis elegans Nematode. Michelle Callaway[1], Erika Perez[1], John D. Alocilja[2], Evangelyn Alocilja[2], **Sylvia A. Lopez-Vetrone[1]**. 1) Biology, Whittier College, Whittier, CA; 2) Biosystems and Agricultural Engineering, Michigan State University, Lansing, MI.

Nanoparticles are being developed and applied for a variety of applications in energy, medicine, safety, and defense products. The anthrax bioterrorism attacks of 2001 have prompted attention to the employment of nanoparticles and nanotechnologies to develop novel methods of detecting bacterial and viral pathogens, but little is known about their toxic effects on multicellular organisms. Interestingly, the nematode Caenorhabditis elegans (C. elegans) may serve as a good animal model to study the possible toxicological effects of nanoparticles on organisms, as it has been widely used in many toxicological studies. Therefore, in this study we investigated the potential toxicological effects of three magnetic nanoparticles (amine, carboxyl, and poly-aniline) that are commonly used in biosensors using C. elegans. Briefly, N2 Bristol wild type C. elegans nematodes were grown and cultured on nematode growth media plates seeded with OP50 bacteria food source. Starting at the egg stage experimental groups were exposed to single nanoparticle types (mixed into their food source) or to food source alone (controls), and assessed for longevity, fertility, metabolism, and response to increased oxidative stress. The results from these studies demonstrate that exposure of C. elegans to single nanoparticle types do not correspond with a statistically significant toxic effect on fertility, metabolism, longevity or their ability to respond to oxidative stress. Although these results suggest that exposure to single nanoparticles may not be harmful, but future studies should be conducted to determine if combined nanoparticle exposure would result in similar findings, as it is rare that contact would be limited to only one type.

290A. Drift of ELT-2 as a cause of aging in C. elegans. **Frederick G. Mann[1]**, Eric Van Nostrand[2], Ari Friedland[3], Stuart Kim[1]. 1) Dev Biol, Stanford Univ, Stanford, CA; 2) Cellular and Molecular Medicine, UCSD, San Diego, CA; 3) Genetics, Harvard University, Boston, MA.

Previous work has shown that approximately 1000 genes change expression during the normal aging process of C. elegans. We hypothesized that some of these gene expression changes cause aging, and that the regulators of these 1000 genes may also regulate the lifespan of the worm. We screened the modENCODE ChIP seq database and found that the ELT-2 GATA transcription factor is enriched for binding to age-regulated genes, suggesting that elt-2 may be a regulator of the aging transcriptome. elt-2 is essential for the development of the intestine, and is known to regulate the expression of nearly all of the intestinal-specific genes. We have shown that ELT-2 reporters decrease expression during aging. Knocking down elt-2 with RNAi shortens lifespan and results in a young worm with a transcriptomic profile resembling that of an old worm. Overexpression of elt-2 lengthens lifespan. Together, these results indicate that the decreasing levels of elt-2 expression during aging result in changes in expression of hundreds of intestinal genes. The age-related changes in the elt-2 transcriptional network limit lifespan and explain part of the normal aging process.

291B. Modulation of Excitotoxic Neurodegeneration in *C. elegans* by the Cell Stress-Resistance Signaling Pathway. Nazila Tehrani[1,2], Moises Dominguez[1], **Itzhak Mano[1,2]**. 1) Physiol. Pharm. & Neurosci., City College, The City University of New York; 2) Program in Neurobiology, the Graduate Center of the City University of New York.

Ischemic stroke causes excitotoxicity, where over-excitation of neurons by the neurotransmitter Glutamate (Glu) leads to necrotic neurodegeneration. Aging is a critical risk factor in stroke, where it has a role not only in increasing the *chances of having* an ischemic event, but also in *worsening the prognosis* following a given insult. Studies suggest that there is an age-dependent decline in cell resistance to ischemic insult. However, the molecular mediators of the excitotoxic neurodegenerative process or the factors that reduce susceptibility to neurodegeneration by increasing stress resistance remain a mystery. We are studying a nematode model for excitotoxicity by deleting the Glu transporter gene glt-3 in a sensitive background. Our recent findings indicate that blocking the conserved Insulin/IGF signaling pathway (and the resulting nuclear translocation of DAF-16) increases cell stress resistance and reduces susceptibility to excitotoxicity. We are now studying the factors that might regulate cell stress resistance upstream and downstream of the core insulin/stress resistance pathway. We focus on a protein complex identified in other animals and found to regulate insulin-controlled metabolism. We find that interrupting this complex decreases suitability to excitotoxic neurodegeneration, expanding its suggest role from that of controlling metabolism to the modulation of neuroprotection. We hope that a better understanding of the effect of cell stress resistance in excitotoxicity will help us gain further insights into processes of neuroprotection and neuronal vulnerability in neurodegenerative conditions related to brain ischemia.

292C. Vitamin D3 slows aging in *c. elegans*. **Karla Mark[1]**, Dipa Bhaumik[1], Milena Price[1], Birgit Schilling[1], Bradford Gibson[1], Michael Holick[2], Gordon Lithgow[1]. 1) Buck Institute, Novato, CA; 2) Boston University School of Medicine, Boston, MA.

Vitamin D3, a fat-soluble secosteroid functions in the body to regulate bone mineral homeostasis, and other aspects of metabolism through genomic and nongenomic mechanisms. The principle action of vitamin D3 is thought to be mediated through binding to the nuclear vitamin D3 hormone receptor (VDR). Moreover, in the presence of vitamin D3, VDRs are known to form heterodimer complexes which in turn bind to vitamin D responsive elements (VDREs), and enhance the transcription of target genes. Although multiple studies report that low serum levels of vitamin D3 are associated with an increased risk of several central nervous system (CNS) diseases including multiple sclerosis, Alzheimer's and Parkinson's disease, there is little information as to the mechanisms for why this occurs. In this study, we show that vitamin D3 treatment suppresses the aggregation of proteins in several adult *Caenorhabditis elegans* (*c. elegans*) models, such as, the amyloid fragment, $Ab_{3\text{-}42}$. Furthermore, we show that vitamin D3 prevents a large number of proteins which normally become insoluble during aging in the worm. Using Mass Spectrometry, we have extended these data to identify the specific proteins affected by vitamin D3 during aging. Another interesting finding from this study is that vitamin D3 also extends lifespan, a trait commonly seen for compounds that promote protein homeostasis. Currently, we have identified several nuclear hormone receptor candidates in the worm, and will be following up on these to determine the signaling pathways involved in vitamin D3's effect on lifespan and protein aggregation. The use of vitamin D3 supplements in the elderly population is controversial. A number of large human clinical trials are underway or about to begin. The discovery that vitamin D3 can suppress age-related pathology (protein aggregation) and slow aging is therefore likely to be highly significant.

293A. Insulin/IGF-1 Signaling Regulates Proteasome Activity through the Deubiquitinating Enzyme UBH-4. **Olli Matilainen[1,2,4]**, Leena Arpalahti[1,2,4], Ville Rantanen[1,3,4], Sampsa Hautaniemi[1,3,4], Carina I Holmberg[1,2,4]. 1) Research Programs Unit; 2) Translational Cancer Biology Program; 3) Genome Scale Biology Program; 4) Biomedicum Helsinki, University of Helsinki, FI-00290 Helsinki, Finland.

The proteasome executes most of the controlled protein degradation in the cell. It has important role in regulation of protein homeostasis and dysfunctions in proteasomal degradation have been linked to many severe disorders, including several neurodegenerative diseases and cancers. Although the importance of the proteasome for cellular viability is well recognized, it is still not known how proteasome activity is regulated in a multicellular organism. Insulin/IGF-1 signaling (IIS) is a well-characterized signaling pathway that regulates e.g. protein homeostasis and lifespan in many organisms. Here we have studied if IIS regulates proteasome activity by using in vitro assays, our previously established in vivo photoconvertible UPS activity reporter system as well as our novel in vivo polyubiquitin reporter. Our results show that reduced IIS enhances proteasome activity in a DAF-16 dependent manner. Furthermore, we demonstrate that DAF-16 represses expression of the proteasome-associated deubiquitinating enzyme ubh-4, which we propose to function as a tissue-specific proteasome inhibitor and a modulator of lifespan. The role of UBH-4 appears to be well-conserved, as downregulation of its mammalian ortholog, UCHL-5, increases proteasome activity and degradation of proteotoxic proteins in mammalian cells. Altogether, our results establish a novel molecular mechanism linking IIS to proteasome activity and add one more branch to IIS mediated maintenance of cellular protein homeostasis.

294B. A redox sensor as a potential regulator of ROS signaling in C. elegans. **Katie McCallum[1]**, Antonio Miranda-Vizuete[2], Danielle Garsin[1]. 1) University of Texas Health Science Center-Houston, Houston, TX; 2) Instituto de Biomedicina de Sevilla (IBIS-CSIC), Seville, Spain.

The ability of an organism to maintain redox homeostasis is critical for its survival. At the cellular level, exposure to oxidative insult can irreversibly damage DNA, proteins, and lipids, all of which can lead to cell apoptosis or necrosis. To counteract such oxidative insults, organisms have developed the ability to sense and respond to oxidative stress, termed the oxidative stress response. SKN-1 is the major transcription factor that coordinates the C. elegans oxidative stress response. Our lab has recently demonstrated that, in C. elegans, intestinal infections stimulate the production of reactive oxygen species (ROS) by Ce-Duox1/BLI-3. While this response has protective effects, it challenges the host's intracellular redox homeostasis, resulting in the simultaneous up-regulation of intracellular detoxification enzymes. This response occurs through the activation of SKN-1, in a p38 MAPK pathway dependent manner. However, it is still currently unknown how BLI-3-generated ROS is capable of activating SKN-1. This question is of broad interest because ROS can serve as an important signaling molecule not only in the immune response, but in the regulation of other vital processes such as cell growth and differentiation. We propose that activation of the SKN-1 is regulated by a redox sensor, which is sensitive to the ROS generated by BLI-3.

295C. Does iron dyshomeostasis drive ageing? **Gawain McColl**[1], B.R. Roberts[1], S.A. James[2], R.A. Cherny[1], A.I. Bush[1]. 1) The Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia; 2) Australian Synchrotron.

Iron has been implicated in the ageing process as a causative factor in the free radical theory of ageing through mechanisms of oxidative chemistry. To explore iron homeostasis in an ageing model, we employed novel analytical approaches to characterize age-related iron changes in Caenorhabditis elegans. Using live animals we imaged iron during ageing via x-ray fluorescence microscopy. We observed dramatic intestinal-iron accumulation with age, which correlated with increased reactive oxygen species. Insulin-like signaling modulates these effects, so that long-lived daf-2 mutants are resistant to these age-related changes, while short-lived daf-16 mutants show more marked effects. To identify the iron-protein complexes that are changing with age, we developed a native liquid chromatography and mass spectrometry technique. We observed that wild-type life span requires the ability to store iron in ferritin, however, iron storage is not required for long-lived phenotype of daf-2 mutants. When challenged with exogenous iron, wild-types can safely store this iron with minimal effects on longevity. When daf-2 mutants are exposed to equivalently elevated iron we observed a significant reduction in lifespan, indicating daf-2 mutant longevity is associated with altered iron homeostasis. These data indicate a causal role of iron homeostasis in longevity.

296A. Exploring the Flexibility of NAD+ Biosynthesis in *C. elegans*. **Melanie R. McReynolds**, Wendy Hanna-Rose. Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA.

NAD+ is an essential co-enzyme. It is necessary for electron transport in many metabolic reactions and it functions as a substrate for several enzymes known as NAD+ consumers. NAD+ biosynthetic pathways are well elucidated; however, the developmental and physiological roles of these pathways are not known. We focus on understanding the developmental and physiological role of NAD+ biosynthetic pathways as well as the questions: How is NAD+ metabolism controlled?, How do changes in NAD+ metabolism influence physiology? and How can NAD+ metabolism be manipulated for therapeutic benefit? Our previous studies have shown that mutations in the (nicotinamide) NAM to NAD+ salvage pathway enzyme, PNC-1, caused developmental and functional defects in the reproductive system of *C. elegans*. The developmental defects are more severe under nutritionally stressed conditions where animals are fed dead E. coli. We measured metabolite levels in wild-type and mutant animals and found that while NAM levels increased significantly and NA levels decreased significantly as expected, there was little change in NAD+ levels between *pnc-1* mutants and wild-type. Therefore, we hypothesize that there are mechanisms compensating for the production and/or consumption of NAD+ in *pnc-1* mutants. To address this hypothesis and to more fully investigate the relative contributions of all NAD+ biosynthetic pathways in development, we are studying the nicotinamide/nicotinic acid riboside (NR/NaR) pathway for the production of NAD+. We measured the transcription level of nrk-1 from N2 or *pnc-1* worms grown on live and dead OP50. Our (q)RT-PCR analysis shows that while nrk-1 mRNA levels do not change in *pnc-1* mutants when growing on live OP50, there is an up-regulation of nrk-1 transcription in the presence of nutritional stress. This data suggests that the NR/NaR pathway has a significant role in the compensation of NAD+ under stressed conditions. We are currently in the process of examining expression of nrk-1 other stress conditions. We are also creating nrk-1 transcriptional and translational GFP reporters, in order to visualize its gene expression and protein localization.

297B. Sugar Stress Reduces Fertility in *C. elegans* via Multiple Mechanisms. Marjorie R. Liggett, Amanda K. Engstrom, Uyen Ho, Michael Mastroianni, **Michelle A. Mondoux**. Biology Dept, College of the Holy Cross, Worcester, MA.

High glucose diets have been linked to obesity, diabetes, and cardiovascular disease, and high galactose diets are implicated in liver failure and neurotoxicity. Previous studies using *C. elegans* as a model for high sugar diets have shown that exposing hermaphrodites to high glucose decreases fertility and delays reproductive timing. We have further characterized the glucose stress response and tested whether this response is sex-specific and/or glucose-specific. We find that glucose stress perturbs reproduction in both sexes at multiple points in the lifecycle. In hermaphrodites, the reduction in fertility and reproductive delay are separable phenotypes dependent on glucose stress during adulthood and development, respectively. We hypothesize that glucose decreases gamete survival via an increase in germline apoptosis, as *ced-3* caspase mutants show no loss of fertility in the presence of glucose stress. Male fertilization success was also decreased on high glucose in an adult-specific manner. We found an increased sensitivity of males to glucose stress, as male fertilization success was compromised at glucose concentrations that had no effect on hermaphrodite fertility. The reduction in male fertility was not due to defects in courtship, as mating behaviors and the number of successful mating events were unchanged on high glucose. In addition to decreased brood size, we found a significant reduction in male progeny in the male-hermaphrodite cross, suggesting that glucose decreases the competitiveness of male sperm and thus gamete quality. We tested whether this response was glucose specific by assaying fertility in response to a high galactose diet. We found that *C. elegans* is sensitive to galactose, as fertility was decreased and reproductive timing delayed; however, worms were less sensitive to galactose than to glucose. Together, our data suggest that multiple types of sugar stress reduce fertility by reducing both gamete quality and survival in *C. elegans*, but that the mechanisms are different in the different sexes and sensitivities to different sugars vary.

298C. A novel kinase regulates dietary restriction-mediated longevity in *C. elegans*. **Arnab Mukhopadhyay**, Manish Chamoli, Anupama Singh. National Institute of Immunology,New Delhi, India.

Dietary restriction (DR) increases life span in most model systems tested. In mammals, it is associated with health benefits including reduced risk of cancer, cardiovascular diseases and diabetes. In spite of such positive impact, DR is easier to implement in an experimental model as compared to human beings. In this context, it will be beneficial to have an internal model of DR (iDR) where, irrespective of the calorie intake, an organism may enjoy its beneficial effects. We will discuss one such model in *C. elegans*.

In *C. elegans*, DR is studied after restricting food intake by using either genetic or non-genetic manipulations. The *eat-2* gene represents a well-studied genetic model of DR where a mutation in a nicotinic acetylcholine receptor subunit leaves the mutant worms with defective pharyngeal pumping and consequently, lower bacterial intake. Non-genetic methods for DR use either serial dilutions or complete deprivation of bacteria. However, these different DR regimes activate distinct pathways. For example, *eat-2* mutants and liquid DR regimes require *pha-4* and *skn-1* transcription factors, being independent of the FOXO homolog, *daf-16*. On the other hand, bacterial dilution protocol on solid media requires *daf-16*. In this context, identification of genes that induce a DR-like state when manipulated, without the confounding effects of dietary intake, is likely to provide fundamental insights into the mechanisms of DR.

Signalling components within an organism sense low nutrient availability to signal onset of a DR response. In this study, we characterized a novel kinase that qualifies as an important component of the nutrient sensing pathway and a DR response initiator. Knocking down the kinase seems to convince the worms of an imminent nutrient crisis that initiates a state of DR, although food is plentiful, and dramatically increasing life span. We will discuss the molecular mechanism by which this kinase affects longevity and metabolism. This kinase represents a model for DR in mammals without dramatic lifestyle changes.

299A. Regulation of self-renewal and differentiation capacities of germline stem cells during ageing. **Patrick Narbonne**, Jean-Claude Labbé, Paul S. Maddox. IRIC, Université de Montréal, Montréal, QC, Canada.

Stem cells have the amazing capacity to proliferate and expand in numbers almost indefinitely *in vitro*. *In vivo* however, the efficiency of stem cell-dependent processes, such as regeneration/repair and reproduction, declines with age in most organisms. It is thus likely that stem cell capacities decay during ageing *in vivo*, but the mechanisms remain unclear. Yet, understanding this phenomenon could lead to the identification of genes or pathways that could be targeted by drugs to restore stem cell function in ageing humans. This may be useful to treat many degenerative disorders, but also to elucidate how cancer stem cells somehow escape from this regulation and acquire/maintain the ability to expand in numbers within an ageing organism. I use the *C. elegans* germline stem cells (GSCs) as a model to study stem cell activity *in vivo* during ageing. By staining the germ line of unmated hermaphrodites fixed at different ages with an M-phase marker (anti-phospho-H3ser10) and a differentiation marker (anti-HIM-3), I have determined their mitotic index until 10 days of adulthood at 25°C. The mitotic index of GSCs progressively decreases as worms age, leading to a decreased GSC pool size. At a population level, the mitotic index reaches a basal level (below 1%) after 4 days, and then remains more or less stable until day 10. GSC population size follows the same trend, reaching a basal size (~100) after 5 days and remaining stable until day 10. Similarly, mitosis-to-meiosis transition of the GSCs is slower in 4 day-old adults compared to 1 day-old. In continuously mated hermaphrodites, the mitotic index remains above 1% for a longer time, but eventually also declines in older animals, and a similar trend is observed in males. This suggests that GSC division rate is coupled to oocyte demand until a certain age, after which the capacity of GSCs to expand in numbers ceases to respond properly to the demand. This is consistent with stem cell capacities progressively deteriorating with age *in vivo*. I am currently generating mitotic index curves relative to age for a variety of aging, metabolic, epigenetic and developmental mutant animals to find out which genes and genetic pathways are involved in this process.

300B. A role for the insulin signaling pathway in development of neuronal aging markers in a polyglutamine protein aggregation *C. elegans* model. **Courtney Rose Nichols**[1], Elena Vayndorf[1], J. Alex Parker[3], Christian Neri[3], Monica Driscoll[2], Barbara Taylor[1]. 1) Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK; 2) Dept of Molecular Biology and Biochemistry, Rutgers, SUNJ, Piscataway, NJ; 3) Laboratory of Neuronal Cell Biology and Pathology, INSERM, Paris, France.

Neurodegenerative diseases, such as Huntington's, Alzheimer's, and Parkinson's disease, result in the progressive loss of neuronal structure and function with age. Many neurodegenerative disorders are caused by genetic mutations and characterized by toxic aggregation of proteins within neurons. We explored the mechanism of accelerated neuronal aging in a polyglutamine (polyQ) protein aggregation model through genetic manipulation. Caenorhabditis elegans expressing the first 57 amino acids of human huntingtin protein with a toxic polyglutamine chain (polyQ128) fluorescently labeled with CFP in YFP-labeled mechanosensory neurons (Parker et al, 2001) were used to monitor neuronal aging phenotypes. We found an age-associated increase in aberrant neuronal morphology, protein aggregation, and functional impairment of the mechanosensory neurons expressing toxic 128 polyQ. We also tested the hypothesis that the insulin signaling pathway is involved in morphological and functional health of aging mechanosensory neurons using neuron-specific RNA interference (RNAi). Furthermore, we measured levels of endogenous oxidative stress and lifespan of aging animals that contain toxic polyQ128 repeats following RNAi manipulation of the insulin signaling pathway. Overall, we found that DAF-16/FOXO is neuroprotective in this accelerated aging model, which corroborates previous findings on the role of DAF-16/FOXO in polyQ128 young adults (Parker et al. 2005). To further characterize overall muscle and neuronal health, we measured action potentials of pharyngeal muscle contractions and neurons that innervate the pharynx using microfluidic electropharyngeography (EPG). In total, our observations support that insulin signaling via DAF-16/FOXO is a mechanism through which accelerated aging and neurodegeneration occurs in this model.

301C. Oligomeric proanthocyanidins extracts are putative anti-obesity targets in the nematode *Caenorhabditis elegans*. **Yu Nie**[1], Sukhi Bansal[2], Bob Hider[2], Peter Hylands[2], Stephen Stürzenbaum[1]. 1) Analytical and Environmental Science Division, King's College London, London, United Kingdom; 2) Institute of Pharmaceutical Science, King's College London, London, United Kingdom.

Proanthocyanidins are among the most common secondary metabolites in plants and thus are crucial to human diet and therefore health. Oligomeric proanthocyanidins (OPCs, DP £ 5) are thought to be effective in improving lipid homeostasis in rodent animals and humans, which may be attributed to their good bioavailability. The effectiveness of OPCs as an anti-obesity treatment has been tested but poorly described due to the vague understanding of their composition. Besides, an *in vivo* study using purified OPCs is yet to be elucidated, a shortfall we aim to address here. Owing to the rapid screen and high-throughput capacity, *Caenorhabditis elegans* is well positioned to advance the knowledge of OPCs' mode of action and efficiency as an anti-obesity drug. Purification of OPCs extracts from grape seeds was realized on preparative-thin layer chromatography (TLC) coupled with reverse-phase high performance liquid chromatography (RP-HPLC), confirmed by mass spectrometry (MS) and nuclear magnetic resonance spectroscopy(NMR). The growth of age-synchronized N2 worms exposed to OPCs was measured. Lipid stores and lysosomal compartments within the worm bodies were visualized by staining with the hydrophobic fluorescent dye Nile Red and LysoTracker. In parallel, a biochemical assay was applied to quantify the triglyceride level. Statistical significance testing was performed with Prism GraphPad5®. In an attempt to purify crude extracts, pure monomers were collected following a RP-HPLC and verified by spectroscopy. Prep-TLC yielded pure dimers, trimers and tetramers confirmed by MS. Worms exposed to OPCs decreased in size, an effect that was most significant with trimer- to pentamer-enriched OPC fractions. A statistically significant decrease was observed in Nile Red staining. Similarly, triglyceride level decreased most when worms were treated with dimer- and trimer-enriched fractions. Exposure to OPC enriched fractions decrease the volumetric surface area and lipid storage capacity of *C. elegans*, confirming their potential as anti-obesity leads. OPCs trimers are predicted to be the most effective.

302A. HIF-independent processes in hypoxia in *C.elegans*. **Divya Padmanabha**, Young-Jai You, Keith Baker. Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA.

Cellular and systemic oxygen homeostasis is a finely regulated process for organisms of all species. Responses to hypoxia have been extensively studied in the context of the transcription factor hypoxia-inducible factor (HIF), a critical regulator of angiogenic and glycolytic genes in hypoxia. Interestingly, gene expression studies provide clear evidence of HIF-independent pathways that are critical for adaptation to hypoxia. However, relatively little is known about the oxygen sensors and regulatory pathways that mediate transcriptional responses independent of the HIF pathway. The nematode *C. elegans* has proven to be a powerful model system to study evolutionarily conserved signaling pathways that regulate hypoxia responses. Because we were interested in further understanding the nature of HIF-independent hypoxic responses, we conducted an RNAi screen to identify transcription factors regulating HIF-independent genes sensitive to hypoxic treatment and/or the hypoxia mimetic, cobalt chloride. We have found that BLMP-1/PRDM1, a zinc finger-containing transcriptional repressor, plays a role in the HIF-independent response in hypoxia. We show that BLMP-1 is necessary for the HIF-independent pathway and that this activity is not dependent on the prolyl hydroxylase *egl-9*, a critical regulator of the HIF-dependent response. We have also identified some downstream targets of BLMP-1 in hypoxia, including a member of the Hsp70 family of heat shock proteins and a metabolic pathway enzyme.

303B. The inner mitochondrial membrane translocase complex TIM23 modulates mitochondrial biogenesis and function during ageing in *C. elegans*. E. Lionaki[1], **K. Palikaras[1]**, N. Tavernarakis[1,2]. 1) Institute of Molecular Biology and Biotechnology, Heraklion 71110, Crete, Greece; 2) Medical School, University of Crete, Heraklion 71003, Crete, Greece.

Ageing is accompanied by marked changes in mitochondrial biogenesis and physiology. Abnormal mitochondria accumulate during aging, while breakdown of mitochondrial DNA (mtDNA) repair mechanisms cause premature aging in mice. 99% of the mitochondrial proteins are encoded by nuclear genes. Proper targeting of mitochondrial proteins is carried out by a few conserved protein complexes which reside on the outer and inner mitochondrial membranes. We have focused on the role of these mitochondrial protein import pathways in ageing. Studies in *Saccharomyces cerevisiae* have established the TIM23 complex as the translocase of the inner membrane responsible for importing matrix proteins, and single-spanning membrane proteins of the inner membrane. About 2/3 of the total mitochondrial proteome rely on TIM23 for their translocation. TIM23-mediated translocation depends on ATP hydrolysis in the matrix as well as on the electrochemical potential of the inner membrane. We have identified the *C. elegans* ortholog of the channel-forming protein Tim23, hereafter termed TIMM-23. RNAi knock-down of *timm-23* causes morphological and fertility defects, embryonic lethality and larval arrest. However, animals treated with *timm-23* RNAi from the L1 stage onwards display extended lifespan, compared to the control population. Blocking mitochondrial matrix protein import compromises membrane potential and activates the mitochondrial unfolded protein response. The molecular cascade triggered by *timm-23* knock-down does not involve the transcription factors, DAF-16, SKN-1 and HIF-1. By contrast, CEP-1 is necessary for lifespan extension under *timm-23* RNAi. Our results suggest that mitochondrial protein import acts in parallel to well-characterized pathways influencing lifespan, such as caloric restriction and insulin/IGF-1 signaling. Given that CEP-1 localizes to mitochondria, we suggest that differential subcellular localization of CEP-1 could be involved in lifespan determination upon blocking mitochondrial protein translocation.

304C. Coordination of mitophagy and the mitochondrial retrograde response during ageing in *C. elegans*. **Konstantinos Palikaras[1]**, Nektarios Tavernarakis[1,2]. 1) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion, Crete, Greece; 2) Medical School, University of Crete, Heraklion, Crete, Greece.

Mitochondria are essential for energy production and have vital roles in calcium signalling and storage, metabolite synthesis and apoptosis in eukaryotic cells. Thus, maintenance of cellular homeostasis necessitates a tight regulation of mitochondrial biogenesis, as well as, the elimination of damaged or superfluous mitochondria. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradative pathway, by which cells regulate mitochondrial number in response to metabolic state. However, little is known about the role and regulation of mitophagy during ageing. To address this question, we developed an imaging system to monitor mitophagy in vivo, and identified conditions that either induce or suppress mitophagy. We used this system to investigate the involvement of mitophagy in *C.elegans* ageing. Inhibition of mitophagy does not affect the lifespan of otherwise wild type animals. By contrast, inhibition of mitophagy markedly shortens the lifespan of long-lived diapause mutants, germline defective animals, or animals grown under dietary restriction. Similarly, impairment of mitophagy shortens the lifespan of long-lived mutants with compromised mitochondrial function. These findings indicate that mitophagy contributes a large part of lifespan extension under conditions of low insulin signalling, germline removal or mitochondrial dysfunction. Mitophagy-deficient mutants are less resistant under conditions of stress, such as heat or oxidative stress and UV-radiation. Mitophagy deficiency precipitates marked alterations of mitochondrial network morphology, mitochondrial mass, ROS levels, mitochondrial membrane potential and cytoplasmic calcium. Importantly, inhibition of mitophagy activates the retrograde response pathway that links mitochondrial function with nuclear gene expression to maintain mitochondrial homeostasis. Our results indicate that mitophagy and the retrograde response are tightly coupled and coordinately contribute to promote mitochondrial homeostasis and longevity.

305A. Mitochondrial Dynamics And Behavioral Plasticity In Response To Oxygen Deprivation Are Linked Through HIF-1. **Eun Chan Park**, Piya Ghose, Alexandra Tabakin, Nathaly Salazar-Vasquez, Christopher Rongo. Waksman Inst, Rutgers Univ, Piscataway, NJ.

C. elegans can survive extreme oxygen deprivation (anoxia, < 0.1% O_2) by entering into a suspended animation state during which locomotory behavior and development arrest. Upon re-exposure to normoxic conditions (21 % O_2), animals resume their behavior and normal development. The underlying mechanism of this stress response is not well understood. Here we show that neuronal mitochondria undergo fission in response to anoxia co-temporaneously with the onset of suspended animation, followed by re-fusion upon re-oxygenation, suggesting that mitochondrial dynamics might contribute to anoxia-induced suspended animation. We find that the hypoxia response pathway, including EGL-9 and HIF-1, regulates the reconstitution of mitochondria following re-oxygenation. Under conditions of normal oxygen, EGL-9 represses the activity of HIF-1, a transcription factor that promotes the expression of hypoxia-response genes. Loss of function mutations in *egl-9* result in rapid re-fusion of mitochondria and behavioral recovery from suspended animation following re-oxygenation; both phenotypes require HIF-1 activity. In addition, the mitochondria are significantly larger in *egl-9* mutants after re-oxygenation than they were prior to anoxia exposure - a phenotype that resembles the stress-induced mitochondria hyperfusion (SIMH)

that has been observed in mammalian cells. Both the changes in mitochondrial dynamics and suspended animation recovery of *egl-9* mutants can be rescued by expressing wild-type EGL-9 solely in neurons. Mitochondrial hyperfusion and rapid recovery observed in *egl-9* mutants following anoxia are modulated by STL-1, a mammalian stomatin-like protein (SLP2) homologue that is reported to function in SIMH. Our results suggest the existence of a conserved stress response involving changes in mitochondrial fission and fusion, and demonstrate that the behaviors executed by a simple neural circuit can be regulated through changes in mitochondrial dynamics.

306B. Independent genetic pathways for stress response and longevity revealed by experimental evolution in the nematode *Caenorhabditis remanei*. Rose Reynolds[1,2], Kristin Sikkink[1], Catherine Ituarte[1], Janna Fierst[1], John Willis[1], William Cresko[1], **Patrick Phillips[1]**. 1) Institute of Ecology and Evolution, University of Oregon, Eugene, OR; 2) Department of Biology, William Jewell College, Liberty, MO.

Within *C. elegans*, many of the best-studied genes with effects on longevity have been found to function within stress-response pathways, and therefore many mutations that extend lifespan also frequently have a positive influence on stress resistance. This pleiotropic coupling between stress resistance and longevity suggests that these phenotypes may be causally related, with increased stress resistance per se being a key component of extending lifespan. Similarly, a number of genes (e.g., *daf-16, hsf-1, pha-4*) have been found to have broad roles in the successful response to a variety of environmental stressors, including high temperatures, oxidation, pathogens and starvation. Is pleiotropy the rule in stress response and longevity or are these particular genes special cases with especially large pleiotropic effects? We tested this hypothesis using by selecting on natural genetic variation within replicate populations of the obligate outcrossing species *C. remanei* under conditions of both chronic and acute heat and oxidative stress. Resistance to both stressors evolved rapidly under both conditions, but increased resistance to high temperatures did not tend to cause a correlated increase in resistance to oxidative stress and visa versa. For the most part, selection for increased stress resistance did not lead to increased longevity in a non-stressful environment, and in many cases reduced it. The stress-selected lines also displayed significant changes in the expression patterns of thousands of different genes, including many classic heat-shock loci. We are currently using whole-genome re-sequencing to identify the genetic basis of the specific responses. Overall, it does not appear that the universal pleiotropy often observed in mutations of large effect is an important component of the evolutionary response to environmental stress. Experimental evolution may therefore provide a valuable means of identifying genes with more subtle, pathway-specific effects.

307C. Deciphering the microRNA responses to high temperature stress. C. Nehammer[1], **A. Podolska[1]**, K. Kagias[1], S. Mackowiak[2], N. Rajewski[2], R. Pocock[1]. 1) BRIC, University of Copenhagen, Copenhagen N, Denmark; 2) Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Strabe 10, 13125, Berlin.

Living organisms are constantly exposed to changing environments and the ability to maintain homeostasis whilst exposed to stress is essential for survival. Therefore, rapid responses to stress, such as high temperature, are crucial in order to avoid detrimental stress induced effects. MicroRNAs are rapidly acting regulatory elements which can fine-tune gene expression when required. Thus, microRNAs are excellent candidates to study the regulation of stress response pathways in C. elegans We performed RNA-seq analysis to identify microRNAs that are regulated when worms are exposed to high temperature stress. Relevant microRNA mutant strains were subjected to a 32°C heat stress and scored for lethality. Interestingly, we identified 4 heat-resistant and 4 heat-sensitive miRNA mutant strains, including mir-71 and mir-239, that were previously associated with stress responses in C. elegans. Further experiments will reveal the specific function, regulation and expression of heat stress regulated miRNAs.

308A. Folliculin is an evolutionary conserved regulator of AMPK function. **E. Possik[1,2]**, Z. Jalali[1,2], Y. Nouet[1,2], M. Yan[1,2], MC. Gingras[1,2], L. Chotard[1,2], F. Dupuy[1,2], C. Rocheleau[3], D. Hall[4], R. Jones[1], A. Pause[1,2]. 1) Biochemistry department, McGill University, Montreal, Quebec, Canada; 2) Goodman Cancer Research Center, McGill University, Montreal, Quebec, Canada; 3) Department of Medicine, McGill University, Montreal, Quebec, Canada; 4) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York, USA.

Energy imbalance and oxidative stress contribute to the development of many life-threatening diseases including cancer. AMPK is a highly conserved heterotrimeric protein complex and a major cellular energy sensor. Under energy stress, activated AMPK directly phosphorylates downstream metabolic enzymes that inhibit anabolic processes and activate catabolic processes, to generate ATP. For instance, AMPK directly phosphorylates ULK1 and activates autophagy, a “self-eating” process to supply the energy required to maintain cell function. Here we identified folliculin (FLCN) as a conserved novel regulator of AMPK function. Germline loss of function mutations in the FLCN gene predisposes to the Birt-Hogg-Dube hereditary cancer syndrome. To characterize the role of the FLCN/AMPK interaction, we used a genetic approach that employs the model organism C. elegans and FLCN knockout mouse embryonic fibroblasts. We found that (1) FLCN is a novel evolutionary conserved energy stress sensor and a negative regulator of AMPK. In worms and mammals, loss of flcn-1/Flcn leads to chronic activation of AAK-2/AMPK, which induces autophagy and confers resistance to various energy stresses such as heat, oxidative stress, and anoxia, glucose and amino acid deprivation. (2) The enhanced resistance of the flcn-1 mutant worms to stress is independent of the canonical DAF-2/DAF-16 insulin signaling pathway, yet it requires autophagy. (3) Upon low energy conditions, FLCN dissociates from AMPK and facilitates the AMPK/ULK1 complex formation, which activates autophagy. The chronic activation of autophagy upon loss of FLCN modifies the cellular metabolism providing an energetic advantage that is sufficient to survive stress and delay cell death. Here we show a novel evolutionary conserved type of AMPK regulation that is distinct from the previously described mechanisms.

309B. Epigenetic Mechanism of Longevity Regulation in *C. elegans*. **Mintie Pu[1]**, Xiujuan Wang[2], Zhuoyu Ni[1], Haiyuan Yu[2], Siu Sylvia Lee[1]. 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) 2Department of Biological Statistics and Computational Biology and Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY.

Recent studies demonstrate longevity is affected and controlled by histone modifications. The link between histone modification and aging was first demonstrated by the observation that RNAi knockdown or genetic inactivation of histone modification enzymes resulting in extended longevity in model organisms, including *C. elegans*. However how histone modification changes impact aging and how aging impacts histone modifications are not clear. To gain insights into the histone modification pattern during aging, we monitored the genomic locations and abundance of several histone modifications involved in gene transcriptional regulation at different ages using ChIP-seq. Here we surveyed the genomic H3K36me3 profile in *C. elegans* somatic cells

during aging. We found H3K36me3 pattern is largely stably maintained during aging. Importantly, we found H3K36me3 level is negatively correlated with gene expression variation during aging. Our data indicate that genes that are lowly methylated are significantly more likely to show greater changes of mRNA levels with age. Our analyses indicate that this negative correlation between H3K36me3 modification level and gene expression variation is independent of gene expression level, gene length and tissue specificity, suggesting an interesting function of H3K36me3 in maintaining the stability of gene transcription during aging.

310C. The mitochondrial stress machinery protects cells from inhibition of the mevalonate pathway. **Manish Rauthan**, Marc Pilon. Department of Chemistry and Molecular Biology, University of Gothenburg, Gothenburg, Sweden, SE-405 30.

Statins are cholesterol lowering drugs and act by inhibiting HMG-CoA reductase, a key enzyme in the mevalonate pathway required for synthesis of cholesterol, coenzyme Q, dolichols and isoprenoids. The effects of statins on the synthesis of these biomolecules are not well studied except for cholesterol. In addition, statins occasionally cause side effects (myopathy, neuropathy) that are independent of their cholesterol-lowering activity. To identify the genetic components and molecular mechanisms behind the non-cholesterol effects of statins we use *C. elegans* as a model organism since its mevalonate pathway lacks the sterol synthesis branch but retains all other branches. We performed forward genetic screens for statin resistance and isolated four mutants with gain-of-function (*gf*) mutations in the *atfs-1 (activating transcription factor associated with stress-1)* gene. This gene is required for activation of the mitochondrial stress machinery or mitochondrial unfolded protein response (UPR^{mt}), and the *atfs-1 (gf)* mutants have constitutively active UPR^{mt}. These mutants are also resistant to inhibitors of enzymes that act downstream of the HMG-CoA reductase and to gliotoxin, an inhibitor that acts on a sub-branch of the pathway important for protein prenylation. Additionally, these mutant worms have an improved mitochondrial function after statin treatment in comparison to wild-type worms. Furthermore, pre-induction of the mitochondrial stress machinery in wild-type worms using ethidium bromide triggered statin resistance, and similar observations were also made in *S. pombe* and in a mammalian cell line. These findings suggest that the effects of statins on mitochondria are primarily due to impairment of protein prenylation, and that the UPR^{mt} protects mitochondria against inhibition of the mevalonate pathway.

311A. Is mitochondrial fragmentation a bio marker of aging? **Saroj G. Regmi[1,2]**, Barbara Conradt[1]. 1) Cell and Developmental Biology, LMU Biocenter, Munich, Germany; 2) Department of Genetics, Geisel School of Medicine at Dartmouth, Hanover, NH.

Mitochondria are dynamic organelles that undergo constant fusion and fission events. This dynamic homeostasis defines the morphology of mitochondria and it is thought to be important for normal mitochondrial function. Using transgenic animals expressing a matrix-targeted GFP under the control of a muscle-specific promoter (P_{myo-3} *mitoGFP*), we observe that body wall muscles display a tubular inter-connected mitochondrial network that fragments with increasing age. Therefore, we aim to address if mitochondrial morphology is a suitable bio-marker of aging. To that end, we are analyzing mitochondria (mitochondrial length, circularity and area) in animals raised at different temperatures and in short-lived and long-lived animals. We found that animals grown at 15°C display a fragmented mitochondrial morphology later than animals grown at 25°C. Currently, we are characterizing mitochondrial morphology in short-lived and long-lived animals. The rationale being that, if mitochondrial fragmentation is indicative of life-span, compared to wild-type animals, short-lived animals should exhibit mitochondrial fragmentation earlier while long-lived animals should exhibit mitochondrial fragmentation later. Consistent with this hypothesis, short-lived *daf-16* mutants display mitochondrial fragmentation earlier than control animals while long-lived *age-1* mutants display mitochondrial fragmentation later than control animals. Currently, we are analyzing long-lived *clk-1* mutants. In addition, we are measuring mitochondrial morphology in seven day old animals to determine if animals that display a fragmented mitochondrial morphology exhibit a shorter lifespan compared to animals that display a tubular mitochondrial morphology.

312B. Regulation of eIF4E compartmentalization by the heat shock response pathway during ageing in *C. elegans*. **M. Rieckher[1]**, A. Princz[1], N. Tavernarakis[1,2]. 1) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion 71110, Crete, Greece; 2) Medical School, University of Crete, Heraklion 71003, Crete, Greece.

Increased lifespan is often correlated with enhanced resistance against intrinsic and extrinsic stress. The eukaryotic initiation factor 4E (eIF4E) is known to regulate ageing and cellular stress resistance through the control of global protein synthesis. Recent studies reveal that eIF4E performs additional regulatory functions by shuttling specific mRNAs from the nucleus to the cytoplasm, and sequesters mRNAs in cytoplasmic aggregates, predominantly during stress. Genetic studies show that the heat shock transcription factor HSF-1 regulates stress responses and modulates lifespan in *C. elegans*. Here, we present evidence that HSF-1 regulates multiple cellular functions of IFE-2, an isoform of eIF4E expressed in the soma. We find that IFE-2 protein levels decrease during ageing. Loss of HSF-1, but not SKN-1 or DAF-16, results in substantially increased *ife-2* mRNA and protein levels in aged animals. Notably, IFE-2 localizes primarily to the nuclei of most somatic cells in animals lacking HSF-1. Moreover, a significantly higher percentage of IFE-2 was found to reside in the nuclei of young animals, compared to aged nematodes. In these animals, IFE-2 mainly granulizes in the perinuclear space and within the nucleus. Granular formation is also observed throughout the cytoplasm. These granules co-localize with well-characterized sites of mRNA degradation, which have been shown to be involved in transcription regulation in the nucleus. Consistently, we observe decreased localization of IFE-2 in the nucleus, upon depletion of specific mRNA degradation factors. Our findings suggest that HSF-1 modulates IFE-2 function and localization during ageing, and that IFE-2 also serves as mRNA transport protein in *C. elegans*. Thus, IFE-2 likely mediates the effects of the heat stress response on both mRNA translation and degradation to influence ageing.

313C. Systemic control of the cytosolic redox environment in *C. elegans*. **C. Romero**, W. Fontana, J. Apfeld. Systems Biology, Harvard Medical School, Boston, MA.

Maintaining a finely tuned redox environment is critical for the normal function of diverse cellular processes. While much is known about the mechanisms that cause and repair protein oxidation, little is known about the regulation of redox homeostasis in the context of the whole organism. To observe redox processes *in vivo*, we made transgenic animals expressing a genetically encoded redox sensor whose spectral properties change in response to the reversible oxidation of a pair of adjacent cysteine residues on its surface. Using ratio-metric fluorescence microscopy, we quantified *in vivo* the

degree of oxidation of this redox couple.

We find that *C. elegans* tissues differ in their cytosolic redox environments. While sensor oxidation varies widely among different worms, it is highly correlated among the individual muscles in the pharynx of each animal. Despite being connected by gap junctions, pharyngeal muscles exhibit significant differences both in the degree of oxidation of the sensor under normal conditions and in the dynamics of sensor oxidation in response to oxidative stress. To investigate the mechanisms that give rise to spatial differences in protein oxidation levels we examined mutants in the insulin/IGF-1 signaling pathway, which has evolutionarily conserved effects on resistance to oxidative stress. Mutants in the insulin/IGF-1 receptor *daf-2* lower protein oxidation levels in a cell-type specific manner. These effects are mediated by the FOXO transcription factor *daf-16*. Using tissue-specific *daf-16* rescue experiments, we find that this gene functions in pharyngeal muscle to control the degree of sensor oxidation in this tissue. Additionally, *daf-16* action in intestinal cells affects the oxidation level of the sensor in pharyngeal muscle cells lacking *daf-16*. Thus, insulin/IGF-1 signaling controls both cell-intrinsic and cell-extrinsic mechanisms for protein oxidation. We propose a model whereby insulin/IGF-1 signaling regulates communication between tissues to coordinate and direct the balance of cellular protein oxidation and repair mechanisms in response to specific organismic needs.

314A. Developmental Exposure to Ultraviolet C Radiation Results in Altered Energy Production Later in Life in Caenorhabditis elegans. **John P Rooney**, Rakesh Bodhicharla, Amanda Bess, Maxwell Leung, Ian Ryde, Alex Ji, Joel Meyer. Nicholas School of the Environment, Duke University, Durham, NC.

Mitochondrial genomes encode for 13 proteins that are essential components of the electron transport chain (ETC) and are normally present at between 1000 and 100,000 copies per cell. However, this number is greatly reduced during specific developmental stages, representing a potential critical window for mitochondrial genotoxicant exposure. Mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA (nucDNA) to damage by many environmental pollutants, for reasons including absence of Nucleotide Excision Repair (NER). NER is a highly functionally conserved DNA repair pathway that removes bulky, helix distorting lesions such as those caused by ultraviolet C (UVC) radiation and many environmental toxicants. In the current experiment we tested the hypothesis that UVC induced mtDNA damage during larval development would alter energy production throughout the life of the nematode. ATP levels were measured as luminescence using the firefly luciferase expressing PE255 glp-4(bn2) strain (generously provided by Cristina Lagido, University of Aberdeen), and both mtDNA and nuclear DNA copy numbers were measured via quantitative real time PCR. We exposed first larval stage PE255 nematodes to a serial UVC dose that results in the accumulation of mtDNA damage while allowing for repair of nuclear DNA damage, and measured ATP levels, mtDNA and nucDNA copy numbers every 48 hours for 10 days. Immediately after treatment, ATP levels were nearly equal, however at post treatment days 2 through 10 ATP levels were 50-70% lower in UVC treated nematodes. Interestingly, there was no detectable change in either mtDNA or nucDNA copy numbers throughout the time course. These results indicate that mtDNA damage during larval development can alter energy production throughout the life of the nematode, and highlights the potential for a critical window of exposure to mtDNA damage. Furthermore, while it is well established that genetic manipulation of expression of ETC components results in reduced ATP levels and lifespan extension, our data suggest the same phenotypes can be induced via environmental exposures.

315B. Assaying nickel toxicity using nematodes. **David Rudel**, Ian Huffnagle, Chandler Douglas, John Atkinson. Department of Biology, East Carolina University, Greenville, NC.

Nickel occurs as metal alloys, insoluble compounds, and soluble compounds all of which can be taken up by animals from the environment. Nickel exposure from anthropogenic sources results in rashes, respiratory conditions and cancer. Ni (II) and other cationic metals travel through waterways and bind to soils and sediments. We have conducted sediment and water assays using two cosmopolitan nematodes, *Caenorhabditis elegans* and *Pristionchus pacificus*. We assayed the effects of both sediment-bound and aqueous nickel upon growth, developmental survival, lifespan, and fecundity. Uncontaminated sediments were collected from 8 sites in the Midwest of the United States and spiked with nickel. We find that nickel-spiked sediment substantially impairs both survival of animals from L1 larva to adult and adult longevity in a concentration-dependent manner. In contrast, the levels of aqueous nickel we tested do not though we note a decrease in fecundity of treated animals over the duration of the test. This indicates aqueous nickel also negatively impacts nematode physiology. *C. elegans* and *P. pacificus* are quite divergent with molecular and developmental differences and both have disparate ecologies. Nevertheless these nematodes give similar, but not identical, results at higher nickel exposure concentrations in water or in sediment. Intriguingly, *P. pacificus* could be tested successfully in sediments hostile to *C. elegans*; arguing that environmental toxicological studies could gain an advantage by widening their repertoire of nematode species. Lastly, using a *C. elegans ced-1::gfp* translational fusion line, we have shown that Nickel induces apoptosis in the *C. elegans* germ line. In contrast to earlier findings in *C. elegans*, we find that Nickel-induced apoptosis is dependent upon the p53 pathway and thus at least a part of nickel toxicity in nematodes is due to genotoxicity.

316C. D-Aspartate oxidase is involved in the caloric restriction-induced lifespan extension in *C. elegans*. **Yasuaki Saitoh**[1], Mari Okutsu[1], Masumi Katane[1], Masae Sekine[1], Takemitsu Furuchi[1], Taro Sakamoto[1], Takao Inoue[2], Hiroyuki Arai[3], Hiroshi Homma[1]. 1) Dept. of Pharmaceut. Life Sci., Kitasato Univ., Tokyo, Japan; 2) Div. Cell. and Gene Therapy Products, Natl. Inst. Health Sci., Tokyo, Japan; 3) Grad. Sch. of Pharmaceut. Sci., The Univ. of Tokyo, Tokyo, Japan.

Among free D-amino acids existing in living organisms, D-serine (D-Ser) and D-aspartate (D-Asp) are the most intensively studied. In mammals, D-Ser has been proposed as a neuromodulator that regulates L-glutamate (L-Glu)-mediated activation of the N-methyl-D-Asp (NMDA) receptor by acting as a co-agonist. On the other hand, several lines of evidence suggest that D-Asp plays important roles in regulating hormone secretion and steroidogenesis. D-Amino acid oxidase (DAO) and D-Asp oxidase (DDO) are known as stereospecific degradative enzymes that catalyze the oxidative deamination of D-amino acids. Mammalian DAO and DDO are presumed to regulate endogenous D-Ser and D-Asp levels, respectively. Previously, we demonstrated that D-Ser, D-Asp, D-Glu and D-alanine (D-Ala) are present in nematode Caenorhabditis elegans, a multicellular model animal. We also found that C. elegans has at least one active DAO gene and three active DDO genes (DDO-1, DDO-2 and DDO-3), and that the spatiotemporal distributions of these enzymes in the body of C. elegans differ from one another. Furthermore, our previous study showed that alterations of brood size and hatching rate are observed in four C. elegans mutants lacking each gene for the DAO and DDOs. Interestingly, lifespan extension was observed in the DDO-3 mutant. To characterize the mechanism of lifespan extension in the DDO-3 mutant, we performed genetic epistasis experiments to test interactions between the DDO-3 gene and other known longevity pathways. The results suggest that DDO-3 is involved in caloric restriction-induced lifespan extension but not in insulin/IGF signaling pathway,

NAD/sir2 pathway nor mitochondrial electron transport system. We also found that D-Glu and L-tryptophan (L-Trp) accumulate throughout life in the DDO-3 mutant. Now we are investigating the relationship between aging and the accumulations of D-Glu and L-Trp.

317A. Genetic analyses of hypoxia response and the roles of HIF-1. **Jenifer Saldanha**, Dingxia Feng, Korinna Radke, Jo Anne Powell-Coffman. Genetics, Development, and Cell Biology, Iowa State University, Ames, IA.

Adaptation to different types of stress is critical to metazoan life. Stress-responses enable animals to adapt to conditions that may be sub-optimal or even lethal, and the genetic bases for these responses have been the topic of extensive research in *Caenorhabditis elegans*. There are a variety of strategies that the animals use to deal with stress. This involves a number of key genetic pathways and their regulators as well as cross talk between them. Of particular interest to the field are the responses to challenges such as high temperatures, pathogens, reactive oxygen species, and fluctuations in oxygen levels. Our work has specifically focused on hypoxia response in *C. elegans*. The response to moderate hypoxia is mediated largely by the transcription factor Hypoxia Inducible Factor-1 (HIF-1). The activity of HIF-1 and its targets is controlled by a number of factors, and recent work has focused on HIF-1-dependent phenotypes in stress response, behavior and longevity. We have performed detailed transcriptome-based analyses on HIF-1, its regulators and target genes to enable further in-depth investigations. The results we are presenting will elaborate on the findings of interest from these experiments, particularly on the roles of HIF-1 and its regulators in stress-responses and resistance.

318B. The Max/Mlx transcriptional network influences aging in *C. elegans*. David W. Johnson, Jesse Llop, Sara Farrell, **Andrew V. Samuelson**. Biomedical Genetics, University of Rochester Medical Center, Rochester, NY.

We have identified a role for the *C. elegans* Max and Mlx ("Max-like") transcriptional network as a key modulator of aging. Orthologous mammalian transcription factors have been implicated in nutrient sensing and metabolic control, suggesting that this network emerged early in evolution to provide a mechanism coupling nutrient availability to longevity. We hypothesize that the Max-like transcription factors form a conserved regulatory system at the intersection of nutrient sensing and longevity control. Notably, a deletion in the genomic region of one of the human MML-1 homologs is associated with Williams-Beuren Syndrome, which notably causes metabolic dysfunctions, silent diabetes, and symptoms of premature aging. In *C. elegans* the Max-like transcriptional network consists of two heterodimeric transcription complexes: MXL-1 (Max) dimerizes with MDL-1 (Mad) to repress transcription, while MXL-2 (Mlx) forms a dimer with MML-1 (Mondo) to activate transcription. Our data indicate that the Mlx (MML-1:MXL-2) and Max (MDL-1:MXL-1) dimers have opposing functions in longevity, reflecting their antagonistic roles in transcription. Furthermore, our data show that transcriptional activation via the Mlx complex is required for the full longevity conferred through reduced ILS and DR, and that the extended lifespan observed in the absence of the Max complex requires DAF-16/FoxO and PHA-4/FoxA. Interestingly, examination of published ChIP-Seq data revealed extensive transcriptional regulatory overlap between DAF-16, PHA-4, and MDL-1. Taken together our results suggest that Max-like transcription factors reside at a key nexus between and help to coordinate the transcriptional outputs of ILS and DR.

319C. ULP-4 SUMO protease controls HMGS-1 activity in cytosolic and mitochondrial metabolic networks during development, aging, and stress. **Amir Sapir**[1], Assaf Tsur[2], Thijs Koorman[3], Mike Boxem[3], Paul Sternberg[1], Limor Broday2[2]. 1) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA; 2) Department of Cell and Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel; 3) Developmental Biology, Utrecht University, Utrecht 3584, The Netherlands.

The molecular circuits orchestrating metabolic networks to meet the dynamic physiology of organisms are largely unknown. We hypothesize that post translational modifications pathways may be critical regulators of key metabolic network nodes based on their ability to rapidly modify protein interactions, subcellular localization, and stability. We focused on the small ubiquitin-like modifier (SUMO) pathway and identified a predicted SUMO protease, ULP-4, that exhibits a dynamic expression pattern and a striking cytosol-to-mitochondria translocation in various muscles and neurons during development, aging, and upon mitochondrial stress. Screening two-hybrid libraries, we found a strong association between ULP-4 and HMGS-1, the C. elegans ortholog of human 3-hydroxy-3-methylglutaryl CoA synthase proteins that play a role both in the cytosolic mevalonate pathway and in starvation-induced mitochondrial ketogenesis. Immunoprecipitation of tagged HMGS-1 protein demonstrates that HMGS-1 undergoes in vivo sumoylation and that the protein is highly sumoylated in 8-days old worms. Strikingly, HMGS-1::GFP is ectopically expressed and ectopically localized to mitochondria in smo-1 mutant background whereas HMGS-1::GFP signal is abolished in ulp-4 mutants. Like ulp-4, hmgs-1 expression is regulated developmentally and during aging and upon mitochondrial stress. ulp-4 and hmgs-1 loss resulted in age-dependent attenuation of muscle activity and impaired fat accumulation. Our results suggest a novel regulatory circuit in which ULP-4 dynamic expression and cytosol-to-mitochondria translocation regulate the sumoylation state of HMGS-1 during development, aging, and stress. Our study predicts that by modification of human HMGS-1 orthologs, SUMO proteases may control key metabolic pathways such as cholesterol biogenesis and ketone body formation.

320A. Using *C. elegans* to explore the role of presenilin in the pathogenesis of Alzheimer's Disease. **Shaarika Sarasija**, Kenneth Norman. Cell Biology and Cancer Research, Albany Medical College, Albany, NY.

Mutations in the genes encoding Presenilin-1 and Presenilin-2 occur in early onset Familial Alzheimer's Disease (FAD), a rare form of Alzheimer's Disease (AD). However, the role of presenilins in AD has remained elusive. The *C. elegans* genes *sel-12* and *hop-1* encode transmembrane domain proteins orthologous to human presenilins. We are interested in investigating whether mutations in *sel-12* and/or *hop-1* can alter calcium homeostasis in *C. elegans*. We have found that the *sel-12* null mutant is hypersensitive to the muscle cell acetylcholine receptor agonist, levamisole, and the acetylcholine esterase inhibitor, aldicarb, suggesting that the muscle of the *sel-12* mutant is hyper-excitable. Introduction of a ryanodine receptor (RyR) null background into the *sel-12* mutant rescues this hypersensitivity. Additionally, we have observed that *sel-12* mutants are shorter in length, possibly caused by increased muscle tone, a condition commonly observed in FAD patients. To test this possibility, we stimulated GABAergic inhibitory motor neurons and found that *sel-12* mutants have a reduced ability to relax their muscles. Together, these data suggest that *sel-12* mutants have elevated levels of sarcoplasmic calcium, which leads to hyper-excitable muscle. Mitochondria are dynamic organelles and act as a significant cytosolic calcium buffer in cells. We discovered that the organization of muscle mitochondria are structurally disrupted and that reactive oxygen species are poorly metabolized in *sel-12*

mutants upon paraquat exposure. Furthermore, we found that reducing sarcoplasmic calcium levels by introducing the RyR null mutation into the *sel-12* mutant background did not restore mitochondria organization. These results suggest that the mitochondria dysfunction observed in *sel-12* mutants is not a result of calcium overloading, but rather the primary defect. Therefore, we hypothesize that SEL-12 is required for normal mitochondria function and that loss of mitochondria function in *sel-12* mutants leads to elevated calcium levels and improper ROS metabolism. Since signaling mechanisms are well conserved, we believe using *C. elegans* as a model system will provide insight into the role presenilins play in the pathogenesis of AD.

321B. The rare sugar D-psicose extends *Caenorhabditis elegans* lifespan by increasing oxidative stress resistance. **M. Sato**[1], H. Sakoguchi[1], T. Shintani[2], K. Okuma[2], K. Izumori[3]. 1) Department of Applied Biological Science, Kagawa University, Miki, Kagawa, Japan; 2) Matsutani Chemical Industry Co., Ltd., Itami, Hyogo, Japan; 3) Rare Sugar Research Center, Kagawa University, Miki, Kagawa, Japan.

Calorie-restricted diets are known to extend lifespans of model animals, including nematodes, flies, mice and monkeys. It is believed that calorie-restricted diets delay the onset of aging-associated diseases such as diabetes, cancer and Alzheimer's disease, and also prolong human longevity. However, in reality, it is difficult to continue calorie-restricted diets for a long period of time. Therefore, the development of calorie restriction mimetics (CRMs) that have similar effects as calorie-restricted diets is of great interest. Our preliminary study showed that the rare sugar D-psicose, a stereoisomer of D-fructose, extended the lifespan of *Caenorhabditis elegans*. The findings suggest that D-psicose serves as a CRM in *C. elegans*. The extension of *C. elegans* lifespan under calorie-restricted diet conditions is thought to be dependent on the induction of oxidative stress-related proteins, including Cu/Zn-superoxide dismutase (SOD), Mn-SOD and catalase regulated by the insulin/IGF-I signaling pathway. We aimed to elucidate the molecular mechanism of the lifespan extension by D-psicose. Wild-type *C. elegans* N2 was used in this study. Treatment of worms with 28 mM D-psicose increased mean lifespan of N2 by ca. 20% (control: 20.9 days; D-psicose: 25.1days). Using Quantitative real-time RT-PCR, we found that the mRNA expression levels of mitochondrial Mn-SOD (*sod-3*), cytosolic catalase (*ctl-1*) and peroxisomal catalase (*ctl-2*) were enhanced by 1.4, 1.5 and 1.6-fold, respectively, after treatment of D-psicose. In contrast, expression of cytosolic Cu/Zn-SOD (*sod-5*) was unaffected by 28mM D-psicose treatment. Total SOD and catalase enzyme activities in N2 with 28 mM D-psicose treatment were increased by 1.4 and 2.1-fold, respectively. D-psicose was thought to extend the lifespan of *C. elegans* by increasing the oxidative-stress resistance of the nematode. These results indicate that D-psicose is a candidate CRM.

322C. DNA damage responses in development and ageing. Michael Mueller, Laia Castells-Roca, Maria Ermolaeva, Peter Frommolt, Sebastian Greiss, Jennifer Schneider, **Björn Schumacher**. Cologne Excellence Cluster for Cellular Stress Responses in Aging-Associated Diseases (CECAD), Institute for Genetics, University of Cologne, Zülpicher Str. 47a, 50674 Cologne, Germany.

Congenital defects in genome maintenance systems cause complex disease phenotypes characterized by developmental failure, cancer susceptibility and premature aging. In contrast to well-characterized cellular DNA damage checkpoint mechanisms, it remains poorly understood how DNA damage responses affect organismal development and maintain functionality of tissues when DNA damage gradually accumulates with aging. The distinct human disease outcomes of DNA repair defects become particularly apparent in syndromes caused by mutations in nucleotide excision repair (NER): While transcription-coupled (TC-) NER defects lead to developmental growth defects and premature ageing in Cockayne syndrome (CS), global-genome (GG-) NER mutations lead to highly skin cancer prone Xeroderma pigmentosum (XP). In C. elegans, defects in the highly conserved TC-NER branch lead to developmental arrest of somatic tissues, while GG-NER defects give rise to genome instability in proliferating germ cells. We have employed the nematode model to explore the distinct DNA damage responses in germ cells and somatic tissues. We identified a network of insulin-like growth factor signalling (IIS) genes that responds to DNA damage during C. elegans development and show that the FoxO transcription factor DAF-16 is activated in response to DNA damage. We demonstrate that DAF-16 alleviates DNA damage induced growth arrest through differential activation of downstream target genes that contrasts its established role in the starvation response, and even in the absence of DNA repair promotes developmental growth and enhances somatic tissue functionality. We propose that IIS mediates developmental DNA damage responses and that DAF-16 activity enables developmental progression amid persistent DNA lesions and promotes tissue maintenance through enhanced tolerance of DNA damage that accumulates with aging.

323A. Gut-specific regulation of transcription in long-lived *daf-2* mutants. Lamia M. Boukhibar, Zoja Nagurnaja, Nuria Vergara Irigaray, **Eugene F. Schuster**. Inst Healthy Ageing, Research Dept GEE, UCL, London, United Kingdom.

In *C. elegans*, mutations of the *daf-2* insulin-like receptor can result in increased adult lifespan by reducing signalling in the insulin/insulin-like growth factor signalling (IIS) pathway. The lifespan extension is dependent on the DAF-16 FoxO forkhead transcription factor. DAF-16 is expressed in many tissues and expression in the intestine has been shown to be important for the regulation of longevity. The intestine is one of the major organs in the worm and makes up to 1/3 of the somatic mass of the organism. It plays key roles beyond digestion and metabolism and is the major lipid storage site in the worm and also the major site involved in the response to environmental stress, such as toxics and pathogens. To better understand the role of the intestine in lifespan extension of daf-2 mutants we extracted intestine specific transcripts by mRNA tagging. Transgenic worms expressed a FLAG-tagged version of the poly-A binding protein in the intestine from the ges-1 promoter. mRNA was crosslinked to bound proteins and the gut specific transcripts were isolated by immunoprecipitation with ANTI-FLAG magnetic beads. RNA-Seq was used to identify differential expressed gut specific transcripts from N2 and daf-2 strains. Analysis of revealed more than 100 genes that are differentially expressed in the gut including several genes known to be up-regulated in the gut of daf-2 mutants in a DAF-16 dependent manner (e.g. sod-3, lys-7 and mtl-1) and the differentially expressed genes were used to build a better understanding of the role of somatic maintenance, antimicrobial response and energy storage and metabolism in the intestine of long-lived daf-2 mutants.

324B. Environmental Stress Resistance in *exl-1* and *dbl-1* Mutants of *C. elegans*. **Yakov Shaulov**[1], Tasmia Hoque[2], Jun Liang Rice[2], Cathy Savage-Dunn[1]. 1) Biology, CUNY Queens College, Flushing, NY; 2) Borough Manhattan Community College, NY.

Environmental stress leads to applicable cellular changes in all biological systems. Our primary objective for this study is to determine whether the *C. elegans* intracellular chloride channel protein EXL-1 plays a role in stress resistance or longevity. In humans, CLIC4 (*exl-1* homologue) is essential in maintaining membrane potential in organelles. A 2005 study has suggested targeting CLIC4 as a novel molecular target for cancer therapy. Determining how various stresses affect longevity, understanding the role of *exl-1* will bring pertinent knowledge to cancer cell therapy. Our secondary objective is to

determine if there are possible interactions of EXL-1 with the TGFb pathway. We performed lifespan assay tests using standard protocols. Three out of four independent trials testing longevity at 20° C showed *exl-1* mutants had no significant difference from control (wild type) N2. Although the *dbl-1* and *dbl-1;exl-1* strains lived shorter (3-4 days) than N2 and *exl-1*, they did not greatly differ from each other. We next determined resistance to heat stress by placing the worms in 32° C environment. Two out of three trials showed *exl-1* mutants died faster than N2 (*dbl-1* strains not tested). Our current data leads us to believe that *exl-1* plays an important role in stress resistance, but not in longevity under favorable conditions. Future experiments will include repeat testing of these four strains under stress, shedding light if EXL-1 interacts with the TGFb system.

325C. Mitoflash is an Early Predictor of Lifespan in *C. elegans*. **Enzhi Shen[1,2]**, Chunqing Song[1,2], Yuan Lin[3], Wenhong Zhang[2], Peifang Su[4], Wenyuan Liu[2], Pan Zhang[2], Jiejia Xu[3], Na Lin[3], Cheng Zhan[2], Xianhua Wang[3], Yu Shyr[4], Heping Cheng[3], Mengqiu Dong[1,2]. 1) China Agricultural University, Beijing, China; 2) National Institute of Biological Sciences, Beijing, China; 3) Peking University, Beijing, China; 4) Vanderbilt Center for Quantitative Sciences, Vanderbilt University, Nashville, USA.

Mitochondria are pivotal to bioenergetics, free radical metabolism and cell death. Mitochondria take a central position in many prominent aging theories. To better understand the physiology of mitochondria during the process of aging in *C.elegans*, and in the hope of identifying an early biomarker of aging, we used in vivo fluorescence imaging to characterize a physiological phenomenon directly related to superoxide production inside mitochondria. This phenomenon, called mitoflash, is an intermittent, quantal mitochondrial activity that is linked to energy metabolism and free radical production. We have found that mitoflash can serve as a novel predictor of lifespan in *C.elegans*. The mitoflash activity in wild-type animals was highly sensitive to changes in metabolic states and oxidative stress, and displayed a robust early peak on adult day 3 and a later one on day 9. Surprisingly, genetic mutations in diverse signaling pathways inversely modified lifespan and the mitoflash activity on adult day 3 (R^{2}**3** 0.40, $p<0.001$, cubic spline or linear regression; Spearman's correlation=-0.65). Drug treatments that extended (or shortened) lifespan tended to reduce (or enhance) the day-3 mitoflash (R^{2}**3** 0.40, $p< 0.005$, cubic spline or linear regression; Spearman's correlation=-0.64). Furthermore, the day-3 mitoflash activity also negatively correlated with the lifespan of individual wildtype worms ($R^2=0.28$, $p<0.001$, linear regression).This is the first time that an early biomarker has been found that can predict the average lifespans of different mutants of the same species, the average population lifespans of animals of the same genetic makeup but under different environmental conditions, and lifespans of individual animals from an isogenic population in the same environment. The unexpected power of mitoflash as an early lifespan predictor suggests that the mitochondrial status, metabolic or oxidative or both, is intimately linked to the process of aging.

326A. The role of autophagy in lipid and mitochondrial homeostasis. **Melissa J Silvestini[1,2]**, Hannah Hong[2], Alicia Meléndez[2]. 1) The Graduate Center, City University of New York, New York; 2) Queens College, Department of Biology, Flushing, New York.

Autophagy is a highly conserved mechanism that maintains cellular homeostasis by recycling long-lived proteins and damaged organelles. It is characterized by the de novo formation of a double-membrane autophagosome that sequesters cytoplasmic material to be degraded by the lysosomal machinery. Previous work from our lab and others, have shown that autophagy is induced and required for lifespan extension in several longevity models including germline-less *glp-1/Notch*, dietary restricted *eat-2*, and *daf-2* insulin/IGF-1 receptor mutants. Recently, we have shown that autophagy is required to maintain normal lipid levels during development in wild-type animals. Additionally, autophagy is required for the increase in lipid accumulation observed in long-lived *glp-1/Notch* loss of function and *daf-2* insulin receptor mutants. Even though autophagy is required for the longevity phenotype and for the proper accumulation of lipids in long-lived animals, the components degraded by autophagy to prolong lifespan are not known. One possibility is that lipophagy is required, as an increase in lipase activity observed in *glp-1/Notch* animals is also autophagy dependent. Interestingly, we have found that genetically reducing autophagy gene function also disrupts mitochondrial homeostasis in body wall muscle cells in *C. elegans* and results in defects similar to the loss of *pink-1*, a cytosolic protein kinase responsible for inducing the selective degradation of mitochondria (mitophagy). Since lipid droplets and mitochondria are both required for energy production and storage, and their homeostasis is autophagy dependent, we propose that this is part of the mechanism by which autophagy is required for longevity. Our lab is interested in determining the connection between lipid turnover or storage, mitochondria and the different longevity pathways.

327B. Assessing drug induced mitochondrial toxicity using *C. elegans*. **Reuben L Smith[1]**, Richard de Boer[1], Winnok H de Vos[2], Erik M Manders[3], Stanley Brul[1], Hans van der Spek[1]. 1) Swammerdam Institute for Life Science, MBMFS, University of Amsterdam, The Netherlands; 2) Cell Systems and Imaging Research Group and NB-Photonics, Department of Molecular Biotechnology, Ghent University, Belgium; 3) van Leeuwenhoek Center for Advanced Microscopy, University of Amsterdam, The Netherlands.

Objective: Mitochondrial dysfunction is a common consequence of therapeutic drug use, especially with drugs used to treat HIV infected individuals. Most research has been done in patient- or cell culture studies, which pose limitations on the experiments that can be performed. Progress in this field is highly dependent on the development of a robust and accurate model system. **Methods:** To address fundamental questions concerning drug-induced mitochondrial dysfunction, *Caenorhabditis elegans* was applied as a model organism. Mitochondrial DNA (mtDNA) copy number was measured using quantitative Real Time PCR. Oxygen consumption rates were measured using a Neofox fiber optic oxygen sensor. Reactive oxygen species (ROS) production was quantified in a self-developed novel strain using a Biotek Synergy Mx plate reader. Mitochondrial morphology in body wall muscle cells was visualized in transgenic *glo-1(zu391) X* animals using mito::GFP expressed from the *myo-3* promoter. Image analysis was performed using ImageJ freeware. **Results:** We show a concentration dependent decline in mtDNA copies when cultured in the presence of various anti-retroviral drugs. Moreover, exposure to these drugs resulted in altered aerobic respiration, increased ROS production and/or a quantifiable disruption of the mitochondrial morphological network. The severity of the observed effects is drug-specific and concentration dependent. Interestingly, the observed biochemical and morphological effects are not necessarily provoked by the same compounds and some of the effects can be alleviated by providing supplementation with anti-oxidant compounds. **Conclusion:** Our observed effects in *C. elegans* closely resemble the side-effects of drugs in patients on anti-retroviral therapy. We conclude that *C. elegans* is a highly suitable model organism to study drug induced mitochondrial dysfunction.

328C. Effect of chronic treatment with amphetamine in an experimental model using Caenorhabditis elegans. Tássia Fontana Lehmen[1], Bruna Puntel[1], Priscila Gubert[1], Roselei Fachinetto[2], **Félix A A Soares[1]**. 1) Dep of Chemistry, UFSM, Santa Maria, RS, Brazil; 2) Dep of Pharmacology, UFSM, Santa Maria, RS, Brazil.

Caenorhabditis elegans, a free-living soil nematode, has already been explored as a valuable bioindicator, since it is one of the best-characterized animal models at the genetic, physiological, molecular, and developmental levels. It has also been proved that C. elegans serves as an excellent candidate for studying the development and functions of nervous system and neurotoxicology. The worms have well characterized dopaminergic system, as well as eight neurons of this system located along the body. Amphetamine (AMPH) is a stimulant drug of the central nervous system that acts broadly on various behaviors. Whereas multiple lines of evidence implicate dopamine receptors in the mechanism of action of AMPH and other abused psychostimulants, the degree to which different receptors support AMPH-induced behaviors has not been completely elucidated. Because of this we decided to investigate the effects of amphetamine on the behavior of nematodes.L1 larva stage worms of wild type strain were treated with AMPH 1mM or water (control group) in agar plates until adulthood. We analyzed pharyngeal pumping rate, defecation cycle length, body bends and oviposition and egg production rates in adult worms using a microscope. Treatment with AMPH 1 mM promoted a reduction in the rates of pharyngeal pumping, oviposition and egg production. Our data demonstrates that the concentration of amphetamine tested can interfere the behaviors mentioned above without, however, changing the defecation cycle length and body bends, acting probably by different routes. However, more studies are needed to elucidate the different mechanisms of action that may be involved, using, for example, knockout strains to dopamine transporters and/or receptors.

329A. ROS and antioxidant interaction in *C. elegans*. **Paul Neal Stein**, Craig W. LaMunyon. Biological Sciences, Cal Poly Pomona, Pomona, CA.

Reactive oxygen species (ROS) are thought to be partially responsible for aging and senescence, but the mechanisms by which ROS participate in aging are not entirely clear. Antioxidants may neutralize harmful ROS, and they have been promoted as a means of therapy. In order to explore the relationship between ROS and antioxidants, we assessed protein damage, antioxidant linked ROS suppression and native antioxidant expression under various treatment conditions. We had earlier shown that worms harboring the *uaDf5* mitochondrial DNA deletion produced elevated hydrogen peroxide and experienced shorter lifespans. The lifespan deficit was reversed by treatment with the antioxidant EUK-134 but not by coenzyme Q10. Here, we tested both antioxidants on uaDf5 worms and worms treated with the ROS-inducing mitochondrial toxin paraquat. We detected no effect of the treatment on protein damage measured as the quenching of fluorescence of a Pmyo-3::GFP transcriptional fusion. We did find changes in hydrogen peroxide production, assayed using the fluorescent dye DCF. The expression of a Psod-3::GFP transcriptional fusion did not change with treatment, although changes in transcript abundance of a host of sod genes were detected by RT-PCR. Our combined results show that sod gene transcription responds to variation in ROS and treatment with EUK-134.

330B. A genetic screen for stress resistance combined with next-generation sequencing reveals new longevity candidates. **Nadia J. Storm**, Martin S. Denzel, Adam Antebi. Max Planck Institute, Biology of Ageing, Cologne, NRW, Germany.

Ageing is associated with the progressive loss of cellular homeostasis, including protein homeostasis, which comprises all processes involved in maintaining a functional proteome. The endoplasmic reticulum (ER) is the major site of protein synthesis for secreted and membrane proteins and thus essential for cellular function. Additionally, the ER is an important point of protein quality control. ER protein folding fidelity and capacity depend on N-glycosylation of nascent proteins, and regulation of these pathways have shown been to be involved in ageing and disease. We therefore hypothesized that improving protein quality control in the ER could result in enhanced health and lifespan. Using the drug tunicamycin (TM) to disrupt the synthesis of N-glycans, we performed a forward genetic screen, selecting mutants that were able to develop in the presence of normally lethal concentrations. We screened more than 200,000 haploid genomes and isolated 358 mutants that were TM resistant. Of these, 109 were long-lived, defined as a minimum 15% increase in median lifespan compared to WT animals, revealing a substantial enrichment of long-lived strains through this selection. Of the 109 long-lived mutants, 62 showed defects in the sensory cilia, as revealed by DiI staining, a phenotype previously associated with stress resistance and longevity. The high proportion of chemosensory deficient mutants suggests a close link between environmental sensing and cell-nonautonomous regulation of protein homeostasis in the ER. From the 47 long-lived mutants that showed no defects in the sensory cilia, we found 4 independent mutations in the gene glucosamine-fructose 6-phosphate aminotransferase (gfat-1), all leading to a gain-of-function mutation. These mutants showed increased lifespan, enhanced levels of autophagy and improved ER function measured by aggregation of proteins in the ER. GFAT-1 regulates the rate-limiting step of the hexosamine pathway that synthesizes precursors for N-glycans, suggesting a direct link between N-glycosylation and heath- and lifespan.

331C. Quantitative analysis of IR-induced effects on locomotion in *Caenorhabditis elegans*. **Michiyo Suzuki[1]**, Tetsuya Sakashita[1], Toshio Tsuji[2], Yuya Hattori[3], Yasuhiko Kobayashi[1]. 1) Microbeam Radiation Biology Gr., Japan Atomic Energy Agency, Gunma, Japan; 2) Dept. of System Cybernetics, Hiroshima University, Hiroshima, Japan; 3) Radiation Effect Analysis Gr., Japan Atomic Energy Agency, Ibaraki, Japan.

Background and purpose: *C. elegans* is a good in vivo model system to examine radiobiological effects. We have previously examined the effects of ionizing radiation (IR) on locomotion in *C. elegans* using 'body bends' (the number of bends counted in the anterior body region at 20-s intervals) [1] and reported an IR-induced reduction in motility [2],[3]. However, the degree of motility in each region of the body has not been established. In the present study, we employed a video-based analysis and investigated the IR-induced effects on locomotion in more detail. ***Irradiation and video-based analysis:*** Young adult wild-type *C. elegans* were placed on a NGM plate with a bacterial lawn (food) and irradiated with graded single doses (0-1.5 kGy) of ^{60}Co g-rays. Immediately after irradiation, animals were transferred to a NGM plate, either with or without food. The movements of five or more animals placed on each plate were video-recorded. The video images were analyzed off-line based on a previously published method [4]. Briefly, after binarization and denoising, the body line was skeletonized and evenly divided into 12 segments; X- and Y-coordinates of each point on the body were subsequently acquired. To evaluate the motility of each point on the body, the moving distance of each of 13 points over a 5 s period was calculated using the X- and Y-coordinates. In addition, we introduced a novel standard, namely the straight distance from head to tail, to evaluate the body form. ***Results:*** Under the -food condition, the moving distance of irradiated animals was reduced in a dose-dependent manner at each point on the body, and there was no difference between the effects on each region of the body. The dose-dependent reduction in locomotion was also observed in animals under the +food

condition. Furthermore, we evaluated body form and found that IR-induced quantitative changes in body form. ***References:*** [1] Sawin, E.R., *et al.* (2000) Neuron 26, [2] Sakashita, T., *et al*. (2008) J. Radiat. Res. 49, [3] Suzuki, M., *et al*. (2009) J. Radiat. Res. 50, [4] Hattori, T., *et al*. (2012) Neural Comput. 24.

332A. Mitochondrial dynamics in response to oxygen deprivation. **Alexandra L. Tabakin**, Piya Ghose, Eun Chan Park, Nathaly Salazar-Vasquez, Christopher Rongo. The Waksman Institute/Department of Genetics, Rutgers University, Piscataway, NJ.

Mitochondria play a critical role in the oxygen-dependent generation of cellular energy. Many aerobic organisms encounter oxygen-deprived environments and thus must have adaptive mechanisms to survive such stress. How mitochondria are regulated in the face of oxygen deprivation stress is not well understood. Here we examine mitochondrial dynamics, including organelle fission and fusion, in C. elegans neurons in response to anoxia. We find that anoxia triggers mitochondrial fission, and that DRP-1, a key component of the mitochondrial fission machinery, is required for this fission. Subsequent reoxygenation results in mitochondrial refusion. We observed that mutations in egl-9, a prolyl hydroxylase oxygen sensor, resulted in mitochondrial hyperfusion (fusion beyond the normal levels present prior to anoxia) upon reoxygenation. This hyperfusion phenotype requires the hypoxia inducible factor-1 (HIF-1), the canonical substrate of EGL-9. Hyperfusion also requires STL-1, a poorly characterized mitochondrial scaffolding protein. Finally, we found that anoxia triggers a behavioral arrest (suspended animation), and that normal neurological behavior was restored upon reoxygenation. Mutants for egl-9 demonstrated a rapid recovery from suspended animation compared to wild type; this rapid recovery also required HIF-1 and STL-1. Our results suggest that C. elegans neurons modulate mitochondrial fission and fusion in response to stress to help organisms adapt to low oxygen environments and to modify neurological function; moreover, this modulation is regulated and rapidly reversible.

333B. Quiescence of entomo-phoretic nematode *Caenorhabditis japonica*. **R. Tanaka**[1], Y. Hirooka[1], T. Kikuchi[2], N. Kanzaki[1]. 1) FFPRI; 2) Miyazaki university.

Entomo-phoretic nematodes are not parasitic to insects but use insects for their transportation. *Caenorhabditis japonica*, a bacteria-feeding nematode, has a species-specific phoresy with a shield bug, *Parastrachia japonensis*, and its lifecycle is synchronized to the bug's life. The active dauer larvae (DL) show intensive host-seeking, high sensitivity to oxidative stress and less than 15 days of longevity without attaching the bug. On the other hand, quiescent DL on the bug survive more than 11 months. Thus, the quiescence associated with the bug seems an essential factor for nematode survivability. In the present study, survivabilities of quiescent and active DL were compared under several different conditions to examine the involvement of the bugs in nematode's longevity. Then transcripts and proteins were analyzed to compare gene expression between quiescent and active DL. The quiescent DL on the bug and active DL were kept in a container with 85% (dehydrated condition) or 97% (lightly dehydrated condition which immobilize the DL) of relative humidity (RH). The most active DL died in one week because of desiccation (85% RH) or fungal infection (97% RH). On the other hands, quiescent DL on the bug showed significantly higher survival rate under the same conditions. Further, the survivability of surface-sterilized active DL kept in 97% RH was almost the same as quiescent DL. Therefore, the bugs are likely to work as the shelter from dehydration, and are also providing anti-microbe activity to DL. The expressed genes and proteins also differed between quiescent and active DL. Expression of genes and proteins involved in several stress resistance, metabolic regulation and cuticle formation were significantly higher in quiescent DL. On the other hand, expression of genes and proteins involved in several metabolic related (activity regulation) were significantly higher in active DL. Our results suggest *C. japonica* DL use their host bug not only for transportation, but also as shelter from environmental stresses and microbe infection. Further, the quiescent stage-specific gene expression allows the extraordinary longevity of this stage.

334C. Fructose accelerates neuronal aging in *C. elegans*. **Marton Toth**, Leena Shah, Monica Driscoll. Department of Molecular Biology & Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ.

Dietary sugar intake has significantly increased in the human population as monosaccharides like glucose and fructose are used in large quantities to sweeten food products. However, there are profound differences between the physiological effects of glucose and fructose. In higher organisms, glucose can be metabolized in every cell and most of it is converted into inert glycogen until broken down in glycolysis to cover cellular energy demand. In contrast, fructose can only be metabolized in the liver and it is converted into lipid droplets and VLDLs. High fructose turnover may cause liver and muscle insulin resistance. Using morphological assays to score aging of individual neurons, we found that excess glucose can be neuroprotective in *C. elegans*. However, even modest additional fructose intake can accelerate the onset of morphological aging features in neurons. We will discuss genetic requirements for fructose toxicity. Our results call attention to the differential effects of dietary sugars and provide clear evidence that underscores concerns about the use of fructose as a food source.

335A. Calcium Exerts Critical Functions in Adult Neuronal Maintenance. **Marton Toth**, Ivana Ganihong, Khushboo Patel, Kelli Gaul, Camisha DuBose, Steven Kim, Saurabh Patel, Wenying Zhang, Jian Xue, Monica Driscoll. Department of Molecular Biology & Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ.

Human brain aging and cognitive decline are associated with synaptic changes and aberrant sprouting, rather than neuronal death. We have had a long-term interest in the genes and environmental factors that influence healthy tissue aging, using the facile experimental model *C. elegans* to decipher the basic biology of healthspan (the period of healthy maintenance prior to detectable functional or structural decline). Our initial study of how *C. elegans* tissues age indicated that, like in human brain aging, neither neuronal death nor axon degeneration is a significant feature of the aging nematode nervous system. However, specific *C. elegans* neuronal types can exhibit dramatic morphological changes (novel branching from processes, new outgrowth from cell bodies, wavy appearance) that increase in frequency with age. We have also shown that synaptic structures deteriorate in the aging *C. elegans* nervous system. Our findings support that *C. elegans* is a relevant model in which to study basic questions of nervous system aging, with anticipated findings likely informative on conserved mechanisms. The calcium-binding EF Hand motif can be found in a large number of protein families. This protein domain confers calcium regulation of proteins functions that include cytoplasmic calcium buffering, signal transduction, and muscle contraction. In a recent screen for genes that can influence the maintenance of adult neuronal integrity we discovered several previously uncharacterized EF Hand motif proteins with either neuroprotective or neurotoxic roles. RNAi knockdown of EF hand gene expression could accelerate or decrease neuronal aging up to 8 times, measured by our recently established branching and outgrowth markers of neuronal aging. We propose that EF Hand motif proteins and calcium

play a substantial role in the maintenance of neuronal integrity in aging neurons.

336B. Regulation of organismal proteostasis by trans-cellular chaperone signaling. **Patricija van Oosten-Hawle**, Robert S. Porter, Richard I. Morimoto. Molecular Biosciences, Northwestern University, Evanston, IL.

A major challenge for metazoans is to ensure that different tissues each expressing distinctive proteomes are, nevertheless, well protected at an organismal level from proteotoxic stress. We have examined this and show that expression of endogenous metastable protein sensors in muscle cells induces a systemic stress response throughout multiple tissues of *C. elegans*. Suppression of misfolding in muscle cells can be achieved not only by enhanced expression of *HSP90* (DAF-21) in muscle cells, but as effective by elevated expression of *HSP90* in intestine or neuronal cells. This cell-non-autonomous control of *HSP90* expression relies upon transcriptional feedback between somatic tissues that is regulated by the FoxA transcription factor PHA-4. This trans-cellular chaperone signaling response maintains organismal proteostasis when challenged by a local tissue imbalance in folding and provides the basis for a novel form of organismal stress sensing surveillance.

337C. Protein homeostasis dysregulation drives aberrant morphology of aging mechanosensory neurons. **Elena Vayndorf[1]**, Courtney Nichols[1], Cyrena Parker[1], Skyler Hunter[1], Marton Toth[2], J. Alex Parker[3], Christian Neri[3], Monica Driscoll[2], Barbara Taylor[1]. 1) Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK; 2) Department of Molecular Biology and Biochemistry, Rutgers, SUNJ, Piscataway, NJ; 3) Laboratory of Neuronal Cell Biology and Pathology, INSERM, Paris, France.

In both *C. elegans* and mammals, the aging nervous system is characterized by decreased synaptic activity, deteriorating short-term and long-term memory, and altered neuronal morphology. We sought to elucidate the functional consequences of altered neuronal morphology by focusing on protein homeostasis in individual neurons. We examined the effects of disrupting proteostasis on the integrity of neuronal cytoarchitecture using a transgenic model with an excessively high neuronal protein load. We found that animals expressing the first 57 amino acids of the human huntingtin gene and an expanded polyglutamine CAG tract (128Q) in mechanosensory neurons accumulate significantly more neuronal aberrations and more protein aggregates, and have a significantly greater decline in function with age, compared to animals that express the non-toxic (19Q) number of repeats, or those that express no repeats. We identified specific morphological alterations, in particular extreme outgrowths in the soma of ALM mechanosensory neurons, as well as a wavy phenotype in processes of PLM neurons, as the major aberrant morphological types in this transgenic background. Our RNAi studies suggest that targeting genes expressed in organelles associated with the maintenance of proteostasis, in particular the proteosome, lysosome and endoplasmic reticulum, alters neuronal morphology and accelerates aging in wild-type animals. Taken together, these results suggest that protein homeostasis is critical for maintaining neuronal integrity and that disrupted proteostasis contributes to morphological abnormalities that increase in frequency with age.

338A. Genome-wide study of stress-responsive microRNA and mRNA transcriptomes in C. elegans. Maria C. Ow, **Isana Veksler-Lublinsky**, Victor Ambros. Molecular Medicine, Univ Mass Medical Sch, Worcester, MA.

All organisms must cope with stress and have evolved various means to respond to detrimental changes in their environment. Because most of the pathways involved in stress response are conserved throughout evolution, studying stress response in C. elegans is likely to result in crucial insights in understanding the pathology and treatment of human disorders. MicroRNAs (miRNAs) are an abundant class of small non-coding RNAs that regulate gene expression by imperfectly base-pairing to the 3'UTR of their target mRNAs. MiRNAs are intimately involved in broad aspects of animal development. Their misregulation can result in the onset of a number of pathophysiological conditions, including heart disease, diabetes, and cancer. Given the prominent role of miRNAs in nearly all aspects of biology, we predict that they also play a role in stress response. In this study we employ genome-wide methods, including small RNA deep sequencing and mRNA transcript microarrays, to study the response of C. elegans to various stresses including oxidative stress, heat stress, and exposure to bacterial pathogens. We employ bioinformatics to determine stress-responsive miRNAs and mRNAs and identify stress-mediated gene regulatory patterns. Our analyses suggest the existence of regulatory patterns common to all stresses as well as stress-specific sub-systems.

339B. Investigating progranulin in aging and neurodegeneration. **Julie Vériène**, J. Alex Parker. CRCHUM, Montreal, Canada.

Progranulin (PGRN) is a growth factor with important roles in development, proliferation, regeneration and immunity, while haploinsufficiency of PGRN is linked to frontotemporal dementia. Recent progress in genetics has shown that genes causative for a particular neurodegenerative disease may in fact influence other neurologic disorders. We are interested whether or not PGRN is a modifier of other forms of late-onset neurodegeneration. To investigate this possibility we turned to C. elegans and the PGRN orthologue PGRN-1. We are using loss-of-function mutations of pgrn-1 to determine its role in polyglutamine, TAR DNA-binding protein 43 (TDP-43) and fused-in-sarcoma (FUS) neuronal toxicity. We are also investigating the function of pgrn-1 in aging and the cellular stress response. PGRN-1 is a secreted protein but very little is known about PGRN-1 receptors. Furthermore the two known PGRN-1 receptor in mammals (Sortilin and TNF-a Receptor) do not have strong orthologues in C. elegans. We will identify PGRN-1 protein interactors using proteomic analysis. Our work will help us better understand the contribution of PGRN to age-dependent neurodegeneration. An update of our findings will be presented.

340C. Age-dependent Proteome Turnover Changes in C. elegans. **K. Vukoti[1]**, X. Yu[2], J. Feng[3], A. Hsu[2], M. Miyagi[1]. 1) Center for Proteomics and Bioinformatics, Case Western Reserve University, Cleveland, OH; 2) Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 3) Department of Pharmacology, Case Western Reserve University, Cleveland, OH.

C. elegans is a useful model organism for studying the age-dependent turnover of individual proteins as the worm has short lifespan. Isotope Labeling with Amino Acids in Cell Culture (SILAC) is an approach used for in vivo incorporation of a labeled amino acid(s) into proteins. We utilized the SILAC proteomic approach to monitor the proteome turnover (protein turnover on the scale of the proteome) in the C. elegans during its lifespan. E. Coli AT713 was cultured in the minimal media to incorporate the 12C- or 13C-lysine into it, and the C. elegans was fed with the 13C-E. coli for two generations and on the first day of the adult life, they were transferred to the plates containing 12C-E. coli. The worm samples were harvested every other day up to day 16,

digested by Lys-C, and analyzed by LC-MS/MS. The mass spectrometry data clearly showed that the unlabeled peptide peaks increase relative to the labeled peptide peak as the worms age, thus monitoring the synthesis of proteins. We found that the synthesis rates of proteins involved in protein folding, molecular transport, and signal transduction are much faster than those for proteins involved in cellular assembly and organization. Interestingly, the data also revealed that there are proteins whose turnover rates increase significantly in old worms, suggesting that these proteins have important roles in the aging process. This study gives us a glimpse as to what kinds of proteins are synthesized at which stages of an organism's life and such information may be useful to better understand the physiological changes during aging. We are currently performing the lifespan assay on some of the genes whose turnover rates were found to be increased after day 11, and the results will also be discussed.

341A. Direct Regulation of HIF-1 by the Metabolic Network in C. elegans. Robert J. Mishur, Maruf Khan, **Haley M. Willhelm**, Shane L. Rea. The Barshop Institute for Longevity and Aging Studies, & Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX 78240, USA.

A number of pro-longevity mechanisms have been described in the nematode Caenorhabditis elegans. One such mechanism involves disruption of mitochondrial respiration, and long-lived animals that fall under this pathway are collectively termed Mit mutants. Mit mutants display delayed larval development, reduced adult size, and prolonged adult life span. We recently discovered that Mit mutants generate a common metabolic footprint enriched in a-hydroxyacids and a-ketoacids. These compounds are structurally related to a-ketoglutarate, an essential co-substrate for a broad class of enzymes called the a-ketoglutarate-dependent hydroxylases, suggesting they may be biologically relevant. One potential target is the a-ketoglutarate-dependent prolyl-hydroxylase EGL-9, which negatively regulates hypoxia factor-1 (HIF-1), a transcription factor necessary for the lifespan extension of Mit mutants. Here we show that treatment of wild type C. elegans with metabolites found in the Mit metabolome indeed causes stabilization of HIF-1. In addition we observed a dose-dependent delay of growth and under some conditions extension of lifespan. Provision of these same compounds to mouse fibroblasts also resulted in increased amounts of transcriptionally competent HIF. We discuss our findings in light of recent studies that show a-ketoglutarate-dependent hydroxylases and their modulation by a-hydroxyacids are important players in the etiology of cancer and obesity and now also lifespan.

342B. Intermittent hyperoxia-induced hormesis decreases aerobic respiration via ins/IGF-1 and p53/CEP-1 signalings in *C. elegans*. **Sumino Yanase**[1,3], Tetsuji Shoyama[2], Hitoshi Suda[2], Naoaki Ishii[3]. 1) School of Sports & Health Science, Daito Bunka University, Higashi-matsuyama, Saitama, Japan; 2) School of High-Technology for Human Welfare, Tokai University, Numazu, Shizuoka, Japan; 3) School of Medicine, Tokai University, Isehara, Kanagawa, Japan.

When a PI3-kinase mutant *age-1*, which has a long lifespan in *C. elegans*, was treated with intermittent hyperoxia, it extends the lifespan by the hormetic effect. We have previously reported that the hormetic lifespan extension is related to not only the inducing of antioxidant genes but also the decreasing of mitochondrial superoxide radical level via an ins/IGF-1 signaling pathway. In general, strains reduced the ins/IGF-1 signaling, such as *age-1* and *daf-2*, have an elevated stress resistance, lower respiration rate, and an increased ATP concentration. It is suggested that intracellular metabolic shift to glycolysis from mitochondrial aerobic respiration occurs in the individual worm of the mutants compared to wild-type animals.

We measured a change in the oxygen consumption level of the long-lived *age-1* mutant in the hyperoxia-induced hormesis. Oxygen consumption level in *age-1* animals significantly decreased after the intermittent hyperoxia exposure. In addition, expression of an antioxidant *gst-4* gene, which is regulated by a homologue CEP-1 in *C. elegans* of a mammalian tumor suppressor p53, decreased in the hormetic *age-1* mutant. Therefore, activity of p53/CEP-1, which is associated with the regulation of intracellular metabolic balance, was nontranscriptionally decreased because the induction of the p53/CEP-1 genes expresseion was not observed in the hormetic *age-1* mutant..

Here, we described that the hyperoxia-induced hormetic lifespan extension in *age-1* animals may be dependent on the metabolic shift to glycolysis from mitochondrial aerobic respiration via not only the ins/IGF-1 but also p53/CEP-1 signalings.

343C. Genetic mechanism of carotenoid nanoparticle-induced health promotion effects in *Caenorhabditis elegans*. **Ji Suk You**[1], Yeong Hun Kim[3], Sang Ho Koo[3], Shin Sik Choi[1,2]. 1) Deptartment of Energy and Biotechnology; 2) Deptartment of Food and Nutrition; 3) Deptartment of Chemistry, Myongji University, Yongin, Gyeonggido 449-728, South Korea.

Carotenoids are natural pigments abundant in vegetables and fruits, which are consumed as various forms including foods, beverages and nutraceuticals for the prevention of aging-related diseases and promotion of human health. However, carotenoids show a low bioavailability and quickly lose their activities by oxygen, heat, and light. In this study, we investigated a genetic mechanism of carotenoids-induced health promotion effects in *Caenorhabditis elegans* using novel carotenoid nanoparticles with functional groups onto the isoprene backbone. In wild type adult worms, carotenoid nanoparticles improved body growth and brood size resulting in significant reduction of reactive oxygen species (ROS) level. In order to elucidate genetic mechanism of carotenoids, the genes required for larger growth and reproduction were screened using mutations in fat metabolism, gap junction, and oxidative stress-related regulators.

344A. *C. elegans* SIRT6/7 homolog SIR-2.4 promotes stress response and longevity via distinct mechanisms. Wei-Chung Chiang[1], **Xiaokun Yu**[2], Daniel X. Tishkoff[3], Bo Yang[3], Tsui-Ting Ching[2], David B. Lombard[3], Ao-Lin Hsu[1,2]. 1) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Department of Internal Medicine, Division of Geriatric Medicine, University of Michigan, Ann Arbor, MI; 3) Department of Pathology, University of Michigan, Ann Arbor, MI.

Sirtuins comprise a unique class of NAD+-dependent enzymes that modify multiple protein substrates to execute diverse biological functions. The sirtuin family is evolutionarily conserved from bacteria to eukaryotes and has been shown to regulate a broad range of processes, including transcription, metabolism, cancer and neuron-degeneration. Here we report that *Caenorhabditis elegans* sirtuin SIR-2.4, a homolog of mammalian SIRT6 and SIRT7 proteins, promotes longevity under dietary restriction, and also promotes re-localization of DAF-16 under various stress conditions, including heat shock, oxidative insult, and proteotoxity. Upon stress, FoxO transcription factor DAF-16 moves to the nucleus to regulate gene expression to ensure organismal survival. We found that SIR-2.4 negatively modulates DAF-16 acetylation, which is associated with the nuclear localization of DAF-16. However, this

modulation does not depend on the catalytic activity of SIR-2.4, suggesting the potential involvement of other deacetylase or acetyltransferase. And we confirmed that SIR-2.4 functions antagonistically with acetyltransferase CBP-1 to modulate DAF-16 acetylation. By contrast, SIR-2.4 is largely dispensable for DAF-16 nuclear activation in response to reduced insulin/IGF-1-like signaling, which is known to influence longevity in worms. Alterations in the expression levels of SIR-2.4 do not appear to influence longevity in the wild type background. Interestingly, we found that SIR-2.4 is required for the longer lifespan of dietary restriction, suggesting that SIR-2.4 may regulate stress response and longevity via distinct mechanisms.

345B. The *Rosmarinus officinalis* extract protects against oxidative stress and increase *C. elegans* lifespan. **D. C. Zamberlan**[1], G. P. Amaral[1], S. T. Stefanello[1], R. L. Puntel[2], F. A. A. Soares[1]. 1) UFSM, Santa Maria, RS, Brazil; 2) UNIPAMPA, Uruguaiana, RS, Brazil.

Oxidative stress has been associated to many genetic and acquired disorders and ageing processes. The *Rosmarinus officinalis* L. is known for it uses in food recipes, but it has also gaining interest by its pharmacological properties. The ethanolic extract of *R. officinalis* L. (eeRo) exhibited significant antioxidant, vasodilator and antiinflammatory properties. The aim of this study was to analysis the possible protective propriety of eeRo in the nematodeo *C. elegans*. The *Rosmarinus officinalis* leaves were subjected to an alcoholic extraction. HPLC fingerprinting of eeRo revealed the presence of the chlorogenic acid (12.2 mg/g), caffeic acid (7.63 mg/g), rutin (3.07mg/g), rosmarinic acid (38.5 mg/g), quercetin (5.10mg/g), kaempferol (2.53 mg/kg), and carnosic acid (26.4 mg/g). The worms were treated with the extract (1, 2.5 or 5 mg/ml) added to de plate and kept at 20ºC. The wildtype strain N2 survival was analyzed under oxidative stress and heat stress. The transgenic strain TK22, wich is oxygen sensitive and has a shorter lifespan compared to the wild type was also used. The effects of eeRo on TK22 lifespan and reactive oxygen species (ROS) production were analysed. In lifespan assay the worms were scored as dead or alive by touch evoked movement every two days until the last worm dies. The ROS production was measured by CM-H2DCFDA. The extract in all concentration was able to decrease the ROS production in N2 and TK22 worms with 2.5 mg/ml being the most efficient. The treatment with eeRo 2.5 mg/ml also protected the worms against oxidative and heat stress increasing the worm survival by 37% and 27.8% respectively. Moreover, the worms treated with eeRo 2.5 and 5 mg/ml showed an increase in the mean and total lifespan of TK22 mutant strain when compared with untreated controls. The results demonstrate that the eeRo has antioxidant activity in vivo, inducing decreased of the ROS, increase of stress surviving, besides provides thermotolerance. However, the exact mechanism by which it exerts its protection remains unclear.

346C. Neutral cholesterol ester hydrolase 1 is protective against a-synuclein-induced toxicity in *C. elegans*. **S. Zhang**, K. A. Caldwell, G. A. Caldwell. Biological Sciences, The University of Alabama, Tuscaloosa, AL.

Aging is the most identified risk factor for Parkinson's disease (PD). In *C. elegans*, we model a cellular aspect of PD by overexpressing a-synuclein (a-syn), a protein that forms inclusions in the brains of PD patients. Nematodes overexpressing a-syn::GFP in the body wall muscles exhibit age-dependent protein aggregates. We can greatly reduce these a-syn-induced inclusions by introducing a mutation in the worm insulin-like signaling receptor gene, *daf-2*. To identify components in the *daf-2* pathway relevant to a-syn accumulation, we performed an RNAi screen for genes that, when knocked down, resulted in enhanced a-syn misfolding in *daf-2* mutants. In total, 60 candidates were identified; one of these was an open reading frame Y43F8A.3. We were curious to know if the corresponding gene product would also modulate a-syn toxicity in a neuronal model of PD. *C. elegans* expressing a-syn in the dopaminergic (DA) neurons of the nematode exhibit age- and dose-dependent neurodegeneration. DA expression of Y43F8A.3 rescues a-syn-induced neurodegeneration. Y43F8A.3 is predicted to encode an ortholog of human neutral cholesterol ester hydrolase 1 (NCEH1). In general, cholesterol ester hydrolase can convert esterified cholesterol to free cholesterol and fatty acids. Considering that neuroprotection by Y43F8A.3 may be associated with a change of cholesterol levels, we further hypothesize that genetic manipulation of a cholesterol regulatory transcriptional factor will result in similar effect on a-syn inclusions. SBP-1 is the worm homolog of human SREBP1 (Sterol Regulatory Element Binding Protein 1). SREBP1 is the transcription factor regulating targets involved in cholesterol homeostasis, such as lipid synthesis and cellular trafficking genes. When knocked down, *sbp-1* also enhanced a-syn toxicity in the DA neuron PD model. Additionally, varying concentrations of cholesterol in the medium have different effects on a-syn toxicity on DA neurons. Lastly, we find that knockdown of genes involved in cholesterol trafficking also enhanced a-syn toxicity on DA neurons. Collectively, cholesterol levels may have an impact on a-syn toxicity in *C. elegans* DA neurons, thus representing a putative metabolic effector of neurodegeneration.

347A. Identification of genes that regulate the ribotoxic stress response in Caenorhabditis elegans. Yan Qi[1], **Xinrui Zhang**[1], Natalia Kirienko[1], Peter Breen[1], Holli Rowedder[1], Gary Ruvkun[1,2]. 1) Department of Molecular Biology, Mass General Hospital , Boston, MA; 2) Harvard Medical School, Boston, MA.

A wide range of bacteria and fungi produce potent xenobiotics that inhibit the growth of other organisms in ecological conflict. In defense, the target organisms have evolved extensive countermeasures to detect and detoxify the foreign toxins, as well as to repair the damage caused by the insults. The ribosome represents a common target of many natural toxins of diverse chemical nature. For example, a small-molecule drug hygromycin produced by the bacterium *Streptomyces hygroscopicus* and a protein toxin exotoxin A produced by the human bacterial pathogen *Pseudomonas aeruginosa* can cause ribotoxic stress in *C. elegans* by inhibiting the translation elongation step of the worm ribosome. Using hygromycin-induced developmental arrest as readout, a genome-wide RNAi screen was carried out to identify genes that regulate the ribotoxic stress response. We show that many hits from this screen are also involved in defense against *Pseudomonas* infection in worms. Microarray and small RNA deep-sequencing experiments were performed to profile the mRNA and small RNA expression levels under ribotoxic stress. We find that many innate immunity, detoxification and metabolism genes are differentially regulated in worms under ribotoxic stress, and the level of differential regulation of these genes is attenuated by inactivation of the genes identified in the hygromycin RNAi screen. Our findings suggest a novel model of xenobiotic detection and detoxification, in which decrement in the essential cellular functions serves as a general signal of xenobiotic stress. Understanding the genetic network behind xenobiotic detection, detoxification, and surveillance of essential cellular functions will provide insights into human response to medicines, drug resistance, and stress response.

348B. Delineating AMPK and TOR longevity. **Yue Zhang**, Ianessa Morantte, William Mair. Department of Genetics and Complex Diseases, Harvard School of Public Health, Boston, MA.

AMP-activated protein kinase (AMPK) and target of rapamycin (TOR) are two critical regulators of cellular nutrient-sensing pathways that mediate the aging process. Although mammalian AMPK and TOR are known to be linked mechanistically, whether this is also true in C. elegans - the model of choice for

the bulk of the aging research - and whether AMPK and TOR function together or separately to increase longevity remains unclear. In mammals, AMPK and TOR negatively regulate each other: AMPK acts upstream of TOR, inhibiting TOR complex 1 (TORC1) indirectly via the TSC complex and directly by phosphorylation of the TORC1 component Raptor. However, recently TORC1 was also shown to act upstream of AMPK: TOR suppresses AMPK via the TORC1 target S6 Kinase, which phosphorylates AMPK alpha and inhibits its subsequent activation. AMPK and TORC1 also have antagonistic effects on lifespan: genetic or pharmacological activation of AMPK promotes C. elegans longevity, while inhibition of TOR extends lifespan. Here, we will examine whether AMPK and TOR modulate lifespan via shared or separable mechanisms, and whether lifespan extension via one, requires altered activity of the other. Suggesting that AMPK acts downstream of TORC1 to modulate aging, we show that lifespan extension via TOR/let-363 and S6 Kinase/rsks-1 inhibition requires the AMPK catalytic subunit AAK-2. Furthermore, we show that phosphorylation of the downstream longevity target of AMPK, CREB-regulated transcriptional coactivator-1 (CRTC-1), is required for lifespan extension by loss of S6K. Our results therefore suggest that the AMPK-CRTC-1 axis is a longevity-specific regulator downstream of the TOR pathway.

349C. A quantitative proteomic analysis of aging in C. elegans. **Stephanie M. Zimmerman**[1], Izumi V. Hinkson[2], Joshua E. Elias[2], Stuart K. Kim[1,3]. 1) Department of Genetics, Stanford University, Stanford, CA; 2) Department of Chemical and Systems Biology, Stanford University, Stanford, CA; 3) Department of Developmental Biology, Stanford University, Stanford, CA.

One way to understand how organisms age is to define the differences between young and old. To identify proteins that are differentially regulated during normal aging in C. elegans, we performed quantitative proteomics by tandem mass spectrometry. In total, we quantified expression of 1797 proteins in at least two of three biological replicates. Of these, 53 proteins show significant changes in levels with age; specifically, 40 were increased and 13 were decreased in old age. These proteins are enriched for expression in the intestine. The proteins that are age-downregulated are strongly enriched for downregulation at the mRNA level, suggesting that decreased RNA expression may be responsible for decreased protein levels in old age. In contrast, the proteins that increase with age do not tend to have age-upregulated mRNAs. This may be due to protein accumulation, or changes in translation or degradation rate with age. Therefore, it may be possible to identify regulators of protein expression (such as miRNAs) that are responsible for driving the changes in the aging proteome that are distinct from the aging transcriptome.

350A. Analysis of the CRTC1 pathway in fat regulation of C. elegans. **Sravya Challa**, Rebecca Hintz, Alexander van der Linden. Biology, University of Nevada, Reno, Reno, NV.

Obesity can result from dysregulation of signaling between centers that regulate feeding in the brain, and sites of fat storage within the body. It is poorly understood how the brain coordinates fat storage in remote tissues. The CREB-regulated transcription coactivator 1 (CRTC1) is a critical metabolic regulator, and is known to act in the brain to control fat storage remotely in peripheral tissues. However, the communication method, such as neuroendocrine factors between the brain and fat tissues, under CRTC1 regulation is unknown. We are studying the CRTC1 pathway in *C. elegans*, and how this pathway controls fat storage. Using lipid-staining techniques and triglyceride quantification methods, our preliminary findings demonstrate that mutations in *crtc-1* (CRTC1) have an increased amount of fat. When we combined mutations between *crtc-1* and *kin-29*, which encodes a Salt-Inducible Kinase (SIK) and potential upstream regulator of CRCT1, we found an even larger amount of fat. This result suggests that *crtc-1* and *kin-29* have synergistic interactions to regulate the amount of fat. Since both *kin-29* and *crtc-1* are expressed in the nervous system and in the intestine (the main site of fat storage), we are further exploring the *crtc-1* and *kin-29* interaction and function in fat regulation in different tissues. Therefore, we are performing rescue experiments by expressing *crtc-1* or *kin-29* in the nervous system or intestine. In addition, we are further investigating the specific changes in the fat content (lipid morphology and classes) of *crtc-1* mutants. Our work will provide a better understanding of how CRTC-1 works to regulate fat at the organismal level, and may ultimately provide therapeutic targets to control human obesity.

351B. Multiple Insulin-like Ligands Regulate the Insulin/IGF Receptor Activity to Prevent Dauer Formation. **Jyothsna D. Chitturi**[1,2], Wesley L. Hung[2], Ying Wang[2], Mei Zhen[1,2,3]. 1) IMS, University of Toronto, Toronto, ON, Canada; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada; 3) Department of Molecular Genetics, University of Toronto, Ontario, Canada.

*JDC and WLH contributed equally to this work

In *C. elegans*, insulin/IGF signaling governs dauer formation, an alternative developmental pathway for animals under stress and starvation (reviewed in Hu, 2007). Conditional alleles of the sole insulin receptor gene, *daf-2*, lead to constitutive activation of dauer program (Gems et al, 1998; Vowels and Thomas, 1992). With 40 insulin-like protein genes, they are proposed to function redundantly to modulate DAF-2 activity level, upon which the animal determine its developmental program (Pierce et al., 2001; Li et al., 2003). However, the cohort of insulin-like ligands in this process is determined. In this study, we report the identification of seven insulin-like ligands that constitute the major insulin signaling inputs through which DAF-2 determines dauer formation. We have deciphered their maturation processes, cellular origins, as well as sites of action. We show that these ligands are processed by two different proprotein convertases, function through both overlapping and non-overlapping cells, and cannot functionally replace each other. Their differential functions and processing suggest multiple levels of regulation for dauer formation.

Gems, D., Sutton, A.J., Sunermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.,L., Larsen, P.L., and Riddle, D.L. (1998). Genetics, 150, 129. Hu, PJ (2007) Wormbook. Li, W., Kennedy, S.G. and Ruvkun, G. (2003) Genes Dev., 17, 844. Pierce, S.B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S.A., Buchman, A.R., Ferguson, K.C., Heller, J., Platt, D.M., Pasquinelli, A.A., Liu, L.X., Doberstein, S.K. and Ruvkun, G. (2001). Genes Dev., 15, 672. Vowels, JJ and Thomas, J. (1992) Genetics 130, 105.

352C. Regulation of Metabolism by TGF-b Signaling in C. elegans. **James F. Clark**[1,2], Vanessa Almonte[1], Cathy Savage-Dunn[1,2]. 1) Biology, Queens College, City University of New York, Flushing, NY; 2) Biology, The Graduate Center, City University of New York, Manhattan, NY.

Transforming Growth Factor -beta (TGF-b) is a large family of peptides that control cell functions such as differentiation, proliferation, and regulation of the immune system. Misregulation of TGF-b has been implicated with a number of diseases. Understanding the pathways that lead to such disorders is integral to finding potential targets for drug therapy. Research has shown that DBL-1, a ligand in the TGF-b super family, plays a major role in body size

regulation in C. elegans. A microarray analysis of genes regulated by the DBL-1 pathway has also identified a number of genes related to insulin signaling and fat metabolism. Insulin involvement in fat metabolism is well documented. However, a function of insulin in growth regulation of C. elegans has not been elucidated. Our goal is to explore the cross talk between TGF-b and Insulin in growth regulation. Four genes were chosen for study, fat-6 and fat-7, D-9 fatty acid desaturases, and ins-4 and ins-7, insulin-like peptides, from the microarray, in addition to dbl-1, lon-2, an inhibitor of dbl-1, and daf-2, an insulin receptor. Double mutants were also obtained to further understand the genetic interactions. Body size is measured throughout the organism's lifespan to understand effects on overall growth. Measurements are taken every 24 hours over over 96 hours. In addition to body size, Oil Red-O, a lipophilic dye, is used to observe the level of lipid storage in the organisms. N2 is used as the control. So far, it was observed that the single mutants, dbl-1, fat-5, fat-6, and fat-7, and the double mutants, fat-5;fat-6 and fat-5;fat-7, have less fat than N2. The fat-6 and fat-7 strains were paradoxically observed to be longer than N2, but this may be due to compensating increases in expression of the unmutated homolog. Future experiments will include the lipid markers: BODIPY 493-503 and a DHS3::GFP strain developed by Zhang et al. in Beijing. These markers will be used analyze and quantify fat storage and metabolism. The goal is to understand how alterations in these signaling pathways affect an organism's development and growth.

353A. Post-embryonic control of DAF-2 insulin-like signaling by the conserved dosage compensation protein DPY-21. **Kathleen Dumas[1]**, Colin Delaney[1], Stephane Flibotte[2], Donald Moerman[2], Gyorgyi Csankovszki[3], Patrick Hu[1,4]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109; 2) Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3; 3) Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109; 4) Departments of Internal Medicine and Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109.

During embryogenesis, an essential process known as dosage compensation is initiated to equalize gene expression from sex chromosomes. Although much is known about how dosage compensation is established, the consequences of modulating the stability of dosage compensation post-embryonically are not known. Here we define a role for the *C. elegans* dosage compensation complex (DCC) in the regulation of DAF-2 insulin-like signaling. In a screen for dauer regulatory genes that control the activity of the FoxO transcription factor DAF-16, we isolated three mutant alleles of *dpy-21*, which encodes a conserved DCC component. Mutations in *dpy-21, dpy-28,* and *sdc-2*, as well as RNAi depletion of other DCC components, suppressed dauer arrest, implicating the DCC in dauer regulation. In *dpy-21* mutants, expression of several X-linked genes that promote dauer bypass is elevated, including four genes encoding components of the DAF-2 insulin-like pathway that antagonize DAF-16/FoxO activity. Accordingly, *dpy-21* mutation reduced the expression of DAF-16/FoxO target genes by promoting the exclusion of DAF-16/FoxO from nuclei. Thus, dosage compensation enhances dauer arrest by repressing X-linked genes that promote reproductive development through the inhibition of DAF-16/FoxO nuclear translocation. This work is the first to establish a specific post-embryonic function for dosage compensation. The influence of dosage compensation on dauer arrest, a larval developmental fate governed by the integration of multiple environmental inputs and signaling outputs, suggests that the dosage compensation machinery may respond to external cues by modulating signaling pathways through chromosome-wide regulation of gene expression.

354B. A novel role for the p120RasGAP family member GAP-3 in dauer regulation. **Kathleen Dumas[1]**, Joseph Kruempel[1], Stephane Flibotte[2], Donald Moerman[2], Patrick Hu[1,3]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109; 2) Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z3; 3) Departments of Internal Medicine and Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, Michigan 48109.

p120RasGAP family members antagonize Ras/MAP kinase (MAPK) signaling by accelerating GTP hydrolysis by Ras GTPases. In *C. elegans*, the p120RasGAP family member GAP-3 antagonizes Ras/MAPK signaling in vulval and germline development. Here we describe a novel role for GAP-3 in the control of dauer arrest. To identify new regulators of the FoxO transcription factor DAF-16, we have embarked upon a search for suppressors of the *eak-7;akt-1* dauer-constitutive phenotype (*seak* mutants). One spontaneous *eak-7;akt-1* suppressor contained a missense mutation in the GTPase-activating domain of GAP-3. Two independent loss-of-function *gap-3* alleles also suppress *eak-7;akt-1* dauer arrest, confirming *gap-3* as a bonafide *seak* gene. Mutations in *gap-1* and *gap-2*, which encode the other predicted RasGAPs in the *C. elegans* genome, did not suppress *eak-7;akt-1* dauer arrest. Surprisingly, a *let-60/Ras* gain-of-function mutation did not phenocopy *gap-3* mutation, suggesting that GAP-3 controls dauer arrest in a LET-60/Ras-independent manner. However, deletion of *lip-1*, which encodes a dual-specificity MAP kinase phosphatase, suppressed the *eak-7;akt-1* dauer-constitutive phenotype to the same extent as *gap-3* mutation, suggesting that MAP kinase activation is sufficient to suppress *eak-7;akt-1* dauer arrest. Our findings implicate a novel LET-60/Ras-independent MAPK signaling pathway in dauer regulation that may act by regulating DAF-16/FoxO activity.

355C. Synthetic cannabinoids influence dauer formation via insulin peptides. Neale Harrison[1], Pedro Reis-Rodrigues[1], Mark Lucanic[2], Jason M. Held[2], Gordon J. Lithgow[2], Thomas Gallagher[3], Young-Jai You[3], **Matthew S. Gill[1]**. 1) Metabolism & Aging, The Scripps Research Institute - Scripps Florida, Jupiter, FL; 2) Buck Institute for Research on Aging, Novato, CA; 3) Virginia Commonwealth University, Richmond VA.

N-acylethanolamines (NAE) are a group of bioactive lipids that include the mammalian endocannabinoid arachidonoyl ethanolamine (AEA, or anandamide). We have previously identified a number of NAEs in *C. elegans* and found that eicosapentaenoyl ethanolamine (EPEA), which is structurally most similar to AEA, can suppress dauer formation in dauer constitutive insulin receptor mutants. Here we examine the effects of synthetic cannabinoids (CB) on dauer formation and find that the CB receptor antagonist / inverse agonist AM251 prevents dauer formation via a mechanism that requires the insulin peptides *daf-28* and *ins-6*. Dauer formation in *daf-2(e1368)* insulin receptor mutants was suppressed by a panel of CB antagonists / inverse agonists, including AM251. The effect of AM251 was inhibited by the presence of the CB receptor agonist O-2545, leading to the observation that O-2545 alone was capable of inducing dauer formation at semi-permissive temperatures in *daf-2(e1368)* mutants. Exposure to AM251 around the time of the dauer decision was sufficient to promote reproductive growth suggesting the involvement of insulin peptides. This was confirmed by the inability of AM251 to rescue an *ins-6; daf-28* double mutant. Co-incubation with EPEA and AM251 lead to a synergistic suppression of dauer formation which was prevented by the addition of the CB receptor agonist O-2545. Finally, EPEA also failed to rescue dauer formation in a *ins-6;daf-28* double mutant. In conclusion, synthetic cannabinoids have potent effects on dauer formation in *C. elegans* that appear to be mediated via changes in insulin peptide availability. These effects overlap with the effects of the endogenous NAE, EPEA, and since worms lack obvious CB receptors it suggests that there may be

a non-canonical CB receptor in *C. elegans*.

356A. Metabolic effects of manganese in the nematode *Caenorhabditis elegans*.. **Priscila Gubert[1,2]**, Bruna Bruna[3], Tassia Lehmen[3], Daiana Avila[4], Felix Soares[3], Michael Aschner[1,2]. 1) Department of Pediatrics, Vanderbilt University, Nashville, TN; 2) Center for Molecular Toxicology, Vanderbilt University, Nashville, Tennessee, United States of America; 3) Departamento de Quimica, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brasil; 4) Universidade Federal do Pampa, Uruguaiana, Rio Grande do Sul, Brasil.

Manganese (Mn) is an essential metal since acts as a cofactor for many enzymes. However, exposure to high levels of this metal may lead to the development of psychomotor disorders , concomitant with dopaminergic (DAergic) neurodegeneration. Furthermore, Mn can interfere with lipid and carbohydrate metabolism. The sensitivity of the DAergic system to Mn is also evident in the nematode, *Caenorhabditis elegans*. Notably, the DAergic system is associated with the control of fat storage in the worm. Our aim was to evaluate whether Mn alters *C. elegans* fat storage and to identify putative pathways involved. Wild-type, dat-1::GFP and daf-16::GFP worms grown under normal conditions were exposed for 4h at larval stage L4 to NaCl or manganese chloride (MnCl2). Next, we determined worm behaviors as well as lipid storage (Nile Red vital labeling). Mn (45 ($p<0.0001$) and 60 mM ($p<0.05$)) caused an increase in Nile Red fluorescence absent DAergic neurodegeneration. Furthermore, Mn (45 and 60 mM) significantly reduced the worms' survival to 70% and 65%, respectively. The body bends and defecation cycles were not affected by Mn. Mn (30, 45 and 60 mM) also caused a significant decrease in pharyngeal pumping, egg-laying and egg-production behaviors. The Fork head transcription factor DAF-16 when activated can inhibit egg fertilization. Based on this observation we investigated DAF-16 localization in response to Mn. Mn led to nuclear translocation of DAF-16 immediately upon treatment, and at 24h post treatment DAF-16 was predominantly in the cytoplasm (inactivated) ($p<0.05$). Our results establish that Mn increase lipid storage in *C. elegans* and delays its reproduction. These effects may be associated with DAF-16 activation. Additional studies are necessary to confirm these hypotheses and verify changes in the lipid accumulation by alternative methods.

357B. Glycosphingolipid mediates clozapine-induced developmental delay and lethality in C. elegans. **Limin Hao**, Bruce Cohen, Edgar Buttner. Mailman Research Ctr, 331A, McLean Hosp, Belmont, MA.

Our aim is to define the mechanisms of action of antipsychotic drugs (APDs), using C. elegans as a genetic model. While some of the direct and immediate effects of APDs are well known, the events which mediate their delayed and long-term effects are not well documented. Exposure to APDs early in development causes dose-dependent developmental delay and lethality in C. elegans. A genome-wide RNAi screen for suppressors of clozapine-induced developmental delay and lethality yielded 40 candidate genes, including sms-1, which encodes a sphingomyelin synthase. This enzyme converts ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol. Since these lipids are known to play important roles in signal transduction, our findings may be relevant to the molecular pathways by which APDs produce their long-term effects. One sms-1 isoform is expressed in the C. elegans pharynx, and its transgene rescues the sms-1 mutant phenotype. We examined pharyngeal pumping and discovered that APD-induced inhibition of pharyngeal pumping requires sms-1, a finding that may explain the role of the gene in mediating APD-induced developmental delay and lethality. By analyzing all the enzymes directly involved in sphingolipid metabolism, we identified ceramide as a key molecule that likely mediates APD effects. We demonstrated that total lipids or sphingolipids extracted from wild-type animals, as well as mixtures of commercially available ceramide-containing compounds, rescue APD-induced developmental delay and lethality. Further investigation revealed that glucosylated ceramide is specifically involved in mediating APD-induced developmental delay and lethality. Using liquid chromatography tandem mass spectrometry (HPLC MS/MS), we found that clozapine treatment reduced ceramide levels in C. elegans. Translating our results to a mammalian model system, we used HPLC MS/MS to show that clozapine also reduced ceramide levels in cultured PC12 cells. The results may lead to new targets for the development of more effective APDs.

358C. Regulation of fat and body growth by SIKs and class II HDACs. **Rebecca Hintz[1]**, Sravya Challa[1], Faye Schilkey[2], Alexander van der Linden[1]. 1) Biology, University of Nevada, Reno, Reno, NV; 2) Genome Sequencing Center, National Center for Genome Resources (NCGR), Santa Fe, NM.

Body size and fat storage of C. elegans are regulated by sensory perception, suggesting that sensory inputs may be involved in energy homeostasis and the allocation of energy for growth and fat storage. Previous work has shown that mutations in *kin-29* (a homolog of Salt-Inducible Kinases) eat normally but have a starved-appearance, a small body size and they hyperforage; all phenotypes indicative of sensing and transducing food incorrectly. Interestingly, *kin-29* acts specifically in chemosensory neurons to regulate body size and sensory gene expression through the action of *hda-4* (HDAC4) and *mef-2* (MEF2). Using triglyceride quantification methods and Coherent Anti-stokes Raman Scattering (CARS) microscopy, we now show that mutations in *kin-29* also cause animals to store more fat. These data together lead to the hypothesis that the inability of *kin-29* animals to correctly perceive sensory inputs from food, causes these mutant animals to grow smaller and store more fat even in the presence of ample food. CARS microscopy also shows that *hda-4* and *kin-29; hda-4* mutants increase the amount of fat stored. Mutations in *HDAC4* resulting in haploinsufficiency have been associated with obesity in humans. We propose that a KIN-29/HDA-4 pathway is necessary to coordinate sensory inputs from food to control body size and fat stores in remote peripheral tissues. To further explore this hypothesis and to better understand how KIN-29 and HDA-4 regulate body size and fat stores, we are using a method to isolate RNA and sequence transcripts specifically from *odr-4*-expressing sensory neurons in wild-type and *kin-29* mutants. Preliminary analysis suggests that *rbg-3* expression is highly increased in *kin-29* mutants. *rbg-3* encodes a Tubby-interacting membrane trafficking protein (RabGAP), and, like *tub-1* is exclusively expressed in the sensory system. Our results propose a model in which KIN-29 activity modulates the Tubby/RBG-3 pathway in sensory neurons to control fat abundance by either neuronal vesicle transport or by secretion of neuroendocrine signals. We are currently exploring these questions.

359A. The Mediator subunit MDT-15 is required for maintenance of membrane lipid unsaturation and ER homeostasis. **Nicole S. Hou[1]**, Stefan Taubert[2]. 1) Cell and developmental biology, University of British Columbia, Vancouver, BC, Canada; 2) CMMT, University of British Columbia, Vancouver, BC, Canada.

Transcriptional regulation is fundamental for the proper control of cellular processes. The Mediator is a transcriptional co-regulator complex that is required for the expression of most genes, yet some Mediator subunits selectively affect biological processes. In C. elegans, the Mediator subunit MDT-15 is required for the transcription of fatty acid metabolism genes. Fatty acids are common components of most lipids, and individual fatty acids profoundly

impact membrane properties and other cellular functions. In this study, we find that inactivating mdt-15 severely disrupts endoplasmic reticulum (ER) homeostasis. In particular, depletion or mutation of mdt-15 results in an overall shift from unsaturated to saturated fatty acids in most lipid species, although the overall abundance of individual lipid species is not altered. The most significant changes in fatty acid distribution occur in phosphatidylcholine, a major membrane phospholipid. The altered membrane lipid fatty acid compositions in mdt-15 worms is accompanied by severe ER stress as judged by upregulation of the ER chaperone hsp-4/BiP, the spliced RNA encoding the ER stress effector XBP-1, and the phosphorylation of the translation initiation factor eIF2a. Surprisingly, dietary supplementation of mdt-15 worms with unsaturated fatty acids only partially alleviates the chronic ER stress, suggesting that mdt-15 maintains ER homeostasis by controlling membrane lipid fatty acid components as well as other unidentified processes. We test whether mdt-15 is directly involved in the unfolded protein response, a conserved feedback mechanism that monitors ER proteostatic status. We show that inactivation of mdt-15 does not render worms hypersensitivity to ER stress or impair the induction of unfolded protein response genes. On the contrary, inactivation of mdt-15 constitutively activates the unfolded protein response. Together, we demonstrate that mdt-15 is required for ER homeostasis, specifically through regulation of proper membrane lipid unsaturation and potentially other unidentified processes.

360B. The *C. elegans* Insulin Signaling Response to Glucose Stress Requires Unique Regulators. **Michael James Hoy**[1], Brian Ganley[1], John A. Hanover[2], Michael W. Krause[2], Michelle A. Mondoux[1]. 1) Biology Dept, College of the Holy Cross, Worcester, MA; 2) National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

In humans, glucose consumption and insulin signaling are intimately linked, as are the consequences of excess glucose (obesity) and impaired insulin signaling (diabetes). However, it is not understood how the cellular responses to glucose change as concentration increases. Using *C. elegans* as a model, a number of insulin-dependent processes sensitive to glucose stress have been identified, including fertility, lifespan, and dauer formation. Glucose stress suppresses dauer formation in a *daf-2* insulin receptor mutant, and we have used this genetic response to identify gene products that regulate insulin signaling specifically in excess glucose. We screened ~11,000 genes by RNAi and identified 151 genes necessary for glucose-induced *daf-2* dauer suppression. Next, we tested these candidates in two secondary screens to determine specificity. To determine glucose specificity, we assayed suppression of *daf-2* dauer formation on control levels of glucose. To determine insulin specificity and eliminate general dauer enhancers, we assayed suppression of dauer formation in a *daf-1* TGF-b receptor mutant. We found that 46% of the candidates were glucose specific, 49% were insulin specific, and 31% (44 genes) were glucose and insulin specific. A DAVID analysis suggests an enrichment of genes involved in growth, ribosome biology, and hermaphrodite sex development. Other categories expected from our data set, like genes involved in aging, were not enriched or had p-values above 0.05. Many of the glucose-specific regulators (~25%) are uncharacterized proteins. Interestingly, although the insulin-signaling pathway is well studied, few candidates (< 5%) have been previously implicated in insulin regulation, suggesting that insulin signaling responds differently to glucose stress than it does to “normal” levels of glucose. We are currently validating the top candidates, testing whether they are important for other glucose stress responses, and beginning to elucidate how they regulate insulin signaling in response to glucose stress.

361C. A CaMK signaling cascade modulates pheromone-mediated developmental plasticity. **In-sok Hwang**[1], Zhi Fang[1], Piali Sengupta[2], Kyuhyung Kim[1]. 1) Department of Brain Science, DGIST, Daegu, South Korea; 2) Department of Biology, Brandeis University, Waltham, MA, United States.

Animals regulate their development in response to changing environmental conditions and their internal metabolic status. This developmental plasticity is mediated by changes in gene expression, but the exact mechanisms by which environmental signals are transduced and integrated with internal status to affect developmental programs are poorly understood. *C. elegans* responds to conditions of overcrowding, limited food and high temperature by arresting development as a dauer larva. High levels of secreted ascaroside pheromone serve as the primary signal to trigger entry into the dauer stage (Golden & Riddle, 1982; Jeong et al., 2005; Butcher et al., 2008). We and others previously showed that pheromone exposure down-regulates expression of a *daf-7* TGF-b and a subset of putative G-protein coupled chemosensory receptor genes such as *str-3* in the ASI neurons; this downregulation influences dauer formation (Nolan et al., 2002; Peckol et al., 1999; Kim et al., 2009). To identify the signaling pathways and genes required for this pheromone-regulated gene expression and dauer formation, we performed a genetic screen and also examined candidate genes (Kim et al., 2009). These analyses showed that the *cmk-1* (CaM kinase I) and *ckk-1*(CaM kinase kinase) genes are required to downregulate *str-3* GPCR expression upon pheromone exposure. As expected, *cmk-1* mutants are also defective in the pheromone-mediated dauer decision. Additionally, we found that *crh-1* (CREB) is required for *str-3* expression even in the absence of pheromone. Surprisingly, CMK-1 and CRH-1 act non-autonomously in the ASE/AWC chemosensory neurons to regulate *str-3* expression in the ASI neurons, suggesting that *cmk-1* and/or *crh-1* may act in these neurons to transmit environmental signals to ASI. The phenotypic analyses of double mutants indicate that the CMK-1 and CRH-1 may act in parallel pathways. Current experiments are aimed at identifying the sensory cues that regulate *str-3* expression, and investigating the mechanisms by which information is transmitted from the AWC/ASE to the ASI neurons.

362A. Epigenetic regulation of stress response in C. elegans. **Moonjung Hyun**[1], Catherine Dumur[2], Young-jai You[1]. 1) Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA; 2) Department of Pathology, Virginia Commonwealth University, Richmond, VA.

In harsh environment, C. elegans undergoes major developmental changes to become a dauer, a dormant form to endure stress.Here we report that BLIMP-1/PRDM-1 is essential for worms to become dauers and that two known dauer-regulatory pathways, insulin (daf-2) and TGFb (daf-7), differentially regulate dauer formation employing distinct epigenetic processes by BLIMP-1/PRDM-1. An RNAi screen of histone modification and nucleosome remodeling factors revealed lin-40, a homolog of mammalian MTA (Metastasis Associate) protein, interacts with BLIMP-1/PRDM-1 to specifically promote dauer formation in the absence of TGFb signaling, whereas, histone acetylation plays a critical role in the absence of insulin signaling. In daf-7 mutants, our results show that BLMP-1 and LIN-40 interact to regulate common targets such as nhr-23 or ptr-4 to regulate dauer formation. However, in daf-2 mutants, HDAC RNAi and HDAC inhibitors prevent dauer formation. Our finding suggests new epigenetic mechanisms to regulate stress response in worms that can be differentially employed depending on the upstream signal.

363B. Targeting Obesity: a new perspective from the worm's point of view. **Tom Janssen**, Lise Peeters, Kevin Van Calster, Nick Suetens, Liliane Schoofs. Functional Genomics and Proteomics Unit, Department of Biology, KULeuven, Belgium.

Obesity is one of the greatest public health challenges of the 21st century. More than one-third of U.S. adults (35.7%) and approximately 17% of U.S. children are obese (CDC 2013). Despite the prevalence of obesity and its related diseases, the signaling pathways for appetite control and satiety are still not clearly understood. Members of the cholecystokinin (CCK) peptide family and their cognate receptors, are known to play an important role in the regulation of feeding behavior (food satiety) and energy homeostasis. Regrettably, despite many efforts, it has been shown that CCK receptor monotherapy is ineffective against obesity. It seems that compensatory mechanisms come into play in long-term studies that are not seen in short-term experiments. To solve this, a better understanding of the mechanisms by which cholecystokinin regulates orexigenic pathways in the body is essential. The *C. elegans* homologue of this hormone system, the *nlp-12*/*ckr-2* signalling system, shares a high degree of structural similarity with its vertebrate counterpart and also displays similar biological activities with respect to digestive enzyme secretion and fat storage. It directly or indirectly affects the expression of several hundred genes, many of which are involved in sugar and fat metabolism and transport, reproduction and the response to starvation. These effects seem to be highly dependent on the animal's feeding status. The expression of the CKR-2 system itself is also reversibly upregulated under starvation and during the dauer stage. In addition, food-consumption tests showed that worms defected in this system display less food satiety than wild type animals, which complies with their function in vertebrates and arthropods. Whether this neuropeptide signaling system is linked to the satiety-induced quiescence pathway is currently under investigation. We have also determined the core receptor activating peptide and have begun testing peptidomimetic agonists. The fundamental study of this cholecystokinin like signaling system in *C. elegans* could prove very helpful in the search for new targets for and the development of novel metabotherapeutics for controlling appetite-related disorders such as obesity.

364C. Unfolded protein response and *enpl-1* depletion sensitize *C. elegans* to the anti-cancer drug cisplatin. Balasubramanian Natarajan[1], Rahul Gaur[2], Oskar Hemmingsson[1], **Gautam Kao[2]**, Peter Naredi[2]. 1) Surgery, Umeå University, Umeå Sweden; 2) Surgery, Sahlgrenska Cancer Center, Gothenburg, Sweden.

Background: Cisplatin is an essential chemotherapeutic drug in the treatment of many cancers. Cisplatin acts both in the nucleus and the cytoplasm to exert its cytotoxic effect. Its use however is limited by the development of resistance in most tumors. The ability to re-sensitize resistant tumors could significantly strengthen cisplatin therapy in patients. We had previously shown that the ATPase ASNA-1 has similar roles as a factor governing cisplatin sensitivity in mammalian tumor cells and *C. elegans*, in a manner that is distinct from its role in the promotion of insulin secretion. **Results:** Here we study the endoplasmic reticulum (ER) resident chaperone ENPL-1/GRP94 and find that its depletion, like that of ASNA-1 makes worms sensitive to cisplatin. Both *asna-1* and *enpl-1* mutants have elevated unfolded protein response (UPR) levels, but it is considerably higher in enpl-1 mutants. While both mutants are cisplatin sensitive, the enpl-1 mutants are much more so with an LC50 that is significantly lower than that of asna-1 mutants. We also find that wild-type worms, which are intrinsically cisplatin resistant, become sensitive when UPR is elevated by tunicamycin treatment or by depletion of IRE-1 activity. **Conclusion:** We conclude that *enpl-1* is a cisplatin sensitizing factor and suggest that manipulation of its levels or of UPR activity will enhance the effects of cisplatin based cancer therapy.

365A. Bacterial fatty acids influence dauer recovery. **Tiffany Kaul[1,2]**, Ifedayo V. Ogungbe[1], Frank C. Schroeder[3], Matthew S. Gill[1]. 1) Aging and Metabolism, Scripps Research Institute, Jupiter, FL; 2) Harriet L. Wilkes Honors College, Florida Atlantic University, Jupiter, FL; 3) Boyce Thompson Institute, Chemistry & Chemical Biology, Cornell University, Ithaca, NY.

Upon entry into the stationary phase, *E.coli* express the enzyme cyclopropane fatty acid synthase (*cfas*) which catalyzes the formation of cyclopropane fatty acids (CFA) from monounsaturated fatty acids (MUFA). *C. elegans* obtain a significant portion of their fatty acids from their bacterial diet and this includes C16 CFA and C18 CFA. Little is known about the metabolic fate or biological function of CFA in worms, although they can be readily detected in both triglycerides and phospholipids. To examine how CFA may influence entry and exit from the dauer stage we grew *daf-2(e1368)* mutants on *E. coli* K12(*cfas*) mutant bacteria, which lack CFA. Bacterial fatty acid profiles confirmed the absence of C16 CFA and C18 CFA in K12(*cfas*) and showed a concomitant rise in C16 and C18 MUFA. Worms grown for several generations on K12(*cfas*) also lacked CFA compared with worms grown on the K12 parent strain. Growth on K12(*cfas*) did not alter the fraction of animals that arrested as dauers at 25°C, but following a shift to 20°C, dauers raised on K12(*cfas*) showed an increased recovery rate after 24h at the permissive temperature. This effect appeared to be due to sensory perception since dauers formed in the presence of K12 showed increased recovery when shifted to K12(*cfas*) at 20°C, and dauers formed on K12(*cfas*) had reduced recovery on K12. Furthermore, increased recovery was promoted by the addition of exogenous MUFA to K12 bacteria, while the addition of phytomonic acid, a CFA from *Lactobacillus*, to K12(*cfas*) bacteria lead to a reduced recovery rate. These data suggest that worms can sense the presence of fatty acids in the environment and that an increased MUFA to CFA ratio promotes dauer recovery.

366B. FGT-1 is a mammalian GLUT2-like facilitative glucose transporter in *Caenorhabditis elegans*. **Shun Kitaoka[1]**, Anthony Morielli[2], Feng-Qi Zhao[1]. 1) Department of Animal Science, University of Vermont, Burlington, VT; 2) Department of Pharmacology, College of Medicine, University of Vermont, Burlington, VT.

Caenorhabditis elegans (*C. elegans*) is an attractive animal model for biological and biomedical research because it permits relatively easy genetic dissection of cellular pathways, including insulin/IGF-like signaling (IIS), that are conserved in mammalian cells. To explore *C. elegans* as a model system to study regulation of the facilitative glucose transporter (GLUT), we have characterized the GLUT gene homologues in *C. elegans*: *fgt-1, R09B5.11, C35A11.4, F53H8.3, F48E3.2, F13B12.2, Y61A9LA.1, K08F9.1* and *Y37A1A.3*. Exogenous expression of these gene products in *Xenopus* oocyte showed the transport activity to unmetabolized glucose analogue 2-deoxy-D-glucose only in FGT-1. The FGT-1-mediated transport activity was inhibited by specific GLUT inhibitor phloretin and exhibited a Michaelis constant (K_m) of 2.8 mM. Mannose, galactose and fructose were able to inhibit FGT-1-mediated 2-deoxy-D-glucose uptake ($P < 0.01$), indicating that FGT-1 is also able to transport these hexose sugars. GFP fusion protein of FGT-1 was seen only on the basolateral membrane of digestive tract epithelia in *C. elegans*, but not in other tissues. The expression was seen from early embryonic stages. Knockdown or mutation of *fgt-1* resulted in increased fat staining in both wild-type and *daf-2* (mammalian insulin receptor homologue) mutant animals. Other known

common phenotypes of IIS mutant animals, including dauer formation and brood size reduction, were not affected by *fgt-1* knockdown in wild-type or *daf-2* mutants. Our results indicated that in *C. elegans*, FGT-1 is mainly a mammalian GLUT2-like intestinal glucose transporter and is involved in lipid metabolism.

367C. Neuronal endoplasmic reticulum stress promotes dauer entry through the activation of the Unfolded Protein Response. **Warakorn Kulalert**, Dennis H. Kim. Department of Biology, MIT, Cambridge, MA.

The maintenance of protein folding homeostasis in the endoplasmic reticulum (ER) is critical for cellular function and survival. Cell autonomous functions of the Unfolded Protein Response (UPR) activated by ER stress include chaperone biosynthesis, protein degradation and attenuation of translation. UPR signaling in response to ER stress may also trigger cell death, as occurs in neurodegenerative conditions and models of insulin-dependent diabetes. The physiological cell non-autonomous effects of ER stress and UPR signaling are not well understood. Here, we report the characterization of a Caenorhabditis elegans mutant in which ER stress in a single pair of neurons activates UPR signaling to regulate larval development. The *daf-28(sa191)* mutant was previously shown to carry a dominant mutation in a neuronally-expressed insulin gene, conferring constitutive entry into dauer diapause, an alternative larval developmental state that includes enhanced stress resistance and extended longevity. We observed that the *daf-28(sa191)* mutation causes ER stress in two neurons, the ASI pair of chemosensory neurons. The ER stress in the ASI neurons does not compromise survival of the neurons, but leads to activation of the eIF2a kinase PERK/PEK-1, which functions to promote entry into dauer diapause. We find that PEK-1 functions in the ASI neuron pair to promote dauer entry and functions independently of previously characterized insulin signaling and TGFb signaling pathways that lead to dauer formation. Our data demonstrate how the activation of the PERK/PEK-1 branch of the UPR by ER stress in a pair of sensory neurons can have cell non-autonomous organismal effects on larval development.

368A. ATGL-1 and LID-1 : Key Players in Fasting-Induced Lipolysis in Caenorhabditis elegans. **Junghyun Lee[1,3]**, Jinuk Kong[1,3], Ju Yeon Jang[1], Junho Lee[2], Jae Bum Kim[1,2]. 1) School of Biological Sciences, Institute of Molecular Biology and Genetics, Center for Adipose Tissue Remodeling, Seoul National University, Seoul, Korea; 2) Department of Biophysics and Chemical Biology, Seoul National University, Seoul, Korea; 3) These authors contributed equally to this work.

Fasting induces various physiological changes including lipid hydrolysis to provide energy source. Lipolysis is a delicate process, accompanied with various signaling cascades and enzymatic changes. However, it has not been thoroughly understood which genes are involved in and how they are regulated in fasting-induced lipolysis. In. C. elegans, we discovered that LID-1 is localized at the surface of lipid droplets in the intestinal cells and is required for the lipolytic activity of lipase ATGL-1. Moreover, we found out that both LID-1 and ATGL-1 are regulated by PKA upon fasting. These data suggest that LID-1 and ATGL-1 would play essential roles in response to nutrient deprivation, indicating the importance of concerted action of lipase and lipid droplet protein in the process of fasting-induced lipolysis.

369B. Absolute Quantitation of *C. elegans* Dafachronic Acids during Development and in Daf Mutants. **Tie-Mei Li[1]**, Jie Chen[2], Xiangke Li[1], Xiao-Jun Ding[1], She Chen[1], Xiaoguang Lei[1,2], Meng-Qiu Dong[1]. 1) National Institute of Biological Sciences, beijing, China; 2) Tianjin University, Tianjin, China.

Under favorable conditions, *C. elegans* larvae grow into reproductive adults after a series of molting cycles. When the environmental condition is harsh, they arrest as dauer larvae. The steroid hormone dafachronic acid (DA) has been shown to be critical for the suppression of dauer arrest. To understand the regulation of endogenous DA levels during development, we set out to develop a sensitive and accurate quantitation method of DAs by the use of LC-MS. We blocked the DA carboxylate group with 2-picolylamine, resulting a 100-fold increase in MS signal. Different MS methods, including Selective Reaction Monitoring (SRM) on a triple-quadruple mass spectrometer and targeted-MS2 or Selected Ion Monitoring (SIM) on a Q-Exactive orbitrap mass spectrometer, were compared for DA quantification. The SIM method consistently exhibited the lowest S/N ratio, thanks to its high mass accuracy and high resolving power. Overall, the method allowed us to detect and quantify the presence of as low as 0.5 ng of DA in 1 mL of packed worms. Using the LC-MS method established as above, we measured the DA levels at different developmental stages of N2 worms. The results showed that DA increased from 0.05 ng/mg total proteins in the L1 stage to 0.8 ng/mg total proteins in the L2 stage and remained high in the L3 stage before dropped to below 0.1 ng/mg total proteins in the L4 and the young adult stages. In Daf-c mutants representing diminished insulin, TGF-b or cGMP signaling (*daf-2*, *daf-7* and *daf-11* mutants) the DA levels remained low at the L2d stage (below 14% of that in WT L2s). Consistent with a previous study [1], exogenous supplement of DA rescued the Daf-c phenotype of these Daf-c mutants. In summary, we have developed an accurate, simple and robust method to measure the absolute quantity of DA in *C. elegans*. This method enabled us to obtain direct biochemical evidence that sufficient DA is essential for L2 larvae to enter reproductive development, and place insulin, TGF-b, and cGMP signaling squarely upstream of DA synthesis. References: 1.Motola, D. L. *et al.*, Cell 124, 1209-23 (2006).

370C. Effect of Repeated Starvation on Fat Content in *C.elegans*. **Shinya Matsumoto[1]**, Kosuke Kato[2], Yasuki Matsumura[2], Nao Sato[1], Yukari Yamamoto[1], Akari Sawanaga[1]. 1) Department of Food and Nutrition, Kyoto Women's University, Kyoto, Kyoto, Japan; 2) Graduate School of Agriculture,Kyoto University,Gokasho,Uji Japan.

It is widely considered that regular feeding rhythm is important to maintain our health in good condition. However, irregularity in feeding rhythm such as late supper and skipping meals can often occur due to busy modern life. If and how such irregular feeding pattern can affect our life is not always clear. The aim of this study is to evaluate the effect of irregular feeding rhythm on the various physiological aspects using *C.elegans* as model organism. Feeding patterns of human and worm are different; while human eat intermittently, worms eat continuously. Therefore, repeated starvation was introduced as the model of irregular feeding rhythm, because the regimen disturbs normal feeding pattern of worm. The cycle of 6 h starvation - 18 h feeding was introduced twice in adult worms, and its effect on fecundity, life span, mobility and fat accumulation was evaluated. No obvious effect was observed on fecundity, life span and mobility. However, fat content, assessed by Nile Red staining, in worm which experienced repeated starvation was shown to increase compared to that of worms which did not experience starvation. To further support the result, the expression of genes involved in fat metabolism such as fatty acid synthase (*fasn-1*), acetyl-CoA carboxylase (*pod-2*), O-acyl transferase (*mboa-2*) and worm sterol regulatory element binding protein

(SREBP-1) ortholog (*sbp-1*) is currently under investigation by RT-PCR. The increment of fat in starvation-experienced worms may have resulted by the fat metabolic shift toward accumulation rather than consumption to prepare the food shortage.

371A. Overexpression of an orphan gene in *Pristionchus pacificus* causes complete inhibition of dauer formation. **Melanie G. Mayer**, Ralf J. Sommer. Max Planck Institute for Developmental Biology, Tübingen, Germany.

Evolutionary ecology investigates how the developmental response to the environment and the ecological interactions of an organism shape the evolution of new phenotypes. Under harsh environmental conditions, *Pristionchus pacificus* can arrest its development and form dauer larvae. The association of *P. pacificus* with scarab beetles is exclusively in the dauer stage, indicating that the dauer stage is essential not only for enduring unfavorable conditions but also for dispersal. Dauer pheromone prepared from the supernatant of *P. pacificus* liquid cultures has dauer-inducing activity and contains two groups of small molecules, ascarosides and paratosides (Bose et al., 2012). We have investigated the ability of *P. pacificus* to enter the dauer stage by extracting dauer pheromone from 16 strains and testing for natural variation in dauer formation (DF) with cross-reactivity assays. Surprisingly, 13 of 16 strains produce pheromones that induce the highest DF in individuals of other strains, showing cross-preference rather than self-preference of pheromones (Mayer et al., 2011). The reference strain PS312 and the strain RS5134 show one-sided cross-preference since RS5134 forms more dauers in response to the PS312 pheromone than in response to its own pheromone. To identify the molecular basis of this difference in DF, we generated recombinant inbred lines and tested them with both pheromones. Using quantitative trait loci mapping, we identified a 5 kbp region with only one gene, Contig44-snap.18, which is a novel non-conserved “orphan” gene. Transgenic lines overexpressing the PS312 or RS5134 version of snap.18 show complete inhibition of DF in response to both pheromones, suggesting that the orphan gene represents a concentration-dependent inhibitor of DF. Indeed, snap.18 is located in a part of the genome for which the PS312 coverage is twice as high as for the adjacent regions, and this is not the case for RS5134. We hypothesize that the duplication of the orphan gene resulted in the low DF phenotype of PS312 and that overexpression of the orphan gene is sufficient to completely inhibit DF. (references: Bose et al. 2012 *Angew. Chem. Int. Ed.* 51:12438; Mayer et al. 2011 *Proc. R. Soc. B* 278:2784).

372B. Identifying transcription factors that regulate fat metabolism and body size. **Akihiro Mori**, Marian Walhout. Systems Biology, UMMS, Worcester, MA.

Over the last decade, several studies have identified transcription factors (TFs) that regulate fat metabolism. In those studies, however, either manual observation or computational approaches have been applied to a limited number of genes and/or images to quantify genetic effects in fat metabolism. This is because expansive identification of genes involved in fat metabolism is experimentally time consuming and there is no user-friendly program which allows researchers to precisely quantify a massive amount of images. Thus, identification of genes that regulate fat metabolism and body size is a challenging problem in both fields of fat metabolism and computational recognition. Here we describe a rigorous and targeted method to comprehensively identify TFs that regulate fat metabolism and body size in C.elegans. We have systematically screened 883 TFs representing 94.4% of all TFs in worms using RNA interference. We have employed Oil-red-O staining to measure the fat content and developed an automated image analysis tool to accurately quantify the effect of each TF knockdown. We have identified ~210 TFs that cause increased fat content (fat) and ~50 TFs that cause decreased fat content (lean) upon knockdown. Additionally, we showed that knockdown of ~90 individual TFs lead to enlarged body size (big) while ~70 TFs are involved in diminished size (small). Overall, we have found that 37.8% of all worm TFs are involved in either fat metabolism or size control. Interestingly, nuclear hormone receptor and zinc finger C2H2 families of TFs are strongly associated with “fat” and “big” phenotypes while homeobox TFs have the opposite phenotypes. Progress will be discussed in conference.

373C. Bis (2-ethylhexyl) phthalate regulates cytochrome P450 (DAF-9) and a specific C4 methylase (STRM-1) towards dauer formation in *C. elegans*. **S. Mukherjee**, T. Paul, M. Guria, A. Nag, J. Bandyopadhyay. Department of Biotechnology, West Bengal University of Technology, Kolkata, West Bengal, India.

Phthalate esters are endocrine disruptors that are implicated to possess severe reproductive toxicity in *C. elegans*. In the present study we show that *C. elegans* interrupts its reproductive life cycle and enters diapause in the presence of bis (2-ethylhexyl) phthalate (DEHP) as a stress-resistant dauer larva in a temperature and concentration dependent manner. Dafachronic acid (DA) is a sterol-derived hormone, whose levels are modulated through the C-4 sterol nucleus methylation by a methyltransferase, Sterol A-ring methylase-1 (STRM-1). The sterol 4-C-methyltransferase inhibits the dauer formation by binding to a nuclear hormone receptor DAF-12. In order to study DEHP toxicity on multiple toxic endpoints reported previously and more particularly the process of dauer formation involving the sterol biosynthetic pathways, the present research has been undertaken to explore the plausible mechanism of action of DEHP in obstructing the normal nematode development. We observed dauer phenotype in the parental generation after exposing *C. elegans* to sub-lethal dose (s) of DEHP. Dauer formation was found to be more pronounced at 15°C and 25°C in comparison to 20°C growth condition at a concentration range of 15-20 mg/L. Furthermore, it is crucial to study the steroid metabolism in *C. elegans* through identification of the activity of STRM-1, as a function of dauer promoting element along with DAF-9, for reproductive development. Subsequently assessing the abundance of DA in DEHP treated worms through NMR studies and GC-MS analysis will enable us to correlate with the DAF-9 activity. In concurrence with other reports the expected higher activity of STRM-1 with increasing concentrations of lophenol (a 4-methyl sterol) are predicted to correspond to a significant decline (almost nil) in the levels of DA and to the activity of DAF-9 as well. The results may subsequently establish the direct role of DAF-9 and STRM-1 in determining the fate of metabolism in *C. elegans*. Phenotypic analysis of *daf-9* and *strm-1* mutants shall further confirm their role(s) in steroid metabolism.

374A. Using Stable Isotope Tracers to Understand the Role of Membrane Maintenance in Aging. Shaw-Wen Chen, **Carissa Perez Olsen**. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

The fatty acid composition of an animal’s phospholipids has a great impact on the properties of the membrane including its permeability, fluidity, and susceptibility to damage. In fact, altered membrane makeup has been associated with a number of diseases as well as with natural aging. The membrane’s integrity is also heavily influenced by its capacity to remove or repair damage; however, little is known about how membrane maintenance mechanisms impact membrane quality over time. Using dietary stable isotope tracers in C. elegans, we can quantify fatty acid replacement in membranes by following the dietary carbon into phospholipids via gas chromatography/mass spectrometry. These studies have found that in young sterile adults about a third of

the membrane fatty acids are replaced or remodeled within a 6 hour period. This significant amount of new fatty acid indicates that the membrane needs a continual infusion of new lipids for replacement of consumed and damaged molecules. Additionally, we have found that, in these young adults, the majority of the new fatty acid tails are directly derived from dietary fat. Currently, we are defining how other metabolic pathways such as lipid synthesis influence the maintenance of membranes by conducting a targeted RNAi screen in conjunction with stable isotope labeling. Over aging, the composition of the membrane changes with a specific loss of polyunsaturated fatty acid species. This loss is predicted to be a result of damage as this group of fatty acids is particularly susceptible to reactive oxygen species attack. We hypothesized that reduced membrane maintenance may contribute to the altered membrane structure observed with natural aging. Indeed, we have found that as nematodes age, the amount of dietary resources funneled into the membrane is dramatically reduced. We are currently examining the relationship between membrane maintenance and longevity in different genetic backgrounds. This effort combined with our studies in young adult populations will allow us to identify important regulatory pathways for the maintenance of appropriate membrane composition and to further understand the role of membrane maintenance in aging.

375B. Quantity proteome analysis of dauer by using iTRAQ in *Caenorhabditis elegans*. **Takehiro Oshime**[1], Yukako Toshato[1], Toshiya Hayano[2], Masahiro Ito[1]. 1) Depart. of Bioinfo, College of Life Sci, Ritsumeikan Univ, Shiga 525-8577, Japan; 2) Depart. of Biomed. Sci., College of Life Sci, Ritsumeikan Univ, Shiga 525-8577,Japan.

Under rich food condition and optimum temperature, *Caenorhabditis elegans* (*C. elegans*) develops successively from embryo to adult in 3 days and can live for about 30 days. Under restricted food, high population density, or high temperature condition, *C. elegans* develops dauer larvae (dauer) via a switch in the development pattern of L1 larvae (L1). Dauer can live for more than 60 days under restricted food condition and is resistant to high temperature, but lacks reproductive potential. Recent studies reported increased expression of sod-3, sod-4, and sod-5 in dauer, and these genes have been thought to influence longevity. This study aimed in identifying the proteins that influence longevity. We performed quantity proteome analysis of dauer, L1, and L3 larvae (L3) by using shotgun proteome analysis and isobaric tags for relative and absolute quantitation (iTRAQ). We compared the protein expression profiles of dauer and L3 and classified the identified proteins into 3 groups—high-expression, low-expression, and unchanged-expression groups—by using logarithm ratio of life stage. Unchanged-expression group included proteins that were not a part of the high-expression and low-expression groups. The proteins of the high-expression group were further divided into 2 groups: specific expression group and non-specific expression group. The proteins of the specific expression group were classified according to the function by using DAVID Bioinformatics Resources. We identified 1,054 proteins by shotgun proteome analysis using iTRAQ. Compared with other groups, the specific expression group included many proteins of unknown function; hence, we focused on this group. Few classified proteins of known function were related to heat response, adult life-span determination, and superoxide metabolic process. These functions are characteristic of dauer. These proteins included *sod-4*, *mtl-1*, and *dao-3*. We found that the expression of *sod-4* and *mtl-1* was higher in dauer, and that *dao-3* influenced specific functions of dauer. Our study provides a basis to understand the function of proteins in dauer.

376C. Comparative metabolomics reveals endogenous ligands of the nuclear hormone receptor DAF-12 regulating *C. elegans* development and lifespan. Parag Mahanti[1], Neelanjan Bose[1], Joshua Judkins[1], Axel Bethke[1], Joshua Wollam[2], **Oishika Panda**[1], Kathleen Dumas[3], Anna Zimmerman[1], Patrick Hu[3,4], Adam Antebi[2,5], Frank Schroeder[1]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA; 2) Max Planck Institute for Biology of Aging, Gleueler Str. 50a, D-50931, Cologne, Germany; 3) Life Sciences Institute, University of Michigan; 4) Departments of Internal Medicine and Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI 48109, USA; 5) Huffington Center on Aging, Dept of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030, USA.

Small-molecule ligands of nuclear hormone receptors (NHRs) play central roles in the regulation of development, cell differentiation and metabolism in metazoans. Although small changes in ligand structures may strongly affect NHR transcriptional activity, the endogenous ligands of many metazoan NHRs remain poorly characterized. Using comparative metabolomics, we identified the endogenous ligands of the *C. elegans* NHR DAF-12, which regulates larval development and adult lifespan. The identified steroid derivatives include only one of two previously predicted DAF-12 ligands, the dafachronic acids (DAs), and have structural motifs reminiscent of mammalian bile acids. Using Alphascreen assays, we confirmed direct binding of DAs to DAF-12. Comparative metabolomics of mutants of steroid-modifying enzymes provides evidence for differential regulation of the biosyntheses of different ligands, indicating that they may serve partially divergent functions. We further developed a flexible synthesis for these ligands and their masked derivatives that enable precise temporal control of *C. elegans* development. We introduced photocleavable amides of 5-methoxy-N-methyl-2-nitroaniline (MMNA) as biocompatible probes to investigate DAF-12 ligand action *in vivo*. Our results show that previous, classical genetics-based hypotheses about NHR signaling in *C. elegans* need to be revised and demonstrate the utility of unbiased comparative metabolomics coupled with elegant synthetic techniques for the identification of metazoan NHR ligands.

377A. Identification of a diet induced signaling pathway for mitochondrial adaptation during aging. **Shanshan Pang**[1], Sean P. Curran[1,2,3]. 1) Leonard Davis School of Gerontology, University of Southern California, Los Angeles, CA; 2) Dornsife College of Letters, Arts, and Sciences, Department of Molecular and Computational Biology, University of Southern California, Los Angeles, CA; 3) Keck School of Medicine, Department of Biochemistry and Molecular Biology, University of Southern California, Los Angeles, CA.

Diet can have a great impact on animal physiology and complex phenotypes such as aging. Animals must be able to utilize distinct strategies to adapt to different food sources that change drastically in the wild. However, the cellular mechanisms underlying the ability to adapt are still largely unknown. Here, we report a complementation group of genetic mutants, which are characterized by a Food Dependent Aging (Fda) phenotype. We have identified three recessive alleles *lax102*, *lax105*, and *lax219*, which define this complementation group. These mutants exhibit a shortened lifespan phenotype when fed *E.coli B* strains but live a normal lifespan when fed an *E.coli K-12* diet. The short-lived phenotype is not due to general sickness as these animals have normal developmental timing and wild type reproductive output until day 2 of adulthood. After the second day of adulthood however, Fda mutants age rapidly, with a maximal lifespan of about 16 days. Fda mutant animals fed an *E.coli B* strain diet exhibit abnormal mitochondrial morphology, reduced ATP levels and increased ROS production in adulthood, which directly lead to lifespan reduction. In addition, host *nmur-1* signaling acts upstream of mitochondrial function to sense diet-derived cue(s) from the pro-aging *E. coli B* strain of bacteria. Together, these results reveal a novel mechanism

underlying how animals adapt to different food sources, and link food type to mitochondrial function during adult aging.

378B. Nematoil - a novel secreted lipid that coats the outer surface of the dauer larva of Pristionchus pacificus. **Sider Penkov**[1], Akira Ogawa[2], Ulrike Pässler[3], Margit Gruner[3], Hans-Joachim Knölker[3], Ralf Sommer[4], Teymuras Kurzchalia[1]. 1) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; 2) Laboratory for Developmental Dynamics RIKEN Quantitative Biology Center Kobe, Japan; 3) Department of Chemistry, Technical University of Dresden, Dresden, Germany; 4) Department for Evolutionary Biology, Max-Planck-Institute for Developmental Biology, Tuebingen, Germany.

The dauer larva formed by multiple nematodes is a specialized developmentally arrested stage for survival and dispersal from unfavorable environment. The survival abilities of dauer larvae are determined by their specific morphology, metabolism, and enhanced stress resistance. A major morphological feature of dauer larvae is the remodeled body surface - they are effectively sealed off by a dauer-specific cuticle that restricts the chemical exchange with the environment. Although the dauer formation is genetically well investigated in several nematode models, there is no much information about the chemical means by which dauer larvae resist to the various kinds of environmental stress. We have found that the nematode Pristionchus pacificus synthesizes dauer stage specific lipids that form a hydrophobic film covering the entire outer surface of the animal. Detailed observation showed that the synthesis and the secretion of the lipids are simultaneously executed late in dauer differentiation, shortly preceded by the molt to dauer larva. The hydrophobic film is a complex mixture of several lipids and advanced chemical analysis revealed that its major component is a very long-chain polyunsaturated wax ester that we name Nematoil. The lipid coat alters the surface properties of the animals - they tend to congregate in tight “dauer clumps” consisting of up to hundreds of individuals, which supposedly enhances their impermeability. Thus, P. pacificus dauer larvae have the biochemical means to enhance their stress response by counteracting collectively.

379C. Isolation of N-acylethanolamine resistant mutants using a forward genetic screen for resistance to synthetic cannabinoids. **Pedro R. Rodrigues**, Neale Harrison, Jitendra Mishra, Thomas Bannister, Matthew S. Gill. The Scripps Research Inst, Jupiter, FL.

In mammals, endocannabinoids (EC) and synthetic cannabinoids (CB) mediate their effects through CB receptors. We have previously demonstrated that eicosapentaenoyl ethanolamine (EPEA), an N-acylethanolamine (NAE) which is structurally most similar to the mammalian EC anandamide, prevents dauer formation in dauer constitutive mutants. Despite the biological activity of endocannabinoids in worms, there is abundant evidence that they do not possess orthologs of the canonical CB receptors. We have therefore performed a forward genetic screen to identify components of NAE / cannabinoid signaling pathways in the worm. EPEA prevents dauer formation in daf-c mutants at semi-permissive temperatures, but it is not fully penetrant, thus limiting its utility in a forward genetic screen. However, we have found that the CB receptor inverse agonist AM251 rescues dauer formation in *daf-2(e1368)* mutants at the fully restrictive temperature (25°C) and acts synergistically with EPEA. Conversely, the CB1 receptor agonist O-2545 induces dauer formation in daf-2(e1368) at semi-permissive temperatures and competes with AM251 and EPEA at the restrictive temperature, suggesting shared molecular targets. While performing structure activity studies, we identified an AM251 derivative, SR10589, which not only prevents dauer entry at lower doses but also causes a fully penetrant L1 arrest phenotype at higher concentrations. Importantly, the L1 arrest is competed by O-2545, and SR10589 at low doses acts synergistically with AM251 and EPEA to prevent dauer formation. We have conducted an F1 forward mutagenesis screen and isolated mutations that suppress the L1 arrest of SR10589 treated animals. The mutants have different degrees of resistance to SR10589, and, interestingly, all the mutants characterized so far also display resistance to AM251 and EPEA, indicating that these molecules act through common factors. We are currently cloning the mutations and believe that their identification will help understand the non-canonical actions of N-acylethanolamines and cannabinoids in worms and higher organisms.

380A. Folate metabolism and the rescue of folate deficiency by thiamine supplementation. **Jason A. Rothman**, Craig W. LaMunyon. Dept. of Biological Sciences, California State Polytechnic Univ, Pomona, CA.

Folate deficiencies have been implicated in many metabolic diseases and syndromes that affect humans. Here, we use Caenorhabditis elegans to investigate the biochemical and metabolic role of folate, as well as to determine the effects of folate deficiency. The C. elegans gene folt-1 is an ortholog of the human reduced folate carrier gene. The FOLT-1 protein has been shown to transport folate across cell membranes in worms. A knockout mutation of the gene, folt-1 (ok1460) causes sterility, shortens lifespan, and slows metabolism. We have rescued the sterility phenotype of the folt-1 worms by supplementation with various combinations of biotin, folate, niacin, oxaloacetate, riboflavin and thiamine. We also partially replicated the folt-1 sterility phenotype by exposing N2 worms to various combinations of methionine, homocysteine, copper chloride and thymidylate synthase RNA interference (RNAi). Indeed, exposing wild-type worms to a combination of homocysteine and thymidylate synthase RNAi completely replicated the folt-1 knockout phenotype. Folate is a major source of methyl groups for epigenetic methylation. We have found that histone H3K9 methylation is reduced in folt-1 mutants but partially restored in folt-1 mutants raised on thiamine. Although the pathway is unclear, it appears that thiamine can supply methyl groups needed for histone methylation. We suggest that our results with C. elegans provide novel insights into the metabolism of folate and other vitamins and may form the basis for future studies for therapeutic treatment of vitamin deficiencies and metabolic diseases.

381B. Worms as chemical masterminds: complete control with small molecules. **Frank C. Schroeder**. Boyce Thompson Inst, Cornell Univ, Ithaca, NY.

Worms are amazingly skilled chemists: using simple building blocks from conserved primary metabolism and a strategy of modular assembly, they create complex molecular architectures to regulate almost every aspect of their development and behavior. Phenotypes regulated by this combinatorial library of small-molecule signals include dauer formation, adult phenotypic plasticity, adult lifespan, attraction of the other sex, aggregation, dispersal, and other behaviors. Many, but not all of the identified compounds are based on the dideoxysugar ascarylose, which serves as a scaffold for attachment of moieties from amino acid, carbohydrate, neurotransmitter, lipid, and nucleoside metabolism, creating signaling molecules that can be active at femtomolar concentrations, such that encountering just a few molecules per minute is sufficient for worms to respond.

Motivated by this unexpected structural and functional diversity, we have embarked on a systematic characterization of the *C. elegans* metabolome. We find that most small molecules in *C. elegans* remain undescribed and that even important primary metabolite classes may include non-canonical compounds. For example, up to a quarter of the entire *C. elegans* lipid content is not represented by triglycerides, phospholipids or fatty acids, but instead

consists of very long-chain ascaroside lipids in which the ascarylose sugar takes the place of conventional glycerol. Our screen has produced evidence for several thousand different metabolites of yet undetermined structures, ranging from simple lipids, amino acid derivatives, and nucleosides to complex modular assemblies. Their identification and subsequent quantification in genome-wide mutant screens will, akin to transcriptional profiling, provide a new basis for the study of *C. elegans* metabolism and associated signaling pathways, enabling re-interpretation of many metabolic, developmental, and behavioral phenotypes.

382C. Mechanistic studies on the regulation of fat accumulation by the TOR pathway in *C. elegans*. **Ming Sheng**[1], Josefin Friberg[1], Philip McQuary[3], Rahul Gaur[1], Staffan Lundstedt[2], Malene Hansen[3], Simon Tuck[1]. 1) UCMM; 2) Dept. of Environmental Chemistry, Umeå University, Sweden; 3) Program of Development and Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA.

Fat accumulation in *C. elegans* is regulated by many signalling pathways including those involving insulin/insulin-like or TGFb-family ligands, nuclear hormone receptors, or the nutrient sensor, TOR. The mechanisms by which these pathways affect fat accumulation, and the way in which the different pathways interact is incompletely understood. To address these questions, we have studied fat regulation in mutants lacking *rsks-1*, which encodes S6 kinase. Vertebrate S6 kinase is a major target of TOR protein kinase. We find that *rsks-1* mutants have dramatically increased fat accumulation in the intestine. Quantification of fat accumulation by TLC/GC revealed that the mutants have more than two-fold higher levels of triglycerides than wild type and disproportionately high levels of C16:1n9 and C18:1n9. Genetically *rsks-1* acts either downstream or in parallel to the insulin and TGFb pathways to affect fat levels. The *rsks-1* defect is partially rescued by expression of *rsks-1* in the germline, where *rsks-1* is required to promote fertility (1). Thus the increase in fat accumulation in the mutant may partly be the result of redirection of resources from the germline to the soma. Metabolomics analyses revealed that the mutants have increased levels of many amino acids and of certain acylcarnitines, molecules required for the transport of fatty acid intermediates into mitochondria during b-oxidation. In genomics-and proteomics-based approaches, we have identified genes and peptides that are differentially regulated in *rsks-1* mutant worms. We have performed genetic screens for mutations that suppress the increased fat accumulation in the mutant. Two allelic suppressor mutations map close to the right of *unc-73* and are strongly phenocopied by RNAi of a gene in this region, R12E2.2, which encodes a conserved protein with a SAD/UNC domain. Two other suppressor mutations are alleles of *aex-5*, which encodes a calcium-dependent serine endoprotease. 1. Korta et al. Development. 2012;139:859-70.

383A. EGL-8, a phospholipase C beta homolog, is a novel regulator of dauer arrest. **Hung-Jen Shih**[1], Andrew Polzin[1], Kathleen J. Dumas[1], Stephane Flibotte[2], Donald G. Moerman[2], Patrick J. Hu[1]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Zoology, University of British Columbia, Vancouver, Canada.

We have identified the phospholipase C beta (PLCb) homolog EGL-8 as a novel regulator of dauer arrest in *C. elegans*. To identify new regulators of the FoxO transcription factor DAF-16, we performed a genetic screen for suppressors of the *eak-7;akt-1* dauer-constitutive phenotype (*seak* mutants). Three independent mutants harbored distinct mutations in *egl-8*, which encodes the sole *C. elegans* PLCb family member. *egl-8(dp14)*, which is a nonsense mutation in the N-terminal pleckstrin homology domain, suppresses *eak-7;akt-1* dauer arrest, as does the independently isolated null allele *egl-8(sa47)*. Therefore, *egl-8/PLCb* is a bonafide *seak* gene. *egl-8(sa47)* suppresses the dauer-constitutive phenotypes of the insulin-like pathway mutants *daf-2(e1368)* and *akt-1(ok525)* but does not influence the dauer-constitutive phenotype of the TGFb-like pathway mutant *daf-1(m40)*, suggesting that EGL-8/PLCb specifically influences dauer regulation by DAF-2 insulin-like signaling. Our findings suggest that EGL-8/PLCb may be a novel regulator of DAF-16/FoxO activity.

384B. Genetic requirements of the pentose phosphate pathway for the intestinal granule formation in *C. elegans*. **Hirohisa Shiraishi**, Takato Oikawa, Maya Ishibashi, Mari Tanabe, Yumi Asanuma, Reiko Aoyama, Kenji Nishikori, Takahiro Tanji, Ayako Ohashi-Kobayashi. Department of Immunobiology, School of Pharmacy, Iwate Medical University, Yahaba, Iwate, Japan.

We have focused on intestinal granular organelles observed from late larval to early adult stage in *C. elegans* since a major subset of them dynamically emerge or evanesce in an age- and nutrient-dependent manner (Nishikori K., *et al.*, *C. elegans* Topic Meeting 2012, Madison, WI). Such organelles are non-acidic granules to which HAF-4 and HAF-9, ABC transporters homologous to a mammalian lysosomal peptide transporter ABCB9, co-localize. The deletion mutants for *haf-4* and *haf-9* exhibited the loss of the HAF-4/HAF-9-positive granules and some physiological defects such as slow growth and reduced brood size, suggesting that the granules play important roles in the storage and supply of some nutritional components during reproductive period.

To uncover the physiological roles and the regulatory mechanisms of the intestinal granules, we have performed comprehensive RNAi screening using *C. elegans* RNAi Feeding Library (Open Biosystems). To achieve the quick and reliable evaluation of RNAi effects, we adopted a high-powered digital microscope for observation. This system magnifies live worms on the rearing agar plate up to 5,000 times, which facilitates screening for the presence of intestinal granules. So far several tens of candidates with reduced granular number have been identified among approximately 2,000 genes tested.

In this meeting, we report *tkt-1*, an orthologous gene for mammalian transketolase (TKT), as one candidate. TKT acts in the pentose phosphate pathway (PPP), which contributes to fatty acid synthesis and oxidative stress prevention via NADPH production as an alternative to glycolysis pathway. We found that RNAi of some PPP-related genes and the glutathione-disulfide reductase gene results in the reduction of the HAF-4::GFP-positive granules and the DHS-3::GFP-positive lipid droplets, but not of the GLO-1::GFP-positive lysosome-related organelles. The NADPH-producing oxidative pathway of PPP might be necessary for the normal formation of specific subsets of the intestinal granules in *C. elegans*.

385C. Overexpression, purification and characterization of *C. elegans* glyceraldehyde-3-phosphate dehydrogenase isozymes. **Ruth L. Silimon**[1], Derek Schwabe-Warf[1], Valeria S. M. Valbuena[1], Justin W. Spengler[2], Megan K. Gautier[1], Katherine M. Walstrom[1]. 1) Division of Natural Sciences, New College of Florida, Sarasota, FL; 2) Ctr. for Biofilm Eng., Montana State Univ., Bozeman, MT.

Glyceraldehyde-3-phosphate (G3P) dehydrogenase (GPD) is the glycolytic enzyme that adds inorganic phosphate to its substrate so that net ATP production is possible. *C. elegans* has 4 different GPD isozymes. Embryonic GPD-1 and GPD-4 are nearly identical, while the homologous GPD-2 and GPD-3 are expressed in postembryonic worms (Yarbrough and Hetch, JBC 259, 14711, 1984; Huang et al., 1989, JMB 206, 411). GPD-3 is involved in protection

from anoxia (Mendenhall et al., 2006, Genetics 174, 1173) and upregulated in dauers and *daf-2* mutants (McElwee et al. 2006, Mech. Age. Dev. 127, 922). We subcloned, overexpressed, and purified both GPD-1/4 and GPD-3 and performed enzyme kinetics studies with all three substrates under the optimal reaction conditions at pH 8.0-8.5. Both isozymes exhibited substrate inhibition at high concentrations of phosphate and NAD^+, but the embryonic form was inhibited at lower concentrations of phosphate or NAD^+ than GPD-3. GPD-1/4 appeared to have a higher specific activity than GPD-3. We determined that the K_M values for NAD^+, phosphate, and G3P for GPD-3 were 0.3 mM, 0.4 mM, and 2.6 mM, respectively. The K_M values for NAD^+ and phosphate for GPD-1/4 were 1 mM and ~0.6 mM. The K_M values from a partially purified GPD preparation from a mixed population of *C. elegans* had NAD^+ and G3P K_M values of 1 mM and 0.3 mM, respectively. Based on the results of Yarbrough and Hetch, we expected the enzyme activity of the adult GPD-2 and GPD-3 enzymes to predominate in the endogenous GPD mixture purified from the worms. Based on our current kinetics results, the endogenous protein mixture had a K_M for NAD^+ more similar to the GPD-1/4 enzyme. We are continuing to compare the activities of the *C. elegans* GPD enzymes to determine which isozyme(s) are active in the endogenous GPD mixture.

386A. Diet Another Day: *agl-1* (glycogen debranching enzyme) embryonic lethality depends on maternal diet. **Jeff Simske**. Rammelkamp Ctr, Cleveland, OH.

Mutations in *agl-1* result in embryonic lethality due in part to reduced activity of AMPK, presumably though inhibition of AMPK via branched glycogen (limit dextrin) binding to the AMPKb b subunit. *agl-1* embryonic lethality is partially penetrant, and escapers develop into fertile adults, albeit with shortened lifespans. Embryonic lethality depends on maternal diet: *agl-1* mutants grown on live or UV-treated OP50 display no embryonic arrest or almost 100% arrest, respectively. Similar *agl-1* phenotypes are observed when grown on glucose, sucrose or glycerol plates with live OP50. Treatment with Metformin, a known AMPK activator, partially rescues *agl-1* lethality and restores AMPK activity, but only on UV or glucose treated bacteria. Metformin alters the metabolism of live OP50 bacteria, resulting in increased *agl-1* lethality. Metformin and attenuated bacteria can expand lifespan via dietary restriction (DR), suggesting that *agl-1* phenotypes may result from DR. To test this, *agl-1* doubles with eat mutations that induce DR were constructed; these double mutants display increased lethality on live OP50, suggesting that a significant fraction of *agl-1* lethality is due to DR. Dietary requirements were further tested by growing *agl-1* on other bacteria. To test whether bacteria itself was providing b-amylase activity to circumvent *agl-1* loss, bacteria mutant for debranching enzyme were fed to *agl-1* mutants; no lethality was observed. HT115 bacteria, which have elevated carbohydrates, did not induce *agl-1* lethality, even after UV treatment (this explains puzzling feeding RNAi results). To test whether *agl-1* diet affects other glycogen metabolic mutants, deletion alleles of glycogen phosphorylase, branching enzyme, and a-amylase were examined, and none displayed diet-dependent lethality. a-amylase mutants showed no embryonic lethality; phosphorylase and branching enzyme mutants were embryonic lethal, but their terminal phenotypes differed from *agl-1*. These results differed from glycogen synthase lethal phenotypes, which more closely match those of *agl-1*. These results suggest that *agl-1* phenotypes result from altered AMPK-dependent and independent pathways, due to changes in glycogen metabolism and diet, respectively.

387B. Novel secreted proteases regulate systemic heme homeostasis in C. elegans. **J. Sinclair**, K. Meng, K. Pinter, I. Hamza. Animal & Avian Sci, Univ Maryland, College Park, MD.

Heme is an iron-containing prosthetic group that plays an essential role in a number of biological processes, including oxidative metabolism, signal transduction, and microRNA processing. Although cells synthesize and regulate heme autonomously, several lines of evidence suggest that cell non-autonomous mechanisms of regulation also exist in which distally located proteins signal systemic heme requirements to an inter-tissue heme trafficking network. The nematode Caenorhabditis elegans is unique among free living organisms because it is a heme auxotroph. It relies solely on environmental heme to survive and reproduce. Therefore, the worm serves as an excellent animal model to explore how cell non-autonomous signals direct intercellular heme distribution without interference from cell autonomous heme synthesis. To uncover the genes involved in systemic heme homeostasis in C. elegans, we analyzed results from Affymetrix microarrays and genome-wide RNAi screens. The combination of these experiments resulted in the identification of two conserved cathepsin-like proteases, which we termed HRG-7 and HRG-8. Here, we show that under low heme conditions, hrg-7 and hrg-8 are upregulated in the worm intestine and depletion by RNAi results in an intestinal heme-deficiency signal without perturbing intestinal heme uptake. Furthermore, knockdown of either gene results in a reduced number of viable progeny, indicating that HRG-7 and HRG-8 are required for normal growth and reproduction. Although HRG-7 and HRG-8 are synthesized in the intestine, both proteins are secreted from the intestinal basolateral membrane and are subsequently internalized by neurons. We propose that HRG-7 and HRG-8 are involved in communicating heme status between the intestine and neurons in C. elegans. Interestingly, humans have over thirty cathepsin and cathepsin-like proteins, with recent data suggesting that this class of enzymes has greater function at the systemic level than previously predicted. Therefore, this work may uncover an unanticipated role for cathepsins in regulating systemic heme homeostasis in mammals.

388C. Metabolism of benzimidazole anthelmintics in Caenorhabditis elegans, and the ruminant parasite, Haemonchus contortus. **Susan J Stasiuk**[1], Gillian MacNevin[2], Dae-Qyun Ro[2], John S Gilleard[1]. 1) VetMed, UofC, AB, Canada; 2) Chemistry, UofC, AB, Canada.

Widespread anthelmintic resistance among animal parasites and concerns regarding its emergence among human parasites makes the understanding of how parasites metabolize and eliminate anthelmintics an important goal. We have previously shown that albendazole, an anthelmintic commonly used in humans and livestock, is metabolized to a glucoside derivative in C. elegans. Glycation of drugs is rare in mammals and had not been previously reported for the benzimidazoles. We have investigated whether other members of the benzimidazole (BZ) family were also glycosylated in C. elegans and in H. contortus, a parasitic nematode that is a model for anthelmintic drug discovery and resistance research. Using HPLC and LC/MS we have characterised the metabolites produced by C. elegans following exposure to albendazole, mebendazole, thiabendazole, oxfenbendazole, and fenbendazole. Each of the BZ were modified by the addition of a glucose (±other) moiety. This glycation appears to be nematode specific, since mammals have been shown to metabolize these substances by sulfo-conjugation reactions only. Furthermore, the metabolites were found mainly in the media suggesting they are efficiently eliminated from the worms. We have shown that the parasite H. contortus metabolizes the BZs to the same glucoside derivatives as C. elegans. One approach to identifying the enzymes responsible for the metabolism is to examine the transcriptomic response. We have shown earlier that a small number of C. elegans genes are up-regulated in response to albendazole (including cyp-35C1, cyp-35A2, cyp-35A5, ugt-16, ugt-63 and gst-5). We have

tested the specificity of this response using QRT-PCR and have found that these genes were up-regulated by the other BZs, although the relative levels of up-regulation differed between drugs. By manipulating the expression levels of these genes in C. elegans we will test the effect on BZ metabolism and potency. In summary, metabolism of BZs differ dramatically between mammals and nematodes but is very similar in C. elegans and H. contortus suggesting C. elegans is a valid model for the functional analysis of these processes in strongylid parasites.

389A. PAQR-2 Regulates Fatty Acid Desaturation During Cold Adaptation in *C. elegans*. **Emma Svensk**[1], Marcus Ståhlman[2], Carl-Henrik Andersson[1], Maja Johansson[1], Jan Borén[2], Marc Pilon[1]. 1) Dept Chem Mol Biol, University of Gothenburg, S-405 30 Gothenburg, Sweden; 2) Sahlgrenska Academy, University of Gothenburg, S-405 30 Gothenburg, Sweden.

Adiponectin is an adipokine with insulin-sensitizing and antiatherogenic actions, which also influences energy homeostasis via the hypothalamus (Kadowaki *et al.* 2008). The human adiponectin receptors AdipoR1 and AdipoR2 are PAQR proteins: 7-transmembrane domain proteins with topologies reversed that of GPCRs, i.e. their C-terminus is extracellular (Tang *et al.* 2005). We have previously shown that a loss of function mutant of the *C. elegans* adiponectin receptor homolog *paqr-2* is unable to adapt to growth at 15°C (Svensson *et al.* 2011). We have now leveraged the *paqr-2* cold adaptation defect in a suppressor screen with the aim of identifying downstream receptor targets. The screen produced nine suppressor mutations that have all been identified through whole genome sequencing and experimental verification. The nine mutants fall into two categories: 1) Mutations that decrease synthesis of phosphatidylcholine and 2) Mutations that influence fatty acid metabolism. The two categories are tied together by phosphatidylcholine being a regulator of the fatty acid metabolism transcription factor *sbp-1*, a homolog of the mammalian SREBP (Walker *et al.* 2011). Consequently we have been able to connect all nine *paqr-2* suppressor mutants into one pathway, the *paqr-2* target pathway. Also, *fat-6* or *-7* RNAi can completely abolish the suppression in mutants from both categories pointing towards an increase in fatty acid desaturation as the common suppressive mechanism.

We conclude that *paqr-2* regulates cold adaptation and that this process is highly dependent on the D9 desaturases. However, despite having identified the *paqr-2* target pathway, the direct action point of this receptor remains unknown.

References: Kadowaki T. *et al.* (2008) FEBS Lett 582:74-80; Svensson E. *et al.* (2011) PLoS One 6:e21343; Tang Y. *et al.* (2005) J Mol Evol 61:372-380; Walker A. *et al.* (2011) Cell 147:840-852.

390B. Functional analysis of the acylpyruvase FAHD1 in *C. elegans*. **A. Taferner**[1], H. Pircher[1], N. Tavernarakis[2], P. Jansen-Dürr[1]. 1) Institute for Biomedical Aging Research, University of Innsbruck, Innsbruck, Austria; 2) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology - Hellas, Heraklion, Greece.

The newly described mitochondrial enzyme FAHD1 (FAH domain containing protein 1) is conserved in evolution from yeast to human. However, its cellular function is still unknown. On the biochemical level, it was shown that FAHD1 exhibits acylpyruvase activity, demonstrated by the hydrolysis of acetylpyruvate and fumarylpyruvate *in vitro*. The enzyme was found expressed in most murine tissues, with highest expression in pancreas and kidney (Pircher et al., 2011). By bioinformatics analysis, we could identify the *C. elegans* gene ZK688.3 as the ortholog of human FAHD1. It was subsequently renamed *fahd-1*. The project aims to clarify the physiological function and regulation of FAHD1, as well as the nature and regulatory mechanisms of possible FAHD1 interaction partners on an organismic level in the multicellular organism *C. elegans*. In *C. elegans*, FAHD-1 is expressed in many cell types, especially in metabolically highly active organs like intestine, pharynx, and vulva. Like its human pendant, it is localized in mitochondria. The depletion of FAHD-1 by RNAi leads to a slight reduction in the mean lifespan of *C. elegans*. To further examine the physiological function of FAHD-1 in the worm, a *fahd-1* mutant *C. elegans* strain was obtained. Although there is still only a minor change in lifespan in these worms, *fahd-1* animals exhibit a strong phenotype. *fahd-1 C. elegans* have a slightly reduced body size and a lower fat content compared to wild-type worms. *fahd-1* worms also have a reduced brood size. Most striking is the uncoordinated movement of these animals, a phenotype that can be rescued by transgenic expression of full-length FAHD-1 in mutant worms. The findings suggest an important function for FAHD-1 in *C. elegans*. We hope that with our work we will be able to clarify the physiological function and regulation of FAHD1, as well as its possible involvement in the aging process of *C. elegans*.

391C. Biochemical and Genetic Analysis of Lipid Droplets in *C. elegans*. **Tracy L. Vrablik**[1], Vlad Petyuk[2], Olga Shiva[1], Jennifer L. Watts[1]. 1) School of Molecular Biology, Washington State University, Pullman, WA; 2) Biological Sciences Division, Pacific Northwest National Laboratory, Richland, WA.

Lipids define the boundaries of cells and organelles, serve as an important fuel source, and participate in a variety of signaling pathways. Disrupted lipid metabolism underlies metabolic syndrome and type 2 diabetes, conditions that are reaching pandemic levels globally. Inside cells, lipids for energy storage are deposited into organelles called lipid droplets. Lipid droplets are comprised of a core of neutral lipids, such as triacylglycerol (TAG), which is surrounded by a phospholipid monolayer. Lipid droplets were previously considered static sites for fat storage, but recent studies suggest they are dynamic and highly regulated organelles. However, our knowledge of the roles of lipid droplets in the context of the development and physiology of a whole organism is still limited.

C. elegans is an ideal model to probe the dynamic roles of lipid droplets. We first sought to biochemically characterize these organelles in wild type (N2) and high fat, *daf-2(e1370)*, mutant worms. We isolated lipid droplets from synchronized young adult worms by mechanical disruption and sucrose gradient ultracentrifugation. Lipidomic analysis confirmed that *C. elegans* lipid droplets are TAG-rich structures with small amounts of phospholipid. Lipid droplets isolated from *daf-2* worms have an increased TAG to phopholipid ratio. The phospholipid composition of both N2 and *daf-2* lipid droplets is predominantly phosphotidylcholine (PC) and phosphotidylethanolamine (PE), with PC in higher abundance. Proteomic analysis of lipid droplets revealed a unique protein composition that agrees well with lipid droplet proteomics results subsequently published by Zhang et al.(2012) Mol & Cell Proteomics, 11(8):317-328. In addition, we found that the lipid droplet proteome was highly similar between N2 and *daf-2* worms. Candidate lipid droplet proteins are currently being assessed for defects in fat storage and development. The dynamic roles of lipid droplet proteins are being explored using translational fluorescent protein fusions.

392A. Genome wide responses to methyl donor availability reveal effects on metabolic, stress response and germline function genes. Wei Ding[1], Michael Irwin[1], Malene Hansen[2], **Amy K. Walker**[1]. 1) UMASS Medical School, Worcester, MA; 2) Sanford-Burnham Medical Research Institute, La Jolla, CA.

Animals must coordinate nutritional input and metabolism with growth and reproduction. Small molecules, such as s-adenosylmethionine (SAMe) produced by the 1-carbon/folate cycle (1CC) and used as the major methyl donor, can modify protein activity and contribute to phospholipid biosynthesis. In *C. elegans*, reducing function of *sams-1*, a SAMe synthase, increases lifespan and lipid droplet accumulation while reducing fertility. We hypothesize that SAMe levels provide signals reseting physiology to compensate for altered nutritional inputs. SAMe is used for methylation of proteins, such as histones in epigenetic modification, but is also critical for production of phosphatidylcholine (PC), a major membrane phospholipid. Indeed *sams-1(RNAi)* animals have low levels of PC and lipid accumulation, fertility and lifespan return to wild-type levels when methylation-independent pathways of PC production are supported through dietary addition of choline. However, links between SAMe levels and direct changes of gene expression are unknown. To separate SAMe-specific effects from combined SAMe/PC functions, we compared global gene expression levels in *sams-1(RNAi)* animals with *sams-1(RNAi)* rescued by choline. Major functional groups of genes increasing with *sams-1(RNAi)* included metabolism (1CC, lipid biosynthesis, carbohydrate modification) and stress responses. However, decreased genes grouped in distinct subsets of metabolic pathways (carbohydrate catabolism, amino sugar metabolism and lipid binding), as well as genes important for germline function and RNA processing. We found that more than 90% of *sams-1(RNAi)* up- or downregulated genes returned to wild-type levels with choline supplementation. Interestingly, genes not returning to wild-type levels clustered in metabolic pathways such as the 1CC, suggesting potential for PC-independent SAMe effects. However, essentially all germline function genes were rescued. This correlates with the near complete rescue of fertility by choline, and suggests coordination of brood size with metabolic function acts through SAMe/PC pathways in these animals.

393B. New path to NAD^+: In addition to salvage biosynthesis, NRK and *de novo* NAD^+ synthesis contribute to NAD^+ recycling in *C. elegans*. **Wenqing Wang**, Matthew R. Lynes, Wendy Hanna-Rose. Penn State Univ, University Park, PA.

NAD^+ is a vital molecule in cellular redox reactions and acts as a cosubstrate for NAD^+ consuming enzymes, which are critical to aging, stress response and energy metabolism homeostasis. NAD^+ salvage biosynthesis is an important pathway to recycle the nicotinamide (NAM) liberated by NAD^+ consumers to rebuild NAD^+ reservoir. Mutation of PNC-1, a nicotinamidase in the NAD^+ salvage pathway in *C. elegans*, results in a series of defects that are individually linked to NAD^+ insufficiency or NAM accumulation[1, 2]. Specifically, NAD^+ insufficiency in *pnc-1* causes gonad developmental delay. Our recent mass-spectrometry data showed that there is a drastic 18-fold increase in NAM levels following *pnc-1* loss of function. However, NAD^+ levels in *pnc-1* mutant only decrease by 30%. These results led us to hypothesize that there are other NAD^+ synthesis pathways contributing to NAD^+ synthesis in *C. elegans*. Here we tested two potential pathways that have been studied in yeast: the NRK pathway and *de novo* NAD^+ synthesis. The NRK pathway uses nicotinamide riboside kinase (NRK) to convert nicotinamide riboside (NR) from the diet to nicotinamide mononucleotide, which can be processed into NAD^+. Mutation of NRK-1 exacerbates gonad developmental delay in *pnc-1*. In addition, supplementing NR rescues gonad developmental delay in *pnc-1* mutants, but not in *pnc-1;nrk-1* double mutants. NAD^+ can also be synthesized *de novo* from tryptophan in yeast. By BLAST, we found that *C. elegans* lacks a key enzyme in *de novo* NAD^+ synthesis, quinolinic acid phosphoribosyl transferase (QPRTase). Surprisingly, supplementing quinolinic acid (QA), the substrate of QPRTase, to *pnc-1* mutants rescues gonad developmental delay. Moreover, RNAi of tryptophan 2,3-dioxygenase, another enzyme in the *de novo* NAD^+ synthesis pathway, exacerbates gonad developmental delay. In conclusion, our results suggest that both NRK and *de novo* NAD^+ biosynthesis pathways actively contribute to NAD^+ synthesis in *C. elegans*. Despite the fact that a QPRTase homologue is missing in *C. elegans*, it is possible that a novel mechanism converts QA into NAD^+. We are working to identify this new mechanism. Reference: 1. Vrablik et al. Development 2009 2. Vrablik et al. Dev Biol. 2011.

394C. *natc-1* mediates stress resistance and dauer formation as a downstream effector of the insulin/IGF-1 signaling pathway. **Kurt Warnhoff**, John T. Murphy, Daniel L. Schneider, Michelle Peterson, Simon Hsu, Kerry Kornfeld. Washington University in Saint Louis, St. Louis, MO.

Animals are exposed to a wide variety of environmental stresses, and they may employ both specific and general strategies to tolerate such stresses. To identify mechanisms that permit *C. elegans* to tolerate high levels of dietary zinc, we screened for mutations that enhanced growth and survival in high levels of supplemental zinc. We identified a *C. elegans* N-terminal acetyltransferase (NAT) gene in this screen, which we named *natc-1*. *natc-1* encodes a protein that is homologous to the vertebrate NAA35, an auxiliary subunit of the NatC complex. NATs function in complexes to catalyze the N-terminal acetylation of proteins, which is a widespread modification that affects the majority of eukaryotic proteins. However, relatively little is known about the biological functions of NATs. We demonstrated that mutations in *natc-1* cause resistance to a broad spectrum of physiologic stressors, including heavy metals, heat, and oxidation. *natc-1* was identified based on resistance to zinc excess, yet the stress resistance conferred by *natc-1* loss-of-function is protective against a wide range of environmental stresses. The *C. elegans* FOXO transcription factor DAF-16 is a key component of the insulin/IGF-1 pathway that mediates general stress resistance and has been predicted to directly bind the *natc-1* promoter. To characterize the role *natc-1* plays in insulin/IGF-1 signaling, we analyzed molecular and genetic interactions with key components of the insulin/IGF-1 pathway. We demonstrated that *natc-1* is transcriptionally repressed by DAF-16 and that mutations in *natc-1* are epistatic to *daf-16* mutations with respect to stress resistance. Furthermore, *natc-1* inhibits dauer formation in a sensitized genetic background. Based on these findings, we hypothesize that *natc-1* functions as a downstream effector of the insulin/IGF-1 signaling pathway to mediate stress resistance and dauer formation. These studies identify a novel biological function for *natc-1* as a modulator stress resistance and dauer formation and define a functionally significant downstream effector of the insulin/IGF-1 signaling pathway.

395A. Screening receptor guanylyl cyclase genes for roles in fat regulation in *C. elegans*. **Emily Witham**, Supriya Srinivasan. The Scripps Research Institute, La Jolla, CA.

The nervous system regulates body fat content and energy balance in response to environmental and endogenous cues through neuroendocrine pathways that are conserved across many species. While the complexity of the mammalian brain makes it difficult to investigate the genes involved in neural circuits that regulate metabolism, the nematode *C. elegans* is a powerful model system with which to study neuronal expression of specific genes that may be related to energy balance, due to its genetic tractability and established neuronal architecture. Studies from our lab suggest that the conserved second messenger cyclic guanosine monophosphate (cGMP) is a regulator of fat storage. We find that the primary effectors of cGMP signaling, the cGMP-dependent protein kinases (PKGs), also regulate intestinal fat. cGMP levels are regulated by two classes of guanylyl cyclases (GCs): soluble and receptor (membrane-bound). Our group has previously studied the effects of the soluble GCs on fat regulation, but the role of the receptor GCs in body fat

has not yet been investigated. *C. elegans* contains an usually high number of receptor GCs (27, whereas only 7 are expressed in mammals), most of which are expressed in sensory neurons. This indicates that these GCs may regulate cGMP levels based on environmental sensory stimuli. The purpose of this work is to identify receptor GCs that may have a role in controlling body fat in *C. elegans*. We are currently conducting a screen of available mutants of the receptor GC genes (*gcy*-1, 3-8, 10, 13-20, 22-27) to test their effects on intestinal fat content by employing Oil Red O staining. Identifying which receptor GCs influence fat stores will begin to illustrate the way in which cGMP signaling regulates fat content, and will ultimately allow us to understand the mechanisms by which environmental cues translate to metabolic regulation.

396B. ATGL-1 requires CGI-58 to Localize to Lipid Droplets Where it Controls Droplet Morphology by Regulating Lipid Exchange and Hydrolysis. **Meng Xie**, Richard Roy. W5/20 Biology, McGill University, Montreal, Quebec, Canada.

The accumulation of excess body fat is associated with a number of major diseases such as type 2 diabetes and certain forms of cancer. C. elegans provides an efficient model to address how fat is stored and hydrolyzed due to its transparent cuticle and its amenability to genetic analysis. Using this model in the past we have shown that AMP-activated protein kinase (AMPK), a metabolic master switch of energy homeostasis that is conserved from yeast to humans, directly phosphorylates and inhibits the activity of adipose triglyceride lipase (ATGL) to protect energy reserves for long term usage. To further understand the consequence of ATGL phosphorylation by AMPK, we generated an antibody against C. elegans ATGL-1 to assess the levels of endogenous protein. We found that ATGL-1 levels were significantly higher in AMPK mutant dauer larvae compared to control daf-2 dauers. We show that AMPK regulates ATGL-1 by signaling its polyubiquitylation and subsequent proteasome dependent degradation. In addition to this level of control, AMPK directed phosphorylation also generated a 14-3-3 binding site on ATGL-1, which was recognized by PAR-5, the C. elegans 14-3-3 protein homologue, which sequesters ATGL-1 away from cellular lipid droplets, thereby reducing its accessibility to substrate. ATGL has been found to function with its co-activator CGI-58 in mammalian cells therefore we tested whether C. elegans CGI-58 could impinge on either or both of these aspects of ATGL-1 function. Mutant dauer larvae that lack AMPK exhibit several metabolic phenotypes that are corrected by removing ATGL-1 and removal of CGI-58 also ameliorates these defects suggesting that these gene products function together in these processes. We provide evidence that CGI-58 physically interacts with ATGL in vivo at both the dauer and adult stages, where they function cooperatively to maintain the appropriate morphology of the lipid droplets. We suggest that CGI-58 affects regulated exchange between the droplets and molecular characterization of the mechanism that underlies the regulation of lipid droplet morphology and lipid transport could potentially shed light on new treatment targets for lipid associated diseases.

397C. Development of a Global-scale Metabolic Network Model of C. elegans. **Lutfu S Yilmaz**, Albertha J Walhout. Program in Systems Biology, UMass Medical School, Worcester, MA.

Life history traits of any organism are dependent upon the conversion of nutrients to energy and biomass via metabolic reactions. While global metabolic reaction networks of many model organisms have been reconstructed and used for the predictive simulations of their metabolism and growth, such a model is as of yet unavailable for C. elegans. Our goal is to develop a metabolic network model of C. elegans that adequately represents its metabolic state and predicts life history traits such as developmental rate and fecundity, by calculating biomass production as body mass or progeny.

The metabolic model is developed in three stages: (1) reconstruction of a reaction network with gene-enzyme-reaction associations based on genomic annotations and literature, (2) conversion of this network to a mathematical model, and (3) application of flux balance analysis (FBA), a method that calculates the flux of all biochemical reactions at the steady state, for the calibration of the model with experimental observations. Initially, we reconstructed a core metabolic network model of C. elegans with 8 central carbon pathways. To develop an animal model, this metabolic network was compartmentalized into three domains representing intestine, germline, and other cells, with an intercellular domain to allow material exchange. FBA simulations were able to reproduce the growth rate during postembryonic development and the reproductive rate at the adult stage, given the bacterial uptake rate (3.2 g bac./g nematode/d). This provided a proof-of-principle for the animal model approach taken. The next step is the extension of the core network to global-scale, for which, 95 metabolic pathways are being considered. The latest generic model involves 770 genes, 450 enzymes, 1334 reactions, and 599 compounds, reconstructed with the help of more than fifty articles for pathway confirmation or identification. The growing metabolic model of C. elegans is expected to become a comprehensive resource that integrates the knowledge on this organism's metabolism and allows the systematic analysis of genotypic and phenotypic information at an unprecedented level using mathematical procedures.

398A. Investigating the role of neuropeptides in regulating body fat levels in *C. elegans*. **Yorke Zhang**, Supriya Srinivasan. Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

The nervous system is a major regulator of fat levels and energy balance across many different species. While several studies in *C. elegans* have defined the molecular signaling mechanisms within neural circuits that govern organismal energy balance, the signaling mechanisms that act between the nervous system and the intestine (the major site of fat storage in *C. elegans*) remain largely unknown. Because no neurons are known to synapse directly onto intestinal cells, the players that mediate communication between the nervous system and intestine are likely long-range neuroendocrine signaling molecules. Neuropeptides are excellent candidates for these unknown signaling molecules. To more comprehensively investigate the role of neuropeptides in regulating fat levels, we are currently conducting a screen of available mutants of neuropeptide genes (*nlp*, *flp*, *ins*) and assessing their intestinal fat content by Oil Red O staining. Additionally, we are assessing the fat phenotypes of the neuropeptide processing genes. Identifying which neuropeptides are involved in regulating fat levels and where they are expressed will allow us to bridge the gap in our understanding of how neural circuits regulate intestinal fat stores.

399B. Biological function of PUFA-derived eicosanoids in *Caenorhabditis elegans*. **Yiwen Zhou[1]**, Jingjuan Ju[2], Erik Nehk[1], Lihong Yin[2], Chrisitan Steinberg[1], Ralph Menzel[1]. 1) Department of Biology, Humboldt-Universität zu Berlin, Germany; 2) Key Lab. Environ. Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, China.

Mammals and *C. elegans* share similar basic mechanisms of cytochrome P450 (CYP)-dependent eicosanoid formation. These eicosanoids are sets of regioisomeric epoxy- and hydroxy-derivatives derived from, typically, 20-carbon polyunsaturated fatty acids (PUFA). So far as is known from GFP

expression experiments, *C. elegans* produces its eicosanoids in the marginal cells. Here we tested the hypothesis that these signaling molecules known to modulate the contractility of cardiomyocytes and vascular smooth muscle in mammals serve as regulators of muscle activity in the nematode, too. In mammals diet primarily controls PUFA and eicosanoid pattern, whereas *C. elegans* can synthesize all PUFA *de novo*. Because this synthesis requires the activity of several specific desaturases, their loss of function were found to cause dramatic changes in derived eicosanoid pattern. First, we examined the frequency of pharyngeal pumping of wild type and several mutant strains under different conditions, with and without food and in response to neurotransmitters (serotonin, glutamate, octopamine, and acetylcholine). The obtained results suggest that eicosanoids seem to be not essential for the reactions to neurotransmitters or food availability. Second, we analyzed the effect of supplementing the medium with different eicosanoids, both in the wild type and PUFA- or CYP-deficient mutant strains. Here we found a clear rescue of impairments of pharyngeal pumping as well as locomotion of the *fat-3(wa22)* strain, not able to produce any 20-carbon eicosanoid, and *emb-8(hc69)*, characterized by an almost complete depletion of active NADPH-dependent CYP-reductase, an essential part of each CYP monooxygenase system in *C. elegans*. The CYP-derived metabolism of PUFA to eicosanoids is also strictly dependent on sufficient oxygen supply. In this respect we could show that eicosanoid pattern modulated *C. elegans*' response to anoxia. The removal of oxygen resulted in a significant higher initial sensitivity of eicosanoid-free or -depleted strains in comparison to the wild type, but became marginalized after long-time exposure.

400C. Neuronal serotonin signaling through G proteins negatively regulates the *C. elegans* immune response. **Alexandra Anderson**, Henry Laurenson-Schafer, Rachel McMullan. Department of Life Sciences, Imperial College London, London, United Kingdom.

In order to overcome pathogenic threats to its survival *C. elegans* uses neuro-immune communication, integrating signals from the nervous system and an appropriate immune response. Infection of *C. elegans* with the coryneform bacterium *Microbacterium nematophilum* results in a tail swelling known as the dar phenotype, which forms part of the innate immune response. This swelling response is associated with changes in the morphology of cells in the anal region of the animal, including the rectal epithelial cells and correlates with an increased ability to clear the pathogen from this region. Using the interaction between *C. elegans* and *M. nematophilum* as a model, we have investigated the role of serotonin-GOA-1 signaling on the innate immune response to infection. Addition of exogenous serotonin during *M. nematophilum* infection suppresses the dar phenotype with a consequent reduction in the animal's ability to clear the pathogen. Conversely reduction in the activity of tryptophan hydroxylase 1 (TPH-1), the rate-limiting enzyme in the biosynthesis of serotonin increases the ability of *C. elegans* to clear *M. nematophilum* from the anal region. Specific neuronal rescues reveal the amphid ADF sensory neuron as the site of action mediating serotonin's effect on the immune response. The regulator of G protein signaling EGL-10, which specifically negatively regulates GOA-1 is required in the rectal epithelium for the dar phenotype to occur in response to infection. Loss-of-function mutations in EGL-10 can suppress the dar phenotype, even in animals with loss-of-function mutations in TPH-1. This suggests that serotonin acts non-autonomously to regulate GOA-1 signaling in the rectal epithelium. Our data highlight the complex neuro-immune cross talk involved in innate immunity.

401A. Microsporidia infection regulates ubiquitin- and chromatin remodeling-associated gene expression in the *C. elegans* intestine. **Malina A. Bakowski**[1], Chistopher A. Desjardins[2], Tiffany L. Dunbar[1], Christina A. Cuomo[2], Emily R. Troemel[1]. 1) Division of Biological Sciences, University of California San Diego, La Jolla, CA; 2) The Broad Institute of MIT and Harvard, Cambridge, MA.

Microsporidia comprise a large phylum of ubiquitous intracellular pathogens that can infect almost all animals, including humans. These obligate pathogens cannot grow independently yet grow very rapidly after invading host cells. Little is known about the pathogenic mechanisms used by microsporidia for growth and infection, or about the host response to these pathogens. The model organism *C. elegans* is a natural host for the microsporidian species *Nematocida parisii*, which causes a lethal intestinal infection. We used RNAseq to analyze *C. elegans* gene expression response to *N. parisii* at distinct stages of infection, and found a unique gene expression pattern. First, microsporidia infection induced expression of *C. elegans* E3 ubiquitin ligase components, specifically the greatly expanded family of F-box domain-encoding genes postulated to act as substrate adapters that target proteins for ubiquitylation. Moreover, infection promoted ubiquitylation in the host intestine and may represent an immune response of *C. elegans* to intracellular infection. Second, we also discovered that infection with *N. parisii* mimicked gene expression that occurs upon perturbation of synMuvB pathway components, which encode chromatin and transcriptional regulators. We used promoter-GFP fusions to demonstrate that some of these genes are induced in the intestine upon infection. The synMuvB pathway controls many aspects of *C. elegans* development including the germline-soma differentiation of tissues during embryogenesis, and cell proliferation. Thus, microsporidia infection may reprogram host cells into a less differentiated state, which may facilitate efficient replication of the parasite and/or promote host survival.

402B. Genetic architecture underlying variation in Caenorhabditis elegans host resistance to a natural pathogen. **Keir M. Balla**[1], Erik C. Andersen[2], Leonid Kruglyak[3], Emily R. Troemel[1]. 1) Division of Biological Sciences, UCSD, La Jolla, CA; 2) Molecular Biosciences, Northwestern University, Evanston, IL; 3) Lewis-Sigler Institute for Integrative Genomics, Princeton, NJ.

Animals are embedded in complex co-evolutionary relationships with pathogens, and the specific genetic consequences associated with most of these interactions remain undefined. Microsporidia are fungal-related obligate intracellular pathogens of all animal phyla, yet the genetic architectures underlying host resistance mechanisms are unknown. We demonstrate natural variation among strains of Caenorhabditis elegans in their ability to resist Nematocida parisii, a microsporidian species isolated from wild-caught nematodes. Resistance varies between the N2 and CB4856 strains post-invasion, mediating the growth of the pathogen and host survival. Furthermore, resistance is dominant, which suggests that it is due to an enhanced immune response. We utilize quantitative genetic analyses to map the genetic architecture of resistance to at least five quantitative trait loci. Two of these loci are confirmed contributors to resistance or susceptibility to N. parisii, and act additively in mediating resistance. These results exhibit the complex genetic nature of resistance mechanisms that have evolved under a ubiquitous host-pathogen interaction, and pave the way for establishing the molecular bases of these interactions.

403C. The tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induces mitochondrial and nuclear DNA damage in Caenorhabditis elegans. **Rakesh Bodhicharla**[1], Joel Meyer[1], Prasad G.L[2]. 1) Nicholas School of the Environment, Duke University, Durham, NC 27708; 2) R.J. Reynolds Tobacco Company, PO Box 1487,Winston-Salem, NC 27101.

The metabolites of the tobacco-specific carcinogen NNK form DNA adducts in animal models. One report indicates that NNK could cause damage to the mitochondrial as well as nuclear genome in rats (Stepanov and Hecht, 2009 Chem. Res. Toxicol. 22: 406-414). Using a different DNA damage detection technology, we tested whether this could be repeated in the nematode Caenorhabditis elegans; we also evaluated whether mitochondrial function would be affected. We treated N2 strain (wild-type) nematodes with NNK in liquid culture. Quantitative PCR was applied to analyze NNK-induced nuclear and mitochondria DNA damage. This assay has the advantage of measuring all DNA lesions that inhibit the DNA polymerase, and normalizes results to mitochondrial DNA copy number (Hunter et al., 2010 Methods 51:444-451). Our results confirm that NNK causes both nuclear and mitochondrial DNA damage, but surprisingly nuclear DNA damage was greater than mitochondrial DNA damage in C. elegans. To test whether the mitochondrial DNA damage was associated with mitochondrial dysfunction, we used a transgenic nematode strain that permits in vivo measurement of ATP levels and found lower levels of ATP in NNK-exposed animals when compared to the unexposed controls. To test whether the lower levels of ATP were due to the inhibition of respiratory chain components we investigated oxygen consumption in whole C. elegans and found reduced oxygen consumption in exposed animals when compared to the unexposed controls. Our data suggest a model in which NNK causes damage to both nuclear and mitochondrial genomes, and support the hypothesis that the mitochondrial damage is functionally important. These results also represent a first step in developing this genetically tractable organism as a model for assessing NNK toxicity.

404A. Enhanced RNA virus susceptibility results from defects in lipid metabolism. **Gina Broitman-Maduro**[1], Saige Pompura[2], Stephanie Coffman[3,4], Yuanyuan Guo[3], Francisco Carranza[2], Shou-Wei Ding[3], Morris Maduro[1]. 1) Dept Biol, Univ California, Riverside, Riverside, CA; 2) MarcU star,Univ California, Riverside, Riverside, CA; 3) Dept Plant Pathology and Microbiology,Univ California, Riverside, Riverside, CA; 4) Genetics, Genomics and Bioinformatics,Univ California, Riverside, Riverside, CA.

Prior work has shown that C. elegans can support replication of (+) strand RNA viruses, including the Flock House Virus (FHV), and the Orsay virus. We have previously reported that transgene expression of FHV RNA1, which encodes the viral RdRP and a viral suppressor of RNAi (B2), can direct its own replication (Lu et al., 2005). By replacing the coding region of B2 with that of GFP (FR1gfp) we can visualize viral replication in backgrounds that are virus-susceptible. In an RNAi screen for factors that influence replication of FR1gfp (see abstract by Stephanie Coffman), we identified genes that are known to affect storage or metabolism of lipids, such as nhr-68 (Ashrafi et al., 2003). To evaluate the relationship between lipid storage and RNA virus replication, we examined fat stores using Oil Red O in chromosomal mutants for other genes identified by the screen, and directly assessed viral replication in strains that are known to have fat storage defects. Our results confirm that conditions known to cause increases in fat stores, such as the germlineless glp-4(bn2) background, result in a significant increase in viral replication. As lipid storage defects have been recently shown to be correlated with autophagy (Hansen et al., 2013), we have tested the role of autophagy in lipid storage-mediated viral susceptibility. Overexpression of the lipase lipl-4 appears to be sufficient to increase viral susceptibility, in a manner that is partially dependent on the autophagy gene atg-1. The connection between lipid storage and susceptibility to RNA virus replication is not immediately obvious, and our data suggest that the interactions are complex, though one possibility is that autophagy regulates some components of the RNAi machinery (Gibbings et al., 2012). Results of characterization of viral replication (both FR1gfp and Orsay virus) in various mutant backgrounds will be presented.

405B. Antagonistic cGMP Signaling Pathways Regulate a Heritable Developmental Response to Pathogens. **Nicholas Burton**, Bob Horvitz. HHMI Dept. Biology, MIT, Cambridge, MA.

Recently a number of studies have linked environmental stress to changes in the physiology of an individual's progeny. To test whether maternal infection affects progeny, we fed C. elegans either Pseudomonas aeruginosa (PA14) or Serretia marcesens (DB10). We found that wild-type parental (P0) worms slow their rate of development when fed either PA14 or DB10 in comparison to OP50. In the following generation, when again exposed to pathogen, the progeny (F1) exhibit a dramatically enhanced slowing of development compared to that of naïve worms. However, when the progeny of infected worms are fed OP50, they exhibit no difference in developmental rate compared to naïve worms, indicating that both the P0 and F1 require exposure to pathogen to result in enhanced slowing. We showed that developmental slowing requires the guanylyl cyclase GCY-36 and the cGMP-dependent kinase EGL-4 as well as the insulin-like peptide INS-7. In contrast to worms unable to slow their development, worms lacking either the guanylyl cyclase GCY-33 or the serotonin biosynthetic enzyme TPH-1 exhibit an enhanced slowing of development. In the case of *gcy-33*, this enhancement can be suppressed by mutations in *gcy-36* and *egl-4*.

(Zimmer et, al. Neuron 61 865, 2009), showed that *gcy-33* acts together with *gcy-31* to mediate responses to low oxygen and that *gcy-36* acts together with *gcy-35* to mediate responses to high oxygen. They also showed that *gcy-33*, *gcy-35* and *gcy-36* promoters drive expression in AQR, PQR and URX neurons. The *gcy-31* promoter, however, does not. Interestingly, we observed that mutations in *gcy-31* do not affect development in response to PA14. Furthermore, we found that genetic ablation of AQR, PQR and URX results in a similar phenotype to that of *gcy-33* mutants. These results suggest that the defect in developmental rate of *gcy-33* mutants is separable from the defect in the response to low oxygen. In summary, we have found that parental bacterial infection influences the development of progeny and that antagonistic cGMP signaling pathways regulate this response at least in part through the AQR, PQR, and URX neurons.

406C. Host-finding strategies of mammalian-parasitic nematodes. **Michelle Castelletto**, Ryo Okubo, Anastassia Tselikova, Elissa Hallem. Microbio, Immuno, Mol. Genetics, UCLA, Los Angeles, CA.

Soil-dwelling nematodes use environmental sensory cues to locate resources and potential mates. Many parasitic nematodes also use these cues to locate appropriate hosts. The human parasite *Strongyloides stercoralis* and the rat parasites *Strongyloides ratti* and *Nippostrongylus brasiliensis* are skin-penetrating nematodes that spend a portion of their life cycle in the soil. We are interested in how these worms use host-derived odorants to search for hosts. We examined the responses of *S. stercoralis*, *S. ratti,* and *N. brasiliensis* infective juveniles (IJs) to carbon dioxide (CO_2), an important host cue for

entomopathogenic nematodes (EPNs) as well as many hematophagous insects. We found that mammalian-parasitic IJs are not attracted to CO_2, suggesting they rely instead on host-specific cues. We then examined the responses of these IJs to a large panel of human odorants, and found that many of these odorants attract parasitic IJs. Moreover, many of the odorants that elicited the strongest responses from *S. stercoralis* have also been shown to attract mosquitoes, suggesting that human-parasitic nematodes and mosquitoes use similar olfactory cues. These results raised the possibility that insect repellents might be effective against parasitic nematodes. However, we found that some insect repellents do not repel mammalian-parasitic nematodes, and in fact *S. stercoralis* and *S. ratti* are attracted to the insect repellent DEET. A comparison of the odor response profiles of the mammalian-parasitic IJs to those of EPN IJs and *C. elegans* dauers revealed that olfactory preferences reflect host range rather than phylogeny, suggesting an important role for olfaction in the evolution of host specificity among parasitic nematodes. Finally, we found that *S. ratti* IJs respond differently to odorants than *S. ratti* non-infective larvae and adults, indicating that the olfactory preferences of at least some parasitic nematodes are stage-specific. We are now expanding these studies to additional species of parasitic nematodes, including some that are passively ingested and some that have more diverse host ranges. We are also investigating the neural basis of odor-driven host-seeking behaviors in mammalian-parasitic nematodes.

407A. Characterization of *Vibrio parahaemolyticus* virulence factors using *Caenorhabditis elegans*. **Hediye N. Cinar**[1], Surasri N. Sahu[1], Sungji Kim[1,2], Augusto A. Franco[1], Christopher Grim[1], Justin Hahn[1,3], Ben D. Tall[1], Mahendra Kothary[1], Atin Datta[1]. 1) Div Virulence Assessment, Food & Drug Admin, Laurel, MD; 2) Kyungpook National University (KNU), Daegu, South Korea; 3) University of Maryland, College Park, MD.

Vibrio parahaemolyticus (Vp), a Gram-negative marine bacterium, is an emerging pathogen causing gastroenteritis, wound infection, and septicemia. Gastroenteritis caused by Vp is associated with the production of thermostable direct (TDH) and TDH-related hemolysins (TRH), and the inflammatory responses are produced by effector proteins secreted by a type III secretion system (T3SS-2a, or b) contained within pathogenicity islands (1). In order to study innate immune responses to known virulence factors, and to discover new virulence factors, we used *Caenorhabditis elegans* as a host organism. Vp isolates, previously serotyped and characterized by PCR for the presence of T3SS-2, TRH, TDH, and urease genes, were tested using a *C. elegans* lethality assay for their virulence. Six groups of Vp strains, each representing the following genotypes, were tested in the *C. elegans* model: 1) *urease+ trh+ tdh+* T3SS-2b+, 2) *urease+ trh+ tdh-* T3SS-2b + , 3) *urease+ trh- tdh-* T3SS-2b + , 4) *urease+ trh- tdh+* T3SS-2a+ , 5) *urease- trh- tdh-* T3SS-2- , 6) *urease- trh- tdh+* T3SS-2a + . Median survival rates of worms tested against Vp isolates ranged between five and sixteen days. Vp strains with functional *urease* gene loci resulted in shorter median survival rates of *C. elegans*. Our data suggest a strong correlation between the presence of Vp gene and *C. elegans* lethality. These results taken together with those previously reported by Honda et al.(2) that urease-positive, TDH- and TRH-negative strains were also capable of causing intestinal fluid accumulation in the rabbit ligated ileal loop assay, suggest a significant role for urease in disease development. Alternatively, urease might be needed to activate other virulence factor/s or a marker linked with a currently unknown virulence factor gene(s) in Vp. Further molecular and functional characterization of urease in *C. elegans* infection, is in progress. (1) Ham H, Orth K, 2012 (2) Honda S, et al. 1992.

408B. Role of OCTR-1 expressing neurons during pathogen infection. **Argenia L. Doss**, Alejandro Aballay. Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

Recent studies from our laboratory showed that OCTR-1, a G protein-coupled catecholamine receptor, down-regulates genes required for innate immunity. Thus, *octr-1(ok371)* animals exhibit enhanced resistance to killing by the human opportunistic pathogen *Pseudomonas aeruginosa*. While OCTR-1 is expressed in five neurons, data suggest that OCTR-1 functions in chemosensory neurons ASI and/or ASH to suppress innate immunity. However, the specific role of ASI and ASH neurons is unclear. Therefore, we examined the role of ASI neurons during infection by measuring the susceptibility of an ASI-ablated strain to *P. aeruginosa*-mediated killing. Our experiments revealed that animals lacking ASI neurons exhibited enhanced susceptibility to *P. aeruginosa*-mediated killing when compared to wild-type animals, indicating that ASI is protective against infection. Genetic ablation of ASI cells also suppresses the enhanced resistance to *P. aeruginosa*-mediated killing of *octr-1(ok371)* animals, suggesting that OCTR-1 may suppress innate immunity by inhibiting ASI neurons. Ongoing experiments to determine the immune function of animals expressing OCTR-1 in ASH or ASI neurons will help us to further address the role of ASI and ASH neurons during pathogen infection. Thus far, our data suggest a role for ASI neurons in the control of innate immunity and further elucidates the mechanism by which OCTR-1 regulates the process.

409C. Tissue Tropism and Infection Characterization of Novel Viruses in Caenorhabditis Nematodes. **Carl J. Franz**[1], Hilary Renshaw[1], Lise Frezal[2], Yanfang Jiang[1], Marie-Anne Félix[2], David Wang[1]. 1) Molecular Microbiology, Washington University, Saint Louis, MO; 2) Institute of Biology of the Ecole Normale Supérieure, CNRS UMR8197, Inserm U1024, Paris, France.

Orsay, Santeuil and Le Blanc viruses were recently discovered, enabling for the first time the study of virus-host interactions using a natural pathogen in the well-established model organism Caenorhabditis elegans and its relative Caenorhabditis briggsae. All three viruses share less than 50% amino acid identity and are most closely related to nodaviruses, which are positive sense RNA viruses with bipartite genomes. Comparison of their complete genomes demonstrated unique coding and noncoding features absent in known nodaviruses. Le Blanc virus, similar to Santeuil virus, was capable of infecting wild C. briggsae isolates but not the AF16 C. briggsae laboratory reference strain nor any tested C. elegans strains. We characterized the tissue tropism of infection in Caenorhabditis nematodes by all three viruses. Using immunofluorescence assays targeting viral proteins, as well as in situ hybridization, we demonstrated that viral proteins and RNAs localized primarily to intestinal cells in larval stage Caenorhabditis nematodes. The viral proteins could be detected in one to six of the 20 intestinal cells present in Caenorhabditis nematodes. In Orsay virus-infected C. elegans, viral proteins could be detected as early as six hours post infection. Furthermore, the RNA-dependent RNA polymerase and capsid proteins of Orsay virus exhibited different subcellular localization patterns from each other. Collectively, these observations broaden our understanding of viral infection in Caenorhabditis nematodes.

410A. *Caenorhabditis elegans* as a Model to Study Parasite-Induced Alterations in Host and Gut Microbiota Interaction. **Teklu Gerbaba**[1], Xin Wang[1], Kevin Rioux[2], Dave Hansen[1], Andre Buret[1]. 1) Biological Sciences , University of Calgary, Calgary, Alberta, Canada; 2) Department of Medicine, University of Calgary, Calgary, Alberta, Canada.

Increasing evidence suggests a role for changes in gut microbiota in the pathophysiology of Irritable Bowel Syndrome (IBS) and Inflammatory Bowel

Diseases (IBD). Acute gastroenteritis caused by enteric pathogens (including *Giardia* sp.) has been implicated in the onset or exacerbation of pathophysiology of these disorders, but the mechanisms remain obscure. The aim of this study is to develop a model system to investigate how an enteropathogen, *Giardia*, may lead to loss of gut homeostasis via alterations of host and gut microbiota interaction. *Giardia* and non-pathogenic *E. coli* (*E. coli* OP50, *E. coli* HB101) together resulted in paralysis and killing of *C. elegans*, while individually they had no effect on worm survival. *Giardia* conditioned media and *E. coli* are sufficient to kill *C. elegans* suggesting that killing is induced by secreted/excreted factor(s) released from *Giardia*, but worm killing induced by *Giardia* requires *C. elegans* direct contact with *E. coli*. Heat treatment of *Giardia* conditioned media failed to inhibit *C. elegans* killing, and *Giardia* only induced *C. elegans* killing in the presence of live *E. coli*. The effect of *Giardia* can also be observed in the presence of attenuated-virulence strains of *C. rodentium* (ESPF and MAP mutants), healthy individual gut microbiota, and inflamed site UC (Ulcerative Colitis) patient's colon microbiota, but *Giardia* has no effect in the presence of non-inflamed site UC patient's colon microbiota suggesting functional difference in the microbiota obtained from the two sites. The results mirror the synergistic effect of Giardia and normal gut micorbiota observed in germ free mice studies. Hence, *C. elegans* may be a useful model system for the study of *Giardia*, gut microbiota, and host interactions. In addition to providing new insights into the contribution of gut microbiota in the development of gastroenteritis, this system will provide a novel approach for the study of the role of gut microbiota in idiopathic functional gastrointestinal disorders.

411B. A key role of mitochondrial dynamics, cytochrome c release and IP3R activity in muscular dystrophy. Jean Giacomotto[1,2], Nicolas Brouilly[1], Marie-Christine Mariol[1], Laurent Segalat[1], **Kathrin Gieseler[1]**. 1) Claude Bernard University Lyon1, CGphiMC, CNRS-UMR5534, Villeurbanne, France; 2) Brain and Mind Research Institute, Sydney Medical School, University of Sydney, NSW 2050, Australia.

Duchenne Muscular Dystrophy (DMD) is a severe muscle degenerative disease caused by mutations in the dystrophin gene. The cellular mechanisms that trigger muscle degeneration are still poorly understood, and to date no curative treatment is available. Using a C. elegans model for DMD in a chemical genetics approach, we found that Cyclosporine A reduces dystrophin-dependent muscle degeneration at low dose but loses its positive effect at high dose. Our data indicate that its beneficial effect at low doses involves a C. elegans mitochondrial cyclophilin D homolog, CYN-1, suggesting an implication of mPT in muscle degeneration in C. elegans. This finding provides the first indication that mPT can occur in C. elegans. Furthermore, we found that mitochondrial dynamics is dramatically affected at the early stages of muscle degeneration and that the knockdown of the fission promoting gene drp-1 reduces both mitochondrial fragmentation and muscle degeneration. This observation suggests i) that mitochondrial fragmentation observed in dystrophic muscles is due to increased mitochondrial fission, and ii) that mitochondrial fission plays a key role in the early processes of muscle degeneration. We also discovered that cytochrome c and its interplay with the calcium channel Inositol trisPhosphate receptor (IP3R) contribute to the abnormal mitochondrial fission and are critical for the progression of dystrophin-dependent muscle degeneration. Thus, our study unveils for the first time, to our knowledge, that in C. elegans cytochrome c is involved in muscle cell death by acting, at least partially, through an interaction with IP3R/ITR-1.

412C. Transcriptome analysis of ALS-associated Mutants *fust-1* and *tdp-1*. **Patrick K. Gonzales**. Integrative Physiology, University of Colorado Boulder, Boulder, CO.

ALS is an adult-onset neurodegenerative disorder characterized by premature degeneration of upper and lower motor neurons eventually leading to fatal paralysis. Approximately 10% of disease instances are familial, with several dominant mutations identified. TDP43 (TAR DNA-binding protein 43) and FUS (fused in sarcoma), are two RNA binding proteins whose mutations are present in a subset of familial ALS cases. Interestingly, the two proteins share structural and functional similarities. Both contain RNA recognition motifs, have glycine rich regions, and both are associated with multiple steps of RNA processing including transcription, RNA splicing, RNA transport and translation. ALS-associated mutations in TDP-43 and FUS suggest defects in RNA processing could result in ALS. In order to understand which RNA processing defects are central to the disease process, it is important to determine which RNA transcripts depend on both proteins for correct abundance and processing. To answer this question we analyzed the transcriptome of strains deleted for the C. elegans homologs of TDP-43 and Fus, *tdp-1* and *fust-1*. Deletion alleles, *tdp-1(ok803)* and *fust-1(tm4439)*, are viable and display only mild phenotypes. Previous high throughput sequencing analysis from our lab showed that *tdp-1(ok803)* preferentially binds transcripts with potential dsRNA structure and limits this structure, particularly within introns. In this study we asked if *fust-1* mutants have a similar expression profile as the *tdp-1* mutant. We used high throughput sequencing of N2, *tdp-1(ok803)*, and *fust-1(tm4439)* to characterize altered transcripts in both mutants from total RNA and from RNA captured using a dsRNA specific antibody (J2). The results of this comparison are reported.

413A. Identification of molecular networks that modulate intestinal *ilys-3* activity in response to danger signals and bacterial challenges. **Maria J. Gravato-Nobre**, Suzanne Jordan, Sophie Andrews, Jonathan Hodgkin. Dept Biochemistry, Univ Oxford, Oxford, Oxfordshire, United Kingdom.

In multicellular organisms cellular damage may induce alarm signals that trigger innate immune responses. Here we used the model bacterial pathogen, *Microbacterium nematophilum* to investigate the responses that lead to a chronic colonization of the digestive tract of *C. elegans*. In the nematode, infection by this Gram-positive pathogen activates intestinal expression of immune effectors such as invertebrate lysozymes; most dramatically *ilys-3* but also *ilys-1, ilys-2* and *ilys-6*, a newly annotated member of this family encoded by W03D2.7.We show that *ilys-3* is required for normal pharyngeal grinder function and defense against pathogen infection. Following *M. nematophilum* colonization, the intestinal induction of *ilys-3* transcription is regulated through the pharyngeal action of ERK MAP kinase signalling pathway in a cell non-autonomous manner. Such intercommunication suggests a mechanism whereby pharyngeal cells sense and propagate danger signals and further instruct antimicrobial responses in distal tissues. In addition to the pathogen-induced pathway, we found that *ilys-3* promoter activity is highly responsive to nutrient depletion. Arrested L1s, or L4s that were removed from food for 24 hours, show enhanced intestinal expression of an *ilys-3* reporter. However, and in contrast to the bacterial-mediated response, inactivation of ERK/MAPK did not block induction of the reporter in the intestine. To dissect the molecular mechanisms that modulate dietary and immune responses, and to investigate the signals that control the expression of *ilys-3*, we developed a hunger sensor strain. This has an integrated array with GFP expression under the control of the *ilys-3* promoter. We performed a preliminary forward genetic screen to identify genes that can alter *ilys-3* transcriptional activity on the standard OP50 or on *M. nematophilum* diets. Two categories of mutants were identified. The first group includes the mutants that exhibit increased constitutive intestinal GFP expression in animals reared on OP50. The second group includes mutants that failed to induce *ilys-3* expression on the

pathogen.

414B. Whole genome expression analysis of *C. elegans* upon recovery from *Salmonella enterica* infection. **Brian P. Head**, Alejandro Aballay. Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC.

C. elegans are capable of mounting an immune response to a variety of pathogens. In the last decade, several major steps in the *C. elegans* innate immune response have been dissected including: 1) host surveillance pathways detect a pathogen and/or loss of homeostasis, 2) various intracellular signaling modules are activated in distinct cell types, and 3) immune effector molecules are produced at the site of infection (primarily the intestinal and hypodermal epithelia). Moreover, stress response pathways are activated to cope with cellular damage from both host immune effectors and pathogen-derived molecules.

Much less is known about the physiology of the animal during recovery from an infection. Thus, we are utilizing the *C. elegans*-pathogen model to study recovery from infection by *Salmonella enterica*. After generating a "mild" *Salmonella* infection in the worm, we alleviate this infection by a combination of antibiotic treatment plus a change in food source. We demonstrate that "recovered" worms have a reduced bacterial burden and enhanced survival. To better understand the transcriptional response during recovery, we have utilized whole genome microarray technology. A variety of expected and novel gene clusters are altered during recovery. Representative genes from several clusters and other interesting candidates were confirmed by qRT-PCR. We are in the process of testing these genes for effects on recovery using standard reverse genetic techniques.

415C. Observation of *Legionella pneumophila* infectious cyst-like forms in the host model *Caenorhabditis elegans* . **Jacqueline Hellinga**, Ann Karen Brassinga. Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada.

The environmental bacterium *Legionella pneumophila* is an intracellular parasite of aquatic protozoa. *Legionella* exhibits a distinct dimorphic lifecycle that alternates between vegetative replicative form (RF) and infectious cyst-like form (CLF). Upon completion of its life-cycle, release of *Legionella* back into the water is facilitated by the lysis of the protozoan host cell. Inadvertent inhalation of aerosolized *Legionella*-laden water droplets by susceptible individuals may lead to infection of alveolar macrophages causing the atypical pneumonia Legionnaires' disease. Upon infection of macrophages, *Legionella* employs mechanisms to alter and evade the macrophage innate immune response. The Dot/Icm type IV protein secretion system is a key virulence system for *Legionella* as secreted bacterial effector molecules specifically interact with the macrophage cellular processes. To date, some of the more than 200 effector molecules have been identified to delay cell death and remodel the host cell cytoplasm for establishment of an intravacuolar replicative niche. To further delineate the mechanisms by which *Legionella* alters the innate immune response, we utilize the nematode *C. elegans* as a host model to investigate *Legionella* immunopathogenesis. Core components of the *C. elegans* innate immune system are functionally conserved with those comprising the macrophage innate immune system. Here we show by germline apoptosis counts that Dot/Icm secreted effector molecules interact with the *C. elegans* PCD pathway. Furthermore, Differential Interference Contrast (DIC) microscopy of live nematodes infected with *Legionella* revealed intravacuolar niches containing motile forms morphologically similar to CLFs. The majority of these niches have been observed to be located in the coloemocytic space, and the gonad, as well as expelled from the vulva. Transmission Electron Microscopy (TEM) revealed cyst-like forms of *Legionella* crossing the epithelia layer of the intestine. Taken together, these findings provide insight on the plausible evolutionary origin of the ability of *Legionella* to manipulate the macrophage innate immune system considering the lack of a defined innate immune system in its natural protozoan host cell.

416A. PTR-15/BUS-13: A patched-related protein affecting surface properties and susceptibility to multiple surface pathogens. Dave Stroud[1], Alexis Tchaconas[1], Patricia Kuwabara[2], **Jonathan Hodgkin[1]**. 1) Dept Biochem, Univ Oxford, Oxford, United Kingdom; 2) Dept Biochem, Univ Bristol, Bristol, United Kingdom.

PTR-15 is one of 24 *C. elegans* proteins with homology to the Hedgehog receptor Patched. PTC and PTR proteins carry both sterol-sensing and RND domains; previous work (Zugasti et al. 2005) showed that several of the PTR proteins are involved in molting. However, RNAi knockdown of *ptr-15* revealed no obvious defect. Whole Genome Sequencing has now revealed that a mutation in a pathogen-sensitivity gene, *bus-13 (e2710)*, is a missense alteration in *ptr-15.* The *bus-13* mutant was isolated by virtue of its resistance to the surface pathogen *Microbacterium nematophilum*. *bus-13* mutants also show variable alterations in staining with multiple fluorescent lectins (Gravato-Nobre et al. 2005). Response of *bus-13* to other surface pathogens is unusual. Most *bus* mutants are killed by either of two complementary Leucobacter strains (Verde1 and Verde2), but not both. In contrast, *bus-13* mutants are rapidly killed by both. The lethal sensitivity to Verde1 provides an excellent assay for *ptr-15* activity: a transgene expressing *ptr-15(+)* allows *bus-13* mutants to grow well in the presence of Verde1, confirming that *bus-13* is the same as *ptr-15*. Many genes affecting the cuticle surface display seam-cell expression (Gravato-Nobre et al. 2011). Surprisingly, the intestine and pharynx, and not the seam cells, are the main sites of expression of a *ptr-15p::ptr-15::rfp* operon rescuing construct. A C-terminal translational fusion construct also exhibited some gut expression, but failed to rescue. Further translational constructs are in progress. Also surprisingly, selection for mutations that allow *bus-13* to grow on Verde1 failed to yield mutations in any of the known and expected Verde1-resistance loci (~ 8 genes); instead, an apparent intragenic partial revertant of *bus-13* was obtained. Similarly, selection for mutations that would allow *bus-13* to grow on Verde2 did not yield many mutations in the expected Verde-2 resistance loci (~ 20 genes). These observations suggest that the pathogen-susceptibility of the *ptr-15/bus-13* mutant is unusual and distinct from that of most other surface-affecting genes.

417B. Using *C. elegans* to identify the nematode phosphorycholine transferase. **Kevin J Jenson**, Patricia M Berninsone. Biology, UNR, Reno, NV.

Parasitic nematodes afflict an estimated 120 million humans worldwide, and at least one billion more are at risk of infection in endemic areas. Available chemotherapeutics target only distinct larval stages and show severe side effects. Furthermore, reported resistance to certain anthelminthics indicates the need for development of new drugs. Nematode-specific biomolecules and the enzymes involved in their biosynthesis provide a rich field for drug target discovery. Phosphorylcholine (PC), a small haptenic molecule, is found linked to glycans in many parasitic nematodes, but not in mammals. PC modification of proteins by pathogens has been implicated in mediating host-pathogen interactions through immunomodulation. This is done by inducing a Th2-type anti-inflammatory response while simultaneously inhibiting T- and B- cell production. Thus, immune attention is diverted away from fighting the parasite. Because phosphorylcholination is not utilized by mammalian cells, the PC transferase catalyzing this reaction makes an attractive anthelminthic drug

target. A major barrier to exploring the PC modification as a drug target is that no protein catalyzing the PC transferase reaction has been identified in eukaryotes.

The free-living nematode *C. elegans* synthesizes PC-modified glycans and thus offers an attractive model to investigate the biology of PC-modified molecules. PC-modification in nematodes occurs when a putative transferase resident to the Golgi utilizes phosphatidylcholine as a PC donor to decorate terminal N-acetylglucosamine (GlcNAc) residues of N-linked glycoproteins. However, the enzyme catalyzing the addition of PC to N-linked glycoproteins remains unknown. The goal of this project is to identify the phosphorylcholine transferase in *C. elegans*.

We have performed bioinformatic analysis and found that eight *C. elegans* genes have homology to key domains of known bacterial PC transferases. We have also compiled a list of Golgi-localized proteins that have no known function. It is our hypothesis that the PC transferase lies within these lists.

418C. Development of a reverse genetics system for a novel nematode virus. **H. Jiang**, D. Wang. Molecular Microbiology Department, Washington University in St. Loius, St. Louis, MO.

Virus host interaction study in genetically tractable model organism C. elegans has been a long term interest for the scientific community. Recently, the identification of three novel viruses that can infect nematode C. elegans and C. briggsae has opened the possibility of study virus host interactions in a natural virus infection system. In virus host interaction studies, genetic manipulations of the host and the virus are equally important. C. elegans is facile to genetic manipulation and high throughput screening as of its simplicity. Thus, a reverse genetics system is demanded for the genetic modification of the novel nematode virus. Here, we report the development of a reverse genetics system for the novel nematode virus. A novel nematode virus reverse genetics was created by 1) cloning the viral cDNA into an expression vector, 2) establishing stable transgenic worms through microinjection of two viral RNA encoding plasmids along with a pRF4 phenotypic marker, 3) harvesting virus through homogenizing and filtering the transgenic worms. Viable virus production was assayed by qPCR, western blot and immunofluorescence assay on de novo transgenic homogenates infected worms. A genetic marker was further introduced into the virus genome and virus production from transgenic animals was further confirmed by Sanger sequencing of the de novo infected worms. This is the first time report of a novel virus reverse genetics created based on a whole metazoan organism. The nematode virus reverse genetics will serve as a fundamental tool in both virus characterization and virus host interaction study in model organism C. elegans.

419A. Identification of disease-causing mechanisms and potential therapeutic targets of Hereditary Spastic Paraplegias using *C. elegans*. **Carl Julien**[1,2,3,6], Arnaud Tauffenberger[1,2,3,6], Dina Aggad[1,2,3,6], Patrick A. Dion[1,2,4,6], Guy A. Rouleau[1,2,4], Pierre Drapeau[3,5,6], J. Alex Parker[1,2,3,6]. 1) CRCHUM, Montréal, QC, Canada; 2) Centre of Excellence in Neuromics, Montréal, QC, Canada; 3) Département de pathologie et biologie cellulaire, Montréal, QC, Canada; 4) Research Center, CHU Sainte-Justine, Montréal, QC; 5) Groupe de Recherche sur le Système Nerveux Central; 6) Université de Montréal, Montréal, QC, Canada.

Hereditary spastic paraplegias (HSPs) are rare neurological disorders with onset ranging from early childhood to late adulthood that can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. HSPs form a large and diverse group of genetic disorders characterized by progressive lower limb spasticity and weakness. Since the spasticity is caused by a progressive distal axonopathy, we investigated in *C. elegans* the mechanisms underlying axon maintenance and function using mutated genes/orthologs previously identified to be involved in HSPs. The genes/orthologs we are studying include *lad-2* (L1CAM), *spas-1* (SPAST), *Y54G2A.2* (ATL1) and *nipa-1* (NIPA1). Using assays to measure neuronal function and degeneration we hope to better understand pathogenic mechanisms and to potentially identify novel therapeutic targets for HSPs. Our preliminary investigations have uncovered a number of small molecules that restore degenerative phenotypes in our HSP models. An update of our findings will be presented.

420B. An integrative *D. discoideum*, *C. elegans* and *D. rerio* approach to assess developmental and reproductive toxicity. **E. Kerkhof**[1], R. Pieters[2], C. Lokman[1], A. Woollard[3], C. Croes[2], M. Teunis[2], R. Bosch[1], M. Wildwater[1]. 1) BioCentre, University of Applied Sciences, Laan van Scheut 2, 6525 EM, Nijmegen, The Netherlands; 2) Department of Innovative Testing in Life Sciences & Chemistry, University of Applied Sciences Utrecht, F.C. Dondersstraat 65, 3507LG, Utrecht, The Netherlands; 3) Biochemistry Department, Oxford University, South Parks Road, OX1 3QU, Oxford, United Kingdom.

An important goal of toxicity testing is to assess and identify health risks associated with exposure to existing and newly introduced chemicals. Of particular importance is developmental and reproductive toxicity (DART), because of the long-term and delayed characteristics of the toxicity. DART testing often involves time- and money-consuming animal studies. Much research activity has been focused on development of alternative test strategies in non-animal systems like single cells, or in non-mammalian multicellular organisms, but these studies showed that data often do not provide a complete picture of DART. We use the combinatorial power of three distinct non-mammalian organisms (*D. rerio*, *C. elegans* and *D. discoideum*) and embryonic stem cells (ES). Developmental pathways in these test systems are well defined but not yet properly related to DART. Combinatorial bioinformatics and laboratory approaches are used to assess inter-species conservation of molecular pathways involved in DART. DART datasets are analysed for affected molecular pathways/profiles, indicative of the functionality of key developmental and reproductive processes such as cell migration, differentiation and pattern formation. Conservation of those pathways in our test systems are assessed. This survey will illuminate conserved molecular mechanisms/pathways that are crucial for DART assessment and lead to identification of proper biomarkers. Our combinatorial approach will support the elucidation of novel DART testing pipelines in non-mammalian organism based systems.

421C. A C. elegans-P. aeruginosa Liquid Assay Identifies Novel Small Molecules with Anti-Infective Properties. **Daniel Kirienko**[1,2,3,4], Natalia Kirienko[1,2], Frederick Ausubel[1,2]. 1) Molecular Biology, Mass. Gen. Hospital, Boston, MA; 2) Genetics, Harvard Medical School, Boston, MA; 3) Infectious Diseases, Alpert Medical School, Brown University, Providence, RI; 4) Infectious Diseases, Miriam Hospital, Providence, RI.

Pseudomonas aeruginosa is a Gram-negative bacterium that causes serious, life-threatening infections for patients with burns, compromised immune systems, medical implants, or cystic fibrosis. Problematically, the bacterium is ubiquitous and exhibits, or readily acquires, resistance to many antimicrobials. These factors make it a serious medical concern, and development of novel anti-infectives is a major human health priority. *C. elegans* is a useful model host for *P. aeruginosa* infection. Many innate immune pathways are shared by *C. elegans* and humans, despite the relative simplicity of the innate immune system of the former. Moreover, many of the same *Pseudomonas* virulence mechanisms are used during infection of *C. elegans* and

humans, making it an attractive option for studying host-pathogen interactions. We developed a liquid-based, *C. elegans-P. aeruginosa* pathogenesis assay to permit high-throughput screening of small molecules. Approximately 86,000 compounds were tested for the ability to ameliorate *C. elegans* killing. 202 hits were recovered, giving an overall hit rate of 0.23%. 93 hits were known antibiotics, 32 were molecules with known (but non-antibiotic) functions, and 70 were compounds with unknown functions. Using additional assays, we further investigated 55 of the 70 compounds with unknown functions. Interestingly, 21 of these compounds also rescued *C. elegans* from a lethal infection with *Enterococcus faecalis*, a Gram-positive pathogen. Furthermore, 32 of these compounds rescued at concentrations substantially lower than what was necessary to inhibit bacterial growth. The compounds appear to act via several separable mechanisms, as they exhibit differential abilities to rescue infections of *P. aeruginosa* on solid media, *E. faecalis* infections in liquid media, and *P. aeruginosa*-mediated pathogenesis in liquid. Several of these molecules are likely to promote immune stimulation, which may translate to mammalian pathogenesis.

422A. Deciphering the function of the ALS/FTD causing genes TDP-43 and C09ORF72 in *C.elegans*. **P Kratsios**[1], J Kerk[1], A Vidal-Gadea[2], J Villarin[1], J Pierce-Shimomura[2], O Hobert[1]. 1) Biochemistry, Columbia University, New York, NY; 2) UT Austin, Neuroscience, Austin, TX.

The human TAR DNA-binding protein-43 (TDP-43) and C09ORF72 genes have been recently identified as being mutated in patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Despite great interest, the normal and pathological function of TDP-43 and C09ORF72 is poorly understood. Exploiting the specific strengths of the *C.elegans* model, we aim to define the normal function of TDP-43 and C09ORF72. The *C.elegans* genome contains single orthologs for TDP-43 (*tdp-1*) and C09ORF72 (*F18A1.6*). We have called *F18A1.6* "*alfa-1*" for ALS/FTD Associated gene homolog. Deletion alleles for *tdp-1(ok803, ok781)* and *alfa-1(ok3062)* were made available to us through the *C.elegans* knockout consortium. Animals lacking *tdp-1* or *alfa-1* are viable, fertile, and appear superficially normal. We have back-crossed the *tdp-1* and *alfa-1* mutants four times to wild-type worms (N2 strain) and performed behavioral analysis of motor function. We observed an age-dependent decline in locomotory activity of *tdp-1* and *alfa-1* mutants, as assessed by thrashing and crawling velocity assays. These locomotory defects are unlikely to be caused by synaptic transmission defects, as *tdp-1* and *alfa-1* mutants did not display any aldicarb sensitivity defects. In addition, we have begun to determine the expression pattern and subcellular localization of *tdp-1* and *alfa-1* by generating isoform-specific fosmid-based translational reporters for TDP-1 (isoforms a-c) and ALFA-1 (isoforms a-b). We have observed that the long *tdp-1* isoforms (TDP-1a and TDP-1c) display a rather broad, nuclear expression pattern, including head neurons, muscle cells, and intestine. In contrast, *alfa-1* expression appears cytoplasmic and restricted to pharyngeal cells and non-neuronal tail cells. We are currently analyzing in further detail the expression pattern of *tdp-1* and *alfa-1* isoforms to design tissue-specific rescue experiments for the locomotory defects. Defining the normal function of *tdp-1* and *alfa-1* in a simple animal model may hold the key for understanding how mutations in these loci cause neurological defects in humans.

423B. Shiga-like toxin 1 confers the full pathogenicity of Enterohaemorrhagic *Escherichia coli* and activation of the p38/MAPK pathway in *Caenorhabditis elegans*. **Cheng-Ju Kuo**[1,3], Ting-Chen Chou[1], Hao-Chieh Chiu[2], Wan-Jr Syu[4], Wen-Tai Chiu[5], Chang-Shi Chen[1,3]. 1) Department of Biochemistry and Molecular Biology, National Cheng Kung University, Tainan, Taiwan; 2) Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan; 3) Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan; 4) Institute of Microbiology and Immunology, National Yang Ming University, Taipei, Taiwan; 5) Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan.

Enterohaemorrhagic *Escherichia coli* (EHEC), a major foodborne pathogen, is responsible for life-threatening diseases in humans as a consequence of the production of Shiga-like toxins. Currently, there is no specific treatment for EHEC infection and the use of antibiotics is contraindicated. Moreover, lack of a good animal model system hinders the study of EHEC virulence with systematic methods *in vivo*. To these regards, we applied the genetic tractable animal model, *Caenorhabditis elegans*, as a surrogate host to study the virulence of EHEC as well as the host innate immunity to this human pathogen. Our results show that *E. coli* O157:H7, a serotype of EHEC, infects and kills *C. elegans*. We also demonstrate that *E. coli* O157:H7 colonizes and replicates in *C. elegans*, stimulates the ectopic expression of microvillar actin, and induces the characteristic attaching and effacing (A/E) lesions in the intact intestinal epithelium *in vivo*. Moreover, our genetic analyses corroborate that the Shiga-like toxin 1 (Stx1) of *E. coli* O157:H7 is required partly for its toxicity. The *C. elegans* p38 MAP kinase signaling pathways, an evolutionally conserved innate immune signaling pathways, are activated and mediated in the regulation of host susceptibility to EHEC infection in a Stx1-dependent manner. Given that the bacterivore *C. elegans* and bacterium, in this case *E. coli*, may have generated a diverse arsenal for both parties to defend each other during the long-term evolution of this predator-prey relationship, our results suggest that this EHEC-*C. elegans* model is suitable for future comprehensive genetic screens for both novel bacterial and host factors involved in the pathogenesis of EHEC infection.

424C. The canonical WNT pathway is an important regulator of *C. elegans* innate immunity against the pathogen *staphylococcus aureus*. **sid ahmed LABED**, Amanda WOLLENBERG, Anna ALVES, Javier IRAZOQUI. Department of Pediatrics, Massachusetts General Hospital, Harvard Medical School 55 Fruit Street, GRJ1416, Boston, MA 02114.

Our lab is interested in dissecting the molecular mechanisms *C. elegans* use to mount an intestinal immune response against the pathogen *staphylococcus aureus*. WNT pathway is a conserved signaling cascade that controls a variety of cellular processes. *bar-1* is one of the *C. elegans* b-catenin transcription cofactor acting downstream of the canonical WNT pathway. *bar-1* is suggested to play an important and conserved role in innate immune defense. Here we report an essential role in immunity for WNT signaling components through *bar-1*. Using mutant and RNAi, we show that four WNT ligands (*lin-44*, *egl-20, cwn-2* and *mom-2*) and three Frizzled receptors (*lin-17*, *mig-1* and *mom-5*) were significantly more susceptible to killing by *S. aureus*. The delivery of LIN-44 at the time of infection using a heat shock promoter is sufficient to rescue *lin-44* mutant defect in adult worms. Moreover, we found that several WNT ligands and receptors effected the expression of candidate-antimicrobial genes including the C-type LECtin CLEC-60 and the LYSozyme ILYS-3. We also demonstrate that *cam-1* (Ror), together with *pry-1* (Axin) and *gsk-3* (glycogen synthase kinase) negatively regulated the expression of *clec-60*. Treatment of adult worms with Lithium chloride, an inhibitor of GSK-3, also leads to increased *clec-60* expression in a *bar-1* dependent manner. Our results suggest that canonical WNT pathway is an important regulator of *C. elegans* innate immune response against *S. aureus*.

425A. Olfactory Plasticity in Entomopathogenic Nematodes. **Joon Ha Lee**, Elissa Hallem. Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA.

Olfaction is a critical sensory modality that allows an organism to process and respond to the surrounding chemical environment. Despite the importance of olfaction for organismal survival, how olfactory behavior is affected by environmental conditions is poorly understood. We are using *Steinernema carpocapsae*, a widely distributed entomopathogenic nematode (EPN) that uses olfactory cues to locate insect hosts, to investigate the context-dependent modulation of olfactory behavior. Using chemotaxis assays, we found that temperature has a strong influence on the olfactory behavior of the infective juvenile (IJ) stage of these parasites. In particular, the cultivation temperature of IJs has a dramatic effect on their olfactory responses, and inducing temperature changes alters their olfactory preferences. We then extended the analysis of temperature's influence on olfactory behavior to other EPN species and found that the extent to which temperature affects olfactory preferences varies widely among EPN species with different climate and geographical distributions. We are now investigating the neural mechanisms that mediate temperature-dependent changes in EPN olfactory behavior. We are also investigating whether these changes reflect differences in host preference or host-seeking strategy at different temperatures. Our work with the developmentally-arrested IJs suggests environmental temperature is sufficient to induce neural plasticity and may pave the way for enhancing the efficacy of EPNs as biocontrol agents.

426B. A non endosomal role for ESCRT-II proteins in neuron and muscle physiology. Emmanuel Culetto[1], Christophe Lefebvre[1], Xavier Manière[2], Ivan Matic[2], **Renaud Legouis[1]**. 1) CNRS- CGM, Universite Paris-Sud 91198 GIF sur Yvette, France; 2) INSERM U1001, 75014 Paris, France.

The Endosomal Complex Required for Transport (ESCRT) proteins are essential for endosome maturation. During this process, the four complexes ESCRT-0, -I, -II and - III are sequentially recruited to the endosome membrane. Beyond this canonical role, ESCRTs complexes have been also involved in membrane abcission during cytokinesis, viral budding and polarized mRNA transport. We have been interested in deciphering how ESCRT components inactivation could affect developmental signaling in *C. elegans*. Our previous phenotypic analysis of ESCRT mutants had revealed very variable developmental phenotypes (1), and we now focussed our work on ESCRT-II developmental function. ESCRT-II is an heterotetrameric complex made of VPS-22, VPS-36 and VPS-25. *C. elegans vps-36* and *vps-22* mutants display similar defects including L4 larval arrest development, enlarged endosomes and uncoordinated phenotype. The locomotion behavior of *vps-36* and *vps-22* mutants has been further analyzed using electrotaxis assay and revealed a progressive alteration in locomotion during larval development. To better understand the molecular basis of the locomotion phenotype we then investigated the sub-cellular localization of ESCRT-II components. For this purpose VPS-22::GFP, VPS-25::GFP, VPS-36::GFP worms and VPS-36 antibody have been generated. Confocal microscopy observations indicate that ESCRT-II proteins are highly expressed in motor neurons and body wall muscle (BWM) cells throughout the larval development and during adulthood. Furthermore, co-localization experiments using specific muscle markers indicate that VPS-36 are restricted to the BWM anchoring structures and the myofilament lattice. However, electron microscopy analyses of *vps-36* mutant did not reveal major alteration of the BWM architecture. Interestingly, a genome-wide RNAi screen performed in drosophila suggests that Vps25 and Vps36 are required for formation and function of the muscle. Altogether, these results indicate that ESCRT-II complex performs a novel evolutionary conserved role in muscle. (1) Djeddi J.Cell Science, 125, 2012.

427C. Identification of TDP-43 modifying kinases in a *C. elegans* model of TDP-43 proteinopathy. **Nicole Liachko[1,2]**, Pamela McMillan[1,2], Chris Guthrie[1], Thomas Bird[1,2], James Leverenz[1,2], Brian Kraemer[1,2]. 1) VA Puget Sound Health Care System, Seattle, WA; 2) University of Washington, Seattle, WA.

Frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) and amyotrophic lateral sclerosis (ALS) are severe progressive neurodegenerative diseases characterized by lesions containing aggregated, hyperphosphorylated TDP-43. In addition, mutations in TDP-43 have been shown to cause some cases of ALS and FTLD-TDP. To study the cellular, molecular, and genetic underpinnings of TDP-43 mediated neurotoxicity in a tractable model system, we have developed a *C. elegans* model of TDP-43 proteinopathy. Expression of familial ALS-mutant TDP-43 in all *C. elegans* neurons causes severe motor dysfunction, and recapitulates some characteristic features of ALS and FTLD-TDP including decreased lifespan, neuronal degeneration, hyperphosphorylation and ubiquitination of TDP-43, and accumulation of detergent insoluble aggregates. We have shown that in *C. elegans*, phosphorylation of TDP-43 at serine residues 409/410 drives mutant TDP-43 toxicity. To identify kinases involved in the pathological phosphorylation of TDP-43, we have assembled and screened an RNA interference (RNAi) library targeting the majority of *C. elegans* kinases. 456 predicted kinases were individually inactivated by RNAi and tested for modification of TDP-43 dependent behavioral phenotypes. 12 kinase-inactivating RNAi treatments suppressed TDP-43 driven motor phenotypes. Null mutant analysis of these candidates in *C. elegans* identified 2 kinases whose loss of function suppresses TDP-43 movement defects and reduces TDP-43 phosphorylation *in vivo*. We have demonstrated that the human homolog of one candidate TDP-43 kinase, *cdc-7*, directly phosphorylates TDP-43 *in vitro* and in mammalian cells. Further, we have demonstrated that treatment with a CDC-7 inhibitor decreases TDP-43 phosphorylation *in vitro* and prevents neurodegeration *in vivo*. The *C. elegans* model of TDP-43 proteinopathy serves as a powerful system to explore the biology of human disease and rapidly test interventions. TDP-43 specific kinases identified using this system may represent effective therapeutic targets for TDP-43 proteinopathies such as FTLD-TDP and ALS.

428A. Searching for therapeutic compounds for Machado-Joseph disease: a *C. elegans*-based screening. A. Teixeira-Castro[1,2,3], A. Jalles[1,2], M. Araújo[1,2], A. Miranda[1,2], C. Bessa[1,2], R. Morimoto[3], **P. Maciel[1,2]**. 1) Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, 4710-057 Braga, Portugal; 2) ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3) Department of Molecular Biosciences, Northwestern University Institute for Neuroscience, Rice Institute for Biomedical Research, Northwestern University, Evanston, IL 60208.

Despite the many efforts that are under way to develop therapeutic strategies, no preventive treatment is yet available for any of the polyglutamine diseases. Machado-Joseph disease (MJD) is one of the polyQ disorders caused by the expansion of a polyQ tract within the C-terminal of the ataxin-3 (ATXN3) protein. Mutant ATXN3 acquires the ability to self-associate and enter an aggregation process, which is associated with several pathophysiological consequences for neurons. The lack of therapeutic strategies that effectively prevent neurodegeneration in MJD patients prompted us to search for compounds that modulate mutant ATXN3-related pathogenesis. Recent data from our lab have shown that many aspects of MJD can be properly modeled in the round worm *Caenorhabditis elegans*. This study is based on the idea that our *C. elegans* MJD model can be used to perform large-scale drug

screenings, in which the identification of effective drugs can be accomplished by looking simultaneously at protein aggregation in the live neuronal cells, and on its impact on neuron-regulated behavior of the whole-animal. Our goal was to screen a library of ~1200 mainly FDA-approved out-of-patent compounds for their ability to prevent or delay the formation of fluorescent mutant ATXN3 aggregates and neurological dysfunction. We excluded the small molecules that were found to be toxic or cause developmental delay to the *C. elegans*. Ten percent of the non-toxic compounds significantly reduced the locomotion deficits of the animals, three of which made mutant ATXN3 expressing worms perform like wild-type animals in the motility assay. The hits are FDA-approved compounds or are currently in clinical trials for other neurological disorders. We should be able to identify efficacious compounds that can be tested in higher organisms and eventually enter clinical development.

429B. Role of natural genetic variation in the control of susceptibility to bacterial infections in Caenorhabditis elegans. **Natalia Martin**, Alejandro Aballay. Molecular Genetics and Microbiology, Duke University Medical center, Durham, NC.

Our laboratory has taken advantage of the simple and well-studied nervous and immune systems of *C. elegans* to study the mechanisms involved in neural-immune communications. Overall, our studies showed that specific genes and neurons in the nervous system of *C. elegans* control immune responses, indicating that cell non-autonomous signals from different neurons act on non-neural tissues to regulate immune responses at the organismal level (1-4). To study the role of natural genetic variation in the organismal control of immune pathways, we studied *C. elegans* wild strains isolated from different parts of the world that carry the same mutation in the *npr-1* gene, which encodes a G-protein coupled receptor that participates in a neural circuit that controls not only avoidance to bacterial pathogens but also microbial killing mechanisms that are controlled by the p38/PMK-1 MAPK immune pathway (1). Interestingly, our studies indicate that 9 wild isolates have evolved mechanisms to compensate for the increased susceptibility to pathogens because of their reduced NPR-1 activity. This highlights the importance of NPR-1 in the control of immunity and provides an opportunity to address which genes and pathways that control immunity in a cell non-autonomous manner may harbor sufficient genetic variation to be targets of natural selection. In order to map and characterize the mechanisms that compensates the effect of the *npr-1* mutation in wild isolates, we are using a combination of whole genome sequencing with a very fine-grained single nucleotide polymorphism mapping strategy (5). Using this approach we rapidly identified a 1 mega base pair locus in chromosome I of one of the environmental isolates that is responsible for the compensatory phenotype. The identification of the responsible gene among the potential candidates is underway. References: 1.Styer et al. Science 322, 460 (Oct 17, 2008); 2.Sun et al. Science 332, 729 (May 6, 2011); 3. Sun et al. EMBO reports 13, 855 (Sep, 2012); 4. Singh and Aballay. J Biol Chem 287, 33191 (Sep 28, 2012); 5. Doitsidou et al. PLoS One 5, e15435, doi:10.1371/journal.pone.0015435 (2010). *Italic Text*.

430C. *fshr-1* provides a connection between the oxidative stress response and innate immunity in *C.elegans*. **Elizabeth V Miller**, Jennifer R Powell. Gettysburg College, Gettysburg, PA.

The initial response to infection is directed by the innate immune system, which identifies and kills invading microbial pathogens. Canonical immune defense is comprised of the induction of antimicrobial peptide effectors in response to pathogen detection by cell-surface receptors. In addition, many animals generate reactive oxygen species (ROS) upon the detection of microbes. ROS are highly reactive, and can interact with most cellular components, disturbing cellular function, and ultimately causing cell death. The same underlying mechanisms that make ROS an effective defense response against bacterial pathogens also make ROS dangerous for normal cellular functions because they are toxic to living cells. Thus, when ROS are generated as part of an immune response, detoxification enzymes must also be produced as part of an oxidative stress response so that the organism itself is not damaged by the ROS immune response. The G-protein coupled receptor FSHR-1 is an important component of the *C.elegans* innate immune response to infection by diverse pathogens. FSHR-1 acts in the intestine of the worms, the site of exposure to ingested pathogens. In *C.elegans*, *fshr-1* is required for the induction of a range of antimicrobial compounds induced by *Pseudomonas aeruginosa*. Because ROS may also be critical in the defense against infection by microbial pathogens, we are investigating a connection between the *fshr-1* response to *P.aeruginosa* with the oxidative stress response. We have analyzed the requirement for *fshr-1* in the production and detoxification of ROS, and have established a dual role for FSHR-1 in oxidative stress and the innate immune response in *C.elegans*.

431A. Update: Screening Potential Anthelmintic Compounds for Novel Activity. Megan Gross[1], Michael Smith[2], Aaron Monte[2], **Jennifer Miskowski[1]**. 1) Bio Dept, Univ Wisconsin-La Crosse; 2) Chem Dept, Univ Wisconsin-La Crosse.

Parasitic worms, called helminths, infect plants, animals, and humans worldwide leading to a decreased food supply, economic hardship, and significant levels of morbidity and mortality. Anthelmintics that combat these infections are represented by only five major classes of compounds. Misuse of these pharmaceuticals has contributed to widespread anthelmintic resistance in worms that infect livestock and emerging drug resistance in human-infecting helminths. The identification of new means to target helminths is imperative. The non-parasitic nematode Caenorhabditis elegans has long been a model system for helminths. Previously, our group screened a series of novel, synthetic compounds for anthelmintic activity in C. elegans. These compounds are derivatives of a natural product stilbene that have generated great interest due to their broad anti-microbial effects. Two microassays were used: the motility assay that screened for paralysis and the developmental assay that screened for developmental delays, developmental arrests, a decrease in fecundity, or death. Six compounds demonstrated significant anthelmintic activity and were prioritized for further study. This research project aims to determine if these six stilbenoid derivatives act via a novel mechanism. To this end, the compounds are being tested against mutant C. elegans that are resistant to existing anthelmintics using the two assays described above, along with an NGM plate-based assay. Work to date has primarily focused on the compound CL-5, which showed the strongest activity. Ivermectin is one of the most widely used anthelmintics today, and ivermectin resistance has been documented in helminth strains. Interestingly, CL-5 affects ivermectin-resistant C. elegans in a dose-dependent manner, similar to wildtype worms. Whereas ivermectin acts on ligand-gated chloride channels, the anthelmintic benomyl disturbs the microtubule cytoskeleton. Benomyl-resistant C. elegans are also sensitive to CL-5 and exhibit developmental defects similar to CL-5-treated N2 worms. We are currently testing mutant strains that are resistant to levamisole and emodepside. In addition, we are pursuing some other interesting effects of CL-5 treatment.

432B. The discovery of intestinal intracellular microbes in the soil nematode collected from the field of the Tohoku district, northeastern Japan. **Kenji Nishikori**, Eisuke Kuroda, Takahiro Tanji, Hirohisa Shiraishi, Ayako Ohashi-Kobayashi. Dept Immunobiol, Sch Pharm, Iwate Med Univ.

A part of microbes possesses the ability of intracellular infections and establishes parasitic, commensal or mutual relationships with their hosts, which often cause cellular and systemic alterations on the host physiology. The studies on the naturally-occurred associations in cells of multicellular hosts will yield key insights into the understanding of symbiotic evolution and infectious diseases.

To obtain a research model, we searched microbe-containing soil nematodes that meet following criteria: (i) being transparent, (ii) being able to grow by feeding with *E.coli* and (iii) reproducing parthenogenetically like *C. elegans*. We collected soil samples from eastern Japan and found that the worm sampled from Morioka, the capital city of Iwate Prefecture in the Tohoku district, harbored microbe-like structures in their intestinal cells. The structure contained a DAPI-positive content, indicating that the structure is the infected microbe. The infection in the intestinal cells sustained through passage cultures at a rate of 54%. The 18S rDNA sequences from the worms coincided with that from *Oscheius tipulae*, which belongs to a family Rhabditidae, whereas the 16S rDNA sequences obtained from the worms were assigned to several bacteria. Among them a sequence of the beta-proteobacteria genus *Burkholderia* was obtained from all tested individuals.

To test whether the microbe can infect horizontally, the microbes prepared from the homogenate of the infected worms were spread on NGM plates seeded with *E. coli* OP50 and fed to the uninfected animals. As a result, we observed the microbial infection in the intestinal cells. The infection was not observed when fed to *C. elegans* regardless of the existence of the microbe within the intestinal lumen, indicating the host specificity of the microbe. These results show that the system between the worms and the microbes can be a good model for the future researches.

433C. Does the *C.elegans* glycosylation gene *bus-8* undergo translational frameshifting to generate different protein isoforms? **Delia M. O'Rourke**[1], Martin Cullen[1], Dave Stroud[1], Mark Pavlyukovskyy[2], Frederick A Partridge[3], Jonathan Hodgkin[1]. 1) Biochem, Univ Oxford, Oxford, UK; 2) Mol Biology, Princeton Univ, Princeton, USA; 3) Univ Manchester, Manchester, UK.

We have previously shown that *C. elegans* with missense mutations in *bus-8* (a nematode-specific glycosyltransferase) are resistant to infections by *M.nematophilum* and *Leucobacter* Verde2, but hypersensitive to infection by *Leucobacter* Verde1. *bus-8* worms are also drug-sensitive, skiddy, defective in mate recognition and bleach-sensitive due to changes in the surface coat of the worm. *bus-8* is additionally essential for ventral enclosure during embryonic morphogenesis and is expressed in cells underneath the ventral epidermis in the embryo and post-embryonically in seam cells (Partridge *et al*. 2008). One *bus-8* allele, *lj22*, is a point mutant in the 5'UTR that contains a conserved putative ORF out of frame with the downstream *bus-8* gene. EST and RNA seq data indicate that the ORF is transcribed and spliced with the downstream *bus-8* transcript. The 5'UTR ORF encodes a possible transmembrane domain that could localise the BUS-8 glycosyltransferase to membrane compartments. We hypothesize that this region is translated with the rest of the gene (which would require +1 translational frameshifting). To investigate this we constructed a TAP-tagged version of BUS-8 and transformed mutant *bus-8* worms with this construct. TAP-tagged BUS-8 is functional as it can rescue both mild and severe *bus-8* mutations. We have purified BUS-8 using the tags and used mass spectrometry analysis to look for peptides corresponding to the 5' UTR encoded ORF. We have also made a number of transgenic worms to investigate the functional roles of the BUS-8 isoforms. We have used genomic mutations in *bus-8* that affect only the 5'UTR *(lj22)* or the main part of the gene *(e2883, tm1410)* with transgenic constructs containing modified versions of *bus-8*. In this way we can investigate which isoforms of BUS-8 can rescue the *bus-8* phenotypes. The sensitivity of both *bus-8 (lj22)* and *e2883* to Verde1 infection has allowed us to conduct suppressor screens. Using this approach we have identified 5 suppressors for each allele, which are currently being characterised. Partridge *et al* (2008) Dev Biol 317 549.

434A. Pathogen induced diapause formation requires the RNAi machinery. Maria Fernanda Palominos[1], Lidia Verdugo[1], Francisco Chavez[2], **Andrea Calixto**[1]. 1) Center for Genomics and Bioinformatics, Universidad Mayor, Santiago, Chile; 2) Microbiology Laboratory, Department of Biology. Faculty of Sciences, University of Chile.

The survival or demise of a host depends on its dynamic interaction with pathogens in its environment. We are investigating the effects of environmental and genetic variation on the survival and behavior of a host and its progeny, upon bacterial infection. We used *C. elegans*, as a host; and *P. aeruginosa* PAO1, an opportunistic human pathogen, as infectious agent. Environmental variation was performed supplementing or removing inorganic phosphate (Pi) from the growth media. Removal of Pi produces pigment expression and more virulent bacteria. Animals on *P. aeruginosa* PAO1 at high Pi survived better than at low Pi. Genetic variations were produced by mutations in both pathogen and host. We followed the viability of worms on *P. aeruginosa* PAO1 for two generations: Surprisingly, worms formed large amounts of dauers in the F2. We show that dauer formation is not specific of *P. aeruginosa* PAO1 exposure since they also formed dauers on *Salmonella enterica*. Dauer entry upon infection depends on transcriptional activation of DAF-16 but is independent from pheromone production since dauer formation was unaffected in *daf-22* mutants. Worms preferred *P. aeruginosa* PAO1 over *E. coli* on high Pi. On low Pi initially preferred *P. aeruginosa* PAO1 but switch their preference for *E. coli* after the first hour. Systemic host responses, like RNA interference (RNAi) are likely to be most effective for defense against viruses since they spread far from their entry place. However the link between RNAi and bacterial pathogenesis is unknown. Several *C. elegans* RNAi mutants have a wild-type appearance under laboratory conditions when raised on non-pathogenic bacteria. While RNAi mutants are able to form dauers upon starvation, we found that both cell autonomous and environmental RNAi mutants, failed to form dauers on *P. aeruginosa* PAO1. Furthermore, RNAi mutants failed to avoid pathogenic bacteria in acute and chronic experiments. Our findings suggest that pathogen induced dauer formation is RNAi dependent and serves as trangenerational defense mechanism against bacterial infection.

435B. The Mitochondrial Unfolded Protein Response Regulates a Pathogen-Specific Innate Immune Pathway. **Mark W. Pellegrino**, Amrita Nargund, Cole M. Haynes. Cell Biology, Sloan Kettering Cancer Centre, New York, NY.

The innate immune system is the host's first line of defense that acts as a generalized, non-adaptive protection mechanism against foreign pathogens. To initiate the innate immune response, the host must first detect the foreign microorganism. Recently, it has been suggested that the innate immune system can be activated via host cellular surveillance programs that monitor pertubations to essential cellular functions including damage to mitochondria, inhibition to translation, and impaired proteolysis, however the exact mechanisms remain poorly understood. Mitochondrial stress or dysfunction activates a mitochondrial unfolded protein response (UPR^{mt}) that leads to upregulation of cytoprotective genes to re-establish cellular homeostasis. A

central regulator of the UPR^{mt} is the transcription factor ATFS-1 that senses mitochondrial stress based on mitochondrial import efficiency. We found that, in addition to cytoprotective genes, ATFS-1 regulates genes with known roles in innate immunity. Consistent with a functional role for the UPR^{mt} in innate immunity, we demonstrate that the UPR^{mt} is both required and sufficient for the detection of, and protection against, the opportunistic bacterial pathogen *Pseudomonas aeruginosa*. We also show that the UPR^{mt} acts independently from known innate immune pathways including the PMK-1/p38 MAPK pathway. We propose that, in addition to its cytoprotective role, the UPR^{mt} acts as a xenobiotic surveillance program to defend the host against pathogens that target mitochondria.

436C. Suppressors of TDP-1 toxicity in *Caenorhadbitis elegans*. **I. Pena-Gonzalez**[1], CD. Link[1,2]. 1) Integrative Physiology, University of Colorado, Boulder, CO 80309, USA; 2) Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309, USA.

Suppressors of TDP-1 toxicity in *Caenorhadbitis elegans* Ilana Pena-Gonzalez1 and Christopher D Link1, 2 1 Integrative Physiology, University of Colorado, Boulder, CO 80309, USA 2 Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309, USA RNA binding protein TDP-43 forms damaging aggregates in multiple neurodegenerative diseases. We have found that the deletion of *tdp-1*, the *C. elegans* ortholog of TDP-43, leads to increased accumulation of double stranded RNA. TDP-1 is not believed to bind to dsRNA itself; however the marked increase in dsRNA accumulation in worms deleted for *tdp-1* implies TDP-1 normally participates in limiting the stability and structure of dsRNA. Although worms deleted for TDP-1 do not have a severe phenotype, overexpressed nuclear TDP-1 is toxic in worms. One possible explanation for this is that too much TDP-1 leads to excessive disruption of structured RNAs needed for normal RNA metabolism. Making use of this overexpression model we have established a heat shock induction system to help identify components associated with TDP-1's prevention of dsRNA accumulation. Identifying mutations that suppress the neurotoxic effects of overexpressed TDP-1 could help identify others factors contributing to the complex mechanism of disassembling dsRNA. A mutagenesis screen has identified four mutant strains that partially suppress the toxicity of overexpressed TDP-1. We have also tested suppressors of other toxic proteins. Further identifying the pieces of the mechanism regulating dsRNA breakdown could help better explain the pathogenic pathway of TDP-1 and consequently TDP-43 in disease.

437A. From fungal spore adhesion to effector gene transcription. Olivier Zugasti, Shizue Omi, Guillaume Bordet, Vincent Rouger, Carole Couillault, Julien Soule, Jerome Belougne, Didier Marguet, Jonathan Ewbank, **Nathalie Pujol**. CIML, Marseille, France.

We have performed forward and reverse genetic screens for genes that alter the expression of an antimicrobial peptide gene reporter (*nlp-29*p::GFP) that normally increases after infection with the fungus *D. coniospora* [PMID: 22395785, 22470487]. Other than genes involved in pathogen recognition and signal transduction, we expected to find genes involved in the adhesion of fungal spores to the worm's cuticle. We indeed found 2 categories: genes that decrease or increase spore adhesion. These provoke Nipi (no induction of peptide after Drechmeria infection) and Hipi (hyper-induction of peptide after infection) phenotypes, respectively. Interestingly, most of the gene knockdowns that cause a Hipi phenotype do not lead to increased expression of *nlp-29*p::GFP in the absence of infection, suggesting a specific role in governing adhesion.

Among the latter class are *bus-2* and *bus-12* that were orginally isolated by Jonathan Hodgkin and encode proteins predicted to act in surface glycosylation. Interestingly, mutations in *bus-2* and *bus-12* reduce the adhesion of the bacterial pathogen *Microbacterium nematophilum*. In *bus-2* mutants, there is an increased exposure on the cuticle of fucosyl glycan compared with N2 worms [PMID: 17339204, 20385555]. A simple explanation for the increased adhesion of *D. coniospora* would therefore be that fucosyl glycans are a target for spores binding.

In less than one hour after spore adhesion, transcription of antimicrobial peptide genes increases markedly. We have now set up a system to manipulate spores using holographic optical tweezers. Single spores or small numbers of them can be precisely handled and brought into contact with a worm at a precise time and location. We have coupled this with spinning disk confocal microscopy, thus allowing us to follow *in vivo* the earliest steps of infection in the adult epidermis. Monitoring the trafficking of known signaling molecules should provide insights into their function, which in some cases remains enigmatic.

438B. Functional analysis of candidate effector proteins from a natural, intracellular pathogen of C. elegans. **Aaron W Reinke**, Emily Troemel. UC San Diego, La Jolla, CA.

Obligate intracellular pathogens are ubiquitous, but they can be extremely difficult to study because they only grow inside of host cells. Microsporidia comprise one of the largest phyla of such pathogens, and are able to infect a wide variety of hosts, including humans. Microsporidia are medically and agriculturally relevant, yet they are poorly understood and few tools have previously been available to study them. Recently, a species of microsporidia, Nematocida parisii, was discovered to naturally infect the intestine of C. elegans, providing a convenient host system to study these enigmatic pathogens. Predicted secreted proteins are candidate effectors that may hijack C. elegans intestinal cells into providing resources for rapid pathogen growth. Several predicted secreted proteins are conserved among multiple species of microsporidia, as well as a large, expanded family that is species-specific. The function of these candidate N. parisii effector proteins is being investigated by heterologously expressing them in the C. elegans intestine, fused to GFP and an affinity tag. The GFP tag will enable the subcellular localization of these proteins to be determined and the affinity tag will enable the interactions of each secreted protein to be determined by affinity purification, followed by mass spectrometry to identify co-purifying proteins. The function of these identified interactions will be investigated by manipulating levels of host and pathogen proteins. Together, these experiments will elucidate the role of these candidate effectors during infection of C. elegans and will provide some of the first functional data for a microsporidian pathogen.

439C. Epitope-tagging the G-Protein Coupled Receptor *fshr-1*. **Joseph D Robinson**, Jennifer R Powell. Biology, Gettysburg College, Gettysburg, PA.

Innate immunity is the body's first line of defense against infection. To recognize infection, receptors sense conserved pathogen-associated molecular patterns (PAMPs), cellular damage caused by the infection, or perturbations in cellular homeostasis. These receptors trigger a response by activating signal transduction pathways that induce the transcription of various effectors. The G-protein coupled receptor FSHR-1 defines an innate immune signaling pathway in *Ceanorhabditis elegans* that is required for the response to a wide variety of pathogens; however, much of where and how it functions within the cell is still unknown. Determining the subcellular localization and the binding partners of FSHR-1 would give us valuable information about its function.

Unfortunately, attempts at generating a functional GFP-tagged FSHR-1 or raising antibodies against an FSHR-1 peptide have been unsuccessful. Instead, we have used fusion PCR to create a 3' HA-tagged version of *fshr-1* under the control of the endogenous *fshr-1* promoter and 3' UTR. The next step is to use Mos1-mediated Single Copy Insertion to integrate the transgene into *C. elegans*. This valuable tool will enable us to determine the subcellular localization of FSHR-1 via immunofluorescence, and FSHR-1 interacting partners through co-immunoprecipitation. Using the information from these and other biochemical tests, we will gain a better understanding of this innate immune pathway's function.

440A. On methane seeps, worms, and parasitic fungi: microsporidia-infected nematodes reveal another secret of the deep sea. **Amir Sapir**[1], Adler Dillman[1], Benjamin Grupe[2], Jeroen Ingels[3], Stephanie Connon[4], Manuel Mundo-Ocampo[5], John DeModena[1], Lisa Levin[2], James Baldwin[6], Victoria Orphan[4], Paul W. Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA; 2) Center for Marine Biodiversity and Conservation, and Integrative Oceanography Division, Scripps Institution of Oceanography, La Jolla, CA 92093-0218, USA; 3) Plymouth Marine Laboratory, Prospect Place, The Hoe, PL1 3DH, United Kingdom; 4) Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA; 5) CIIDIR-IPN, Unidad Sinaloa, Mexico; 6) Department of Nematology, University of California, Riverside, CA 92521, USA.

The deep sea is earth's largest habitat, yet the nature and level of parasitism in this environment are mostly unknown. Here we report the discovery of a fungus-related parasitic microsporidium, Nematocenator marisprofundi that infects nematodes in methane seeps on the Pacific Ocean floor. This infection is species-specific and has been temporally and spatially stable over two years of sampling, indicating an ecologically relevant host-parasite interaction. N. marisprofundi targets the host's body wall muscles causing cell lysis, and in severe infection cases even muscle filament degradation. Phylogenetic analyses place N. marisprofundi as a separate branch among basal microsporidia lineages, suggesting that microsporidia-nematode parasitism occurred in the deep sea early in microsporidia evolution and that N. marisprofundi belongs to a novel and basal deep-sea microsporidian clade. Our findings present a new perspective on the abundance, nature, and ecological significance of deep-sea parasitism by placing nematodes, one of the most abundant animal phyla in many deep-sea settings, as a host for microsporidia parasites. This demonstrates the complexity of methane seep ecosystems being a hub for inter-kingdom interactions between bacteria, nematodes, and parasitic fungi. Our study adds microsporidia parasitism as a previously unknown characteristic of chemoautotrophic methane seep ecosystems and suggests a role for fungal-mediated pathologies in the deep sea.

441B. A *C. elegans* genome-wide RNAi screen identifies modifiers of mutant TDP-43. **Aleen Saxton**[1,2], Nicole Liachko[2,3], Brian Kraemer[1,2,3]. 1) Seattle Institute of Biomedical and Clinical Research, Seattle, Washington; 2) Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, Washington; 3) Department of Medicine, Gerontology Division, University of Washington.

Aggregated, hyperphosphorylated TAR DNA-binding protein-43 (TDP-43) occurs in multiple neurodegenerative diseases including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration. Transgenic *C. elegans* expressing familial ALS mutant TDP-43 *(ckIs423[Psnb-1::hTDP-43(M337V)]* subsequently, TDP-43^{M337V}) have a variety of abnormal phenotypes including uncoordinated locomotion. Using the Ahringer RNA interference (RNAi) feeding library, we have completed a genome-wide screen for suppressors or enhancers of TDP-43^{M337V} motor defects and fitness in an *eri-1(mg366);lin-15(n744)* RNAi enhancing mutant background. Out of the 16,750 unique RNAi clones in the library, a total of 2,486 potential positive candidates were selected for retesting following the initial screen. Positive candidates have also been tested in non-transgenic *C. elegans* to confirm TDP-43 specific effects of the RNAi on movement and viability. From these candidates, we have identified many candidate suppressors that improved TDP-43^{M337V} movement, and many candidate enhancers that worsened TDP-43^{M337V} phenotypes, causing paralysis, developmental arrest, or death. Validation of hits from the collection of identified RNAi modifiers is ongoing using genetic loss of function studies in candidate genes quantitatively in the TDP-43^{M337V} transgenic animals. Our screening approach promises to uncover conserved genes involved in TDP-43 related neurotoxicity.

442C. Developing *Heterorhabditis* nematodes as an experimental system for the study of mutualistic symbiosis. **Hillel Schwartz**, Paul Sternberg. Division of Biology and HHMI, California Institute Of Technology, Pasadena, CA.

Entomopathogenic nematodes of the genus *Heterorhabditis* are insect killers that live in mutually beneficial symbiosis with pathogenic *Photorhabdus* bacteria. *Photorhabdus* is rapidly lethal to insects and to other nematodes, including *C. elegans*, but is required for *Heterorhabditis* growth in culture and for the insect-killing that defines the entomopathogenic lifestyle. The symbiosis between *Heterorhabditis* and *Photorhabdus* offers the potential to study the molecular genetic basis of their cooperative relationship. We developing tools to make such studies more feasible: we have been studying multiple nematodes of the genus *Heterorhabditis* and developing tools for the molecular genetic analysis of *Heterorhabditis bacteriophora*.

Many species of *Heterorhabditis* and variants of *Photorhabdus* have been isolated; some pairings show specificity in their ability to establish a symbiotic relationship. To better understand these interactions and other variations in the lifestyles of *Heterorhabditis*, we have sequenced *H. indica*, *H. megidis*, *H. sonorensis*, and *H. zealandica*; a *H. bacteriophora* genome sequence is available. A comparison of these closely related species may help us to identify mechanisms that regulate the response to bacterial interactions and to find variations that correlate with differences in lifestyle or bacterial compatibility.

In addition to genomics, we are developing *H. bacteriophora* as a laboratory organism. *H. bacteriophora* grows well on plates, has been reported to be susceptible to RNAi and transgenesis, and can develop as a selfing hermaphrodite, and so should be a powerful system for the molecular genetic study of the aspects of biology to which it is uniquely well suited, most prominently symbiosis. This potential is severely diminished by inconvenient sex determination: the self-progeny of hermaphrodites are mostly females with some males; at low density, their progeny are almost exclusively females. We have screened for and isolated a constitutively hermaphroditic mutant for use in molecular genetic studies of symbiosis. This mutant also offers the opportunity to explore the basis of hermaphrodite sex determination in *H. bacteriophora*.

443A. Key residues of Cry5B structure and function: Mutagenesis by alanine scanning. **Jillian Sesar**, Yan Hu, Hui Fan, Partho Ghosh, Raffi Aroian. University of California, San Diego, La Jolla, CA.

Soil-transmitted helminths (hookworms, whipworms, and Ascaris) infect upwards of two billion people worldwide. Only one drug (albendazole) shows adequate efficacy against STHs under single-dose conditions for mass drug administration. However, it's far from ideal against hookworms and

whipworms, and recent studies have shown instances of low efficacy. New treatments are therefore important. Crystal (Cry) proteins produced from the soil bacterium Bacillus thuringiensis have been used for decades to control insects that destroy crops and transmit human diseases, and studies have shown these proteins to be safe to humans. Our lab has shown that Cry proteins, specifically Cry5B, are able to kill both the free-living nematode Caenorhabditis elegans, as well as parasitic nematodes in vivo (eg. Ancylostoma ceylanicum). We are currently investigating several of these Cry proteins to be safe and effective anthelmintics. Cry proteins intoxicate invertebrates by acting as pore-forming toxins. Several defined steps in their mechanism of action have been suggested from insect studies, but there is still great uncertainty as to the importance of these steps. We believe that the C. elegans-Cry5B system has great potential to unlock mysteries surrounding Cry proteins. I have performed an alanine scan of all 698 amino acids in the toxin domain of Cry5B. Many mutants were isolated and subsequently tested on C. elegans to assess for changes in toxicity levels. These results show mostly a decrease in toxicity against C. elegans compared to wild type Cry5B. However, a few show apparent increased toxicity and my on-going goal is to validate the effectiveness of these. I will discuss the challenges in testing the ability of single mutations to increase toxicity. My longer-term goal is to correlate these changes in activity with specific changes in protein functionality (eg. receptor binding, protease processing, etc) in order to better understand critical steps in Cry protein action. Another application of my work is to identify Cry protein variants that could have potential increases in toxicity against STH parasites, thereby improving Cry protein activity for treating one of the most neglected diseases of our time.

444B. PUFA therapy ameliorates Parkinson disease like symptoms. **Shashikumar Shivaiah**, Rajanikant Golgodu Krishnamurty. School of Biotechnology, National Institute of Technology, Calicut, Kerala, India.

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting about 1 - 2% of the population over the age of 65. PD encompasses a spectrum of core clinical features such as rigidity, bradykinesia, tremor at rest and postural instability. However, PD is a heterogeneous disorder, as many patients also develop cognitive dysfunctions, including anxiety, depression and dementia or abnormalities in olfactory and visual perception. Pathologically, PD is characterized by the specific and massive loss of dopamine (DA) containing neurons in the Substantia Nigra pars compacta and aggregation of protein alpha-Synuclein levels. Poly unsaturated fatty acid (PUFA) are highly abundant in the brain and omega-3 and omega-6 PUFA are essential structural components of the cell membrane phospholipids. Omega-3 fatty acids are dietary essentials, and the current low intakes in most modern developed countries are believed to contribute to a wide variety of physical and mental health problems. In the present study, the chemically induced PD symptoms in transgenic Caenorhabditis elegans UA44 (baln11;Pdat-1::Pdat-1::GFP) exposed to various concentration of 6-Hydroxydopamine (6-OHDA) by exposing from the egg stage to adult larva with and with out supplementation of PUFA Linoleic acid (LA) along with food source. The results were confirmed by various parameters, the decreased locomotion being normalized to like control by counting number of body bends (29 and 42% decrease in locomotion in 5 and 25mM 6-OHDA and 20% protection by LA against 6-OHDA), body thrashing and cognition enhancement by PUFA (LA) supplemented groups are well evidenced. The main markers of PD symptoms alpha Synuclein accumulation and degeneration of dopamine neuron were similarly reduced, as evidenced by fluorescence intensity and microscopic analysis. The mitochondrial enzymes decreased were measured such as Succinate dehydrogenase, complex I and IV, total thiols and cytochrome c activity. In conclusion, the nematode model C. elegans with its simple nervous system and GFP tagged dopaminergic neuron offer an valuable tool for studying the pathogenesis of Parkinson disease and evaluate the anti-Parkinsonian effects of PUFA molecules.

445C. Antimicrobial compound screens using *C. elegans* model system for periodontal pathogens -. **Shahid S. Siddiqui[1,2]**, Fathy A. Faskhani[1], Mohammad Al-Beyari[1]. 1) Northwestern University, Dept of BMBCB, Evanston, IL 60208; 2) UQU-DENT, UQ University, Makkah, SA.

Chronic periodontal disease (PD) is an inflammatory condition that is highly widespread. Periodontal disease is a result of sub-gingival plaque pathogens, leading to alveolar bone loss. PD is also associated with diabetes, obesity, cardiovascular disease, respiratory disease, rheumatic arthritis and pre-term birth. The mechanism of periodontal infection is poorly understood due to the complexity of microbial factors, and highly complex inflammatory and anti-inflammatory signaling between pathogens and the host. New microbial pathogens are emerging that are resistant to conventional antibiotics; thus there is a need for drugs that are new class of antibiotics, and novel compounds that reduce pathogenicity of invading microbes. Pioneering studies in Frederick Ausubel's Laboratory (Harvard Medical School) have shown the effective use of *C. elegans* as a simple and powerful genetic model to screen for antimicrobial compounds, such as the discovery of RPW-24 that protects *C. elegans* from bacterial infections by modulating the p38 MAPK, and ATF7 transcription regulatory pathways [Pukkila-Worley et al. 2012]. We have previously used *C. elegans* to screen chemicals by using the nematode egg laying assay, and have identified novel compounds that affect egg laying behavior in a high throughput screen of commercially available chemical library [Siddiqui et al., *C. elegans* meeting UCLA, 2011]. We are screening chemical libraries in *C. elegans* for novel compounds that may inhibit the growth of oral pathogens such as *Pseudomonas aeruginosa,* found in the periodontal infections; and look for modulators of NSY-1, SEK-1, PMK-1 Mitogen Activated Protein (MAP) Kinase pathway that is comparable to the mammalian ASK-1(MAP kinase kinase kinase)/MKK3/6 (MAP kinase kinase) p38 MAP kinase signaling pathway. To validate the discovery of novel compounds in *C. elegans* high throughput screens for periodontal pathogens, we will use *in vitro* PLC (periodontal ligament cell) cell culture screens. Results will be presented on the efficacy of screened compounds on oral pathogens specific to chronic periodontal disease.

446A. Using Caenorhabditis elegans to investigate the genetic and mechanistic basis of cellular defense against bacterial pore-forming proteins. **Anand Sitaram**, Raffi Aroian. Section of Cell and Developmental Biology, University of California San Diego, San Diego, CA.

A major group of virulence factors produced by several significant species of pathogenic bacteria are secreted pore-forming proteins (PFPs) that bind to host cells and form oligomeric pores in the plasma membrane. Intoxicated cells can recognize and respond to these pores, but the relevant genes and the cell biological processes that underlie this response remain unclear. A previously published feeding RNAi screen in wild-type Caenorhabditis elegans revealed over 100 genes required for PFP defense. Here we show that strains with loss of function mutations in three of these genes are also hypersensitive to PFPs expressed by bacteria grown on agar plates or to recombinant PFPs in liquid culture. One gene was chosen for further study based on the strength of its phenotype. This gene was shown by RNAi to be specifically required for PFP defense in the intestine, the target tissue of PFPs produced by ingested bacteria. Furthermore, the requirement for this gene in PFP defense is specific; RNAi knockdown of the gene does not sensitize

worms to heat or heavy metal stress. The mammalian homolog of this gene of interest is a cytosolic adaptor implicated in signaling to stimulate actin rearrangement at the plasma membrane as well as kinase signaling. Because membrane trafficking/endocytosis and kinase signaling have each been previously shown to be critical for PFP defense in C. elegans, this gene is a strong candidate for playing a central upstream role in coordinating cellular defenses against PFPs.

447B. *C. elegans* and mammalian cell cultures: comparison of two different approaches to toxicological screening of silver nanoparticles. **C. Soria**[1], T. Coccini[2], S. Giorgetti[1], L. Marchese[1], I. Zorzoli[3], U. De Simone[2], V. Bellotti[1,4], M. Stoppini[1], L. Manzo[2,3]. 1) Dept. of Molecular Medicine, University of Pavia, Pavia, Italy; 2) Toxicology Division, Salvatore Maugeri Foundation IRCCS and University of Pavia, Pavia, Italy; 3) Dept. of Internal Medicine and Clinical Therapeutics, University of Pavia, Pavia, Italy; 4) Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, Royal Free Campus, University College London, London, United Kingdom.

Understanding the fate and toxicological behavior of nanomaterials *in vivo* has becoming crucial in the study of their biological effects. In recent years, potential health risks resulting from exposure to silver nanoparticles (AgNPs) have attracted much attention based on increasing applications of materials containing these nanoparticles and their presence in a large number of industrial, medicinal and consumer products. Toxicological effects of AgNPs was investigated in parallel by *in vitro* assays using central nervous systems (D384) and pulmonary (A549) cell cultures, and *in vivo* experiments using the ancestral *C. elegans* strain (N2). Cell toxicity of nanoparticles was evaluated by measuring mitochondrial function (MTT assay), membrane integrity and cellular morphology (Calcein AM/Propidium Iodide staining), and cell growth and proliferation (clonogenic test). The effects on *C. elegans* were measured by examining larval metamorphosis, the movement performance of the adult worms and overall survival. The toxic effects evaluated by IC50 values indicated that the *C.elegans* model was more susceptible to changes caused by the test-nanoparticles with 5 to 10 fold increases in sensitivity compared with mammalian cell cultures. Ongoing experiments examining the metabolic pathways affected by AgNPs exposure in cells and *C. elegans*, and comparative analysis of the gene response in the two systems will further validate *C. elegans* as an informative, rapid and inexpensive model suitable for toxicological studies.

448C. *C. elegans* expressing human β2-microglobulin: a novel model for studying the amyloid toxicity. **C. Soria**[1], L. Diomede[2], M. Romeo[2], S. Giorgetti[1], L. Marchese[1], PP. Mangione[1,3], I. Zorzoli[4], F. Romano[5], S. Ramat[5], M. Salmona[2], V. Bellotti[1,3], M. Stoppini[1]. 1) Dept. of Molecular Medicine, University of Pavia, Pavia, Italy; 2) Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy; 3) Centre for Amyloidosis and Acute Phase Proteins, Division of Medicine, Royal Free Campus, University College London, London, United Kingdom; 4) Dept. of Internal Medicine and Clinical Therapeutics, University of Pavia, Pavia, Italy; 5) Dept. of Industrial and Information Engineering, University of Pavia, Pavia, Italy.

Availability of living organisms to mimic key molecular events of amyloidogenesis of human proteins is crucial for elucidating the molecular mechanism of the disease really occurring *in vivo*. Human b2-microglobulin (b2-m) causes systemic amyloidosis in haemodialysed patients. The structure, misfolding propensity, kinetics of fibrillogenesis and cytotoxicity of this protein, *in vitro*, have been studied more extensively than for any other globular protein. However, no suitable animal model for b2-m amyloidosis has been so far reported. We have now established and characterized three new transgenic *C. elegans* strains expressing wild type human b2-m and two highly amyloidogenic isoforms: P32G variant and the truncated form DN6 lacking of the 6 N-terminal residues. The expression of human b2-m affects the larval growth of *C. elegans* and the severity of the damage correlates with the intrinsic propensity to self-aggregate that has been reported in previous *in vitro* studies. We have no evidence of the formation of amyloid deposits in the body-wall muscles of worms. However, we discovered a strict correlation between the pathological phenotype and the presence of oligomeric species recognized by the A11 antibody, that feet well also with the superoxide levels detected in worms. The strains expressing human b2-m exhibit a locomotory defect quantified with the body bends assay. Also a specific software was developed for monitoring the movement during the whole lifespan of *C. elegans*. Here we show that tetracyclines can correct this abnormality confirming that these compounds are able to protect a living organism from the proteotoxicity of human b2-m.

449A. Toxicity of the fungal lectin CCL2 against *C. elegans*. **K. Stutz**[1], A. Butschi[1], S. Bleuler-Martínez[2], T. Wohlschlager[2], M. Aebi[2], M. Künzler[2], M. Hengartner[1]. 1) Institute of Molecular Life Sciences, University of Zurich, Switzerland; 2) Institute of Microbiology, ETH Zurich, Switzerland.

Lectins are non-immunoglobulin, carbohydrate-binding proteins without catalytic activity towards the recognized carbohydrate. Many fungal lectins display toxicity towards *C. elegans* and other organisms and thus may be part of a lectin-mediated defense system of fungi against predators, which might be exploited to fight animal parasitic nematodes. In *C. elegans*, toxicity of lectins is assessed by feeding *E. coli* overexpressing a lectin. This results in inhibition of development and reproduction as well as premature death. We previously showed that the fungal lectin CCL2 is highly toxic to *C. elegans* (Schubert et al., 2012). To characterize the toxicity phenotype of CCL2 on post-L4 larvae, we are performing confocal and transmission electron microscopy. The intoxicated worms show a severely affected intestine and delayed egg laying. To identify the sugar moiety bound by CCL2, we performed a glycan array analysis of recombinant CCL2, which revealed a pronounced carbohydrate-specificity for Fuca1,3GlcNAc-containing glycans. Resistance of *C. elegans* mutants (*bre-1*, *ger-1*, *fut-1*) defective in the biosynthesis of the a1,3-core fucoside confirmed this glycotarget. To find more components involved in the CCL2 toxicity pathway, we performed a *mos-1*- and EMS mutagenesis screen. All but one mutation were again in *bre-1*, *ger-1* or *fut-1*. This confirmed the relevance of these three genes, while the sequencing of the unknown mutation is pending. To identify the proteins carrying the fucoside recognized by CCL2, we performed affinity chromatography and pulled down four proteins that were previously identified as targets of endogenous galectins and shown to be components of the glycocalyx of the *C. elegans* intestine (Maduzia et al., 2011). Their individual mutants are not resistant against CCL2. The involvement of these four proteins in the CCL2 pathway is examined at the moment. Taken together, our results suggest that the nematotoxicity of CCL2 is mediated by binding to glycoproteins of the intestinal glycocalyx. Interestingly, the same glycoproteins are also bound by endogenous lectins.

450B. Interplay of host and pathogen genetics upon RNA virus infection in *C. elegans*. **Melanie Tanguy**, Peter Sarkies, Jeremie Le Pen, Eric A. Miska. Gurdon Institute, Cambridge, United Kingdom.

Understanding antiviral pathways in a model organism such as *Caenorhabditis elegans* can provide an important tool to study host pathogen

interactions. The characterisation of antiviral immunity in *C. elegans* has been hampered by the absence of a known virus infecting the animals in the wild. Since the isolation of the Orsay virus, a RNA virus of the *Nodaviridae* family, in a wild type isolate of *C. elegans* (Felix *et al.* 2011), we are now able to investigate host pathogen genetic interactions in this organism. Previous work carried out in the laboratory characterized the potent role of RNA interference in the host defence toward Orsay virus infection. Here we present the first forward genetic screen conducted to identify novel actors of the antiviral innate immunity pathway in *C. elegans*. We also show a new method to investigate Orsay virus RNA genome diversity in infected *C. elegans* animals. Indeed, RNA virus genome(s) are known to exist as a collection of closely related genomic variants rather than a master sequence associated with few mutated copies. This genetic diversity represents a major source of variability, for example well known to be associated with acquired resistance to antiviral therapy. It is a great challenge to better understand the interaction of this viral mutant "swarm" with host genetic variations. The method we developed is based on high throughput small RNA sequencing of primary siRNAs that are generated from direct dicing of the viral RNA genome. This approach allowed us to identify single nucleotide polymorphisms (SNP) in the Orsay genome and thus we reveal the influence of host genetic variation on the pattern of viral SNPs.

451C. Role of wild-type ataxin-3 and valosin-containing protein/p97 in Machado-Joseph disease: a study in *C. elegans*. **A. Teixeira-Castro**[1,2,3], H. Brignull[3], D. Ribeiro[1,2], R. Morimoto[3], P. Maciel[1,2]. 1) Life and Health Sciences Research Institute (ICVS), School of Health Sciences, University of Minho, 4710-057 Braga, Portugal; 2) ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; 3) Department of Molecular Biosciences, Northwestern University Institute for Neuroscience, Rice Institute for Biomedical Research, Northwestern University, Evanston, IL 60208.

The role of the wild-type proteins in the pathology of polyglutamine diseases remains unclear. There are conflicting reports on whether the expression of the wild-type proteins, as well as their molecular partners should be increased, maintained or abolished in the disease context for therapeutic means. To gain insight into this issue, we have used novel *C. elegans* models that allowed us to simultaneously study the aggregation dynamics of both wild-type and pathological forms of ataxin-3 proteins *in vivo*, as well as its toxic outcomes at the behavioral level of the organism. We show that (i) recruitment of ataxin-3 into polyQ-containing cellular aggregates is polyQ length-dependent, (ii) that wild-type ataxin-3 overexpression leads to increased aggregation and worsens motor behavior of affected polyQ-expressing animals and that (iii) endogenous ataxin-3 knock-out does not aggravate aggregation or motor neuron dysfunction in a *C. elegans* model of ataxin-3 pathogenesis. In contrast, ablation of the ubiquitin-selective chaperone CDC-48, an interactor of ataxin-3, worsens the phenotype. Our findings support the idea that wild-type ataxin-3 alone is unlikely to play a neuroprotective role in MJD.

452A. Potential Roles of Peroxidases in C. elegans Innate Immunity. **George R. Tiller**, Danielle A. Garsin. Microbiology and Molecular Genetics, UTHEALTH Houston, Houston, Tx.

ROS (reactive oxygen species) are produced at mucosal epithelial surfaces, as part of the innate immune response to combat invading pathogens. The proteins responsible for this ROS production belong to the NOX/DUOX (NADPH Oxidase and Dual Oxidase) family and include C. elegans Ce-Duox1/BLI-3. It was previously shown that in response to infection with Enterococcus faecalis, BLI-3 released ROS, and reduction of bli-3 expression caused the worms to be significantly more susceptible. Additionally, DUOXs are known to generate ROS for use by peroxidases, which then generate more potent oxidation products that aid in the host's innate immune response. An example is human lactoperoxidase (hLPO), which uses ROS from hDUOX2 to produce hypothiocyanite to facilitate clearance in the airway epithelia. Based on this information, we hypothesized that BLI-3-generated ROS may act as a substrate for peroxidases to generate antimicrobial agents in the C. elegans immune response. Using bli-3 as the query, WormBase.org was searched for homologous genes. The genes-of-interest were screened for a susceptibility-to-pathogen phenotype in C. elegans using RNAi (RNA interference) to reduce their expression. Genes necessary for full resistance against pathogen were further tested for general fitness defects using longevity assays. F49E12.1 RNAi worms incubated with E. faecalis at 25°C displayed an increased susceptibility phenotype (P-value = 0.0011; median survival: 189 and 165 hours, V.C. and F49E12.1, respectively). As determined by Amplex Red, when infected, F49E12.1 RNAi worms display increased H2O2 levels relative to vector control (P-value = 0.0068) which suggests F49E12.1 utilizes H2O2 for a, currently, unknown purpose. Interestingly, F49E12.1 possesses significant homology to peroxidases that are crucial for innate immunity in crustaceans as well as mammals. Therefore, the current focus is to elucidate how the putative peroxidase F49E12.1 contributes to the C. elegans innate immune response.

453B. The ABC transporter MRP-7 inhibits methylmercury-induced whole animal and dopamine neuron pathology. **Natalia VanDuyn**, Richard Nass. Pharm & Toxicology, Indiana Univ Sch Med, Indianapolis, IN.

Background: Methylmercury (MeHg) exposure from occupational, environmental, and food sources is a significant threat to public health. Recent epidemiological and vertebrate studies suggest that MeHg exposure may contribute to dopamine (DA) neuron vulnerability and the propensity to develop Parkinson's disease (PD). We have developed a novel *Caenorhabditis elegans* (*C. elegans*) model of MeHg toxicity and have shown that low, chronic exposure confers embryonic defects, developmental delays, and DA neuron degeneration, and that the toxicity is partially dependent on the phase II antioxidant transcription factor SKN-1/Nrf2. Statement of Purpose: In this study we asked what genes and molecular pathways are involved in MeHg-induced whole animal and DA neuron pathology. Methods: We utilized a reverse genetic screen, biochemical assays, immunofluorescence, transgenic *C. elegans*, RT-PCR, ICP-MS, Western analysis, and neuronal morphology analysis to characterize expression, localization and the role that SKN-1 and MRP-7 play in MeHg-induced whole animal and DA neuronal death. Results: Over 17,000 genes were screened for whole animal sensitivity to MeHg, and 92 genes were identified (90% have strong human homologues) that affect whole animal and/or DA neuron pathology. Here we report detailed analysis of a previously uncharacterized ABC transporter, MRP-7. MeHg confers increased mortality in *mrp-7(RNAi)* animals relative to WT. Furthermore, exposure of the genetic knockdown animals to extremely low MeHg levels results in 40% of animals displaying DA neuron degeneration. Transgenic animals expressing *mrp-7* transcriptional fusions indicate that *mrp-7* is expressed in DA neurons as well as other cell types. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) studies indicate that *mrp-7(RNAi)* animals contain 2-fold higher Hg levels relative to WT. Conclusions: This study describes a novel whole genome reverse genetic screen that identified MRP-7 as well as a number of other genes involved in MeHg-associated resistance that will likely prove useful in identifying therapeutic targets to inhibit MeHg-induced cellular toxicity in humans. Support: NIEHS ES014459 and ES003299 to RN; and EPA STAR Graduate Fellowship to NVD.

454C. HLH-30/CeTFEB plays a central role in host defense against bacterial infection. **Orane Visvikis**[1], Nnamdi Ihuegbu[2], Anna-Maria Alves[1], Lyly Luhachack[1], Amanda Wollenberg[1], Gary Stormo[2], Javier Irazoqui[1]. 1) Department of Pediatrics - Program of Developmental Immunology, Massachusetts General Hospital, Boston (MA); 2) Department of Genetics - Center of Genome Sciences, Washington University Medical School, St. Louis (MO).

During infection, the host mounts complex defense mechanisms to eliminate the invading microbe and preserve its own integrity. To better understand these defense mechanisms, we analyzed the nature and regulation of the transcriptional response that we showed to be triggered by *Caenorhabditis elegans* when infected by *Staphylococcus aureus*. We used bioinformatics to analyze the promoter of the *S. aureus*-induced genes and found enrichment of the DNA binding sequence of the HLH-30 transcription factor. Phylogenetic analysis identified HLH-30 as the homolog of human TFEB, a master regulator of autophagy and lysosomal regulation. Performing survival assay, we found that *hlh-30* mutants exhibit enhanced susceptibility to *S. aureus* -mediated killing, a phenotype rescued by transgenic expression of HLH-30::GFP. Using RNA-seq, we found that ~80% of the *S. aureus*-induced genes require HLH-30 for their induction, thus revealing the central role of HLH-30 in controlling the host response. Fluorescence analysis of HLH-30::GFP from transgenic animals revealed acute activation of HLH-30, being targeted in cell nuclei early during infection. We next performed gene set enrichment analysis of HLH-30-dependent genes and found an over-representation of cytoprotective genes, as well as immune genes. We verified by qRT-PCR and *in vivo* reporter analysis that HLH-30 induces the expression of autophagy/lysosomal and antimicrobial genes upon infection. Finally, we showed that autophagy itself and expression of specific lysozymes are required for host defense.

Altogether, these results indicate that HLH-30 plays a critical role in *C. elegans* host defense, and suggest that TFEB might play a similar role in mammalian host defense by regulating both autophagy and immune genes during infection.

455A. Genetic basis underlying differential susceptibility to bacteria in *Caenorhabditis elegans*. **Ziyi Wang**[1], Michael Herman[1], L. Basten Snoek[2], Jan Kammenga[2]. 1) Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, Kansas, USA; 2) Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands.

Although *Escherichia coli* OP50 is the standard lab food for *C. elegans*, it is unlikely to be part of its natural diet. In fact, the bacterial environment *C. elegans* naturally encounter could be quite heterogeneous. In the wild, animals must defend against potential pathogens within mixed food sources. One such potential pathogen is *Stenotrophomonas maltophilia*, a ubiquitous bacterium that has been isolated in association with nematodes from natural environments, but can also cause nosocomial infection in humans, especially immune-compromised individuals. For instance, our lab isolate, *S. maltophilia* JCMS, was found surprisingly pathogenic to *C. elegans* in that even *daf-2* mutants that are otherwise long-lived in any other bacteria tested to date, are as susceptible as wildtype animals. More interestingly, we found N2 (Bristol, England) and CB4856 (Hawaii) respond to *E. coli* OP50 and *S. maltophilia* JCMS differently and observed a significant genotype by environment (G×E) interaction, which has been thought to be a major mechanism that maintains genetic variation in natural populations. To understand the evolution of *C. elegans* responses to a heterogeneous bacterial diet it is crucial to identify the underlying genetic variation responsible for differential susceptibility and to characterize the corresponding molecular mechanism. We chose to take a quantitative genetic approach to this question and have mapped quantitative trait loci (QTL) in a N2×CB4856 recombinant inbred panel for post-reproductive lifespan on *E. coli* OP50 and *S. maltophilia* JCMS. We identified a novel QTL on LGX that contributes to the G×E interaction. We then used introgression lines that contain distinct lengths of chromosome X within this QTL region from CB4856 in an otherwise N2 genomic background for fine mapping. We confirmed and narrowed down the position of this locus to a relatively small region that contains reasonable number of candidate genes for further functional tests. We are currently endeavoring to pinpoint the causal genes and will report our progress.

456B. Acyl-CoA synthase-3 and the nuclear receptor NHR-25 regulate innate immunity genes and promote pathogen resistance. **Jordan D. Ward**[1], Carol Couillault[2], Brendan Mullaney[3], Benjamin Schiller[1], Teresita Bernal[1], Sarah Petnic[1], Marc Van Gilst[4], Kaveh Ashrafi[3], Jonathan Ewbank[2], Keith Yamamoto[1]. 1) Cellular and Molecular Pharmacology, UCSF, Mission Bay Campus, San Francisco, CA; 2) Centre d'Immunologie de Marseille-Luminy, UM2 Aix-Marseille Université, Marseille, France; 3) Department of Physiology, UCSF, Mission Bay Campus, San Francisco, CA; 4) Fred Hutchinson Cancer Research Center, Seattle, WA.

We have previously demonstrated that the acyl-CoA synthetase , *acs-3*, negatively regulates the nuclear hormone receptor, *nhr-25*, to promote an endocrine program of lipid uptake and synthesis. Given that NHR-25 is a transcription factor, we performed microarray analysis to identify regulated genes promoting this metabolic program. Much to our surprise, despite the observation that *nhr-25(lf)* suppresses all known *acs-3* mutant phenotypes, loss of function of either *acs-3* or *nhr-25* had a similar effect at the transcriptional level. In both cases, EASE and gene ontology analysis revealed a significant upregulation of genes involved in cuticle formation and pathogen response. qPCR verification of the microarray data suggested that *acs-3* and *nhr-25* are epistatic with respect to pathogen response gene induction. Elevated pathogen response gene expression can be caused by osmotic stress resistance phenotypes and cuticular damage. *acs-3* mutants have: i) a subtle resistance to acute hyperosmotic stress; ii) a partially penetrant sensitivity to hypo-osmotic stress; and iii) an intact cuticular barrier, as exhibited by elevated staining with the cuticle impermeable Hoechst 33358 dye in tail nuclei. *nhr-25* mutants showed essentially wild-type responses in all of these assays. We then performed survival assays with the pathogens *Drechmeria coniospora* and *Pseudomonas aeruginosa* to test whether loss of *acs-3* or *nhr-25* activity affected resistance to pathogens. Loss of *acs-3* activity caused hypersensitivity to *Drechmeria*, while *nhr-25* mutants were sensitive to *Pseudomonas*. Intriguingly, *acs-3* mutation suppressed the sensitivity of *nhr-25* mutants to Pseudomonas. Together, these results highlight a link between the ACS-3-NHR-25 endocrine program and resistance to pathogens.

457C. A unique *Stenotrophomonas maltophilia* strain that evades a major *Caenorhabditis elegans* defense pathway. **Corin Vashoun White**[1], Brian Darby[1,2], Michael Herman[1]. 1) Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, KS, USA; 2) Current Address: Department of Biology, University of North Dakota, Grand Forks, ND, USA.

Stenotrophomonas maltophilia is an aerobic gram-negative bacterium that has been isolated from environmental and clinical locations. In the course of our studies, we discovered a local *S. maltophilia* environmental isolate JCMS that is pathogenic to *Caenorhabditis elegans*. However, neither characterized *S. maltophilia* environmental isolate R551-3 nor clinical isolate K279a were as pathogenic to *C. elegans*. To understand the basis of these differences, we

transformed each strain with GFP to follow their fates within the *C. elegans* gut and conducted titer assays. We found that live JCMS accumulated in the intestine more than *E. coli* OP50 and the other *S. maltophilia* strains. Several pathways that serve to protect *C. elegans* from various pathogenic bacteria have been discovered, including the p38 MAPK, UPR and TGFb-related pathways. Mutants that disrupt numerous components of these pathways are hypersensitive to both JCMS and OP50, suggesting that the functions of these genes are needed for a general bacterial response. Surprisingly, DAF-2/IIS pathway mutants displayed shortened lifespans on *S. maltophilia* JCMS, which is striking as most have long lifespans on other bacterial pathogens. We are using several different strategies to identify candidate genes and/or pathways that might explain this *S. maltophilia* JCMS specific evasion of the DAF-2/IIS defense pathway. We used an unbiased genetic screen to identify two mutants that are specifically resistant to JCMS and one that is hypersensitive. In addition, we identified 457 genes that were significantly differently expressed among pairwise combinations of wildtype nematodes exposed to OP50, JCMS or K279a. Genes differentially regulated in response to *S. maltophilia* JCMS as compared to *S. maltophilia* K279a or *E. coli* OP50 might be specific to pathogenicity; whereas those differentially regulated in response to both *S. maltophilia* strains as compared to OP50 might be specific to *S. maltophilia*. Our combined studies aim to elucidate new and existing genes and pathways *C. elegans* employs to respond to *S. maltophilia* K279a and pathogenic JCMS.

458A. The effects of bacteria from rotting fruit on *C. elegans* gene expression and lifespan. **Amanda C Wollenberg**[1], Marie-Anne Félix[2], Javier E. Irazoqui[1]. 1) Developmental Immunology, Massachusetts General Hospital, Boston, MA; 2) Institut de Biologie de L'Ecole Normale Supérieure, Paris, France.

As a natural bacterivore, the nematode *Caenorhabditis elegans* is a tractable system in which to investigate interactions between bacteria and the eukaryotic digestive tract. This system has already yielded insights into the molecular crosstalk between medically relevant pathogens and the host intestine. However, until recent work demonstrated that wild *C. elegans* populations are often found in association with rotting fruit, it was unclear what types of microbial communities would be naturally encountered by *C. elegans*, and what sorts of beneficial or detrimental interactions might occur between *C. elegans* and bacteria in the wild. We are beginning to address these questions using a panel of bacterial isolates that were collected by Dr. M-A Felix at seven microsites in France (e.g. a rotting apple) where *C. elegans* populations were also found. The isolates have been assigned to 18 genera on the basis of 16S rDNA sequencing and represent 2 Gram-positive phyla (Actinobacteria, Firmicutes) and 4 Gram-negative phyla (Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidetes). Our hypothesis is that this collection of bacterial isolates will include not only potential pathogens, but also potentially beneficial microbes able to protect nematodes from more pathogenic bacteria, perhaps via colonizing the intestine or by priming the immune system. We will show that the isolates vary both in their effect on the lifespan of adult animals, and in their capacity to elicit immune responses such as induction of putative antimicrobial genes. Finally, we will present evidence that nematode immune pathways characterized in the context of the response to human pathogens also play a role in responding to these naturally isolated bacteria.

459B. *Enterococcus* infection of *C. elegans* as a model of innate immunity. **Grace J. Yuen**, Frederick M. Ausubel. Molecular Biology, Massachusetts General Hospital, Boston, MA.

Enterococcus is a Gram-positive commensal that is also an important opportunistic pathogen. Most human enterococcal infections are caused by either *E. faecalis* or *E. faecium*. Our laboratory has modeled *Enterococcus* infection in *C. elegans* using both species. We have previously shown that infection with either species leads to gut distention, but only *E. faecalis* is able to establish a persistent and lethal infection in the nematode. We now provide evidence that at least three canonical *C. elegans* immune signaling pathways are important for survival during infection with *E. faecalis* and *E. faecium*. While the lifespan of wild-type worms is unaffected by *E. faecium* infection, mutations in the PMK-1, FSHR-1, and BAR-1 immune signaling pathways lead to an immunocompromised phenotype. This new finding suggests that an active host response is required to keep *E. faecium* infection "in check" in the worm intestine. To further characterize the *C. elegans* host response to *Enterococcus* infections, we used genome-wide transcriptional profiling of nematodes feeding on *E. faecalis* and *E. faecium*, as well as two controls, heat-killed *E. coli* and live *Bacillus subtilis*, a non-pathogenic Gram-positive. We found that relative to *B. subtilis*, *E. faecalis* and *E. faecium* caused the upregulation of 249 and 166 genes, respectively, of which 105 genes were common to both, comprising the *Enterococcus* gene signature. Shared by both *Enterococcus* infection signatures were genes relating to oxidation/reduction, acyl-CoA dehydrogenase/oxidase activity, fatty acid metabolism, and C-type lectins. Additionally, the *Enterococcus* infection gene signature is fairly distinct from the *P. aeruginosa*, *S. aureus*, and *C. albicans* infection signatures. Furthermore, of the 91 genes that are upregulated in *E. faecalis* (more virulent) relative to *E. faecium* (less virulent), 22 are shared with the 77 genes upregulated in worms infected with virulent *Microbacterium nematophilum* relative to *avirulent M. nematophilum* (O'Rourke *et al*., 2006), suggesting that these genes may comprise a "virulence response signature." Studies are underway in understanding the biology of *Enterococcus* infection in *C. elegans* and identifying novel *Enterococcus*-activated pathways.

460C. Effects of Diphenyl Diselenide on transgenic *Caernohabiditis elegans* Alzheimer's disease model. **D. C. Zamberlan**, L. P. Arantes, J. B. T. Rocha, F. A. A. Soares. Chemical Dept, UFSM, Santa Maria, RS, Brazil.

With increasing life expectancy, neurodegenerative diseases have becomeever more common and Alzheimer's disease (AD) the most reported one. AD is mainly characterized by the presence of senile plaques in the brain formed by toxic peptide amyloid-b (Ab). The current explanation for Ab toxicity in AD still remains controversial in view of the fact that the relationship between Ab species and Ab specific behavior has not been defined in vivo. Simple invertebrate models of neurodegenerative diseases offer experimental advantages for addressing basic cellular processes that are conserved among all animals. The diphenyl diselenide (DPDS), an organic selenium compound, is characterized by its potential antioxidant, anti-inflammatory, hepatic and neuroprotective. This study aims to determine the effects of chronic treatment with DPDS in the nematode *C. elegans* model of AD. The wildtype strain N2 and transgenic worms which expresses the Ab_{1-42} constitutively (CL2006) or inducible in the body wall muscles (CL4176) and it's control strain (CL802), inducible neuronal Ab expression (CL2355) and it's control strain (CL2120) were treated with DPDS in different concentrations since eggs to adulthood. Behavioral assays (paralysis, chemotaxisqu and egg laying) and the levels of reactive oxygen species were quantified in order to verify if this compound is able to modulate the toxicity of the peptide by its antioxidant ability. DPDS reduced the levels of reactive species, the mortality induced by juglone and ameliorate the deficits in odorant preference associative learning of the worms. However, the chronic treatment with DPDS also induced a decreased of movement and a raise of the paralysis induced by Ab. This results indicate that despite the protective effects in oxidative stress and memory provided by DPDS, it exacerbate the toxic effects of Ab in the body wall muscles of the transgenic worms, suggesting that the toxicity of this peptide not only occurs by

oxidative stress. Thereby, more studies are necessary to understand how exactly the Ab peptide induces toxicity in *C. elegans* model and how the DPDS acts to exerts its protective effects in the worms.

461A. UNC-119 interacts with ARL-13 and plays a role in ciliogenesis. **Qing Zhang**, Qing Wei, Yuxia Zhang, Jinghua Hu. Mayo Clinic, Rochester, MN.

Mutations in human small GTPase ARL13B cause Joubert syndrome (JBTS), a neurodevelopmental ciliopathy. Uncovering the endogenous effector(s) of ARL13B would facilitate the determination of the pathogenesis underlying JBTS. Here, we demonstrate that ARL-13, the Caenorhabditis elegans homologue of ARL13B, directly interacts with UNC-119, a retinopathy causal protein and also a known effector of ARL-3. Both ARL-13 and UNC-119 localize exclusively to the doublet segment of the cilium. The proper ciliary targeting of UNC-119 is ARL-13-dependent and ARL-13 shows mild mislocalization phenotype in unc-119 mutant worm. Transmission electron microscopy (TEM) experiments revealed that unc-119 mutant shows similar cilia defects as arl-13 mutant does. Moreover, we found that ARL-3, ARL-13, and UNC-119 may exist in one functional complex to execute their ciliary roles. Intriguingly, the ciliogenesis defective phenotype in both arl-13 and unc-119 mutant worms can be partially rescued by ARL-3 depletion, further supporting the functional crosstalk among these three proteins. Our results suggest that UNC-119 could be the shared effector for both ARL-13 and ARL-3, the two small GTPases implicated in either human or mammalian ciliopathy models.

462B. CED-10 Rac signaling pathway regulates axon regeneration via JNK MAPK pathway. **Tanimul Alam**, Kazuya Hirose, Strahil Pastuhov, Naoki Hisamoto, Kunihiro Matsumoto. Div. of Biol. Sci., Grad. School of Sci., Nagoya Univ., Japan.

Axon regeneration is regulated by JNK MAPK pathway, which is conserved among a wide variety of organisms. The C.elegans JNK MAPK pathway is composed of MLK-1 (MAPKKK), MEK-1 (MAPKK) and KGB-1 (MAPK). We have previously identified MAX-2, a mammalian PAK-like kinase, and MIG-2 GTPase as upstream activators of the JNK pathway in heavy metal stress response (Fujiki et al., MCB 30, p995, 2010). We found that *max-2* mutant worms exhibited axon regeneration defect. However, the *mig-2* mutation had no effect on axon regeneration. We found that MAX-2 associated with GTP-bound CED-10, a Rac-type GTPase, and that the *ced-10* mutation was defective in axon regeneration. Furthermore, we found that CED-5, a guanine nucleotide exchange factor of CED-10, was also required for efficient axon regeneration. These results suggest that a Rac signaling pathway, composed of CED-5, CED-10 and MAX-2, acts as one of the upstream activators of JNK pathway in axon regeneration.

463C. The atypical Rho GTPase CHW-1 works downstream of SAX-3/Robo to mediate axon guidance in *Caenorhabditis elegans*. **Jamie K. Alan**[1,2], Erik L. Lundquist[2]. 1) Foundational Sciences, Central Michigan University College of Medicine, Mt. Pleasant, MI; 2) Molecular Biosciences, University of Kansas, Lawrence, KS.

During development, neuronal cells extend an axon towards their target destination in response to a cue to form a properly functioning nervous system. Rho proteins are Ras-related small GTPases that regulate cytoskeletal organization and dynamics along with cell adhesion and motility. Furthermore, Rho proteins such as CDC-42, MIG-2 and CED-10 are known to regulate axon guidance. Despite extensive knowledge about canonical Rho proteins (RhoA/Rac1/Cdc-42), little is known about the *C. elegans* atypical Cdc-42-like family members CHW-1 and CRP-1 in regards to axon pathfinding and neuronal migration. *chw-1*(Chp/Wrch) encodes for a protein that resembles human Chp (Wrch-2/RhoV) and Wrch-1 (RhoU), and *crp-1* encodes for a protein that resembles TC10 and TCL. Here, we show that *chw-1* works redundantly with *crp-1* and *cdc-42* in axon guidance. Furthermore, our experiments suggest that precise levels of *chw-1* expression and activity are required for proper axon guidance. Also, *chw-1* genetically interacts with the guidance receptor *sax-3*. Finally, in VD/DD motor neurons *chw-1* and *sax-3* might act together to control axon guidance. In summary, this is the first studying implicating the atypical Rho GTPases *chw-1* and *crp-1* in axon guidance. Furthermore, this is the first evidence of a genetic interaction between *chw-1* and the guidance receptor *sax-3*. Finally, we show that *chw-1*, but not *crp-1*, might act with *sax-3* in axon guidance.

464A. Postmitotic diversification of olfactory neuron types is mediated by differential activities of the HMG-box transcription factor SOX-2. **Amel Alqadah**[1,3], Yi-Wen Hsieh[1,3], Berta Vidal Iglesias[2], Chieh Chang[1,4], Oliver Hobert[2,4], Chiou-Fen Chuang[1,4]. 1) Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; 2) Columbia University Medical Center, New York, NY; 3) Equal contribution; 4) Co-senior authors.

The nervous system is composed of an immense variety of different cell types. This is especially important in the developing sensory system, which generates a large number of specialized neurons for sensing various stimuli in the environment. However, the molecular mechanisms that regulate sensory neuron diversity are only partly understood.

In the *C. elegans* olfactory system, AWA, AWB, and AWC neurons are dedicated to sensing volatile odorants. Here we reveal a novel role of the highly conserved HMG-box transcription factor SOX-2 in post-mitotic specification as well as alternative differentiation of AWC and AWB olfactory neurons. From a non-biased forward genetic screen, we identified a missense mutation in the HMG domain of the SOX-2 protein that results in a cell fate transformation from AWB to AWC at both molecular and functional levels. This phenotype is similar to that caused by loss-of-function mutations in the homedomain protein LIM-4. Analysis of a *sox-2* null allele reveals functions of *sox-2* in postmitotic cells to specify general identities of both AWC and AWB neurons. We show that SOX-2 functions cooperatively with the OTX/OTD transcription factor CEH-36 to specify the AWC cell fate, and that SOX-2 and LIM-4 function cooperatively to further differentiate AWB from AWC fates. Together, our results suggest a model in which SOX-2 partners with different transcription factors such as LIM-4 or CEH-36 to diversify postmitotic olfactory cell types. Sox2 plays an important role in the maintenance of neural progenitor/stem cell pluripotency and inhibition of neurogenesis in the developing nervous system. Our study demonstrates that *sox-2* is reused in postmitotic cells to regulate alternative differentiation of two olfactory neurons.

465B. An Investigation into the Affect of Neuronal Activity on Proper Neural Connectivity in *C. elegans*. **Kristine E. Andersen**, Benjamin Barsi-Rhyne, Kristine Miller, Christopher Vargas, Joori Park, Emma Holdrich, Miri VanHoven. Department of Biological Sciences, San Jose State University, San Jose, CA.

Neuronal activity has been implicated in the establishment and maintenance of appropriate synaptic connections in vertebrate and invertebrate systems. However, the molecular mechanisms by which activity affects connectivity are poorly understood. Activity may affect axon outgrowth, axon guidance, synaptogenesis, or the maintenance of neural connections. We have utilized the synaptic partner recognition marker Neuroligin-1 GFP

Reconstitution Across Synaptic Partners (NLG-1 GRASP) to label synapses between PHB sensory neurons and AVA interneurons, as well as an mCherry fluorophore expressed selectively in these cells to visualize potential defects in axon outgrowth, axon guidance, and contact between pre- and postsynaptic neurites *in vivo*. Interestingly, we find different roles for different types of neuronal activity in neural development. Loss-of-function mutations in *odr-3*, which encodes a G-alpha subunit required for sensory activity, result in reduced synapses between PHB and AVA neurons. Time course experiments indicate that L1s are unaffected in *odr-3* mutants, suggesting that this molecule is not required for initial establishment of synapses. These results indicate that *C. elegans* may be a powerful model organism for elucidating the molecular mechanisms by which sensory activity mediates synaptic connectivity. Surprisingly, we also found that loss-of-function mutations in *unc-7*, a gap junction component, result in reduced contact between PHB and AVA neurites, but left nerve bundles in the preanal ganglion and other regions largely intact. This indicates that electrical synapses may be required for correct contact between pre- and postsynaptic neurites within a complex nerve bundle. Our future goal is to further characterize the roles of these genes in development and maintenance of neural circuits.

466C. Dendritic arborization in dauer IL2 neurons: Genetic and bioinformatic analyses. **Rebecca J Androwski**[1], Alina Rashid[1], Tom Ritter[2], Nathan E Schroeder[1], Maureen M Barr[1]. 1) Dept. of Genetics, Rutgers Univ, Piscataway, NJ; 2) http://ritter.vg.

Under adverse environmental conditions, *C. elegans* enters a stress resistant dauer stage. We discovered a wild-type dauer-specific phenotype wherein six inner labial sensory neurons (IL2) undergo dramatic reorganization including extensive arborization of the IL2 quadrant (IL2Q) neurons (see N. Schroeder et al abstract). To identify genes involved in dauer-specific IL2 remodeling , we used a candidate gene approach. Representative genes were examined from several molecular pathways: dauer formation, ciliogenesis and intraflagellar transport, Notch signaling, cell fate and axon guidance. For example, both UNC-86 and LIN-32 are transcription factors necessary for IL2 cell fate. Most *unc-86* alleles are defective in IL2 formation. However, *unc-86(n848)* mutants show normal IL2 morphology in non-dauers, but defects in dauer-specific IL2 arbors. Interestingly, *lin-32* mutants that form IL2 neurons show no obvious defects in arborization. The RFX-transcription factor DAF-19, is a master regulator of ciliogenesis. *daf-19(m86)* mutants lack all sensory cilia, but show extranumerary branching in the lateral IL2s suggesting a role for cilia in inhibiting arborization.

Through a forward genetic screen we identified *kpc-1*, a kex2-like proprotein convertase and furin homolog, as required for organized arborization in both dauer IL2 neurons (See N Schroeder et al abstract) and adult PVD and FLP multidendritic neurons (see Rashid et al abstract). To identify potential KPC-1 substrates, we cross referenced genes upregulated in PVDs (1), genes upregulated during dauer (2), and genes containing putative proprotein cleavage sites (3). We have assembled a list of 159 potential regulators of dendritic branching which we are beginning to characterize.

1.Smith et al. Dev Bio. 345 (2010) 18-33.
2.Jeong et al. PLoS One. (2009 Jan) 4(1) e4162.
3.Duckert et al. Protein Eng Des Sel. 17 (2004) 107-112.

467A. Candidate modulators of tubulin and microtubule dynamics during *C. elegans* neural development. **Renee A. Baran**, Hyun S. Kim, Evan Choate, Mealani Kaiser. Biology Department, Occidental College, Los Angeles, CA, 90041.

tba-1 encodes an alpha-tubulin widely expressed during *C. elegans* development. We showed previously that a dominant allele, *tba-1(ju89)*, causes defects in locomotion and motor neuron development. To identify TBA-1-interacting proteins and better understand how microtubule dynamics and function are regulated in developing neurons, suppressors of *tba-1(ju89*) were isolated in a genetic screen based on reversion to wildtype movement. Multiple suppressors were mapped using snip-SNP analysis, and candidate suppressors were identified following whole genome sequencing. Two of the mutant genes identified by this approach were *klp-7* and *zer-1*. *klp-7* encodes a member of the kinesin-13/MCAK family that function in microtubule depolymerization. In addition to early embryonic functions, *klp*-7 is required for regrowth of neurons following laser axotomy (Ghosh-Roy et al., 2012). Experiments are in progress to test if KLP-7 expression and activity is altered in *tba-1(ju89)* mutants. Alternatively, decreased KLP-7 activity in the suppressor may compensate for another defect in microtubule dynamics due to altered TBA-1 function in the *ju89* mutants. *zer-1* encodes a conserved ubiquitin ligase substrate recognition subunit. Similar to the closely related protein, ZYG-11, ZER-1 interacts with *C. elegans* CUL-2 (Vasudevan, et al. 2007). The target substrates of ZER-1 are unknown, and it has not been implicated previously in neural development or function. ZER-1 may play a role in regulating tubulin stability directly or potentially modulate expression of critical regulators of tubulin, microtubules or microtubule-based transport in neurons.

468B. PLR-1, a novel E3 ligase, controls cell polarity and axonal extensions in *C. elegans*. **Jaffar M Bhat**, Jie Pan, Harald Hutter. Biological Sciences , Simon Fraser University, Burnaby , British Columbia , Canada.

During embryonic development neurons differentiate, and grow processes (axons and dendrites) that have to reach their appropriate target. This task is highly regulated and is achieved by using a set of conserved guidance cues and receptors. In *C. elegans* AVG is the pioneer interneuron extending the first axon during the establishment of the ventral nerve cord. The cues used by the AVG axon for its outgrowth and navigation are not known yet as the available mutants for known guidance cues and receptors do not show significant defects in AVG navigation or outgrowth. We carried out a forward genetic screen to identify genes important for the correct outgrowth of the AVG axon. We isolated two mutations *hd129* and *hd128*, both of which turned out to be alleles of *plr-1*. *plr-1* mutants show a polarity reversal of the AVG neuron and also a premature stop and midline crossing defects of the AVG axon. In addition *plr-1* mutants have migration and outgrowth defects in several other classes of neurons as well as a premature stop of the posterior excretory canals. *plr-1* is predicted to encode an E3 ligase, a large and diverse group of proteins, characterised by the presence of a RING finger domain. E3 ligases are enzymes transferring ubiquitin from E2 conjugating enzymes to substrate proteins, which are then degraded by the proteosome complex. Mutations in a gene reducing Wnt-signalling (see also WBPaper00039205) as well as mutations in *unc-53* and *unc-73* suppress the AVG defects, but not the other defects seen in *plr-1* mutants. This places *plr-1* in a Wnt regulation pathway and also suggests that *plr-1* has Wnt independent functions. UNC-53 is a cytoskeleton regulator protein orthologous to human NAV2 and UNC-73 is a guanine nucleotide exchange factor (GNEF). Both have established roles in the guidance of migrating cells and axons. We are currently investigating how *unc-53* and *unc-73* interact with *plr-1* to establish the correct polarity of AVG.

469C. Gaq mediates effects of antipsychotic drugs on C. elegans developmental delay/lethality. Limin Hao, Afsaneh Sheikholfslami, Kristin Harrington, Bruce Cohen, **Edgar Buttner**. Mailman Research Center, McLean Hospital, Belmont, MA 02478.

Our aim is to understand the mechanisms of action of antipsychotic drugs (APDs) using C. elegans as a genetic model. In C. elegans, APD exposure causes developmental delay or lethality, depending on the drug concentration. APDs target neurotransmitter receptors such as dopamine, glutamate, serotonin, and acetylcholine receptors, many of which are associated with Ga proteins. To test whether Ga proteins mediate APD-induced developmental phenotypes in C. elegans, we screened mutants of four types of Ga proteins and found that Gaq (egl-30) reduction-of-function mutants were hypersensitive and a gain-of-function mutant was slightly resistant to clozapine. To confirm that Gaq activity meditates clozapine's effects, we analyzed mutants of Gaq upstream modulators, such as eat-16, ric-8, rsbp-1, rsbp-2, tax-6, cnb-1, egl-10, and unc-43. Our results indicated that increased Gaq activity leads to drug resistance and decreased Gaq activity leads to drug hypersensitivity. We also analyzed downstream components in the egl-30 signaling pathway that regulates acetylcholine release and found that the drug effects are not mediated by these genes. To discover downstream effectors involved in clozapine's actions, we performed a forward genetic screen for suppressors of egl-30(rf) clozapine hypersensitivity. We obtained 15 suppressors, one of which was more resistant to clozapine than wild type. Eight of the remaining mutants were strong suppressors, and six were weak. All of these strains retained the Egl and Unc phenotypes of egl-30(rf). We are now characterizing these mutants to test whether acetylcholine release is affected. We have snip-mapped two suppressors, neither of which mapped to regions overlapping known suppressors of clozapine-induced developmental delay. Snip-mapping of other suppressors is underway.

470A. Mechanisms of age-related decline in axon regeneration. **Alexandra Byrne**, Trent Walradt, Austin Hubbert, Marc Hammarlund. Genetics, Yale University School of Medicine, New Haven, CT.

The ability of injured axons to regenerate declines with age, yet the mechanisms that regulate axon regeneration in aging animals are not well understood. We found that axon regeneration declines in aging *C. elegans* GABA motor neurons. Moreover, *daf-2* mutants, which lack the conserved insulin/IGF1 receptor, maintain their ability to regenerate severed GABA axons significantly longer than wild type animals. The increased regeneration in *daf-2* mutants is mediated by neuronal activity of the forkhead transcription factor DAF-16/FOXO. We further analyzed *daf-16* mosaic animals and found that *daf-16* regulates regeneration independently of lifespan. Thus, decline of axon regeneration in aged animals is an intrinsic, neuron-specific, and genetically regulated process. In addition, multiple lines of evidence suggest that insulin signaling and *daf-16* regulate regeneration in aged GABA neurons via the critical *dlk-1* MAP kinase regeneration pathway.

Further investigation of age-related regeneration revealed that *daf-18*/PTEN, like *daf-2*, inhibits GABA neuron regeneration in *C. elegans*. Surprisingly, *daf-18*'s role in regeneration is not mediated by DAF-16/FOXO, as loss of *daf-16* function does not alter the enhanced axon regeneration of aged *daf-18* mutants. By contrast, Rapamycin (a TOR inhibitor) does inhibit the enhanced regeneration of aged *daf-18* mutants, but does not affect axon regeneration in wild type animals. These data suggest that TOR pathway activation accounts for increased axon regeneration in *daf-18* mutants. Together, our results establish two parallel molecular pathways that act in aging neurons to regulate their response to injury. By investigating the relationships between *daf-16*, *daf-18*, TOR, *dlk-1*, aging, and axon regeneration, we hope to provide a better understanding of how a neuron ages and provide insight into recovery of function after injury.

471B. Mechanisms of VD motor neuron differentiation: UNC-55 expression and repression is determined by isoforms of UNC-62. **R. Campbell**, W. Walthall. Biology Dept, Georgia State University, Atlanta, GA.

The GABAergic D class motor neurons of the ventral nerve cord (VNC) compose a cross-inhibitory neural network that contributes to the animal's sinuous pattern of locomotion. The D motor neurons share a number of similarities that result from the expression of the paired homeodomain transcription factor, UNC-30. The D class can be further subdivided into two subclasses, the DD and VD motor neurons by the expression of the nuclear hormone receptor, UNC-55. UNC-30 is required for the terminal differentiation of the D type motor neurons through the transcriptional activation of GAD/UNC-25 and VGAT/UNC-47 as well as numerous other downstream targets. Expression of UNC-55 in the VD motor neurons suppresses a subset of the downstream targets of UNC-30 and allows the VDs to innervate ventral muscle. While in the DDs, UNC-55 is not expressed and innervation of the dorsal muscle is observed. In previous studies, mutations in unc-55 caused the VD motor neurons to assume a DD like fate, in which the synaptic pattern and expression profile of the VDs is similar to the DDs. Sufficiency studies where UNC-55 was ectopically expressed in the DDs caused the DDs to assume a VD like innervation pattern and gene expression profile. These studies indicate; that in the context of the D motor neurons, UNC-55 is necessary and sufficient to induce a VD motor neuron fate. How then is the expression of UNC-55 regulated? Utilizing a bioinformatics analysis, a list of potential cis/trans-regulatory elements of the unc-55 promoter was generated. Using an unc-55::gfp reporter, each transcription factor was tested for a disruption in unc-55::gfp expression using mutant and RNAi analyses. Here we show that different isoforms of the Pbx/Hox cofactor Meis/UNC-62 are capable of activating and repressing unc-55::gfp expression. Our results suggest that different isoforms of unc-62 differentially regulate the expression of UNC-55 in the VNC. How isoforms UNC-62 cause this differential regulation is the current study of interest.

472C. A role for muscle-skin interactions in shaping PVD sensory dendrites. **Kevin Celestrin**[1], Hannes Buelow[1,2], Zaven Kaprielian[2,3]. 1) Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 3) Pathology, Albert Einstein College of Medicine, Bronx, NY.

Complex dendritic arbors facilitate the reception and transduction/integration of a wide variety of environmental stimuli, such as temperature, touch, and pain. Similarly, the complex dendritic arbors of cortical neurons in the central nervous system allow the cortex to function as the center for reasoning, learning and memory. The loss of these complex dendritic arbors represents a hallmark of psychiatric disorders (e.g. schizophrenia) and neurodegenerative diseases (e.g. Alzheimer's). A wide variety of genes have been implicated in the regulation of dendrite growth including transcription factors, regulators of intracellular transport, and modulators of Golgi-ER and endosomal dynamics. However, the detailed molecular mechanisms and genetic pathways that regulate dendrite branching and dendritic arbor formation remain poorly understood. Specifically, the mechanism of how extracellular factors regulate dendrite development. It has recently been shown that integrins, receptors for ECM proteins, are required for proper

dendrite branching in the Drosophila nervous system supporting a significant role for coordinated tissue interaction in dendritic development. In preliminary studies utilizing the highly branched mechanosensory neuron PVD in Caenorhabditis elegans, We have identified a role for several genes, such as integrin, PINCH (an adaptor protein that interacts with integrin) and perlecan in the organization of PVD. These genes are part of a complex that coordinates tissue interactions between the muscle and skin suggesting that these may play a role in shaping dendrite arbors. We will report on our progress to elucidate how muscle-skin interactions coordinate PVD dendritic arbor formation.

473A. Microtubules-Based Inhibition of RhoA and MAPK Signaling Promotes Synapse Maturation and Axon Branch Maintenance in *C. elegans*. **Chun-Hao Chen**, Yu-Hsien Lee, Chun-Liang Pan. Institute of Molecular Medicine and Department of Chemistry, National Taiwan University, Taiwan.

Connectivity of the neuronal circuit is refined by the dynamic regulation of synapse formation and elimination. While mechanisms that promote synapse formation had been studied in detail, those that maintain established synapses are still poorly understood. We identified two tubulin genes, *mec-12*/a-tubulin and *mec-7*/b-tubulin, as essential for synapse and axon branch maintenance. In the *mec-12* and the *mec-7* mutants, the PLM synapses formed normally but were progressively lost, with concomitant retraction of the PLM axon branch. The synapse and axon branch defects of the tubulin mutants were suppressed by mutations in *rhgf-1*, a RhoGEF with potential microtubule association. Genetic analysis suggested that the GEF activity of *rhgf-1* triggered synaptic and axon branch defects and that the PDZ and the C1 domains may inhibit this GEF activity. We further demonstrated that RHGF-1 acted through the Rho (*rho-1*), the Rac (*ced-10* and *mig-2*) and the Rho kinase *let-502*/ROCK, but independently of the canonical ROCK target MLC-4/myosin regulatory light chain. Instead, it activated a conserved p38/MAPK pathway, consisting of *dlk-1*/MAPKKK, *mkk-4*/MAPKK, and *pmk-3*/MAPK. DLK-1 was required and sufficient to promote PLM branch retraction cell-autonomously through sequential phosphorylation of MKK-4 and PMK-3. These MAPK components were expressed both at the synapse and in the neuronal soma. Interestingly, we found that blocking the dynein-based retrograde transport significantly reduced axon branch defects of the *mec-7* mutants. We propose a model in which microtubules disassembly activates MAPK signaling through ROCK, and this MAPK activity is part of the retrograde signals that induce subsequent axon retraction upon microtubule loss. Our work had identified molecular mechanisms by which microtubules promote synaptic stability and axon branch maintenance in the neurons. This work is supported by the National Science Council, Taiwan (NSC99-2320-B-002-080 and NSC100-2320-B-002-095-MY3) and National Taiwan University (NTU-CDP-102R7810).

474B. Asymmetric Hox Expression by Two Opposing Wnt Signals Drives *C. elegans* Neuroblast Migration through Differential Cell Polarization. **Chung-Kuan Chen**[1], Gian Garriga[2], Chun-Liang Pan[1]. 1) Institute of Molecular Medicine, National Taiwan University, Taiwan; 2) Department of Molecular and Cell Biology, University of California, Berkeley, USA.

Neurons undergo long-range migration during development to reach their future positions, and they polarize towards the direction of movement. In *C. elegans*, descendants of the QL neuroblast migrate posteriorly, and those of the QR neuroblast migrate anteriorly. The Wnt EGL-20 drives expression of the Hox gene *mab-5* in the QL, but not in the QR lineage, resulting in posterior migration of the QL descendants. By contrast, anterior migration of the QR lineage requires the activity of another Hox gene *lin-39*. How Wnt signaling interacts with these two Hox genes is not completely understood. We found that LIN-39 was expressed at high level in both the QL and the QR neuroblasts. Soon after the Q.a/p cells divided, LIN-39 was downregulated in the posteriorly-migrating QL.ap and the QL.pa cells, but remained high in the anterior-migrating QR.ap and the QR.pa cells. The level of LIN-39 in these cells seemed to correlate with the direction towards which these cells polarized. In the *egl-20* or the *mab-5* mutants, the QL descendants polarized and migrated towards the anterior, and LIN-39 level remained high in the QL.ap and the QL.pa cells. We found that mutations in the Wnt *cwn-1* or the Frizzled *mom-5* suppressed the anterior migration or polarization of the QL lineage, and the aberrantly high level of LIN-39 was also attenuated by the *cwn-1* mutation. While *cwn-1* promotes *lin-39* expression in the QL lineage when the *egl-20*/*mab-5* signaling was absent, LIN-39 level in the QR lineage was not affected by Wnts. As a result, mutations in *cwn-1*, *egl-20* and *lin-39* all affected QR anterior migration, and double mutants between any of these three mutations further worsened the phenotypes. We hypothesize that in the QL lineage, an *egl-20*-dependent MAB-5 represses LIN-39 transcription to allow for posterior polarization and migration of the QL.ap and the QL.pa cells. In the QR lineage, the Wnts and LIN-39 act in separate pathways to control QR anterior migration. This work is supported by the National Science Council, Taiwan (NSC100-2320-B-002-095-MY3) and National Taiwan University (NTU-CDP-102R7810).

475C. Autonomous and nonautonomous regulation of Wnt-mediated neuronal polarity by the *C. elegans* Ror kinase CAM-1. **Shih-Chieh Chien**[1], Mark Gurling[1], Gian Garriga[1,2]. 1) Department of Molecular and Cell Biology; 2) Helen Wills Neuroscience Institute, University of California, Berkeley, CA 94720.

Wnts are a conserved family of secreted glycoproteins that regulate various developmental processes in metazoans. Three of the five *C. elegans* Wnts (CWN-1, CWN-2 and EGL-20) and the sole Wnt receptor of the Ror kinase family (CAM-1) are known to regulate the anterior polarization of the mechanosensory neuron ALM.[1,2,3] Here we show that CAM-1 and the Frizzled receptor MOM-5 act in parallel pathways to control ALM polarity. We also show that CAM-1 has two functions in this process: an autonomous signaling function that promotes anterior polarization and a nonautonomous Wnt-antagonistic function that inhibits anterior polarization. These antagonistic activities can account for the weak ALM phenotypes displayed by *cam-1* mutants. Our observations suggest that CAM-1 could function as a Wnt receptor in many developmental processes, but the analysis of *cam-1* mutants fails to reveal CAM-1's role as a receptor in these processes because of its Wnt-antagonistic activity. In this model, loss of CAM-1 results in increased levels of Wnts that act through other Wnt receptors, masking CAM-1's autonomous role as a Wnt receptor. [1]: Hilliard & Bargmann (2006) Dev Cell 10(3):379-90 [2]: Prasad & Clark (2006) Development 133(9):1757-66 [3]: Babu et al. (2011) Neuron 71(1):103-16.

476A. Multi-layers of molecular mechanisms govern dendritic arborization in neurons. **H. Chiu**[1,2], Y. Zou[1], T. Ferreira[1], C.-F. Chuang[1], C. Chang[1]. 1) Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH; 2) Current address: Division of Biology, California Institute of Technology, Pasadena, CA.

PVD and FLP Nociceptive neurons innervate the skin with elaborate dendritic arbors that respond to stimuli of harsh mechanical force or extreme temperatures. Genetic screens were used to identify genes regulating self-avoidance, tiling, and maintenance in dendritic arbors. Multi-layers of molecular mechanisms have been found to govern dendritic arborization of these neurons to ensure maximum sensory coverage of skin that envelops the entire

animal body. We will present our progress in understanding each layer of these mechanisms. In particular, four mutants in different genetic loci display a strikingly similar phenotype of “collapsed menorah” in dendritic arbors, suggesting that they may define a genetic pathway for patterning PVD dendrites. One of them encodes a cell surface protein that guides the growth of the quaternary dendrite by setting up its growth pathway on skins and muscles.

477B. Neuronal fusion induced by *unc-70/b-spectrin* dependent axonal injury requires components important for clearance of apoptotic cells. **Sean Coakley**[1], Brent Neumann[1], Hengwen Yang[2], Ding Xue[2], Massimo Hilliard[1]. 1) Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia; 2) Department of MCD Biology, University of Colorado, Boulder CO, USA.

Axonal regeneration is a major component of neuronal repair after traumatic injury. However, we have a very poor understanding of the mechanisms needed to achieve target reconnection. In *C. elegans*, following transection, regenerating axons can fuse with their distal, separated axonal fragment to reconnect the two processes and restore the original axonal tract. This fusion event is highly specific and requires, at least partially, the fusogen EFF-1. The molecules that regulate the recognition and specificity of this fusion process are unknown. Here we show that loss of UNC-70/b-spectrin induces a novel phenotype of cell-cell fusion between the mechanosensory neuron PLM and nearby PLN, which shares many characteristics with axonal fusion following regeneration induced by laser axotomy. PLM/PLN cell-cell fusion induces a mixing of fluorophores between the two cells, can be modulated by the expression of the fusogen EFF-1, and is regeneration dependent. Using a candidate gene approach, we have discovered that the conserved phosphatidylserine receptor, PSR-1, also plays an important role in PLM/PLN fusion. In animals with mutations in *psr-1* the rate of PLM/PLN fusion is significantly reduced. PSR-1 has been previously shown to bind exposed phosphatidylserine (PS) on the surface of apoptotic cells to mediate recognition and engulfment by phagocytes. We also found similar defects in animals lacking two other molecules involved in cell corpse engulfment, transthyretin-like protein TTR-52 and its binding partner CED-1 (a phagocyte receptor). In addition to reduced PLM/PLN fusion in an *unc-70* induced injury model, animals lacking PSR-1 or TTR-52 have axonal fusion defects following UV laser axotomy. Our results suggest that common molecular machinery mediates axonal fusion and apoptotic cell clearance, through molecules involved in recognition and interaction between damaged axonal compartments and between apoptotic cells and phagocytes.

478C. Characterization of nde-5, a newly identified mutant displaying axon guidance and sprouting defects. **Zhao Hua Ding**[1,2], Cristina Slatculescu[1,2], Antonio Colavita[1,2]. 1) The Graduate Program in Cellular and Molecular Medicine, University of Ottawa, 451 Smyth Road, Ottawa, Ontario, Canada, K1H8M5; 2) University of Ottawa, Ottawa, ON, Canada.

A genetic screen for worms displaying ectopic VC4 and VC5 neurites identified five complementation groups. Four of these were shown to be mutations in Van Gogh (vang-1), prickle (prkl-1), dishevelled (dsh-1) and the beta-subunit of farnesyltransferase (fntb-1) revealing a role for a PCP-like pathway in negatively regulating neurite formation or outgrowth. We are currently characterizing and attempting to clone the fifth gene identified in this screen: neurite outgrowth defective-5 (nde-5). In addition to ectopic VC4/5 neurites, nde-5 mutants display highly penetrant axon sprouting, guidance and outgrowth defects that are not found in vang-1 and prkl-1 mutants. These results suggest the possibility that nde-5 is involved in a PCP -like pathway in VC4/5 but likely plays a more general role in specifying or regulating neuronal morphology. We have mapped nde-5 to a small region of LGI. We will present our data characterizing the neuronal phenotypes of nde-5 and our progress identifying the nde-5 locus.

479A. Early neural specification and the regulation of asymmetric neurogenesis in the C-lineage. **Terry J. Felton**, Solayr Layton-Thomas, Richard J. Poole. Department of Cell and Developmental Biology, University College London, London, UK.

The generation of neurons during development from a fertilised egg requires a cascade of neuronal lineage-specific genes. Proneural genes, a highly conserved family of basic helix-loop-helix transcription factors are key members of this genetic cascade yet the upstream molecular events that directly regulate their expression remain ambiguous. Bilaterian nervous systems are largely left-right (L/R) symmetrical yet certain structures are present asymmetrically. How asymmetric neurogenesis is regulated is unclear. To investigate these problems we are studying the early specification of the unilateral glutamatergic interneuron PVR. PVR is strikingly asymmetrically positioned on right side and derives asymmetrically from the C-lineage, a lineage that otherwise produces left and right sided bilateral pairs of hypodermal and body wall muscle cells. We have found that the proneural transcription factor *hlh-14/achaete-scute*, is a key early regulator in the development of PVR. *hlh-14* is asymmetrically expressed in the neuroblast that gives rise to PVR and in *hlh-14* mutants, terminal markers of PVR fail to be expressed and the cell is transformed into a hypodermal cell, the Cp lineal equivalent.

We have found that maintenance of *hlh-14* expression in the C-lineage is dependent on its dimerisation partner, the daughterless homolog *hlh-2*, suggesting autoregulation. We are now addressing further the *cis*-regulatory logic and *trans*-acting factors that drive asymmetric *hlh-14* expression. To this end we are performing a comprehensive promoter analysis of *hlh-14*, investigating the role of the Wnt-based binary specification module and isolating C-lineage mutants via (i) a forward genetic screen with a strain containing terminal cell fate markers of both PVR and the lineally related interneuron DVC (ii) 4D-lineage analysis of a collection of temperature sensitive lineage mutants kindly provided by Prof Ralf Schnabel and (iii) a candidate approach based on embryonic expression patterns described by the Waterston lab. Our most recent results will be presented.

480B. UNC-68/RyR channels modulate critical sub-cellular calcium signals during normal and optogenetically enhanced neuronal regeneration. Lin Sun[1,2], James Shay[1,2], Samuel Chung[1,2], Christopher Clark[3], Mark Alkema[3], **Christopher V. Gabel**[1,2]. 1) Dept Physiology & Biophysics, Boston Univ School of Medicine, Boston, MA; 2) Photonics Center, Boston Univ; 3) Dept. of Neurobiology, Univ. of Massachusetts Medical School.

Sub-cellular calcium signaling plays an important role in a neuron’s ability to recover from traumatic damage. Employing femtosecond laser surgery, time-lapse microscopy, fluorescent calcium imaging and optogenetic photo-stimulation, we can damage a single neuron, quantify its regeneration, measure sub-cellular calcium signals and dynamically manipulate cell physiology *in vivo*. Extending previous *C. elegans* studies that measured an initial (~5 min) damage induced calcium transient within an axotomized neuron, we observe a prolonged, localized calcium signal within the vicinity of the damage point for 5 h following laser axotomy of the ALM neuron. This signal is eliminated by mutation of *unc-68* encoding the *C. elegans* homologue of the ryanodine receptor (RyR) channel, a calcium release channel in the endoplasmic reticulum membrane. In addition, the same *unc-68(e540)* mutants exhibit a >50% reduction in regeneration outgrowth at 5h and 24h post-lesion as well as severely disrupted regeneration guidance. Employing optogenetic

techniques we periodically stimulate axotomized ALM neurons by photo-activation of the cation channel channelrhodopsin-2 (ChR2). This results in a robust, >30%, increase in regeneration outgrowth over the 24 h following laser surgery. The effect is eliminated in *unc-68(e540)* mutants, or by pharmacologically blocking UNC-68 with dantrolene. Calcium induced calcium release via RyR channels plays an important role in amplifying sub-cellular calcium signals within a neuronal growth cone to modulate axon guidance. In *C. elegans*, UNC-68 is necessary for ChR2 photo-activation of muscle. In our experiments UNC-68 calcium release may amplify the ChR2 initiated signal to stimulate additional outgrowth. Taken as a whole, our results demonstrate an important role for UNC-68/RyR calcium release in stimulating early regeneration outgrowth and point to possible therapeutic control of cellular calcium physiology to enhance neuronal regeneration *in vivo*.

481C. Lesion conditioned regeneration of *C. elegans* amphid sensory neurons is mediated through a reduction of sensory signaling and does not require DLK-1. Samuel H. Chung[1,2], **Christopher V. Gabel[1,2]**. 1) Dept Physiology & Biophysics, Boston Univ Sch Medicine, Boston, MA; 2) Photonics Center, Boston Univ.

Tragically, mammals display weak neuronal regeneration within the central nervous system following traumatic neuron damage. An exciting discovery in neurotherapeutics is that mammalian neurons can strongly regenerate their central axons into and beyond an injury site if a conditioning lesion is made on their peripheral sensory axons. This lesion conditioning effect has been studied in mammals for decades, yet is still poorly understood. Employing subcellular femtosecond laser ablation we observe a strong lesion conditioning effect in multiple types of *C. elegans* amphid sensory neurons, where a dendrite lesion stimulates regeneration of a transected axon. Interestingly, the effect is observed in a *dlk-1(ju476)* mutant background demonstrating that DLK-1 is not necessary for lesion-dependent regeneration. Moreover, pharmacologically blocking or genetic mutation of the L-type voltage-gated calcium channel mimic a dendrite lesion stimulating axon regeneration. This provides a direct link to mammalian lesion-conditioned regeneration, which is mediated by a reduction of electrical sensory activity and specifically through a reduction in L-type voltage-gated channel activity. In addition, we observe a link between *C. elegans* lesion-conditioned regeneration and ectopic axon outgrowth in the same amphid sensory neurons. We find that genetic mutations, previously shown to cause ectopic outgrowth during development, also stimulate lesion-conditioned axon regeneration. Our results indicate that reduction of a sensory activity dependent calcium/calmodulin pathway within the amphid neurons conditions the cell for regeneration. Taken together, we demonstrate direct genetic, molecular and cellular links between three types of axon outgrowth: *C. elegans* lesion conditioning, *C. elegans* ectopic outgrowth and mammalian lesion conditioning, establishing *C. elegans* as a powerful model system for the study of lesion conditioning. Our findings in *C. elegans* are pointing towards a conserved activity-dependent pathway that modulates the nervous system's intrinsic regenerative capabilities.

482A. Menorah-menorah auto-fusion as a mechanism of PVD response to injury. **Tamar Gattegno[1]**, Meital Oren[1,2], Benjamin Podbilewicz[1]. 1) Department of Biology, Technion-Israel Institute of Technology, Haifa 32000, Israel; 2) Departments of Biochemistry and Molecular Biophysics, Columbia University, New York, NY 10032, USA.

The PVDs are two nociceptors localized along C. elegans lateral sides, enabling them to sense strong mechanical stimuli such as pain, temperature and posture. The PVDs develop through a dynamic process which involves extension and retraction of dendrite branches. Though we now better understand C. elegans PVD development, very little is known about PVD regeneration and degeneration following injury. In order to study the genetic pathway guiding PVD response to injury, we use laser microsurgery to severe the PVD and live imaging of GFP reporters. We found that when the PVDs are injured at the 1ry branch, AFF-1 mediated fusion occurs between the terminal branches to bypass the site of injury, followed by EFF-1 mediated pruning and arbor simplification. Degeneration of the distal stump occurred in animals that failed to undergo reconnection of the 1ry severed branch or menorah-menorah fusion. We used Kaede photo-convertible fluorescent protein expressed in the PVD to support the observation that injured PVD has reconnected through auto-fusion. We severed the PVD 1ry branch, recovered the worms and detected reconnection after 6-12 hours. The PVD cell body of recovered animals was than photo-converted from green to red and we analyzed the transfer of the Red-Kaede through the reconnected neuron. We found that PVD reconnection occurs through fusion. We then performed a similar experiment in lin-22 animals which have 2 to 5 extra PVD cell bodies (kindly provided by M. Heiman and C. Yip) to study whether adjacent PVDs connect by fusion. We found that following injury different PVDs can fuse as a response to injury. These experiments show that PVD neurons response to dendrotomy involves four steps: (1) Loss of self-avoidance or tiling. (2) Menorah-menorah fusion mediated by AFF-1 and/or reconnection of injured 1ry branches by auto-fusion. (3) Ectopic sprouting of dendrites from the PVD. (4) Simplification by branch retraction mediated by EFF-1. The outcome of the regeneration process is the recovery of the PVD morphology and if regeneration by auto-fusion fails the distal part degenerates.

483B. Development and Function of RIS, a Caenorhabditis elegans GABAergic interneuron. **Marie Gendrel**, Oliver Hobert. Howard Hughes Medical Institute, Columbia University, Department of Biochemistry and Molecular Biophysics, New York, NY, USA.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate brain and dysfunction of GABAergic neurons can have profound pathological implications. In C. elegans, 26 neurons express conserved GABAergic terminal differentiation markers, such as the enzyme producing GABA (GAD/*unc-25*), the GABA-specific vesicular transporter (VGAT/*unc-47*) and the protein targeting VGAT to the synaptic membrane (a LAMP-like protein/*unc-46*). 25 of these are motoneurons and only one neuron, named RIS, is an interneuron that most closely resembles the dominant type of GABA neuron in vertebrates. Preliminary GABAergic cell fate marker analysis has shown that the RIS neuron fails to express terminal differentiation markers characteristic of GABA fate in *lim-6* and *nhr-67* mutant animals. Loss of LIM-6 -a LIM homeobox transcription factor- suggests that RIS might be involved in the control of metabolism. In parallel, we generated a null allele of *nhr-67* -a Tailless/TLX ortholog- using the MosDEL technique in order to determine its role in RIS development. We hypothesize that these *lim-6* and *nhr-67* broadly control RIS GABAergic fate and that they may do so together. In parallel, to identify cis-regulatory elements necessary for the expression of known RIS terminal differentiation markers (*unc-47*, *unc-25*,*unc-46*, *lim-6* and *nlr-1*), we used mutational analysis of gfp reporter genes. Altogether we should learn whether the RIS terminal differentiation markers are co-regulated through common cis-regulatory elements and trans-acting factors, with either the LIM homeobox gene *lim-6* or the Tailles/TLX ortholog *nhr-67* or the combination of the two. In addition, using the split-caspase system, we have generated transgenic animals in which RIS is genetically ablated to determine

RIS function. We will report on a series of behavioral test undertaken with these animals. Together, these results should give us a detailed picture of a GABA interneuron development and function.

484C. Studying membrane fusion during development and maintenance of *C. elegans* neurons. **R. Giordano-Santini**, M. A. Hilliard. Queensland Brain Institute, The University of Queensland, St Lucia, Australia.

EFF-1 and AFF-1 are highly efficient fusogens known to mediate cell-cell fusion events during the development of different *C. elegans* tissues. Both fusogens are also expressed in neurons, and EFF-1 has recently been shown to play a role in the development and remodelling of neurites[1,2], indicating that cell membrane fusion is an important neuronal event in *C. elegans*. However, very little is known about the molecular mechanisms underpinning membrane fusion within these cells; importantly, it is also unclear how this process is highly restricted to the individual cell, with fusion between neurites of adjacent neurons almost never observed. In non-neuronal tissues where cell-cell fusion occurs, numerous transcription factors and regulators have been identified as being critical for proper cell-cell fusion[3]. Our hypothesis is that similar mechanisms are in place in neurons, controlling fusion of neurites within individual cells and preventing neurites of adjacent neurons from fusing. We used different pairs of tightly associated neurons, AWCR/AWCL (head) as well as PLM/PLN (tail), to test this hypothesis and to study EFF-1 and AFF-1-mediated fusion in neurons. Using transgenic strains and microscopy techniques, we have found that overexpression of EFF-1 or AFF-1 under neuron-specific promoters leads to mixing of cytoplasms between individual neurons. We confirmed that the cytoplasms are indeed connected by using the photoconvertible protein Kaede. We also determined the temporal requirement of the fusogen by using a construct where EFF-1 is under a heat-shock promoter. In addition, using a fluorescent-tagged version of EFF-1, we are characterising the site of fusion between adjacent neurons when the fusogen is overexpressed. Finally, we have started a genetic screen using neuronal-specific RNAi, where genes known as cell-cell fusion regulators are investigated for their capacity to induce mixing of cytoplasms between associated neurons. The results presented here will give us new insights into the molecular mechanisms that control membrane fusion during development and remodelling of *C. elegans* neurons. [1]Oren-Suissa *et al.*, *Science*, 2010, [2]Gosh-Roy *et al.*, *J. Neurosci.* 2010, [3]Podbilewicz, *Wormbook*, 2006.

485A. A transcriptional network controlling reciprocal homeotic transformations of ALM and BDU neurons. **P. Gordon[1]**, O. Hobert[1,2]. 1) Dept Biochemistry, Columbia Univ, New York, NY; 2) Howard Hughes Medical Institute.

The transcription factor unc-86 is expressed in 57 neurons in adult hermaphrodites. UNC-86 has been shown to regulate terminal cell fate in a wide variety of neuron types, including the six mechanosensory neurons. In these cells, unc-86 and its cofactor mec-3 are known to induce and maintain neuronal fate. The mechanisms by which unc-86 regulates the other 51 neurons are less well understood. We have examined the means by which unc-86 differentially induces multiple cell fates by focusing on the mechanosensory ALML/R neurons and their sister cells, the interneurons BDUL/R. unc-86 is expressed in the mother cell of ALM and BDU, and then in both daughter cells throughout the life of the animal. We find that both unc-86 and the transcription factor pag-3 are required for expression of BDU reporter genes. In addition, we have found another gene, ceh-14, that contributes to the regulation of a subset of BDU specific genes. We examine the relationship between the ALM and BDU regulatory factors to determine how asymmetry is established. Some insight into this question also comes from our examination of homeotic transformation in ALM and BDU. Loss of pag-3 has been shown to cause ectopic expression of some ALM genes in the BDU neurons. Here, we show that this transformation of BDU to ALM includes a change in morphology and function of the neuron. In addition, we find that the loss of mec-3 causes the converse fate switch: ALM neurons are converted into BDU, as measured both by reporter gene expression and morphological changes. In addition to examining the developmental consequences of the BDU regulatory network, we have studied the genetic basis of BDU functions. BDU has been shown to play a role in anterior harsh touch response and in guidance of AVM neuron branching in the nerve ring. We show that ceh-14 is important in both functions. Loss of ceh-14 leads to significantly lower harsh touch response in comparison to N2. Because ceh-14 regulates several neuropeptides that are expressed in BDU, this suggests that neuropeptide release may mediate BDU function in the harsh touch response pathway. In addition, loss of either ceh-14 or pag-3 disrupts synaptic connections between AMV and ALML/R in the nerve ring.

486B. EGL-13/SoxD Specifies Distinct O_2 and CO_2 Sensory Neuron Fates. **Jakob Gramstrup Petersen[1]**, Teresa Rojo Romanos[1], Vaida Juozaityte[1], Alba Redo Riveiro[1], Ingrid Hums[2], Lisa Traunmüller[2], Manuel Zimmer[2], Roger Pocock[1]. 1) Biotech Research and Innovation Centre (BRIC), Copenhagen, Copenhagen OE, Denmark; 2) Institute of Molecular Pathology (IMP), Vienna, Austria.

A major question in neurobiology is to understand how the huge diversity of neuronal types is generated. In this study we show that the conserved SoxD transcription factor EGL-13 controls terminal differentiation of the gas-sensing neurons BAG L/R (O_2/CO_2-sensing), URX L/R, AQR and PQR (O_2-sensing). Further, we have identified specific cis-regulatory motifs and trans-acting factors that confer correct expression of *egl-13* in the gas-sensing neurons. These findings extend our understanding of mechanisms of neuronal diversification and the regulation of molecular factors that may be conserved in higher organisms. Four independent alleles of *egl-13* were isolated from forward genetic screens for gas-sensing mutants. We found that EGL-13 acts cell autonomously to ensure expression of terminal neuronal gas-sensing features and loss of *egl-13* causes a failure to respond to changes in O_2 and CO_2. We subsequently found that *egl-13* expression is regulated through two different promoter motifs in BAG and URX respectively. In BAG we found two ETS-5 sites responsible for expression whereas AHR-1 binding sites drive EGL-13 expression in URX. Finally, we established that EGL-13 is sufficient to induce terminal features of the O_2 and CO_2 sensing neurons, in certain cellular contexts, when ectopically expressed under a heterologous neuronal promoter. These data underline the importance of Sox genes during development and specifically show that *egl-13* function is critical for the specification of O_2 and CO_2 sensing neurons.

487C. How to Fix a Broken Neuron. **Julie E Grimm[1]**, Meital Oren[1,2], Benjamin Podbilewicz[1]. 1) Biology, Technion Institute of Technology, Haifa, Israel; 2) Department of Biochemistry and Molecular Biophysics, Columbia, New York, USA.

Recent research in *C. elegans, Drosophila* and mice has aimed at investigating the regenerative ability of neurons. In contrast to previous understanding, studies of dendritic and axonal injury revealed hugely dynamic and moderately successful recovery. Our work seeks to elucidate the elements necessary for dendritic recovery to injury in order to expand our limited view of neuronal plasticity, maintenance and stability. Our model neuron, the

mechanosensory PVD, is the most arborized neuron in C. elegans. The PVD response to dendritic injury follows a certain pattern: bypass of self-avoidance mechanisms followed by reattachment at or near the site of injury, sprouting and simplification of branches. Failure of PVD reattachment results in degeneration of the distal fragment. Our work focuses on the arborization functions of NHR-25 (a nuclear hormone receptor) and EFF-1 (epidermal fusion failure 1, a cell-cell fusion protein) and their roles in dendritic fusion. The nuclear hormone receptor NHR-25 is known to be essential for epidermal and vulva development in C. elegans. We found that NHR-25 is also important for normal arborization, maintenance of dendritic trees and injury response. *nhr-25* deficient worms show excess branching at and around the site of injury up to 50 hours post injury, a time when ectopic branching in response to injury would normally be pruned back. Through epistatic analysis we discovered that this function is consistent with a model in which *nhr-25* positively regulates *eff-1*. By analyzing NHR-25 expression dynamics using an NHR-25::GFP translational reporter we found that NHR-25 expression is reduced in the hypodermis in response to injury . These results suggest that nhr-25 regulates PVD branching and branch fusion non-cell autonomously. We are performing tissue-specific rescue of the *nhr-25* arborization phenotypes to determine whether *nhr-25* functions in PVD repair from the hypodermis. We have previously shown that EFF-1 acts cell autonomously in the PVD, suggesting that *eff-1* and *nhr-25* may act in parallel pathways. Our finding provides a link between the external and internal neuronal environment and the regenerative ability of the neuron itself.

488A. Neuronal microtubule organization in C. elegans. **Martin Harterink**[1], Bart de Haan[1], Sander van den Heuvel[2], Casper Hoogenraad[1]. 1) Cell Biology, Utrecht University, Utrecht, Netherlands; 2) Developmental Biology, Utrecht University, Netherlands.

In polarized cells, such as neurons, the microtubule cytoskeleton offers tracks to transport various cargos along axonal and dendritic projections. Recent data indicate that differences in microtubule organization within axons and dendrites enable molecular motors to sort cargo to specific directions and establish and maintain neuronal polarity. However how these differences are established is hardly understood. C. elegans has relatively simple neurons which offer a great starting point to understand the basics of this microtubule organization. Using GFP tagged end-binding proteins to visualize microtubule growth we have compared several classes of neurons with different characteristics (such as ciliated vs unciliated and branched vs unbrached). Here we will present a preliminary analyses of the neuronal microtubule organization of wildtype as well as in several mutants.

489B. Excluding membrane proteins from the cilia: A role for UNC-101 & LRK-1. **A Holmes**[1], M Doll[2], A Gartner[1]. 1) GRE - CLS, University of Dundee, Dundee, United Kingdom; 2) University of Cologne, Cologne, Germany.

Neurodegenerative diseases represent an increasing practical and financial burden on developed societies. Parkinson's Disease, affecting dopaminergic neurons, is one such disease, affecting ~1% of people over 60 in the UK. While most PD is idiopathic, ~15% is attributable to genetic causes. Of this, a significant proportion is linked to mutations in LRRK2, a Leucine-Rich Repeat Kinase; implicated in diverse cellular processes, from membrane traffic to mitochondria. The highly conserved C. elegans orthologue of LRRK2, *lrk-1*, interacts with *unc-101*, encoding a component of the AP-1 complex - important in post-Golgi transport, to regulate the localisation of synaptic vesicle proteins (SV) (Sakaguchi-Nakashima et al., 2007). We show here that these genes also interact to affect transport to the cilia of dopaminergic neurons. Perturbing *unc-101*, clathrin and *rab-8* misroutes ODR-10, normally localized to the cilia, to all membrane compartments and that there is a requirement for the critical endocytosis proteins, clathrin, *dyn-1* and the AP-2 complex in regulating cilium membrane volume (Dwyer et al., 2001, Kaplan et al., 2010, 2012). Using the dopamine transporter, DAT-1, fused to YFP, we show that it is excluded from the cilia of dopaminergic neurons, contrary to ODR-10, in wild type worms. In *unc-101*, and *unc-16*, a scaffold protein, mutants DAT-1 is misrouted to the cilia. Mutations in AP-2, *dyn-1*, & *rab-8* did not result in DAT-1 misrouting, suggesting a novel role for the AP-1 complex in sorting cargoes away from the cilia or in establishing a membrane diffusion barrier at the base of the cilium. A *lrk-1* mutation enhances the above DAT-1 ciliary misrouting phenotype, in contrast to the latter's rescuing effect on SV proteins. This suggests that *lrk-1* may be a cargo dependent regulator of *unc-101*. Using a LRK-1::GFP we show that it is expressed in dopaminergic neurons and hope to study its localisation and protein interactions to further elucidate function. Additionally, we have performed mutagenesis screens for DAT-1 cilia misrouting phenotypes and have isolated several independent mutations. We hope that these will offer new insight into cilia establishment and maintenance.

490C. A paired-like homeodomain protein UNC-42 specifies the SAA neuron fate in *C. elegans*. **Myeongjin Hong**[1], Leesun Ryu[1], Chris Li[2], Kyuhyung Kim[1]. 1) Department of Brain Science, DGIST, Daegu, South Korea; 2) Department of Biology, City College of New York, New York, NY.

Neuronal specification and differentiation are orchestrated through signaling molecules and transcription factors. However, the mechanisms underlying how specific transcription factors regulate cell fate are not fully understood. Using *C. elegans* as a model system, we are investigating the molecular and cellular mechanisms for neuronal cell-fate specification. In *C. elegans*, the SAA cholinergic interneurons consist of two dorsal (SAADL/R) and two ventral (SAAVL/R) neurons that have been implicated in head foraging via the circuit comprising the RME and SMB motor neurons (White et al., 1986). To examine how the SAA neurons are specified during development, we conducted genetic screens and candidate gene searches utilizing the expression pattern of the *flp-7* neuropeptide gene, which is expressed in the SAA neurons as well as other neurons (Kim et al., 2004). We found that in *unc-42* mutant animals *flp-7* expression was completely abolished only in the SAA neurons, suggesting that *unc-42* is required for the expression of *flp-7* in these neurons. UNC-42 is a paired-like homeodomain protein of the Q50 class homologous to murine Pax-7 (Basch et al. 2006; Baran et al., 1999). To investigate how UNC-42 regulates *flp-7* expression in SAA, we examined the promoter of *flp-7* gene and identified a cis-regulatory motif that is required for *flp-7* expression in SAA. In addition, the promoters of other SAA markers, including *lad-2* (L1 CAM adhesion molecule homolog), are being analyzed. We are also testing whether expression of *unc-42* is autoregulated and whether transgenic worms expressing *unc-42* cDNA under the control of *hsp-16.2* heat shock promoter restores *flp-7* expression in SAA. Finally, we are planning to do further genetic screens to identify other novel genes that control SAA specification.

491A. *tld-1* regulates the synaptic localization of the TIR-1 Ca^{2+} signaling scaffold protein in left-right neuronal asymmetry. **Yi-Wen Hsieh**, Chieh Chang, Chiou-Fen Chuang. Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati , OH.

The *C. elegans* left and right AWC olfactory neurons specify asymmetric subtypes through a stochastic, coordinated cell signaling event. We previously showed that the TIR-1/Sarm1 scaffold protein assembles a Ca^{2+}-regulated signaling complex at synaptic regions in the AWC axons, in a microtubule-dependent mechanism, to specify AWC diversity. We also showed that proper localization of the TIR-1 Ca^{2+} signaling complex at the AWC synapses is

important for precise AWC asymmetry. To identify additional molecules required for the localization of TIR-1 in AWC subtype choice, we performed an unbiased forward genetic screen to identify mutants with defective TIR-1 localization and AWC diversity. From the screen, we identified *tld-1*, *tld-2*, *tld-3*, and *tld-4* (TIR-1 localization defective) with reduced localization of TIR-1 at synapses in the AWC axons, and/or accumulation of TIR-1 in the AWC cell body. We have identified the mutation responsible for the *tld-1* phenotype using whole genome sequencing (kindly performed by Alexander Boyanov in Oliver Hober's lab). We will present genetic analysis of *tld-1* mutants and molecular characterization of the *tld-1* gene in the specification of left-right AWC neuronal asymmetry. Our study of the *tld* genes will provide insights into how cell-specific Ca^{2+} signaling proteins such as TIR-1 are linked to the conserved synaptic assembly in neuronal diversification.

492B. Coordination of small GTPase Arls in cilia signaling in C.elegans. **Zeng Hu**, Yujie Li, Yuxia Zhang, Qing Zhang, Jinghua Hu. Department of Biochemistry and Molecular Biology, Mayo Clinic, Rochester, MN.

Once overlooked as a vestige, primary cilium has emerged as a key sensory organelle in many important physiological and developmental signaling pathways in higher organisms. Defects in cilia contribute to a wide spectrum of human diseases, now termed ciliopathies. Mounting evidences have highlighted the role of various small GTPases in cilia formation and signaling. Among them, 3 Alr proteins, ARL13b, ARL3 and ARL6 (BBS3) are particularly interesting. ARL13b and ARL6 are identified as one of the causal genes for Joubert Syndrome and BBS syndrome, respectively. ARL3-/- mice show classical ciliopathy phenotypes, implicating that ARL3 may be a ciliopathy gene too. Previously, we reported that ARL13b homolog ARL-13 and ARL3 homolog ARL-3 coordinate intraflagellar transport in C.elegans. The function of worm ARL-6 remains poorly understood. Here, we found that ARL-6 could form a complex with the BBSome, and moving along the axoneme. However, In contrast to other reported bbs mutants in which IFT integrity is disrupted, arl-6 show completely normal IFT and cilia morphology. Further analyses indicated that ARL-6 may specifically regulate the ciliary targeting of sensory receptors, and all three small GTPases, ARL-3, ARl-6, and ARL-13, genetically interact with each other in cilia signaling.

493C. UNC-116/KHC Acts with UNC-6/Netrin and UNC-40/DCC to Maintain Sensory Neuron Position in Caenorhabditis elegans. Ben Barsi-Rhyne[1], Kristine Miller[1], Christopher Vargas[1], Anthony Thomas[1], Joori Park[1], Martina Bremer[2], **Jessica Jarecki[1]**, Miri VanHoven[1]. 1) Department of Biological Sciences, San Jose State University, San Jose, CA; 2) Department of Mathematics, San Jose State University, San Jose, CA.

The organization of neurons and the maintenance of that arrangement are critical to brain function. Failure of these processes in humans can lead to severe birth defects, mental retardation, and epilepsy. Several kinesins have been shown to play important roles in cell migration in vertebrate systems, but few upstream and downstream pathway members have been identified. Here, we report that the *C. elegans* Kinesin-1 Heavy Chain (KHC)/KIF5 ortholog UNC-116 functions to maintain neuronal cell body position of the PHB sensory neurons. We find that UNC-116/KHC acts in part with the cell and axon migration molecules UNC-6/Netrin and UNC-40/DCC in this process, but in parallel to SAX-3/Robo. We have also identified several UNC-116/KHC interactors that are required for this process, including the cargo receptor UNC-33/CRMP2, the cargo adaptor protein UNC-76/FEZ and its regulator UNC-51/ULK, and the cargo molecule UNC-69/SCOCO. In addition, we have identified two actin regulators required for this process: UNC-44/Ankyrin and UNC-34/Enabled. Interestingly, all of these genes also act in cell migration and/or axon outgrowth; however, many proteins that function in these processes do not affect PHB position. UNC-116/KHC appears to act in parallel to previously described adhesive mechanisms involving SAX-7/L1CAM and DGN-1/Dystroglycan. Our findings suggest an active UNC-116/KHC-mediated pathway may direct a posterior migration required to maintain proper PHB cell body position and identify several genes required for proper PHB position.

494A. Transcriptome analysis reveals genes regulated by MAB-5/Hox in posterior migration of Q neuroblast descendants. **Matthew P Josephson**, Joel Tamayo, Mahekta Gujar, Stuart Macdonald, Erik Lundquist. Molecular Biosciences, University of Kansas, Lawrence, KS.

The Q neuroblasts QR and QL are born in the same location along the longitudinal axis then migrate in opposite directions exposing QL but not QR to an EGL-20/Wnt signal. This Wnt signal induces expression of the homeobox transcription factor MAB-5/Hox, a determinant for posterior migration, causing the QL descendants (SDQL, PVM, and PQR) to migrate posteriorly. *mab-5* loss of function (lof) results in anterior migration of QL descendants, and *mab-5(e1751)*, a gain of function (gof) allele that expresses MAB-5 in both QL and QR, results in posterior migration of QR descendants. To identify genes regulated by MAB-5 that drive posterior migration, RNA seq was conducted on wild-type, *mab-5lof*, and *mab-5gof*. Genes were found that were upregulated or downregulated in lof or gof backgrounds. Gene ontology term analysis showed that these were significantly enriched for genes encoding secreted and transmembrane molecules that mediate interactions with the extracellular matrix. We used RNAi to perturb genes that were upregulated in *mab-5gof* and downregulated in *mab-5lof*, with the idea that these might be genes that are upregulated by MAB-5 to drive posterior migration. We found that RNAi of approximately a third of these genes (17) modified posterior QR descendant AQR migration in *mab-5gof*, indicating that they are required by MAB-5 to drive posterior migration. Existing mutations within genes were used to confirm RNAi results. The MAP kinase activated protein kinase *mak-1*, and the C. elegans F-spondin homolog spon-1 were both upregulated in mab-5gof and both suppressed the *mab-5gof* posterior AQR migration. Q cell specific RNAi experiments on these genes did not result in suppression, suggesting a non cell-autonomous role in posterior migration. A ChiP-seq study previously identified *mak-1* but not *spon-1* as a direct target of MAB-5 (Niu et al, 2011). The data taken together suggest that *mak-1* is a direct target of MAB-5 required for posterior migration of Q descendants, and *spon-1* may be indirectly controlled by MAB-5 in posterior migration.

495B. Patterning of sexually dimorphic neurogenesis in the ventral nerve cord by HOM-C/Hox and TALE homeodomain transcription factors. **Andrea K. Kalis**, Djem Kissiov, Breanna Tetreault, Emily Kolenbrander, Jennifer Ross Wolff. Carleton College, Northfield, MN.

Sexually dimorphic neurons control reproductive behaviors throughout animal phyla. *C. elegans* ventral cord neurons (VCNs) provide an excellent model of sexual specialization: Pn.aap neuronal precursors undergo distinct cell division, death, and differentiation to generate 6 hermaphrodite-specific neurons (VCs) and 9 pairs of male-specific neurons (CAs and CPs). To better understand sex-specificity of these key developmental events in VCN neurogenesis, we developed a toolkit of markers that allows us to examine sex-specific neurogenesis, asymmetric fates of daughters of a neuroblast division, and regional specification. Previous studies identified roles for the HOM-C/Hox transcription factors LIN-39 and MAB-5 in many of these aspects of Pn.aap fate. Using our toolkit, we expand on this work, demonstrating roles for LIN-39, MAB-5, and the TALE homeodomain proteins CEH-20 and UNC-62 in promoting

survival, differentiation, and regionalization of VCs, CAs, and CPs. We find that *ceh-20* and *unc-62* play sexually distinct roles, contributing to Pn.aap survival in hermaphrodites, but not in males. Instead, in males, *ceh-20* and *unc-62* are required for serotonergic specification of CPs, but not for one aspect of CA fate (*ida-1::gfp expression*) that is shared with hermaphrodite VCs. Expression patterns of VCN markers reveal regionally distinct programs of fate specification along the A-P axis in males, with fates apparently defined by partially overlapping domains of *lin-39* and *mab-5* expression and function. Interestingly, neuronal fates in the region of overlap appear to be reciprocally controlled by *lin-39* and *mab-5*. In addition, we find that the sex determination pathway regulates Pn.aap fates during L1, a time coinciding with the birth of Pn.aap. Given that these Hox/TALE genes are active in both sexes, we predict that they regulate targets in conjunction with the sex determination pathway to bring about sex-specific differentiation of VCNs. These findings shed light on how Hox-mediated cell fate decisions and sex determination intersect to influence development of neuronal sex differences.

496C. Region-specific control of ventral cord neuron fate revealed by *lin-39(ccc16)*. **Andrea K. Kalis**, Maria Carson Sterrett, Jennifer Ross Wolff. Carleton College, Northfield, MN.

The *C. elegans* ventral nerve cord (VNC) is comprised of iterative lineages of motor neurons. A subset of neurons in the VNC are derived from the precursor cell Pn.aap and are sexually dimorphic, consisting of six VCs in hermaphrodites and nine pairs of CAs and CPs in males. In males, previous research has found that HOM-C/Hox genes *lin-39* and *mab-5* are required for survival and division of Pn.aap and *lin-39* is further required for the serotonergic fate of CP1-6. The functions of LIN-39 and MAB-5 are such that the anterior VNC is defined by *lin-39* expression in CA/CP1-4, the middle by overlapping *lin-39* and *mab-5* expression in CA/CP5-6, and the posterior by *mab-5* expression in CA/CP7-9. Thus, regionalization of the VNC along the A-P axis is apparent in the descendents of the Pn.aap lineage.

We performed a genetic screen for defective Pn.aap development using the male-specific serotonergic marker *tph-1::gfp*. We isolated a mutant strain that loses expression of *tph-1::gfp* in CP5-6 but retains expression in CP1-4. Whole genome sequencing revealed a G to A transition in the splice acceptor for the fifth coding exon of *lin-39*. rtPCR analysis indicates that *lin-39(ccc16)* mutants make two abnormal splice isoforms, both likely truncating the protein after the homeodomain. Sequencing also revealed an amino acid substitution in the closely linked *lin-13* locus. Our mutant line is ts sterile, possibly due to the *lin-13* mutation. We are currently investigating whether this mutation also contributes to VNC phenotypes. *tph-1::gfp* expression is absent in *lin-39(null)* mutant CPs and hypomorphic mutations result in random loss of *tph-1::gfp* expression in CPs, suggesting *lin-39(ccc16)* is not simply a weak loss-of-function mutation. Despite the normal expression of *tph-1::gfp* in CP1-4, *lin-39(ccc16)* lacks expression of the CA reporter *ida-1::gfp* in CA1-4, similar to null mutants. Thus, this mutant may reveal the mechanism by which *lin-39* contributes to survival, division, and fate differently in CA/CP1-4 than CA/CP5-6 and may shed light on sex-specific neuronal development in the Pn.aap lineage.

497A. Non-autonomous regulation of neuronal migration by Insulin signaling, DAF-16/FOXO and PAK-1. **Lisa Kennedy**[1], Steven Pham[2], Alla Grishok[2]. 1) Genetics & Development, Columbia Univ, New York, NY; 2) Biochemistry and Molecular Biophysics, Columbia Univ, New York, NY.

Neuronal migration is essential for nervous system development in all organisms and is regulated in the nematode, C. elegans, by signaling pathways that are conserved in humans. We find that the Insulin/IGF-1-PI3K signaling pathway modulates the activity of the DAF-16/FOXO transcription factor to promote the anterior migrations of the hermaphrodite-specific neurons (HSNs) during embryogenesis of C. elegans. When signaling is reduced, DAF-16 is activated and promotes migration, conversely, when signaling is enhanced, DAF-16 is inactivated and migration is inhibited. Specifically, we demonstrate that null mutations in the daf-16 gene as well as inhibition of DAF-16 activity due to a daf-18/PTEN mutation and activation of PI3K signaling cause an HSN undermigration phenotype, while an increase in DAF-16 activity resulting from decreased Insulin/IGF-1 signaling leads to HSN overmigration. Surprisingly, our data indicate that DAF-16 promotes HSN migration cell non-autonomously from the hypodermal tissue. We also implicate pak-1, which encodes a p21-activated kinase, as a downstream effector of DAF-16 activity in the hypodermis during HSN migration. To our knowledge, this is the first example of FOXO and Pak1 acting non-autonomously in nervous system development. As a FOXO-Pak1 pathway was recently shown to regulate mammalian neuronal polarity, our findings indicate that the roles of FOXO and Pak1 in neuronal migration are likely conserved from C. elegans to higher organisms.

498B. Regulation of unc-3, the Terminal Selector Gene of Cholinergic Motor Neurons. **SY Kerk**[1], P Kratsios[2], O Hobert[2]. 1) Doctoral Program in Neurobiology & Behavior, Columbia U, NY, NY; 2) Biochemistry & Molecular Biophysics, HHMI, Columbia U, NY, NY.

The unc-3 gene is the terminal selector gene (TSG) of cholinergic motor neurons (MN) in the ventral nerve cord (VNC). One of the most important defining criteria of a TSG is its constitutive expression in cells in which it functions to maintain their terminal identity throughout the lifetime of the organism. Interestingly, unlike many other TSGs described thus far, unc-3 does not auto-regulate to maintain its own continued expression. In fact, the expression level of an unc-3 reporter is increased when introduced into an unc-3 null mutant worm strain - suggesting that UNC-3 negatively auto-regulates its own expression instead. Our project aims to better understand unc-3 regulation in VNC cholinergic MNs; specifically: 1) how is its expression initiated and maintained and 2) why does it negatively auto-regulate. To address the first question, we are dissecting the cis-regulatory region of unc-3 in hopes of defining binding motif sequences necessary for proper unc-3 expression. Preliminary results demonstrate that different cis-regulatory regions are required for unc-3 expression in the DA, DB, VA, and VB subtypes as a whole as opposed to the AS subtype alone. In addition, the initiation of unc-3 expression during early embryonic stages and the maintenance of this expression after late embryonic stages are also dependent on distinct cis-regulatory regions. Taken together, our data suggest that unc-3 is both spatially and temporally regulated in a modular fashion by various regulatory factors. A finer characterization of the binding motif sequences could potentially lead to the bioinformatic candidate identification of direct upstream regulators that could then be experimentally tested. Simultaneously, we are also performing forward genetic screens to identify such factors in an unbiased manner. In addressing the second question, we are conducting overexpression studies to explore if differential expression levels could affect unc-3 function and/or specificity. As unc-3 also controls the expression of MN subtype-specific terminal differentiation genes, the notion that its specificity is expression level-dependent fits aptly with our observation that unc-3 is modularly regulated in the various MN subtypes.

499C. Specification of SMB motor neuron fate by the C. elegans LIM homeobox protein LIM-4. **Jinmahn Kim**[1], Jihye Yeon[1], Chris Li[2], Kyuhyung Kim[1]. 1) Brain Science, DGIST, Daegu, South Korea; 2) Biology Dept. CUNY, New York, NY.

The expression of specific transcription factors dictates the differentiated features of postmitotic neurons. However, the mechanism of how specific molecules determine or specify neuronal cell fate during development is not fully understood. In *C. elegans*, the cholinergic SMB motor neurons consist of four motor neurons that innervate muscle quadrants in the head, send processes posteriorly down the sub-lateral cords (White et al., 1986), and monitor the amplitude of sinusoidal movement (Gray et al., 2005). To identify factors that specify the neuronal cell fate of SMB, we performed genetic screens and isolated several *lim-4* mutants in which *flp-12* neuropeptide gene expression was completely abolished only in the SMB neurons and not in other *flp-12* expressing neurons. Previously, it was shown that the LIM-4 LIM homeobox protein has a major role in specification of AWB chemosensory neuron identity (Sagasti et al., 1999). We found that the expression of another SMB marker, *odr-2* (GPI-Anchored protein) was also abolished in *lim-4* mutants and LIM-4 maintains its own expression by autoregulation. To investigate the molecular mechanism of *lim-4*, we did promoter analyses and bioinformatics searches with the SMB marker genes, and identified several *cis*-regulatory motifs including putative LIM-4 binding sites. We are currently investigating whether LIM-4 directly binds these motifs and these regulatory elements are sufficient for the expression of the non-SMB marker genes in the SMB neurons. In addition, we expressed *lim-4* cDNA under the control of the heat shock promoter and not only fully restored *flp-12* gene expression in *lim-4* mutants, but also induced the ectopic expression of *flp-12* in other cell types. Therefore, *lim-4* appears to be necessary and sufficient to promote specification of the SMB motor neuron fate.

500A. Dissecting the role of CEBP-1 in axon regeneration. **Kyung Won Kim**[1], Phoenix Ying[1], Thijs Koorman[2], Mike Boxem[2], Yishi Jin[1,3]. 1) Division of Biological Sciences, UCSD, La Jolla, CA; 2) Division of Developmental Biology, Utrecht University, Netherlands; 3) Howard Hughes Medical Institute.

Failure of injured axons to regenerate in the adult central nervous system (CNS) leads to permanent disability. Understanding the molecular basis underlying axon regeneration is of fundamental interest to repair the injured nervous system. Injury signaling in lesioned axons must be transmitted to the nuclei to trigger intrinsic pro-regenerative programs that can accelerate axon regrowth. Importin-dependent retrograde transport is one of the pathways to be activated upon nerve injury. Recent studies have identified multiple transcription factors that undergo retrograde transport induced by injury. Defining the specific targets of such retrograde factors will hold significant interests to the discovery of new candidates to enhance regenerative potential of injured axons. C. elegans is now established as a tractable model system to discover the conserved molecular mechanisms of axon regeneration. Recent studies showed that p38 mitogen-activated protein kinase (MAPK) pathway plays a key role in axon regeneration. A critical downstream regulator is CEBP-1, a member of C/EBP (CCAAT/enhancer-binding protein) of the basic leucine zipper (bZIP) transcription factor superfamily. We find that CEBP-1 is expressed in many types of neuronal cells and localized to the nucleus, supporting that CEBP-1 acts as a transcription factor like other conserved C/EBP homologs. We have identified an Importin protein as a putative binding partner of CEBP-1. We are testing the idea that retrograde transport of CEBP-1 can relay the injury signaling to the nucleus.

501B. Characterizing Ca^{2+} dynamics in the M4 neuron using GCaMP3. **Alena Kozlova**, Sana Hussain, Peter Okkema. Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL. 900 S Ashland Ave, 60607.

C. elegans pharyngeal behavior consists of two distinct types of muscle contraction called pumping and isthmus peristalsis. Peristalsis is a wave-like contraction within individual muscle cells in the posterior isthmus of the pharynx. The cholinergic motor neuron M4 synapses with muscles specifically in the posterior isthmus, and it is essential for this peristaltic contraction. Mutants lacking the M4 specific transcription factor CEH-28 hyperstimulate isthmus peristalses, and they exhibit abnormal and ectopic synapses throughout the pharyngeal isthmus. We are interested in how M4 stimulates isthmus peristalsis in wild-type animals, and how synaptic defects in *ceh-28* mutants contribute to hyperstimulation of isthmus peristalsis. To address these questions we are using the genetically encoded calcium indicator (GECI) GCaMP3 to monitor intracellular Ca^{2+} transients in M4. We have found that pharyngeal muscle contractions correlate with spikes in M4 Ca^{2+} concentrations. In wild-type animals Ca^{2+} transients occur preferentially in the posterior compartment of M4 where it forms synapses with isthmus muscles, and we are currently examining these transients in *ceh-28* mutants. We also plan to characterize Ca^{2+} dynamics in the isthmus muscles in wild-type animals and *ceh-28* mutants. Our results indicate GECIs can be used to monitor activity in M4, and we plan to use these indicators to understand how M4 stimulates this complex peristaltic contraction of the isthmus muscles.

502C. Diversification of motor neuron differentiation programs through a network of evolutionarily conserved transcription factors. **P Kratsios**, J Kerk, R Mourao, O Hobert. Biochemistry, Columbia University, New York, NY.

Vertebrate and invertebrate motor neurons (MNs) are organized into distinct subtypes that display remarkable functional and anatomical diversity. The underlying basis of such diversity is the differential expression of MN subtype-specific gene batteries composed of terminal differentiation genes whose products define the specific properties of a functional MN subtype throughout life (e.g. ion channels, neurotransmitter receptors). The gene regulatory mechanisms that establish and maintain MN diversity by controlling the expression of MN subtype-specific gene batteries are largely unknown. Our work shows that the phylogenetically conserved COE (Collier, Olf, EBF)-type transcription factor (TF) UNC-3 is required for terminal differentiation of the majority of MN subtypes in the *C.elegans* ventral nerve cord (SAB, DA, DB, VA, VB, AS). UNC-3 directly regulates the expression of terminal differentiation genes common to all cholinergic MN subtypes, i.e., acetylcholine (ACh) pathway genes, and terminal differentiation genes found in specific MN subtypes. We hypothesize that UNC-3 achieves MN subtype-specific gene expression by operating together with other TFs (activators/repressors) expressed in specific MN subtypes. Following a candidate approach, we found that the homeodomain TF UNC-4 is an UNC-3 target required for terminal differentiation of the MN subtypes SAB and DA9. This approach further revealed that the posterior HOX cluster gene *egl-5* is also required for DA9 terminal differentiation. However, UNC-3 operates in a distinct manner from UNC-4 and EGL-5 in DA9. UNC-3 directly co-regulates the expression of ACh pathway genes (common to all MN subtypes) and DA9-specific genes (*itr-1, avr-15*, etc), whereas UNC-4 and EGL-5 are specifically required for the expression of DA9-specific genes without affecting ACh pathway genes. The expression of *unc-3, unc-4*, and *egl-5* is initiated during development and maintained to adult stages, suggesting that these TFs not only assign but also maintain MN fate throughout life. These findings uncover a network of evolutionarily conserved TFs that diversify MN fate and reveal a novel role for HOX genes in MN terminal differentiation, which may be conserved across phylogeny.

503A. The effects of aging on dendritic plasticity and PVD-FLP branch coexistence. **V. Kravtsov**[1], M. Oren-Suissa[1,2], B. Podbilewicz[1]. 1) Department of Biology, Technion- Israel Institute of Technology, Haifa, Israel; 2) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, USA.

Self-avoidance, tiling and coexistence are the main mechanisms that enable the best dendritic coverage. C. elegans undergoes aging-associated changes that ultimately lead to decreased functionality of the organism, including its neurological functions. Recent research has shown that the nervous system of C. elegans undergoes changes and alterations during aging including dendritic morphology (Tank et al., 2011). We study dendritic plasticity, aging and spatial dendritic organization of two highly arborized mechanoreceptors in C. elegans, PVD and FLP (Oren-Suissa et al., 2010). PVD dendrites of L4s and young adults show regenerative ability following dendrotomy (laser induced severing of dendrites). Our working hypothesis is that in older ages this ability to regenerate is compromised. Previous studies and our preliminary results indicate that PVD and FLP do not overlap in larval stages (Smith et al., 2010). In addition, dendrites within each bilateral PVD do not overlap through a self-avoidance mechanism (Smith et al., 2012). We found that (1) the coverage fields of the PVD and FLP overlap in adult worms, which indicates coexistence and not tiling. This overlap increases as the worm ages. (2) PVDs show aberrant arborization at the age of 9 days of adulthood. (3) Dramatic increase in self-avoidance defects as animals age. In humans many neurodegenerative diseases as well as generalized cognitive decline are associated with age, aberrant arborization or both (e.g. autism and Alzheimer's disease). However our understanding of how these disorders are triggered and aggravated is scarce. Our research provides an insight into the aging and regeneration process of individual neurons. Oren-Suissa, M., et al. (2010). Science 328, 1285-1288. Smith, C.J. et al. (2010). Developmental Biology 345, 18-33. Smith, C.J., et al. (2012). Nature Neuroscience 15, 731-737. Tank, E.M.H. et al. (2011). Journal of Neuroscience 31, 9279-9288.

504B. kin-20 is required for maintenance of neuronal architecture. **M LaBella**, R Rawson, C Frøkjær-Jensen, N Jorgensen, MW Davis, M Bastiani, EM Jorgensen. University of Utah, Salt Lake City, UT.

Many regulators of neural development and axon outgrowth have been identified, yet very little is known about how neurons maintain their structure throughout adulthood. Defects in neuron maintenance can result in synapse loss and neurodegeneration, a characteristic of neurodegenerative diseases such as Alzheimer's disease. We are interested in how neurons maintain their architecture and connectivity throughout the life of the animal. We identified the gene kin-20, a serine-threonine kinase, in a forward genetic screen for uncoordinated mutants. Interestingly, kin-20 is likely required for neuronal maintenance during adulthood. kin-20 null animals become progressively uncoordinated from late larvae into adulthood. Similarly, axons grow out normally in development but then form spontaneous growth cones at late larval stages, leading to ectopic branching. Since the role of kin-20 in neuron maintenance is not understood, we conducted a kin-20 suppressor screen. We isolated more than 50 kin-20 suppressors that restore both adult neuron morphology and locomotor behavior to near wild-type levels. Unfortunately, complementation tests are not possible because kin-20 and most of the suppressors have a maternal effect. We developed two mapping strains using minimal Mos1 transposon (miniMos) inserts. In these dominant-mapping strains chromosomes are labeled with different GFP and mCherry markers. We successfully used the miniMos mapping strains to map mutations to a specific chromosome. We are currently identifying the genomic location of kin-20 suppressors by whole genome sequencing. This screen will hopefully identify genes downstream of KIN-20 kinase function.

505C. A screen for regulators of anterior-posterior axon outgrowth in *C. elegans* identifies the DNC-1/p150^{Glued} subunit. **Vi Leitenberger**, Angela Lee, Brian Ackley. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

During development axons and dendrites encounter a complex environment of signaling cues that guide the processes in specific directions to enable efficient wiring of neural networks. From a biological perspective we have two questions: molecularly, what cues collaborate with each other to direct axons in a specific direction and cellularly, how do neurons respond to these cues. Here we are focused on signals that regulate the anterior-posterior (A/P) axon outgrowth of the *C. elegans* D-type GABAergic neurons. We have shown that the cadherin-domain containing FMI-1/Flamingo and components of the Wnt signaling pathway, including *mig-5*/Dishevelled, function in parallel to regulate A/P axon guidance (Huarcaya Najarro and Ackley, 2013). To identify molecules that functioned with *fmi-1*, we conducted a screen in a *mig-5(rh94)* sensitized background. Approximately 2000 haploid genomes were screened and 3 mutants *(lh4-lh6)* were isolated that enhanced the penetrance of A/P defects in *mig-5*. *lh5* is a substitution mutation in *dnc-1*, which encodes a dynactin/p150^{Glued} subunit of the dynactin complex. *dnc-1* loss of function causes axon outgrowth defects along the A/P axis, indicating DNC-1 is required for proper axon patterning. DNC-1 functions as part of the retromer complex that regulates MIG-14/Wntless retrograde transport from early endosomes to trans-Golgi network (Coudreuse et al., 2006, Prasad and Clark, 2006). However, *dnc-1;mig-5* mutants have a higher penetrance of defects, compared to single mutants, suggesting DNC-1 function in axon development may be in parallel to Wnt signaling. Temperature shift assays indicate that *dnc-1* activity is required prior to the L3 stage to establish D-type GABAergic motorneurons patterning, suggesting a role in axon formation, rather than maintenance. We are determining whether the role of DNC-1 in A/P axon outgrowth is via its retromer function, or whether it affects other known signaling molecules involved in this process. Given the essential function of DNC-1 in endocytosis and transporting selective cargos to appropriate compartments, we are currently investigating how flamingo, dynactin, and Wnt signaling pathways work together to regulate axon outgrowth.

506A. A Neuron from Mesoderm: A Likely Case of *in vivo* Neuronal Reprogramming. **Shuo Luo**, Bob Horvitz. HHMI, Dept Biology, MIT, Cambridge, MA.

The ability to reprogram non-neuronal cells into neurons opens novel avenues for both the study and treatment of neurodegenerative and neuropsychiatric diseases that affect millions of patients worldwide. However, the reprogramming processes in mammalian systems are slow, inefficient, and highly variable, and our current understanding of molecular pathways and mechanisms that control reprogramming is limited. In the *C. elegans* nervous system, most of the 302 neurons are derived from the ectodermal AB blastomere. However, six neurons are generated from mesodermal lineages. Furthermore, a transgene that expresses RFP under the *hlh-1/CeMyoD* promoter reveals strong reporter expression in the immediate ancestors of the mesodermally-derived neurons (http://epic.gs.washington.edu/genesPage2.html). This finding suggests that these neurons are generated through a mechanism that involves endogenous reprogramming. Therefore, identifying the molecular mechanisms that drive the development of these neurons might generate novel insights into neuronal reprogramming. We have focused on one of the six mesodermally-derived neurons, the I4 neuron, which is

generated as a sister of the pm5 muscle cell during pharyngeal development. Using a transgenic strain that labels I4 with GFP and the pharyngeal muscles with mCherry, we have performed genetic screens and identified 16 mutant isolates in which the I4 neuron is transformed into a muscle-like cell. So far, we have identified three genes defined by those mutants: *let-19*, *dpy-22*, and *hlh-3*. *let-19* and *dpy-22* encode homologs of conserved Mediator subunits, which regulate gene expression by recruiting RNA polymerase II to transcription start site. *hlh-3* encodes the homolog of Ascl1/Mash1, which can help drive mammalian neuronal reprogramming. Genetic analysis indicates that *hlh-3* and the Mediator genes act in parallel pathways to regulate I4 specification: while mutations in either *hlh-3* or the Mediator genes result in low penetrance of I4 transformation, mutations in both *hlh-3* and the Mediator gene causes high penetrance of the phenotype. We are investigating the mechanisms by which *hlh-3* and the Mediator genes regulate I4 neuronal reprogramming.

507B. The *C. elegans* Microtubule Minus-end Capping Homolog, PTRN-1, Stabilizes Synapses and Neurites. **Jana Dorfman Marcette**, Jessica Jie Chen, Michael Nonet. Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO.

Microtubules function in axonal pathfinding and synaptic trafficking, however, the role of regulators of microtubule minus-end dynamics in neuronal function remains largely unexplored. In epithelial cells, CAMSAP proteins cap microtubule minus ends, and are important for the stability of cell adhesions. Here we report that the C. elegans CAMSAP homolog, PTRN-1, localizes to both neuronal processes and synapses, and is required for the morphological stability of axons and synaptic specializations. In ptrn-1 mutants we found neuromuscular behavioral defects including uncoordinated locomotion, pausing during pharyngeal pumping, and egg retention, and morphological defects in multiple classes of neurons. PLM mechanosensory neurons initially extend wild-type axons, and subsequently remodel by overextending axons and retracting presynaptic varicosities and entire synaptic branches. This remodeling of neuronal morphology depends on a functioning DLK-1 MAP kinase pathway, and is likely microtubule-based as microtubule acetylation mutants, known to disrupt higher-order microtubule structures, mimic the overextension phenotype. In ALM mechanosensory neurons, an additional neurite projects posteriorly from the cell body in addition to axonal overextension. Ptrn-1 phenotypes in PLM and ALM neurons are suppressed by different treatments. The axon overextension and retraction phenotype in PLM neurons is suppressed by growing worms at cooler temperatures or by dlk-1. Growing ptrn-1 worms at a cooler temperature, but in the presence of the microtubule-poisoning drug, colchicine, increases the axon overextension phenotype; but suppresses the presence of the additional neurite in ALM neurons. We speculate that these differences are based in context-specific microtubule structures formed in the cell-body vs. the synaptic termini. Our results suggest that destabilization of microtubule structures caused by loss of ptrn-1 is activating a regeneration program leading to changes in neurite morphology. We propose a model whereby local minus-end microtubule stabilization mediated by a functional PTRN-1 is necessary for maintenance of synaptic sites and other axonal termini.

508C. Role of the histone demethylase RBR-2 in neuronal development. **Luca Mariani**, Julien Vandamme, Anna Elisabetta Salcini. BRIC, University of Copenhagen, Copenhagen, Denmark.

Background. Methylation of lysine residues (K) located on histone proteins is believed to be an important regulator of chromatin structure. This modification is associated both to gene expression and repression, depending on the residue modified, and is regulated by two classes of enzymes: Lysine Methyl Transferases (KMTs) and Lysine Demethylases (KDMs). In the last few years, aberrant histone lysine methylation has been associated to neurological and psychiatric disorders, suggesting that such epigenetic mechanisms might play a central role in brain development and functions. Results. We performed a screen using available mutant alleles for selected KDMs, searching for defective axon migration and neuronal cell fates. Among others, we identified RBR-2, a Jumonji domain-containing protein with demethylase activity on tri-methylated lysine 4 of histone 3 (H3K4me3), as a potential factor involved in some aspects of neuronal functions. Analyses of transgenic animals and immunofluorescence with specific antibodies show that RBR-2 protein is expressed in several cell types, including neurons, at embryonic and larval stages, and, accordingly with its function, it localizes in the nucleus. Mutant animals show aberrant axon migration of some neurons that pioneer the ventral nerve cord. These defects arise during embryonic development and can be rescued by re-expressing the wild-type but not the catalytically inactive variant of RBR-2, indicating that its enzymatic activity is essential for the correct guidance of the neurons. Currently, we are measuring genetic interactions with other genes implicated in the process of axon guidance in order to identify in which pathway RBR-2 is acting. Conclusions and Perspectives. These data provide evidences that RBR-2 plays an important role in some aspects of the development of the nervous system in *C. elegans*. Being RBR-2 a demethylase for H3K4me3, an activating mark, thus acting as a transcriptional repressor, future analyses will be directed at identifying target genes directly or indirectly regulated by RBR-2. To this purpose, experiments such as ChIP and RNA extraction followed by deep sequencing, both in wild-type and mutated lines, are planned.

509A. A forward genetic screen identifies modifiers of a voltage- and Ca^{2+}-activated K^+ channel in left-right neuronal asymmetry. **Grethel Millington**, Chieh Chang, Chiou-Fen Chuang. Division of Developmental Biology, Cincinnati Children's Hospital Research Foundation, Cincinnati, OH.

The developing nervous system generates a large diversity of cell types with distinct patterns of gene expression and functions. One way to establish neuronal diversity is to specify neuronal subtypes across the left-right axis. The *C. elegans* left and right AWC olfactory neurons communicate to specify asymmetric subtypes, AWC^{OFF} and AWC^{ON}. The default AWC^{OFF} is specified by a Ca^{2+} regulated kinase cascade that is activated by influx of Ca^{2+} through the voltage-gated Ca^{2+} channel UNC-2/UNC-36. Intercellular communication between the two AWC neurons and other neurons through the NSY-5/innexin gap junction network antagonizes *unc-2/unc-36* Ca^{2+} signaling in the induced AWC^{ON} cell. However, the mechanisms by which *nsy-5* inhibits *unc-2/unc-36* Ca^{2+} signaling is largely unknown.

A previous study implicated a role of SLO-1 (Ca^{2+}-activated large conductance "BK" K^+ channel) in promoting AWC^{ON}. However, how *slo-1* regulates AWC^{ON} choice has not been studied. Activation of vertebrate SLO-1 channels causes transient membrane hyperpolarization, which makes it an important negative feedback system for Ca^{2+} entry through voltage-activated Ca^{2+} channels. Consistent with the physiological roles of SLO-1, our data suggest that *slo-1* acts downstream of *nsy-5* to inhibit *unc-2/unc-36* Ca^{2+} signaling in the specification of AWC^{ON}. To identify the genes required for s*lo-1* function in inhibiting *unc-2/unc-36* Ca^{2+} signaling for promoting AWC^{ON}, we performed a non-biased forward genetic screen to isolate mutants that suppress the *slo-1*(gf) phenotype. *vy11* is one of the mutants identified from this screen. The phenotype of *vy11* mutants is suppressed by loss-of-function mutations in the Ca^{2+} channel gene *unc-36*, suggesting that the *vy11* gene acts upstream of the *unc-2/unc-36* Ca^{2+} signaling pathway. Together, our results suggest a model in which *nsy-*

5 inhibits *unc-2/unc-36* Ca^{2+} signaling through *slo-1* and the *vy11* gene. Our ongoing cloning of the *vy11* gene will elucidate the underlying molecular mechanisms of the model.

510B. Neuropeptides in neuronal development, maintenance and regeneration. **Ellen Meelkop**, Massimo A. Hilliard. Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Neuropeptides consist of short sequences of amino acids and function through G protein-coupled receptors and second messengers such as calcium and cAMP. Their involvement in processes such as learning, memory, and behaviour (e.g. locomotion and social behaviour) has widely been established[1]. However, their role in neuronal development, maintenance and regeneration has only started to be explored. Recent work has shown that the neuropeptide pigment dispersing factor (PDF) is involved in neuronal regeneration in *C. elegans*[2]. Mutant animals lacking PDF-1 peptides showed a 25% decrease of regrowth, 24 hours after axotomy in the mechanosensory PLM neuron. So far, it remains unknown where, when and how these neuropeptides stimulate regeneration. In *C. elegans*, neuronal regeneration after a laser-induced axotomy occurs through regrowth of the proximal fragment (attached to the cell soma), which is capable of reconnecting and eventually fusing with the distal fragment (separated from the cell soma). Whenever reconnection and fusion fail to occur, the distal fragment degenerates in stereotypical pattern. In order to elucidate the involvement of the PDF system in these events in *C. elegans*, we are characterizing the mechanosensory ALM and PLM neurons during development and regeneration in *pdf-1, pdf-2* and *pdfr-1* mutant backgrounds. While ALM and PLM both develop normally, axonal regeneration and reconnection to the distal fragment is particularly compromised in the PLM neurons of *pdf-1* and *pdfr-1* mutant animals. Simultaneously, the distal fragments degenerate at a slightly faster rate. By expressing receptor fusion proteins under the *pdfr-1* promoter, we were able to confirm their expression in the mechanosensory neurons[3,4] and postulate that the observed effects are controlled cell-autonomously. By using *C. elegans* as a model for neuronal development, maintenance and regeneration, we are now able to genetically dissect the pathways through which specific neuropeptides modulate these crucial biological processes.[1] L. Frooninckx *et al.*, Front. Endocrin. 3 (2012). [2] L. Chen *et al.*, Neuron 71, 1043 (2011). [3] A. Barrios *et al.*, Nat. Neurosci. 15, 1675 (2012). [4] T. Janssen *et al.*, JBC 283, 15241 (2008).

511C. Developing a cell contact sensor for tracking neuron-glia interactions in vivo. **Karolina Mizeracka**, Maxwell Heiman. Genetics, Boston Children's Hospital, Boston, MA.

The mechanism by which neurons selectively recognize their glial partners is an important and largely unexplored question in neurobiology. To begin to address this question, we are developing a cell contact sensor designed to report on the interaction of adjacent cells. Inspired by the GRASP system developed by Bargmann and colleagues that can be used to label synaptic contacts by the extracellular reconstitution of a GFP molecule, we aim to develop a contact sensor that will trigger a transcriptional readout in a cell of interest, providing a more permanent mark. In order to translate an external interaction to a change in intracellular gene expression, we have modeled our contact sensor on the well-characterized Delta-Notch ligand-receptor signaling pathway, a naturally occurring system in which cell-cell contact triggers transcriptional activity. In brief, interaction of an artificial ligand-receptor pair on the surfaces of adjacent cells will result in intramembrane proteolysis of the receptor, releasing a cytoplasmic transcriptional effector, and thereby genetically marking the receiver cell. First, we will test our reporter system in S2 Drosophila cells, ensuring that interaction between our artificial ligand and receptor results in cleavage of the receptor and activation of a transcriptional reporter. Next, we will test our reporter system in vivo, focusing initially on the stereotyped and highly selective interactions between the amphid sensory neuron AFD and the amphid sheath glial cell that surrounds the AFD dendrite tip. Specifically, we will express an artificial ligand on the surface of the AFD neuron and an artificial receptor on the surface of the amphid sheath glial cell. Contact between this neuron and its glial cell will result in a transcriptional output, activating a fluorescent reporter in the glial cell. This system will allow us to screen for “miswiring” mutants in which an amphid neuron improperly associates with a non-amphid glial cell, as a means to identify factors that mediate neuron-glia recognition. Once established, this contact sensor could be readily applied to report on any cell-cell interaction, in C. elegans or other animals.

512A. Local and global inhibitory cues define the stereotyped synaptic tiling in *C. elegans*. **Kota Mizumoto**, Kang Shen. Biology, Stanford University, Stanford, CA.

Cellular interactions between neighboring axons and global positional cues are essential for the stereotyped positioning of individual axons and global topographic map formation. On the other hand, little is known about the role of those cues at the level of synaptic pattern formation. To address this question, we utilized *C. elegans* motoneurons as a model system. Nine DA class motoneurons innervate the dorsal musculature in *C. elegans*. Although the axons of DA neurons significantly overlap with each other, each neuron innervates a unique and non-overlapping segment of the muscle field by restricting its synaptic domain to a distinct region of its axon —a phenomenon we term “synaptic tiling”. Using the DA8 and DA9 motoneurons as an experimental system, we found that the precise arrangement of their adjacent but non-overlapping synaptic domains requires a local Plexin (*plx-1*) signaling that is dependent on the axon-axon interaction (Mizumoto and Shen 2013).

While local Plexin signaling is essential for the fine synaptic tiling border formation, we observed only a partial overlap between DA8 and DA9 synaptic domain in the synaptic tiling mutants, suggesting that there are other positional cues that roughly define the DA8 and DA9 synaptic domains. We then focused on two posteriorly expressed Wnts, LIN-44 and EGL-20, which we have reported to inhibit synapse formation in the posterior axonal segment of DA9 neuron. We found that while both DA8 and DA9 showed similar sensitivity to the most posterior Wnt, LIN-44, DA8 is more sensitive to EGL-20, which is expressed slightly anterior to LIN-44, than DA9. We found that an EGL-20 receptor, MIG-1(Frizzled) is expressed in DA8 but not at the detectable level in DA9, suggesting that selective EGL-20 receptor expression makes DA8 more sensitive to EGL-20 gradient. From these results we propose that precise synaptic patterns depend on local axon-axon interaction and global positional cues.

513B. Genes Needed for Neuronal Ensheathment. **Emalick Njie**, Daniel Cabrera, Brian Colbitz, Xiaoyin Chen, Martin Chalfie. Biological Sci, Columbia Univ, NEW York, NY.

Ensheathment, a fundamental feature of development in invertebrates and vertebrates, is a process in which cells wrap around other cells. For instance, glial cells and epidermal cells ensheath neuronal axons and dendrites to facilitate action potential propagation and dendritic tree formation, smooth

muscle pericytes ensheath blood vessels to modulate blood flow, and somatic gonadal cells ensheath germ cells during gonad organogenesis. In C. elegans the ensheathment of touch receptor neurons (TRNs) by hypodermal tissue results in an observable shift in TRN position. We have narrowed down the period in which the TRN process separates from the muscle (a sign of ensheathment) to beginning approximately at the 8th hr of the 11hr L4 stage and ending 9hrs after the animals have finished the adult molt. During this period, the process does not smoothly separate from muscle, but appears to separate at random points, thus resulting in a wavy, rather than a straight, process. Loss of some genes needed for ensheathment, such as mec-5 and fbl-1, resulted in processes resembling this intermediate stage. Loss of other genes, e.g., mec-1 and him-4, resulted in processes that do not separate at all. Deficiencies in focal adhesion components (pat-2/a-integrin, pat-3/b-integrin, pat-6/actopactin, unc-97/PINCH, and unc-112/Mig-2) also resulted in animals in which the TRNs do not separate from muscle. To identify additional ensheathment genes, we generated forty-nine separation defective strains from an ethyl methanesulfonate screen of approximately 36,000 haploid genomes. Preliminary mapping of these strains revealed six new alleles of mec-5, five new alleles of him-4, two new alleles of mec-1, four unidentified genes with at least two alleles, and twenty-five unidentified genes with one allele each. Twelve of the mutants have impaired touch sensitivity. We have identified one of the unidentified genes as D1009.3, which encodes a protein most similar to the mammalian extracellular protein ficolin, FIBDC1 (35% identical, 50% similar). Loss of D1009.3, which results in partially separated TRNs, does not cause touch sensitivity. We plan to further study these mutants to elucidate the molecular basis of ensheathment.

514C. A Neomorphic Mutation of mec-12/alpha-Tubulin Redirects Synaptic Vesicle Transport in C. elegans by Enhancing Dynein Activity. Jiun-Min Hsu[1], Chun-Hao Chen[1], Yen-Chih Chen[1], Kent McDonald[2], Mark Gurling[3], Albert Lee[1], Gian Garriga[3], **Chun-Liang Pan[1]**. 1) Institute of Molecular Medicine, National Taiwan University School of Medicine, Taipei, Taiwan; 2) Electron Microscopy laboratory, University of California, Berkeley; 3) Department of Molecular and Cell Biology, University of California, Berkeley.

Polarized cargo transport into axons or dendrites is mediated by specific molecular motors. Synaptic vesicles (SV) are targeted to the axon by the plus end-oriented motor UNC-104/KIF1A. Since they are also associated with the minus end-oriented motor dynein, it is unclear why they are prevented from entering the dendrite, which contains abundant minus end-out microtubules. The *C. elegans* gene *mec-12* encodes an a-tubulin enriched in the touch neurons that is required for SV transport. We identified a missense mutation of *mec-12*, *gm379*, which not only prevented SV from reaching synaptic regions, but also mistargeted them to the non-axon compartment of the PLM neuron. *gm379* altered a conserved C-terminus glycine residue and behaved as a neomorphic mutation, as SV mistargeting was not seen in the *mec-12* null and could be completely abolished by *mec-12* RNAi. Interestingly, reducing UNC-104/KIF1A function worsened vesicle mistargeting, and excess UNC-104 partially rescued it. By contrast, elimination of the dynein heavy chain DHC-1 suppressed SV mistargeting and significantly rescued vesicle transport defects in *gm379*, mimicking the effects of UNC-104 overexpression. Amino acid substitution at G416 revealed a charge-based principle governing the tubulin selectivity for KIF1A or dynein, with excess negative charge enhanced microtubules-dynein interaction and reciprocally reduced microtubules-KIF1A interaction. Our work extends previous in vitro structural studies and reveals mechanistic insights on the electrostatic interactions between the molecular motors and the C-terminus of alpha tubulin in the regulation of polarized SV transport. This work is supported by a National Health Research Institute Career Development Grant (NHRI-EX101-10119NC), the National Science Council, Taiwan (NSC100-2320-B-002-095-MY3), and NTU (NTU-CDP-102R7810).

515A. Endocannabinoid AEA as injury signal in axon regeneration. **Strahil Iv Pastuhov**, Naoki Hisamoto, Kunihiro Matsumoto. Division of Biological Science, Nagoya University, Nagoya 464-8602, Aichi, Japan.

The endocannabinoid arachidonoyl ethanolamide (AEA) inhibits axon regeneration of D-type motor neurons in *Caenorhabditis elegans* by regulating the activity of the JNK MAP kinase cascade (Pastuhov SI et al (2012), *Nat Commun.* 3:1136). AEA levels are controlled through enzymatic synthesis and degradation. Our previous work demonstrated that inactivation of the AEA degradation pathway inhibits axon regeneration. For example, deletion mutation in the gene for fatty acid amide hydrolase (FAAH), the enzyme that inactivates AEA by hydrolysis, causes defect in axon regeneration due to accumulation of high levels of endogenous AEA. This time, we focused on the synthesis of AEA in axon regeneration. NAPE-1 is a worm homologue of NAPE-PLD, an enzyme involved in AEA synthesis. We found that the *nape-1* null mutation suppressed the *faah-1* defect in axon regeneration. Conversely, overexpression of *nape-1* in D-type motor neurons by the *unc-25* promoter inhibited axon regeneration after laser surgery. These results suggest that the inhibition of axon regeneration by NAPE-1 occurs at restricted area around the injured neuron. Interestingly, when *nape-1* was overexpressed in touch receptor neurons by the *mec-7* promoter, D-type motor neurons were able to regenerate after laser surgery, but their regenerating axons tended to make a sharp turn upon approaching the lateral midline, where axons of PLM touch neurons extend. These results suggest that high local levels of AEA inhibit axon regeneration and that a gradient of lower concentration affects the guidance of the regenerating axon.

516B. Characterization of the VC class motor neurons in Caenorhabditis elegans: molecular mechanisms required for their differentiation. **Laura Pereira**, Oliver Hobert. Dept Biochemistry, Columbia Univ, New York, NY.

Motor neurons are a diverse class of neurons that synapse with muscles to control their activity. In order to mediate processes such as locomotion and reproduction, motor neurons differentiate into subtypes defined by their anatomical features, location, connectivity profile and expression of molecular markers. The mechanisms regulating how the cholinergic VC motor neuron class acquire and maintain their fate to function properly is unknown. Following a candidate gene approach as well as forward genetic screens I am working on identifying the genes required for the development and identity maintenance of the VC motor neuron class in C. elegans. By analyzing the expression of VC-specific markers in mutant backgrounds I have found that in the absence of the msh-like homeobox transcription factor vab-15 there are extra VC-like neurons compared to wild type. The number of post-embryonically generated VA, VB, AS and VD neurons is also defective in vab-15 mutants. The mechanisms by which VAB-15 affects the differentiation of neurons derived from the P neuroblasts is being further examined.

517C. Genetic analysis of cholinergic synaptogenesis in Caenorhabditis elegans. **Marie Pierron**, Bérangère Pinan-Lucarré, Jean-Louis Bessereau. Ecole Normale Supérieure, IBENS,Biology Departement, Paris, F-75005 France.

At the Caenorhabditis elegans neuromuscular junction, acetylcholine receptors (AChRs) form post-synaptic clusters precisely facing acetylcholine release

sites and mediate excitatory transmission. The number and the localization of these receptors are important to regulate the strength of synaptic transmission. To identify novel mechanisms involved in the formation and the maintenance of cholinergic synapses, we performed a novel genetic screen based on the direct visualization of fluorescently-tagged AChRs. Mutations causing abnormal AChR localization were identified using whole genome re-sequencing. Out of 29 mutants isolated from the screen 8 mutants had abnormal AChR distribution patterns that had not been described previously. For each mutant candidate genes were identified using whole genome re-sequencing. Causative mutations were confirmed by complementation test and rescue experiments. In some mutants, presynaptic defects (axonal outgrowth and branching) were most likely responsible for AChR distribution defects (mislocalized clusters or missing clusters). In other mutants, AChR cluster distribution was altered independently of presynaptic defects. Mutants from this group are currently being characterized.

518A. CEH-28 activates *dbl-1* expression and signaling in the M4 pharyngeal neuron. **Kalpana Ramakrishnan**, Paramita Ray, Peter Okkema. University of Chicago, Illinois.

The NK-2 homeodomain transcription factor CEH-28 is specifically expressed in the M4 pharyngeal motor neuron, where it regulates synaptic assembly and pharyngeal peristalsis. We are interested in how CEH-28 controls multiple M4 functions, and we are identifying CEH-28 targets. *dbl-1* encodes a TGF-b family protein expressed in M4 and a subset of other pharyngeal and non-pharyngeal neurons, and we have found *dbl-1* expression is specifically lost in M4 in *ceh-28* mutants. The *dbl-1* promoter contains separable regulatory elements controlling expression in M4 and other neurons. We identified an M4-specific enhancer in the *dbl-1* promoter and found mutation of predicted CEH-28 binding sites strongly reduced enhancer activity. We asked if DBL-1 functions downstream of CEH-28 to regulate M4 synaptic assembly, but *dbl-1* mutants exhibited normal M4 synapses and pharyngeal peristalses, suggesting *dbl-1* mediates a distinct, non-autonomous activity of M4. To test this hypothesis, we examined cells located nearby M4 in *ceh-28* and *dbl-1* mutants. The pharyngeal g1 gland cells extend processes contacting M4, and both *ceh-28* and *dbl-1* mutants exhibit morphological abnormalities in these processes. Similar defects were also observed in *sma-6* and *daf-4* mutants affecting receptors in the Sma/Mab pathway. However, *sma-2* and *sma-3* mutants affecting R-Smads in this pathway have normal gland cells, suggesting DBL-1 affects gland cells through Smad-independent function of the Sma/Mab pathway. We can partially rescue the gland cell defects in *dbl-1* and *ceh-28* mutants by expressing *dbl-1* only in M4. M4 contains dense core vesicles (DCVs) that mediate neuropeptide and protein secretion, but these DCVs do not regulate DBL-1 secretion as *unc-31* mutants defective in DCV release exhibit normal g1 gland cells. Based on these findings, we hypothesize that CEH-28 is a direct activator of *dbl-1* expression in M4, and that DBL-1 secreted from M4 affects gland cell morphology by Smad-independent signaling through the Sma/Mab TGF-b signaling pathway. M4 is a multi-functional cell that stimulates pharyngeal peristalsis and regulates sensory perception under hypoxic conditions, and our work identifies another novel function for this neuron.

519B. KPC-1 protease activity regulates dendritic arborization. **Nelson J Ramirez**[1], Yehuda Salzberg[1], Julius Fredens[2], Niels Færgeman[2], Hannes Buelow[1]. 1) Albert Einstein College of Medicine, Bronx, NY; 2) Department of Biochemistry and Molecular Biology University of Southern Denmark.

Proper dendrite morphogenesis is important for the establishment of functional neuronal circuitry, as evidenced by the defects in dendrite shape seen in human neurological disease and as a result of drug abuse. We are investigating dendrite morphogenesis using the mechanosensory neuron PVD with its elaborate dendritic endings as a model. In a genetic screen for genes required for shaping PVD arbors, we have identified several alleles of kpc-1, which encodes a proprotein convertase homolog of the kex2/furin family. Detailed observations as well as live imaging experiments of different alleles suggest that the normal function of kpc-1 is to promote primary branch extension and to suppress secondary branch formation, likely requiring convertase activity for this process. Cell specific rescue experiments show that kpc-1 expressed specifically in PVD is sufficient to rescue the mutant phenotype and a Pkpc-1::GFP reporter exhibits widespread neuronal expression, suggesting that kpc-1 acts cell-autonomously in PVD. To dissect the pathway by which this convertase influences dendritic architecture, we pursued an unbiased proteomic approach (SILAC) to identify potential KPC-1 substrates and downstream effectors. Reasoning that the absence of a protease may manifest in a changed peptide profile, we compared the proteomes of wild-type animals, kpc-1 mutants and cell-specifically-rescued mutants. We identified 63 candidates that were different in the mutant, but indistinguishable between the rescued strain and wild type animals. Of those, 37 are more abundant and 26 are less abundant. We do not know whether the function of these candidates is positively or negatively regulated by kpc-1. Therefore, we are currently conducting RNAi experiments against the identified genes in wild type and kpc-1 mutant worms to determine whether they recapitulate the kpc-1 mutant phenotype or suppress the mutant phenotype, respectively. We will report on the progress of our studies at the meeting.

520C. Neuron-glia communication in the assembly of the C. elegans nerve ring. **Georgia Rapti**, Shai Shaham. Rockefeller University, ENS-INSERM, New York, NY, USA.

Formation of functional neural circuits requires proper apposition of neuronal partners and fasciculation of neurons directed to similar locations. How neurons recognize each other to form fascicles and how glia support the formation of such complex structures is not well understood. The C. elegans nerve ring (NR) is an excellent setting to study these questions. This CNS-like neuropil consists of about 170 axons navigating in stereotypical tracts. The inner part of the NR is lined up by six mesodermally-derived glial-like GLR cells. The outer NR is enveloped by four CEPsh glia that are required for NR formation and axon guidance. We are taking several approaches to characterize NR assembly. First, we identified reporters to label and follow embryonic neurons to characterize the relative entry of axons and glial processes into the presumptive NR. Our preliminary results suggest an orderly population of the nerve ring, with glial processes appearing relatively early. Second, to identify molecules controlling NR axon guidance and fasciculation we performed a forward genetic screen aimed at identifying animals with defects in amphid commissure axons. We are currently characterizing those mutants and will present progress on mapping. Third, to study the roles of CEPsh glia in NR formation, we undertook strategies to achieve specific embryonic expression as well as specific time-controled cell ablation. These strategies should allow us to image CEPsh development at high resolution, and to precisely address their role in NR formation. Together, these approaches should begin to provide insight into the complex problem of neuropil assembly- a fundamental process in the development of all nervous systems.

521A. Cutting Edge: Expression and function of KPC-1/furin in C. elegans. **A. Rashid**, R. Androwski, N. Schroeder, M. Barr. Genetics, Rutgers University, Piscataway, NJ.

Proprotein convertases (PCs) are responsible for the cleavage of proproteins into their biologically active forms. The human PC family contains seven proteins that cut at dibasic cleavage sites while there are four PCs in *C. elegans* (KPC-1, BLI-4, AEX-5, and EGL-3). KPC-1 is homologous to furin, a PC essential for mammalian development and associated with numerous pathologies. However, *C. elegans kpc-1* mutants are viable allowing us to study the function of this gene in living animals.

We found that *kpc-1* is required for dauer-specific dendrite arborization in four IL2 quadrant (IL2Q) neurons (See N. Schroeder et al. abstract). In *C. elegans* there are three classes of multidendritic neurons: the IL2Qs in dauer and adult PVD and FLP neurons. We examined the PVD and FLP neurons in *kpc-1* adults. Similar to the IL2 neurons in dauers, *kpc-1* mutants show highly disorganized and truncated PVD and FLP neurons, demonstrating that KPC-1 is a general regulator of dendrite arborization. We are currently determining whether *kpc-1* is required for PVD and FLP mediated behaviors such as proprioception and harsh touch response.

kpc-1 expression showed strong temporal control. However, consistent expression was observed in the ventral nerve cord.To assess the effect of *kpc-1* on movement we used both standard body bend assays and thrashing assays. We found that *kpc-1* mutants are sluggish compared with wild-type animals. *kpc-1* expression was also observed in eight vulval precursor cells during the “Christmas tree” stage of vulval development. However, we observed no obvious defects in vulva morphology in *kpc-1* mutants. Finally, we observed temporal expression of *kpc-1* during embryogenesis and a reduced brood size in *kpc-1(gk8)* mutants, suggesting that *kpc-1* plays a role in early development. We conclude that *kpc-1* plays a multifaceted role in development, dendritic branching, and nervous system function.

522B. The role of the C. elegans Shugoshin homolog in sensory neurons. **Bryn Ready**[1], Tiffany Timbers[2], Kunal Baxi[1], Michel Leroux[2], Carlos Carvalho[2]. 1) Biology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada; 2) Molecular Biology and Biochemistry, Simon Fraser University, Canada.

Members of the Shugoshin family of proteins have essential roles in cell division from yeast to humans. Shugoshin is necessary to prevent premature loss of sister chromatid association at the centromere during anaphase. Surprisingly, deletion mutants and RNAi knockdown of the sequence homolog of Shugoshin in C. elegans (sgo-1; C33H5.15) does not show significant loss of viability. Considering that C. elegans chromosomes lack discrete centromeres, these findings pose the question of what, if any, is the function of this protein in the worm. Here we present preliminary evidence for the expression and function of SGO-1 in sensory neurons with cilia, the microtubule-based sensory organelles. Our preliminary findings suggested that the subcellular localization of a GFP::SGO-1 fusion protein is at the base of, or within, cilia. We carried out functional assays to test for a potential role of SGO-1 in ciliary biogenesis, maintenance and/or function using a sgo-1 deletion mutant. We found that sgo-1(tm2443) worms display developmental and behavioral defects also observed in other true ciliary mutants: (1) worms are defective in dauer formation; (2) worms do not disperse on food but rather stay aggregated, (3) worms show a reduced and retarded avoidance response to CO2 and (4) worms fail to chemotax towards the attractant diacetyl. We are currently investigating the biological function of SGO-1 in cilia formation by looking at ciliary structure phenotypes of sgo-1(tm2443) mutants that may be explained by defects in Intraflagellar transport (IFT). IFT is a process involved in cilia biogenesis in which molecular motors transport ciliary cargo bidirectionally from the base of the cilia, along the microtubule-based axoneme, towards the growing end of the ciliary compartment. In light of evidence that suggests that Shugoshin may also have extra-nuclear roles in regulating the assembly or stability of microtubule-related structures such as the centrosome, our findings further reveal a previously unknown function for a Shugoshin homolog in the context of a fully differentiated cell type.

523C. *egl-46* is a novel BAG cell fate modulator. **Alba Redo Riveiro**, Roger Pocock. BRIC, Copenhagen University, Copenhagen, Denmark.

Genetic studies in *C. elegans* attempt to identify molecular components required for neural circuits to function correctly. This study focuses on BAG neurons, which have the major role in oxygen (O_2) and carbon dioxide (CO_2) sensing. Development of BAG neurons is controlled by the conserved transcription factors ETS-5 (Brandt, Aziz-Zaman et al. 2012) and EGL-13 (Petersen et al. 2013-in press). However the effect of *ets-5* and *egl-13* loss of function mutants does not affect all BAG terminal fate markers. Therefore, other genes must act in parallel to these factors to specify BAG fate. In a classical forward genetic screen, we identified *egl-46* as a BAG fate modulator. EGL-46 is a zinc finger transcription factor that regulates terminal cell divisions in the Q lineage where *egl-46* mutants undergo extra rounds of terminal Q cell divisions (Wu, Duggan et al. 2001). The *egl-46* mammalian ortholog *Insm1* has a similar function in determining cell fates. *Insm1* has been implicated in the development of pancreas (Farkas, Haffner et al. 2008), cortex and hindbrain (Jacob, Storm et al. 2009). *Insm1* mutant mice present a thicker layer of proliferative progenitors and a thinner neuro-basal layer in the olfactory ephitelium (Rosenbaum, Duggan et al. 2011). Here we show that *egl-46* mutants have defects in the expression of specific terminal markers in the BAG neurons. We have rescued these defects by transgenically expressing a fosmid containing the *egl-46* locus. Currently, we are performing genetic interaction studies with BAG-fate regulators like *ets-5* and *egl-13*.

524A. Hunting for new genes that function in neuronal maintenance. **James Ritch**, Avery Fisher, Andrea Thackeray, Claire Benard. Department of Neurobiology, UMass Medical School, Worcester, MA.

Neuronal maintenance molecules ensure that neuronal structures, connectivity and function are preserved throughout life. A number of molecules have been identified to date that maintain the precise organization of neural circuits, after their initial establishment, in the face of wear and tear caused by body movements and growth (1). SAX-7, the *C. elegans* ortholog of the L1 family of vertebrate adhesion molecules, functions to maintain the position of neuronal soma, axons and dendrites. While the nervous system of *sax-7* mutant animals develop normally, specific defects arise later in life. A subset of embryonically developed axons of the ventral nerve cord become defasciculated during the first larval stage, and ganglia become progressively displaced later in larval and adult stages (2). SAX-7/L1 is a transmembrane protein of the immunoglobulin superfamily and is believed to function as a cell adhesion molecule. In vertebrates, L1 carries out developmental roles in axon migration, guidance, and fasciculation. In humans, L1 mutations cause neurological disorders including mental retardation and hydrocephalus. Moreover, L1 plays a role in the adult nervous system, since deletion of L1 in the mouse adult brain leads to learning disabilities (3). This indicates that both *C. elegans* SAX-7 and vertebrate L1 function in the adult nervous system to promote its integrity and functionality. SAX-7 functions within the nervous system to maintain the organization of fascicles and ganglia through homophilic and

heterophilic interactions (2). To understand how SAX-7 functions and identify conserved neuronal protection mechanisms, we have carried out a screen for mutations that modify *sax-7* mutants. We expect to discover genes that promote neuronal protection (enhancers) and that antagonize it (suppressors). From screening 6,000 haploid genomes, we isolated and outcrossed 5 suppressors and 2 enhancers. To identify the causal molecular lesion in our alleles, we are currently utilizing whole-genome deep sequencing and the Galaxy platform (4). 1) Benard and Hobert 2009 2) Sasakura et al 2005; Chen et al 2010; Pocock et al 2008 3) Law et al 2003 4) Minevich et al 2012.

525B. Neurobehavioral defects in a C. elegans mutant for an intellectual disability-associated gene. **Ana-Joao Rodrigues[1,2]**, Carlos Bessa[1,2], Filipe Marques[1,2], Bruno Vasconcelos[1], Filipa Pereira[1,2], Adriana Miranda[1], Patrícia Maciel[1,2]. 1) Life and Health Sciences Research Institute (ICVS), University of Minho, Braga, Portugal; 2) ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal.

Intellectual disability (ID) is one of the most frequent and disabling neurological impairments with an estimated prevalence of 1.5-2% in Western countries. Recent technological advances identify an incredible number of novel genetic associations every year. However, these associations often lack functional validation, and for several of the identified genes, their function in the nervous system remains undisclosed. This prompted us to use Caenorhabditis elegans to functionally validate the genetic associations and to better understand the role of a given protein in the nervous system. So far, we have studied 25 mutant strains, that correspond to 18 orthologues of human genes previously linked to ID. One candidate, ID34, presents gross anatomical defects (multivulva, loss of vulva), developmental delay (larval arrest), embryonic lethality and decreased life span. In parallel, this strain presents some degree of uncoordination and mild chemotaxis defects. Crossing ID34 with strains expressing GFP in specific neuronal sub-types, we found that the GABAergic network was strongly affected - abnormal neuronal positioning and migration. The cholinergic circuit also presented some defects, but not so pronounced. In agreement with the anatomical data, animals were more sensitive than the wild-type to Pentylenetetrazol (PTZ - GABA antagonist) and to aldicarb, a cholinesterase inhibitor. We are now assessing these mutants in other behavioral paradigms to assess learning and memory.

526C. Deciphering the molecular mechanisms that regulate the specification of O_2 and CO_2 sensing neurons. **Teresa Rojo Romanos**, Jakob Gramstrup Petersen, Roger Pocock. BRIC, Copenhagen University, Copenhagen, Denmark.

In response to changes in O_2 and CO_2 levels, *C. elegans* is able to mount rapid behavioral responses. The O_2 and CO_2 sensing system in *C. elegans* is predominantly comprised of 6 neurons: BAGL/R, URXL/R, AQR and PQR. The BAG neurons sense both decrease of O_2 and increase of CO_2 levels. The URX neurons respond to increases in O_2 level together with AQR and PQR. However the molecular mechanisms that control the formation and function of this crucial neural circuit are not fully understood. In our laboratory, we have identified several factors essential for correct differentiation of the O_2 and CO_2 sensing neurons. Three of these factors are EGL-13 (BAG, URX, AQR and PQR), ETS-5 (BAG) and AHR-1 (URX, AQR and PQR). To gain a deeper understanding of the molecular interactions that lead to the differentiation of these neurons we are performing a large-scale saturation forward genetic screen for mutants with defects in the development of O_2 and CO_2 sensing neurons. In parallel to the forward genetic screening approach we are performing promoter deletion analysis of the terminal differentiation gene battery of BAG and URX neurons. We aim to identify minimal *cis*-regulatory elements in these promoters that are sufficient to drive O_2 and CO_2 sensing neuronal cell fate. From this analysis we hope to identify the *trans*-acting factors that bind to these minimal *cis*-regulatory motifs. The role of such factors in driving O_2/CO_2 sensing neuronal cell fate will then be analyzed *in vivo*. Preliminary results from the promoter analysis have found short motifs that drive expression in the O_2 and CO_2 sensing neurons. In the analysis of the *flp-8* (URX-expressed) promoter for example we have identified a 600bp fragment that harbors predicted conserved binding sites for *egl-13* and *ahr-1*. Site-directed mutagenesis of these sites in the promoter show reduced expression, indicating the need for both factors for the proper expression of *flp-8* in the URX neurons. Similar promoter deletion analysis studies will be presented.

527A. Modular control of glutamatergic neuronal identity in *C. elegans* by distinct homeodomain proteins. **Esther Serrano Saiz[1]**, Richard J Poole[1,2], Felton Terry[1,2], Hobert Oliver[1]. 1) Dept Biochem, Columbia Univ, New York, NY; 2) Department of Cell and Developmental Biology, University College London, UK.

A key identity feature of an individual neuron type is its neurotransmitter phenotype. Glutamate is a transmitter used by many different neuron types. Terminal steps of glutamatergic neuron differentiation remain little studied. Specifically, it is not known how the expression of the key defining feature of glutamatergic neurons, the vesicular glutamate transporter, VGLUT, is controlled. We show here that the C. elegans VGLUT-encoding gene *eat-4* is expressed in 37 of the 118 distinct neuron classes of the *C. elegans* nervous system. We describe the regulatory mechanisms that control glutamatergic identity of several distinct, VGLUT-expressing glutamatergic neuron classes. Through transgenic reporter genes studies, we show that *eat-4* expression is controlled in a highly modular manner, with distinct cis-regulatory modules driving expression in distinct glutamatergic neuron types. We identify 12 different transcription factors, most of them phylogenetically conserved, that are either alone or in specific combinations required for *eat-4* expression in 19 distinct glutamatergic neuron types, which constitute more than half of all glutamatergic neuron classes. All of these transcription factors are continuously expressed in specific glutamatergic neuron classes and at least some of them control *eat-4* expression directly. Each of these transcription factors also controls a number of additional terminal features, indicating that the adoption of a glutamatergic phenotype is linked to other identity features of a neuron. Ten out of the 12 transcription factors described here to control terminal glutamatergic identity are homeodomain transcription factors and most of them have vertebrate homologs also expressed in mature glutamatergic neurons suggesting conservation of regulatory control mechanisms. Finally we show that the *unc-86*-orthologue *Brn3* mouse gene and the *ceh-14* and *mec-3* orthologous *Lim1* gene are expressed in glutamatergic neurons in the brain stem, in where at least *Lim1* in playing a role in the maintenance of these neurons, suggesting a conserved role of this logic of regulation.

528B. Methods for Studying Nerve Ring Assembly in C. elegans. **Anupriya Singhal**, Peter Insley, Shai Shaham. Rockefeller University, New York, NY.

Glial cells are a major organizing force in developing nervous systems, and play critical roles in the assembly of the nerve ring in C. elegans (Yoshimura et al., 2008). However, little is known regarding the embryonic stages of nerve-ring assembly or the specific roles played by glia. Three important challenges exist. First, most cell-specific reporters are not expressed early enough in embryogenesis to allow time-lapse imaging. Second, while cell identification in larvae and adults is facilitated by reproducible patterns of cell body and process positions, the changing complement of cells in the embryo makes cell

identification challenging. Third, maturation of the nerve ring occurs as embryos begin to twitch, making it difficult to image cells over time without blur. We have been developing methods to address these three problems. To overcome the dearth of reagents marking individual embryonic neural and glial cells, we are using inducible expression of the Cre recombinase to achieve sparse labeling of embryonic cells with GFP reporters flanked by lox sites. We are also developing a method, based on a previously described set-up (Kamei et al., 2009), using an infrared laser to target the heat shock response to individual cells for GFP reporter expression. To identify labeled cells, we are developing a computer-automated method for real-time cell identification relying only on the relative position of cells in the wild-type embryo and not their complete lineal history. This method allows for a substantial reduction in imaging time and analysis compared to existing methods. Finally, we have modified the iSPIM light-sheet imaging methodology of Shroff (Wu et al., 2011) to allow rapid imaging of embryos with little motion blur. We will use these technologies to follow neuronal development at cellular and subcellular resolution, and assess the primary defects that arise upon ablation or inhibition of glia. We want ultimately to integrate these methodologies into a complete imaging and image-processing system for the study of C. elegans nerve ring development, with an eye specifically to understanding the role of glial cells in that development.

529C. Regulation of sensory neuron architecture. **Aakanksha Singhvi**, Christine Friedman, Shai Shaham. The Rockefeller University, New York, NY 10065.

The shapes of neuronal receptive endings are exquisitely tuned to their function. Receptive endings of some sensory cells, including photoreceptors and auditory hair cells, have microvilli that allow dense packaging of receptors in a small space. To understand how sensory neurons form and maintain microvilli, we are studying the receptive endings of the *C. elegans* AFD thermosensory neuron, which are enveloped by the AMsh glia. Using cell ablations and inducible inhibition of secretion, we demonstrated that glia-secreted cues dynamically regulate the shape of AFD microvilli. To identify these glial signals, we performed a forward genetic screen seeking mutants with defects in AFD shape. Of the six mutants identified, we have cloned four. One mutant affects the gene *unc-23*, encoding a BAG2 co-chaperone that functions with *Hsp70* to control protein folding. Microvilli defects in these mutants are progressive and coincident with the appearance of large vesicular structures within glia, suggestive of a secretory block. Intriguingly, mutations in human *Hsp* genes underlie a subset of sensory neuropathies characterized by progressive glial myelin damage and axonal tip degeneration. Our analyses lead us to propose a model where UNC-23 regulates protein folding of a small number of polypeptides in glia by antagonizing the E3/E4 ubiquitin ligase, CHIP/CHN-1. A second mutant we isolated affects the gene *gcy-8*, encoding an AFD-expressed receptor guanylyl cyclase important for thermosensation. Our analyses suggest that deregulated cyclase activity in AFD alters microvilli. Strikingly, receptor guanylyl cyclase mutations result in structurally abnormal microvilli in rod and cone cells of the eye. Finally, our preliminary data also suggest a role for the engulfment pathway in maintaining AFD microvilli. This is intriguing, given that engulfment by retinal pigmented epithelial cells sculpts rod and cone microvilli in mammals. Taken together, our studies provide new insight into how glia-neuron communication controls neuronal receptive ending morphology, and suggest that mechanisms governing receptive ending shape may be conserved.

530A. Regulatory logic of pan-neuronal gene expression. Inés Carrera[1,3], **Nikolaos Stefanakis[1,3]**, Oliver Hobert[1,2]. 1) Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY; 2) Howard Hughes Medical Institute; 3) equally contributed.

Cell fate decisions in the vertebrate nervous system are particularly complex as the nervous system is composed of a remarkably heterogeneous assemblage of cell types. Although a lot is known about how specific transcription factors, or Terminal Selectors (TS), specify different neuronal types by coregulating neuron-type specific terminal differentiation genes, much less is understood about the regulatory programs that control the expression of those neuronal features shared by every neuron, pan-neuronal features. Addressing this question is key to understanding how neuronal fate is determined. In this work, we are dissecting the *cis*-regulatory logic of broadly expressed neuronal genes and also identifying those *trans*-acting factors that regulate them. Promoter bashing analysis of *cis*-regulatory control elements of pan-neuronal genes shows a piecemeal regulation of gene expression in different neuronal types as well as redundant elements. We find that TS are also able to regulate expression of isolated *cis*-regulatory modules of some pan-neuronal genes, although in TS mutants full promoters of these genes are not affected. We are currently conducting genetic screens to identify these redundant transcription factors. Analysis of the temporal and spatial expression of broadly expressed neuronal genes by fosmid reporters show expression in the nervous system as well as in other tissues. Progress towards the understanding of how pan-neuronal gene expression is regulated will be presented.

531B. The CDH-4 Fat-like cadherin is required for anterior-posterior Q neuroblast migrations. **Lakshmi Sundararajan**, Erik Lundquist. Molec Biosciences, KU, Lawrence, KS.

We use Q neuroblasts in *C. elegans* to study neuronal cell migration. The QR and QL neuroblasts undergo initial protrusions anteriorly and posteriorly, respectively. They then migrate in the direction of protrusion and divide to produce three neurons, of which AQR (from QR) migrates anteriorly and PQR (from QL) posteriorly. Our results (Sundararajan and Lundquist, 2012) show that UNC-40/DCC acts in parallel to a pathway including MIG-21 and PTP-3/LAR in directing posterior migration of QR and QL. These proteins have left-right functional asymmetry. In QL, UNC-40 and PTP-3/MIG-21 act redundantly to direct posterior migration. In QR, UNC-40 and PTP-3/MIG-21 mutually inhibit each other's posterior migration and cause QR to migrate anteriorly. Hence, UNC-40, PTP-3 and MIG-21 contribute to signaling systems in QR and QL. We wanted to identify genes that function with UNC-40, PTP-3 and MIG-21 in QR and QL. In a forward genetic screen we identified *cdh-4*, a fat-like cadherin implicated in Q descendant migration (Schmitz et al., 2008). *cdh-4* mutants show defects in QR and QL migration (75% of QR cells migrate posteriorly). To understand how *cdh-4* interacts with *unc-40*, *ptp-3* and *mig-21*, we built double mutants of *cdh-4* with these genes. In QR, both *unc-40RNAi* and *ptp-3RNAi* suppressed the posterior migration seen in *cdh-4* mutants, suggesting that this posterior migration requires functional UNC-40 and PTP-3. The above result shows that CDH-4 has a role in both UNC-40 and PTP-3 pathways, which might explain why *cdh-4* mutants show a high percentage of QR migrating posteriorly. However, in QL *unc-40RNAi; cdh-4* mutants show increased anterior migration, suggesting that UNC-40 and CDH-4 are required in redundant pathways for posterior QL migration. In contrast, *ptp-3RNAi; cdh-4* mutants resembled *cdh-4* mutants alone, suggesting that PTP-3 and CDH-4 might function in the same genetic pathway. Current studies are aimed at understanding the genetic interactions between *cdh-4* and *mig-21* and to identify whether CDH-4 acts in the Q cells or other cells. The *cdh-4* promoter driving GFP is not expressed in the Q cells. Cell specific RNAi techniques are being used to test autonomy of *cdh-4* function in Q descendant migration.

532C. A PCP-like pathway acts to position or properly space (tile) embryonic motor neurons along the ventral nerve cord. **M. Tanner**[1], A. Ghadban[1], C. Slatculescu[1], B. Huang[1], T. Perkins[2], A. Colavita[1,2]. 1) Neuroscience, University of Ottawa, Ottawa, Ontario, Canada; 2) OHRI, Ottawa Hospital, Ottawa, Ontario, Canada.

Planar Cell Polarity (PCP)-like signaling has recently been linked to multiple aspects of nervous system development and wiring. We have found that a PCP-like pathway involving PRKL-1 and VANG-1 is required for DD motor neuron position along the AP axis. The six DD neurons (DD1-6) are normally positioned equidistant from each other in the ventral nerve cord. In *prkl-1* and, to a lesser extent, *vang-1* mutants, DD neurons are shifted anteriorly and display distinctive cell spacing defects. These positioning defects can be rescued by *prkl-1* or *vang-1* expression from a pan-neuronal promoter, suggesting cell-autonomous roles. Conversely, over-expression of neuronally-expressed *prkl-1* results in posteriorly shifted DD neurons. Interestingly, the strong anterior shift in *prkl-1* mutants can be suppressed by simultaneous loss of *vang-1*, suggesting that PRKL-1 normally antagonizes VANG-1 function in DD neurons. A candidate screen of known Wnt and PCP mutants revealed *prkl-1* and *vang-1*-like DD positioning and spacing phenotypes in several other genes including *mom-2/WNT* and *let-502/ROCK*. Ectopic expression of MOM-2, which is normally expressed in the posterior embryo, from the more anteriorly expressing *cwn-1* promoter was sufficient to induce DD positioning defects similar to those of loss-of-function mutants. Taken together, these data suggest a role for a PCP-like pathway in regulating neuronal cell body placement or spacing (tiling) along the ventral nerve cord in the embryo, a process which may involve an instructive role for Wnts along the AP axis.

533A. Transmembrane Collagen COL-99 is Involved in Axon Guidance Along Major Longitudinal Axon Tracts and Ventral Nerve Cord Asymmetry in *C. elegans*. Thomas Unsoeld, **Jesse Taylor**, Harald Hutter. Biology, Simon Fraser University, Burnaby, BC, Canada.

The ventral nerve cord (VNC), the major longitudinal axon tract of *C. elegans*, consists of two fascicles separated by motor neuron cell bodies aligning at the ventral midline. The majority of neurons extend axons into the right tract creating the highly asymmetric structure of the VNC. Specifically, axons leaving the brain decussate into the right VNC tract shortly after descending from the nerve ring in the head. Mutants of known guidance cues and receptors have shown either no or very minor asymmetry defects in the VNC indicating existence of additional molecules involved in creating VNC asymmetry. Forward genetic screens in our lab revealed several candidate mutants displaying partially penetrant symmetrical VNC and midline crossing defects. One mutation, *hd130*, has been identified as an allele of the uncharacterized transmembrane collagen, *col-99*. *col-99* encodes a type II transmembrane collagen consisting of a small intracellular domain and a large extracellular domain containing several collagen domains. Sequencing revealed an early nonsense mutation in *col-99(hd130)* likely resulting in complete loss-of-function. Further characterization of axonal trajectories in *col-99* mutant animals identified significant axonal defects in most VNC neurons. Significant axonal navigation defects were observed in additional longitudinal axon tracts including the dorsal nerve cord and the two dorsal sublateral tracts. During axonal outgrowth in the embryo, *col-99*-GFP reporter expression is prominent in the hypodermis but not the nervous system suggesting a non cell-autonomous role for COL-99. *col-99* interacts genetically with the collagen processing enzyme *dpy-18*, the basement membrane component *nid-1* and the collagen-receptors *ddr-1* and *ddr-2*. We currently explore the idea that the ectodomain of COL-99 is shed by proprotein convertases and localizes to the basement membrane. Our results demonstrate the importance of *col-99* during axon navigation along major longitudinal tracts in *C. elegans*, and suggest a role in the decussation event establishing VNC asymmetry.

534B. Mutations in *C9ORF72/F18A1.6* produce developmental and behavioral defects. **Xin Wang**[1], Edgar Buttner[1,2]. 1) Molecular Pharmacology, McLean Hospital/Harvard Medical School, Belmont, MA; 2) Departments of Neurology and Psychiatry, McLean Hospital/Harvard Medical School, Belmont, MA.

Expanded hexanucleotide repeats in the human *C9ORF72* gene cause frontotemporal dementia and amyotrophic lateral sclerosis (Boeve et al., 2012; Renton et al., 2011). The normal and abnormal mechanisms of action of this human disease gene are not fully understood. We are characterizing phenotypes associated with mutations in *F18A1.6*, the *C. elegans* homolog of *C9ORF72*. We found that feeding RNAi of *F18A1.6* caused developmental delay, while the insertion/deletion allele *ok3062* did not. *F18A1.6(RNAi)* also reduced locomotor behavior in a thrashing assay, compared to empty-vector control. Surprisingly, *ok3062* animals displayed increased thrashing, compared to N2. To better understand the cellular basis of the locomotion phenotypes, we are now conducting pharmacological and neuronal GFP reporter assays. We are also generating transgenic animals to test the ability of *C9ORF72* to rescue *F18A1.6*-associated phenotypes and to test the effects of expressing expanded hexanucleotide repeats in C. elegans. References Boeve, K. B. Boylan, N. R. Graff-Radford, M. DeJesus-Hernandez, D. S. Knopman, O. Pedraza, and P. Vemuri (2012). Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. Brain 135, 765-783. Renton, E. Majounie, A. Waite, J. Simón-Sánchez, S. Rollinson, J. R. Gibbs, J. C. Schymick, and H. Laaksovirta (2011). A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257-268.

535C. Investigation of a novel transition fiber component DYF-20 in C.elegans. **Qing Wei**, Jinghua Hu. Dept Med, Mayo Clinic, Rochester, MN.

Cilia act as motile or sensory devices on the surface of most eukaryotic cells. Dysfunction of cilia has been linked to a wide spectrum of human disorders, now collectively termed ciliopathies. To discover novel genes involved in cilia formation and function, we performed an EMS screening for dye filling defect mutants in C.elegans. We isolated a partially dye filling defect mutant dyf-20(jhu511). DYF-20 is a poorly characterized protein across species. GFP-tagged DYF-20 specifically label cilia base. Double staining with transition zone markers indicated DYF-20 locates below the transition zone, and around transition fiber region. jhu511 animals show defective cilia formation with truncated distal segments. However, not like in another TF mutant dyf-19, no abnormal IFT movement was observed in jhu511. Interestingly, ciliary sensory receptors, including OSM-9 and PKD-2, mislocalize in the cilia of jhu511 mutants, indicating that DYF-20 plays a key role in the proper trafficking of ciliogenic cargos. Taken together, our results reveal a novel TF component and provide insights into the function of TF in regulating the ciliary loading of various sensory receptors.

536A. Transition fibers protein DYF-19 regulates the ciliary entry of assembled IFT complex. **Qing Wei**, Qingwen Xu, Kun Ling, Jinghua Hu. Dept Med, Mayo Clinic, Rochester, MN.

Sensory organelle cilia play critical roles in embryonic development and normal tissue homeostasis. Phylogenetically conserved intraflagellar transport (IFT) machinery, which are composed of motors, IFT-A and IFT-B subcomplexes, and cargos, builds and maintains all cilia. However, how the gigantic IFT

machinery (consisting > 20 polypeptides) gets across the size-dependent permeability barrier at cilia base remains unknown. Here we show that a highly conserved protein DYF-19 localizes specifically on transition fibers (TFs), a poorly understood structure at cilia base, and regulates the ciliary import of assembled IFT machinery. We retrieved and cloned dyf-19 in a whole-genome genetic screen for C. elegans mutants with disrupted IFT integrity. DYF-19 is essential for the ciliary entry of IFT complexes through direct interaction with IFT component DYF-11. DYF-19 deficiency does not affect TF integrity but results in the accumulation of assembled IFT particles below TFs. Furthermore, we found that human homolog of DYF-19 is essential for ciliogenesis and shares conserved localization and function with its worm counterpart. Collectively, these results demonstrate that TFs act as the bona fide ciliary gate for size-dependent permeability of ciliogenic proteins, and DYF-19 is the key functional but not structural TF component that directly interacts and actively facilitates the ciliary entry of gigantic IFT particles.

537B. The SWI/SNF chromatin remodeling complex selectively affects multiple aspects of serotonergic neuron differentiation. **Peter J Weinberg**[1], Nuria Flames[2,3], Hitoshi Sawa[4], Gian Garriga[5], Oliver Hobert[2]. 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Department of Biochemistry and Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, New York, NY; 3) Instituto de Biomedicina de Valencia, IBV-CSIC, Valencia, Spain; 4) National Institute of Genetics, Mishima, Japan; 5) Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Regulatory programs that control the specification of serotonergic neurons have been investigated by genetic mutant screens in the nematode Caenorhabditis elegans. Loss of a previously uncloned gene, *ham-3*, affects migration and serotonin antibody staining of the hermaphrodite-specific (HSN) neuron pair. We characterize these defects here in more detail, showing that the defects in serotonin antibody staining are paralleled by a loss of the transcription of all genes involved in serotonin synthesis and transport. This loss is specific to the HSN neuron class as other serotonergic neurons appear to differentiate normally in *ham-3* null mutants. Besides failing to migrate appropriately, the HSN neurons also display axon pathfinding defects in *ham-3* mutants. However, the HSN neurons are still generated and express a subset of their terminal differentiation features in *ham-3* null mutants, demonstrating that *ham-3* is a specific regulator of select features of the HSN neurons. We show that *ham-3* codes for the C. elegans ortholog of human BAF60, Drosophila Bap60 and yeast Swp73, which are subunits of the yeast SWI/SNF and vertebrate BAF chromatin remodeling complex. We show that the effect of *ham-3* on serotonergic fate can be explained by *ham-3* regulating the expression of the Spalt/SALL-type Zn finger transcription factor *sem-4*, a previously identified regulator of serotonin expression in HSN and of the *ham-2* Zn transcription factor, a previously identified regulator of HSN migration and axon outgrowth. Our findings provide the first evidence for the involvement of the BAF complex in the acquisition of terminal neuronal identity and constitute genetic proof by germline knockout that a BAF complex component can have cell-type specific roles during development.

538C. Developmental Specification of a Polymodal Nociceptor in C. elegans. **Jordan Wood**, Denise Ferkey. Department of Biological Sciences, University at Buffalo, SUNY, Buffalo, NY.

All animals rely on their ability to sense and respond to the environment to survive. Nociception serves an important protective function, eliciting withdrawal and avoidance behaviors in response to potentially damaging stimuli. However, while pain sensation is particularly valuable in helping an organism to avoid potentially harmful stimuli, there are still large gaps in our understanding of the mechanisms that govern nociceptor development across species. In C. elegans, the pair of polymodal nociceptive ASH head sensory neurons responds to a broad range of aversive stimuli, including soluble chemicals, odorants, ions, osmotic stress and mechanosensory stimulation, with ASH detection activating backward locomotion and aversive responses. Importantly, the ASHs are believed to be analogous to nociceptors that detect multiple pain modalities in systems ranging from other invertebrates such as Drosophila to vertebrates. To understand the continuum that connects embryonic neuronal developmental specification with neuronal physiological function in adult animals, we aim to characterize the developmental transcriptional hierarchy that bestows the C. elegans ASH nociceptors with polymodal sensitivity and to determine the extent to which identified modality-specific gene batteries contribute to associated adult animal sensory behaviors. Our central hypothesis is that the paired-like homeodomain transcription factor UNC-42 functions as a “terminal selector” in the developmental specification of ASH cell identity. We will present behavioral and ASH-reporter data examining the role for UNC-42 in coordinating multiple cellular characteristics during development in the specification of the functionally distinct ASH neuronal identity.

539A. SYD-1 mediates ventral axon guidance in the HSN neuron of *C. elegans*. **Yan Xu**, Christopher Quinn. Dept of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, WI.

During development axons navigate towards their targets, giving rise to the neural circuitry that controls behavior. During this navigation process, axons respond to extracellular cues that activate receptors to steer the axon along its trajectory. Guidance receptors are thought to be modular, forming in various combinations that have distinct functions. For instance, the UNC-40 receptor can function alone as a receptor for the UNC-6 guidance cue. Alternatively, UNC-40 and SAX-3 can function together, independently of UNC-6, as a receptor complex for the SLT-1 guidance cue (UNC-40/SAX-3 signaling) [1]. Since these different configurations of UNC-40 have distinct functions, they must have distinct signaling pathways. However, little is known about the pathway that mediates UNC-40/SAX-3 signaling. We have found that UNC-6 independent UNC-40 signaling can be mediated by SYD-1, an intracellular protein that contains a rhoGAP-like domain. We propose that SYD-1 may function with UNC-40 and SAX-3 to mediate UNC-40/SAX-3 signaling to promote the response to the SLT-1 guidance cue. We are currently characterizing the role of SYD-1 in guidance signaling and seeking to determine how it might be regulated to modulate UNC-40/SAX-3 signaling. [1] T.W. Yu et al., *Nat. Neurosci.* 5:1147-1154 (2002).

540B. Understanding cellular mechanisms of selective fasciculation between dendrites. **Candice Yip**[1], Maxwell Heiman[1,2]. 1) Harvard Medical School, Boston, MA; 2) Boston Children's Hospital, Boston, MA.

The organization of axons and dendrites into defined bundles, known as selective fasciculation, is a conserved feature of nervous system wiring from nematodes to mammals. It shapes the development of neural circuits, including the guidance of growth cones along existing axonal bundles in grasshopper embryos, sorting *Drosophila* axons into longitudinal pathways, generating topographic maps of visual space in the *Drosophila* optic lobe, and ensuring vomeronasal axons innervate their correct targets in mice. Defining mechanisms of selective fasciculation has been hindered by two major

obstacles: (1) the challenge of discerning individual processes within a bundle, which may contain hundreds of processes, and (2) the challenge of distinguishing defects in neuron outgrowth or guidance from defects in fasciculation, since these processes are intimately coupled. In order to overcome these challenges, we have turned to the major *C. elegans* sense organ, the amphid, which contains 12 neurons and two glial cells. Classic electron microscopic reconstructions show that each neuron extends an unbranched sensory dendrite that resides in a stereotyped position in the amphid bundle. This stereotypy in dendrite location leads to the hypothesis that dendrites establish and maintain specific adhesive connections with their neighbors. Here, we used three-color fluorescent labelling of individual amphid neurons to recapitulate the classic EM observation that amphid dendrites are located in stereotyped positions relative to one another. We are taking several approaches to elucidate cellular mechanisms underlying this selective fasciculation. First, we are imaging wild-type animals and known mutants to determine when in development and where along the dendrites selective fasciculation occurs. Second, we are using cell ablation to examine the role of each neuron in determining the positioning of its neighbors. Third, we are using cell fate transformations to test if neuron identity, rather than birthplace, specifies dendrite positioning. Understanding mechanisms of selective fasciculation will help elucidate basic developmental processes important for nervous system organization.

541C. Dendrite tiling as an emergent property of self-avoidance. **Candice Yip**[1], Maxwell Heiman[1,2]. 1) Harvard Medical School, Boston, MA; 2) Boston Children's Hospital, Boston, MA.

Dendrite arbors are sculpted into a morphologically diverse array of shapes and sizes, which are critical determinants of neural circuit function and behavior. This elaborate morphogenesis is governed by fundamental organizational principles, including isoneuronal repulsion between dendrites of a single neuron, called self-avoidance, and heteroneuronal repulsion between dendrites of neurons belonging to the same class, called tiling. Here, we took advantage of a *lin-22* mutation that causes the formation of extra PVD neurons to demonstrate that dendrite tiling can arise as an emergent property of self-avoidance. PVDs are mechanosensory neurons that elaborate complex, yet ordered, dendrite arbors through the contact-mediated repulsion of "self" dendrites. In wild-type animals, a single PVD extends dendrites that cover the entire body wall. In contrast, *lin-22* mutants generate a total of five PVDs on each side that are evenly spaced along the length of the animal. We found that each *lin-22* PVD elaborates a smaller, non-overlapping dendrite field, suggestive of tiling. We used laser ablation of the ectopic PVDs to demonstrate that these smaller arbors are not due to defective dendrite growth in the *lin-22* background, but instead reflect mutual repulsion between PVDs, consistent with a tiling model. Next, we tested whether *unc-6*/Netrin, which has been shown to mediate PVD dendrite self-avoidance in wild-type animals, is also required for dendrite tiling between PVDs. Indeed, tiling in *unc-6*; *lin-22* double mutants was severely diminished, suggesting that *unc-6*-mediated self-avoidance is sufficient for generating a tiled array of dendrite arbors in the presence of ectopic neurons of the same subtype. These results suggest that the apparently complex phenomenon of dendrite tiling can, in fact, emerge spontaneously from pre-existing mechanisms of dendrite self-avoidance.

542A. Suppression of microtubules dynamics by DHC-1 is required for an intact cytoskeleton and efficient cargo trafficking in C. elegans dendrites. **Shaul Yogev**, Kang Shen. Dept Biology, Stanford, Stanford, CA.

A hallmark of neuronal microtubules (MTs) is their stable, non-dynamic character. Yet how this stability is achieved, or what purpose it serves is not well understood. Here we show that Dynein Heavy Chain 1 (DHC-1), the main subunit of the cytoplasmic dynein retrograde motor is required to suppress MT dynamics in the dendrites of C. elegans neurons. In the absence of dhc-1, dendritic MTs are more dynamic, suggesting that their stability is decreased. Accordingly, MTs fail to fully support dendrite elongation, leading to shorter processes. More dramatically, dynamic "plus end out" MTs fail to stop at the distal dendrite tip in dhc-1 mutants, and instead loop backwards, giving rise to aberrant cytoskeletal structures. Cargo is not correctly transported through these structures, and accumulates as large ectopic puncta. Previous studies have shown that cargo accumulation at axonal tips in dhc-1 mutants is a consequence of impaired retrograde transport. However, our data indicate that the dendritic dhc-1 phenotypes are not due to a failure in minus-end directed transport. Instead, we show evidence for a model in which cortically anchored DHC-1 captures MTs, thereby stabilizing them. This capture and stabilization couples MT growth to dendrite growth, and yields a straight cytoskeleton on which cargo can be smoothly transported. Thus, our results provide both a mechanism for ensuring a stable MT cytoskeleton in dendrites and illustrate its biological significance.

543B. Identification of APL-1 as a long-range or short-range signaling molecule in *C. elegans* . **Pei Zhao**[1,2], Chris Li[2]. 1) Graduate Center, the City University of New York, 365 Fifth AvenueNew York, NY 10016 USA; 2) Department of Biology, City College of New York, MR526, 160 Convent Avenue, New York, NY 10031 USA.

Alzheimer's Disease (AD) is a progressive fatal neurodegenerative disease. Excessive levels of the amyloid precursor protein (APP) cleavage product, the b-amyloid peptide, are one pathogenesis factor for AD. The limitation of mammalian animal models makes it difficult to explore APP biological function. The orthologous APP gene in *C. elegans* is *apl-1*, which has an essential function in development and aging. Like mammalian APP, APL-1 is cleaved by the a/g-secretase pathway to produce sAPL-1 and APL-1 cytoplasmic domain (AICD). In this research, we try to investigate whether released sAPL-1 might act as a long-range signaling molecule or short-range signal by tagging the N-terminus of APL-1 with GFP and the C-terminus with mCherry. The *in vivo* location of the different fluorochromes will provide clues as to how sAPL-1 travels among organs or tissues and what types of cells act as targets of APL-1. Pan-neuronal expression of APL-1 or the extracellular domain of APL-1 is sufficient to rescue the *apl-1* loss-of-function lethality, suggesting that neurons are a critical source of APL-1. To determine from which neurons APL-1 must be released, we are using different promoters to drive APL-1 expression in different subsets of neurons and test their rescuing ability in *apl-1* knockout animals.

544C. Genetic basis of subtype diversification of Touch Receptor Neurons in *C. elegans*. **Chaogu Zheng**, Margarete Diaz Cuadros, Martin Chalfie. Department of Biological Science, Columbia University, New York, NY.

Although the logic for neuronal cell fate determination has been well accepted, it is not entirely clear how neurons sharing the same fate can further diversify into subtypes that have distinct structures. We found that both intrinsic genetic programs and extrinsic environmental cues are essential for this morphological diversification. The touch receptor neurons (TRN) of *C. elegans* have different cell shapes. Both the anterior (ALM) and the posterior (PLM) TRN have single anteriorly directed processes with a distal terminal branch, but only the PLM neurons have a prominent posterior process. We found that

two posterior Hox genes (*egl-5* and *php-3*), which are only expressed in PLM but not ALM neurons, are responsible for this morphological difference. PLM neurons in *egl-5* or *php-3* mutants have a significantly shortened posterior process, while misexpression of either *egl-5* or *php-3* in ALM neurons could induce the growth of an ectopic posterior process. Genetic studies showed that *egl-5* and *php-3* could replace each other in the function of inducing the posterior process, suggesting that they are both needed for the normal PLM cell shape, but they work in parallel. We also found that mutation of *egl-5* and the other posterior Hox gene, *nob-1*, led to the loss of TRN markers in PLM but not ALM, indicating that the distinct Hox gene expression patterns between the TRN subtypes contribute to their morphological and developmental differences. Moreover, a genetic screen searching for mutants with short PLM posterior process identified *dsh-1*, which encodes a homolog of Drosophila Dishevelled protein, suggesting that Wnt signaling is also important for the growth of PLM posterior process. Unlike the Hox genes, *dsh-1* was expressed in both ALM and PLM neurons and overexpression of *dsh-1* in TRN did not cause the growth of an ectopic ALM posterior process, which indicated that only PLM but not ALM received the external Wnt signal that induced posterior neurite outgrowth. Noncanonical Wnt signaling pathway is responsible for the PLM morphogenesis, as no defect was found in *bar-1*/beta-catenin and *pop-1*/TCF mutants. Thus, TRN subtype differences depend on both intrinsic Hox gene expression and extrinsic signals.

545A. Sink or Swim: Identifying Novel Regulators of Presynaptic Dopamine Signaling. **Sarah Baas**[1], J. Andrew Hardaway[2], Shannon Hardie[1], Sarah Whitaker[1], Tessa Popay[1], Phyllis Freeman[1], Randy Blakely[1,3]. 1) Department of Pharmacology; 2) Neuroscience Graduate Program; 3) Department of Psychiatry, Vanderbilt Unversity, Nashville, TN.

Dopamine (DA) modulates brain circuitry relevant to cognition, reward, motor control, and arousal. Perturbed DA signaling is believed to contribute to addiction, ADHD, schizophrenia, and Parkinson's disease. The presynaptic DA transporter (DAT) is a major control point for DA signaling and increasing evidence indicates that altered regulation of DAT may contribute to risk for these disorders. We have chosen the nematode *Caenorhabditis elegans* as a model system to elucidate novel mechanisms regulating DAT and/or DA signaling. We have demonstrated that DA is important for swimming behavior in the worm, as ablation of the *C. elegans* gene encoding DAT (*dat-1*) results in swimming-induced paralysis (Swip). This behavior is attenuated in animals treated with the vesicular monoamine transporter inhibitor, reserpine, and is dependent on signaling through a postsynaptic, D2-like receptor, DOP-3. To determine novel presynaptic regulators of DA signaling, we undertook a chemical mutagenesis screen to identify reserpine-sensitive Swip animals. Two mutants, *vt31* and *vt34*, do not possess mutations in *dat-1*, fail to complement and map to the same region on LG1. Similar to *dat-1* null animals, these mutants exhibit robust Swip that is suppressed by deletions in *cat-2* or *dop-3*. Over-expression of GFP::DAT-1 does not rescue Swip in these mutants, despite normal trafficking of the protein to the synapse. The mutants are partially resistant to the toxin 6-hydroxydopamine, which requires uptake by DAT-1 to produce DA neuron degeneration, consistent with reduced DAT-1 function. Unlike *dat-1* animals, *vt31* and *vt34* have altered male tail morphology, which we found arises from a premature stop codon in the Runx transcription factor homolog RNT-1. Ongoing experiments will determine whether the *rnt-1* mutation underlies the Swip phenotype in these mutants. Elucidation of the molecular lesion associated with *vt31* and *vt34* may yield important and conserved clues to the presynaptic regulation of DA signaling. Supported by NIH awards MH095044 to RDB, T32 MH065215 (S.B.), F31 MH093102 (A.H.), and MH095044 (R.D.B).

546B. Understanding the function of Cell Adhesion molecules in the *C. elegans* nervous system. Pratima Sharma, Ashwani Bhardwaj, Pallavi Sharma, Vina Tikiyani, Shruti Thapliyal, Nagesh Kadam, **Kavita Babu**. Dept of Biological Sciences, IISER Mohali, Mohali, Punjab, India.

Cell Adhesion Molecules (CAMs) are known to play important roles at synapses, which are the sites of communication between neurons and their targets. They are required for various aspects of synapse function including maintaining the integrity and promoting the stability of the synapse as well as linking the pre-synaptic and post-synaptic membranes. CAMs have also been shown to be required for target recognition and the differentiation of pre- and post-synaptic structures (Reviewed Curr Opinion in Cell Biol. 2003, 15:621-632). Our experiments aim to study the function of Cell Adhesion Molecules (CAMs) on synapse development and activity dependent plasticity at the *C.elegans* neuromuscular junction (NMJ). To identify CAMs that regulate the body wall NMJ we had previously done an RNAi screen for changes in aldicarb sensitivity on a set of cell adhesion molecules picked out from the *C. elegans* genome (Neuron 2011,71(1):103-116). Aldicarb is an acetylcholine esterase inhibitor that causes hypercontraction of muscles in wild type animals. Mutants with defects in synaptic transmission could have altered responses to aldicarb (PNAS 1996, 93(22): 12593-8). We are currently characterizing CAMs that show either enhanced sensitivity or resistance to aldicarb using pre-synaptic and post-synaptic markers as well as soluble GFP markers. We will also present our current work on elucidating the mechanism of functioning of an Immunoglobulin superfamily CAM, RIG-3, that we have previously shown to have an activity-dependent anti-potentiation function at the *C. elegans* NMJ (Neuron 2011,71(1):103-116).

547C. Unraveling mechanisms for the establishment of synaptic connectivity in a simple neural circuit. **B Barbagallo**, M Francis. UMASS Medical School, Worcester, MA.

The establishment of appropriate connectivity within neural circuits is essential for nervous system function. We are using the motor circuit of *C. elegans* as a model to study neural circuit development. At the *C. elegans* neuromuscular junction (NMJ), body wall muscles extend membrane processes to the nerve cord where they form *en passant* synapses with both cholinergic (ACh) and GABA motor neurons (MN). To determine the role of presynaptic ACh MNs in instructing synapse development, we have been studying *unc-3* mutants in which ACh MNs fail to terminally differentiate (1), and a transgenic strain previously characterized in our lab in which the ACh MNs die immediately after hatch (2). Failure of ACh MNs to differentiate or death of ACh MNs disrupted the localization of postsynaptic acetylcholine receptors (AChRs). Even in the absence of ACh MNs, GABA MN morphology remained grossly normal. To evaluate a potential role for ACh MNs in influencing GABA synapse development, we examined the structure and distribution of GABA synapses. We found the apposition of presynaptic release sites and postsynaptic receptors was unchanged by loss of ACh MNs; however, GABA synapse density and localization was dramatically affected. Thus, inhibitory synapse development appeared at least partially dependent upon ACh MNs. How might ACh MNs contribute to inhibitory synapse development? GABA synapses were normal in animals lacking AChRs on body wall muscles (*unc-29;acr-16*), suggesting excitatory transmission onto the muscle is not involved. In contrast, specific expression of tetanus toxin in the ACh MNs produced changes in GABA synapses comparable to those observed in strains lacking ACh MNs, suggesting a requirement for vesicular release from ACh MNs. Finally, mutations in genes broadly required for dense core vesicle release (*unc-31*) and neurotransmission (*unc-64*) did not alter the number or distribution of inhibitory

synapses. The failure of these global manipulations to affect inhibitory synapse development, coupled with the obvious effects of cell-specific manipulations, leads us to propose that normal synaptic connectivity requires a balance of ACh and GABA MN signaling. 1. Kratsios et al., 2012 2. Barbagallo et al., 2010.

548A. Understanding the mechanism of environmental stress induced nervous system plasticity. **Abhishek Bhattacharya**[1,2], Oliver Hobert[1,2]. 1) Dept Biochemistry & Molecular Biophysics, Columbia Univ, New York, NY; 2) Howard Hughes Medical Institute.

Stress induced plasticity of the nervous system is a complex process that ultimately leads to adaptive behavior. Although anatomical and functional properties of the rewiring process have been studied at the level of individual neurons or small circuits in many organisms, it has never been studied at the level of an entire nervous system. We are studying the problem of nervous system rewiring in response to environmental cues by investigating the hibernation-like alternative diapause state of the nematode C. elegans, called the dauer state. Under adverse environmental conditions, mainly starvation, high population density and high temperature, C. elegans larvae molt into dauer state. Dauer animals not only show altered morphology, but also show a remarkably altered responsiveness to various environmental cues and strikingly different locomotory behavior. However, the neuronal circuit responsible for these altered behaviors is largely unknown. Our preliminary data suggests many *innexins* (*inx*)/gap junction genes, which build electrical synapses, dramatically switch their cellular specificity of expression in dauer nervous system. We are currently killing neurons with altered *inx* gene expressions to identify the role of these rewired circuits and the role electrical synapses play in these circuits that ultimately leads to altered dauer behaviors. We anticipate that our molecular, anatomical and behavioral studies together will be able to reveal some of the molecular mechanisms that underlie the nervous system rewiring process, and its ensuing behavioral consequences.

549B. Serotonergic/Peptidergic Cotransmission in the C. elegans Egg-Laying Circuit. **Jacob Brewer**, Michael Koelle. Dept. of MB&B, Yale University, New Haven, CT.

The hermaphrodite-specific neuron (HSN) has long been thought to release serotonin to excite the active phase of egg laying. This idea is based in part on the facts that animals in which the serotonergic HSNs fail to develop are severely egg-laying defective, and that the addition of exogenous serotonin to these animals is sufficient to induce egg laying. However, mutants defective for serotonin biosynthesis are only modestly egg-laying defective. Further, when we use channelrhodopsin to activate the HSN neurons, we see strong stimulation of egg laying even in mutants unable to synthesize serotonin. These results suggest that serotonin released from the HSNs may be sufficient but not necessary to stimulate egg laying. We hypothesized that another neurotransmitter released by the HSN stimulates egg laying in the absence of serotonin. Mutants with defects in neuropeptide signaling have long been known to be egg-laying defective, and at least five neuropeptide genes have been reported to be expressed in the HSN. To test if a neuropeptide could be another neurotransmitter released by the HSN to stimulate egg laying, we have been analyzing knockout mutations and transgenic overexpressors for each of the five HSN-expressed neuropeptide genes to see how they affect egg laying. Our results so far have shown that that overexpressing at least one of these neuropeptide genes, *nlp-3*, causes a profound hyperactive egg-laying phenotype. This is consistent with the idea that one or more NLP-3 neuropeptides are released by the HSN to stimulate egg laying. We therefore propose that the HSN acts to excite the egg-laying circuit by using serotonin and at least one neuropeptide as cotransmitters. We are carrying out a detailed study of this system to understand the purpose and logic of serotonergic/peptidergic cotransmission in the egg-laying circuit.

550C. Multiple dopamine signaling pathways antagonize RhoA signaling in the nervous system. **Kimberley H.R. Bryon-Dodd**[1], Clara Essmann[1], Andrew Porter[1], Rachel McMullan[2], Stephen Nurrish[1]. 1) MRC Laboratory for Molecular Cell Biology, University College London, London, United Kingdom; 2) Imperial College, London, United Kingdom.

Hyperactivation of RHO-1, the solo *C. elegans* RhoA ortholog, in motorneurons (nRHO-1*) causes exaggerated body bends and increased release of the neurotransmitter acetylcholine (ACh). A suppressor screen of the exaggerated body bend phenotype was performed to identify effectors of RHO-1 in the adult nervous system.

One such mutant, *nz99*, was identified as a nonsense mutation in the dopamine reuptake transporter, DAT-1. Both *dat-1(nz99)* and a putative null deletion allele, *dat-1(ok157)*, suppressed the exaggerated body bends of nRHO-1* animals but only partially suppressed the increased ACh release phenotype. A fosmid containing the DAT-1 gene rescued the exaggerated body bends in the nRHO-1*;*dat-1* double mutants.

dat-1 mutations should increase levels of dopamine signaling and mutations that block dopamine synthesis prevent *dat-1* mutations from suppressing RHO-1*. Thus elevated dopamine signalling acts to oppose RHO-1 signalling in neurons. Consistent with these results addition of exogenous dopamine substantially improves the locomotion defects of nRHO-1* animals.

dat-1 suppression of nRHO-1* also requires at least two dopamine GPCRs, DOP-1and DOP-3, demonstrating that activation of multiple dopamine receptors is required to antagonize RHO-1 neuronal signaling. These receptors are differentially expressed in the *C. elegans* nervous system suggesting that multiple dopamine signaling pathways must be activated to efficiently antagonize RHO-1 signalling. We are currently testing the site of action of DOP-1 and DOP-3 for antagonising nRHO-1* effects on locomotion.

551A. Amphetamine and b-Phenylethylamine Activate an Amine-Gated Chloride Channel. Bryan Safratowich[1], Chee Lor[1], Laura Bianchi[2], **Lucia Carvelli**[1]. 1) Pharmacology, Physiology and Therapeutics, University of North Dakota, Grand Forks, ND; 2) Department of Physiology and Biophysics, University of Miami, Miller School of Medicine, Miami, FL.

Amphetamine is a highly addictive psychostimulant, which is thought to generate its effects by promoting release of dopamine through reverse activation of the dopamine transporters. However, some amphetamine-mediated behaviors persist in dopamine transporters knockout animals, suggesting the existence of alternative targets. We demonstrate here the identification of a novel amphetamine target. We show that in C. elegans, a large fraction of the behavioral effects of amphetamine is mediated by activation of the amine-gated chloride channel LGC-55. Furthermore, we demonstrate that the endogenous trace amine b-phenylethylamine (b-PEA) activates the same channels, but more efficiently than amphetamine. Importantly, our data also show that amphetamine acts as a partial agonist, thus preventing full activation of LGC-55 by b-PEA. These findings bring to light alternative pathways

engaged by amphetamine, and urge rethinking of the molecular mechanisms underlying the effects of this highly addictive psychostimulant drug.

552B. Redundant mechanisms for modulation of the serotonergic HSNs by an environmental cue. **Jung-Hwan Choi**, Niels Ringstad. Skirball Institute, NYU School of Medicine, New York, NY.

Egg-laying in worms is regulated by hermaphrodite-specific neurons (HSNs), which stimulate the vulval muscles through serotonin. Previous studies have shown that cholinergic and peptidergic input to HSNs inhibits egg-laying. However, it has been shown that removal of only one of these inhibitory inputs to HSNs cannot induce egg-laying, but removal of both inputs induces precocious egg-laying. BAG neurons have been shown to the primary source of HSN inhibition through the neuropeptide, FLP-17, and its receptor, EGL-6, a G protein-coupled receptor expressed in HSNs. BAG-ablated wildtype animals show normal egg-laying behavior, whereas BAG-ablated unc-17 mutants, which lack the vesicular acetylcholine transporter, have a precocious egg-laying phenotype. This suggests there are redundant inhibitory pathways, one that requires BAG neurons and FLP-17 and another that requires cholinergic transmission. To better understand how the BAG-dependent and BAG-independent pathways contribute to HSN inhibition in vivo, we propose to perform calcium imaging of HSNs in response to BAG neuron stimulation in wildtype and mutant animals which lack cholinergic or peptidergic input.

553C. Understanding how neurotransmitter signaling drives two-state activity of the *C. elegans* egg-laying behavior circuit. **Kevin M. Collins**, Michael R. Koelle. Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

We are interested in understanding how neurotransmitter signaling allows a neural circuit to execute distinct behavior states. *C. elegans* regulates egg laying by alternating between an inactive phase and a ~3 minute “active” state during which clusters of 3-6 eggs are laid. Six VC motor neurons release acetylcholine to excite the vulval muscles, and two HSN motor neurons release serotonin which signals through vulval muscle receptors to promote the active phase. Four uv1 cells release tyramine to inhibit egg laying. We are using GCaMP calcium imaging in behaving animals to understand how the signaling events in the circuit produce its two state behavior. We recently reported that the vulval muscles are rhythmically excited during each locomotor body bend (1). We found the UNC-103 K+ channel depresses muscle excitability below the threshold that drives calcium transients and contraction during the inactive phase, while still allowing signals above threshold during the active phase.

To understand how the HSN, VC, and uv1 neurons regulate vulval muscle excitability, we are manipulating neurotransmitter release from these cells and using GCaMP to record changes in neuron and vulval muscle activity. Activation of serotonin release from the HSNs using Channelrhodopsin induces rhythmic vulval muscle twitching and egg-laying behavior—hallmarks of the active phase. We find that VC neuron activity increases during the active phase, and preliminary results show that egg-laying events mechanically distort the uv1 cells and trigger calcium transients. We have previously shown that TRPV channels, which can be mechanically gated, are required for uv1 to inhibit egg laying (2). We propose that successive egg-laying events increase uv1 calcium signaling, promoting release of tyramine and neuropeptides which terminate the active phase of egg laying.

(1) Collins and Koelle (2013). J. Neurosci. 33, 761-775.

(2) Jose et al. (2007). Genetics 175, 93-105.

554A. Screening for Suppressors of Excitotoxic Neurodegeneration in *C. elegans*: Using Nematode Genetics to Understand the Process of Neurodegeneration in Brain Ischemia. **Anthony O Edokpolo**, Itzhak Mano. Physiol, Pharm & Neurosci, CCNY.

In spite of extensive studies, the molecular mechanisms involved in neuronal loss in stroke and brain ischemia are not well understood, and clinical trials based on our partial understanding of the process ended with disappointment. Genetic screens in simple animal models offer a powerful alternative approach, since screens are unbiased, analysis is facilitated by strong research tools, and cellular mechanisms are highly conserved through evolution. We produced a reliable model for excitotoxicity in the nematode *C. elegans* (using the *Dglt-3;nuIs5* strain) and we now draw on this model to screen for genes whose mutation or inhibition alter the extent of neurodegeneration. We are using two approaches to systematically knock-down/modify *C. elegans* genes and test their involvement in excitotoxic neurodegeneration: a) We are using an RNAi library to knock down each gene in the nematode’s genome. b) We are using EMS to introduce random mutations throughout the genome. In both cases we are monitoring the effect of genetic modification on the level of neurodegeneration. We screened ~2,000 mutagenized genomes and identified 4 mutant strains that show enhanced excitotoxicity and 5 mutant strains that show suppression/decrease in the level of neurodegeneration. These mutants have been isolated, and we are currently characterizing them to identify the most interesting ones. To that end we are carrying out behavioral assays to understand if normal synaptic activity has been modified compared to our original *Dglt-3;nuIs5* strain. For example, we want to understand if the enhanced neuronal survival is due to a specific impairment of post synaptic cell death mechanisms, or if the reduction in excitotoxic cell death seen in these mutants is caused by an overall reduction in synaptic activity. Following the initial characterization of cell death pathway mutants, we will be using whole genome sequencing to identify enhancer or suppressor mutations and study their mechanism of action. Novel insights into molecular mechanisms of excitotoxicity in *C. elegans* are likely to help elucidate conserved processes in higher animals, and possibly help us suggest new intervention strategies to reduce neurodegeneration in stroke.

555B. VAV-1 acts cell autonomously to regulate cholinergic motor neuron presynaptic activity. **A. Fry**, J. Laboy, K. Norman. Ctr Cell Biol & Cancer Res, Albany Med College, Albany, NY.

Synaptic transmission is a fundamental cell-cell signaling process that underlies basic physiological processes such as sensation, movement, and feeding. Deregulation of synaptic transmission occurs in a variety of human diseases or conditions, including Alzheimer’s disease, epilepsy, and schizophrenia. However, the mechanisms that modulate neurotransmitter release are not well understood. We are studying the locomotory behavior of *C. elegans* to better understand neuromodulatory mechanisms that regulate nervous system activity. We have found that mutations in the gene *vav-1*, which encodes an evolutionarily conserved Rac GTPase family guanine nucleotide exchange factor (GEF), result in an elevated rate of locomotion. This and other *vav-1* mutant phenotypes are consistent with elevated acetylcholine release. Nevertheless, *vav-1* null mutants exhibit normal sinusoidal patterns of locomotion and the nervous system develops normally in these animals. We found that restoration of VAV-1 expression in only cholinergic neurons restores the elevated locomotion rate of *vav-1* mutant worms to wild type levels. We also determined that the hyperactive phenotype of *vav-1* mutants is dependent on synaptic vesicle (SV) release. In transgenic animals, cholinergic VAV-1::GFP fluorescence is present at neuromuscular junctions. Furthermore, *vav-1*

mutants have normal distribution of synaptic vesicle-associated proteins GFP::SNB-1 and Venus::RAB-3 at cholinergic neuromuscular junctions, but the fluorescence intensity of these proteins at synapses is altered. This may indicate that the neuronal role of VAV-1 is to organize synaptic release machinery, or to inhibit synaptic vesicle release in a more direct manner, at existing synapses. Currently, we are testing the requirement of enzymatic GEF activity for normal function of neuronal VAV-1, investigating the distribution of further neuromuscular junction proteins in *vav-1* mutants, and determining whether cholinergic expression of VAV-1 rescues abundance of synaptic proteins to wild type levels. Taken together, our results unveil a novel role for Vav proteins in an inhibitory neuromodulatory signaling pathway.

556C. The AP2 clathrin adaptor subunit APM-2 regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. **Steven D. Garafalo**, Caroline L. Dahlberg, Emily Malkin, Peter Juo. Molecular Physiology & Pharmacology, Tufts University School of Medicine, Boston, MA.

Glutamate receptors (GluRs) mediate the majority of excitatory neurotransmission in the central nervous system. Regulation of the postsynaptic abundance of GluRs controls the strength of synaptic transmission which plays a critical role in learning and memory. We are interested in identifying genes and mechanisms involved in trafficking GLR-1 GluRs to synapses in the ventral nerve cord (VNC). GLR-1 is an AMPA-type GluR which is expressed in interneurons and localized to synapses in the VNC. Here, we show that APM-2 (also known as DPY-23), the m2 subunit of the clathrin adaptor AP2, regulates the abundance of GLR-1 in the VNC. AP2 is well known as a regulator of clathrin-mediated endocytosis at the plasma membrane. Surprisingly, we found that *apm-2(gm17)* and *apm-2(e840)* loss-of-function mutants have reduced levels of GLR-1 in the VNC. This effect can be rescued by expression of wild type *apm-2* cDNA in the *glr-1*-expressing interneurons. In addition, expression of *apm-2* under a heat-shock inducible promoter at the L4 stage can rescue the GLR-1 defect observed in *apm-2* mutants, suggesting that APM-2 can function in the mature nervous system to regulate GLR-1. In contrast, mutation of another clathrin adaptin UNC-11/AP180, which regulates endocytosis of GLR-1 at synapses, results in accumulation of GLR-1 in the VNC. Interestingly, *apm-2;unc-11* double mutants exhibit reduced levels of GLR-1 at synapses suggesting that *apm-2* functions prior to GLR-1 endocytosis in the VNC. We hypothesize that APM-2 regulates anterograde trafficking of GLR-1 from the cell body to synapses. Consistent with this idea, GLR-1 accumulates in cell bodies of *apm-2* mutants and genetic double mutant analysis indicates that *apm-2* functions in the same pathway as the kinesin *klp-4*, which is involved in anterograde transport of GLR-1. Our data reveal a novel function for APM-2 in regulating the levels of GLR-1 in the VNC and suggest that APM-2, and possibly the AP2 complex, regulate anterograde trafficking of GluRs. Several possible models of how APM-2 regulates GLR-1 trafficking will be discussed.

557A. Analysis of optogenetically evoked motor neuron activity by Ca^{2+} imaging using RCaMP in muscle. Sebastian Wabnig, **Caspar Glock**, Cornelia Schmitt, Alexander Gottschalk. Buchmann Institute, Goethe University, Frankfurt, Germany.

Understanding how information is computed in the nervous system requires the functional analysis of individual neurons, for example by phenotype or by Ca^{2+} imaging during concomitant manipulation of neuronal activity. With a promoter-defined expression pattern and light as a trigger, optogenetic tools facilitate a comparably non-invasive as well as spatiotemporally specific neuronal manipulation. Previously, we and others targeted specific cells using recombinase systems. As first examples, we expressed channelrhodopsin-2 (ChR2) in the backward command neuron AVA and the aversive neuron ASH, respectively. Thus, we were able to trigger reversal behavior upon blue light stimulation. Since little is known about the neuronal patterns driving muscle activity patterns that underlie backward locomotion, we addressed this by combining photostimulation of sensory or command neurons via ChR2 and Ca^{2+} imaging of muscles via the red-fluorescent RCaMP, by exploiting their independently addressable spectral properties. To facilitate this approach, we immobilized the animals. While this precludes dynamic proprioceptive feedback, required for normal propagation of the body wave, it may enable recording the “pure” neuronally driven activity patterns, since these signals are not overlaid with proprioception-induced depolarization of motor neurons. Photostimulating AVA neurons provoked distinct spatio-temporal muscle activity patterns along the body of the animal. In contrast, ASH activation appeared to elicit more pronounced motor responses and different activity patterns. This may indicate that the sensory neuron, which signals to many neurons in addition to AVA, can evoke more complex activity patterns than the “pure” activation of the backward command neuron, whose main output is to motor neurons. AVA may thus trigger motor neurons not dynamically, but rather “lift” them to a new activity level, while ASH-triggered neurons evoke more complex patterns of motor activity. Furthermore, we currently analyze mutants lacking gap junctions for their role in motor pattern propagation; activity in these animals appears to be feeble and more “chaotic”. This study will contribute to understand the *C. elegans* locomotor system.

558B. Changes in Cellular Circuits for Chemosensation during Development May Underlie Maturation of Chemotaxis Behavior. **Laura A. Hale**, Sreekanth H. Chalasani. MNL-SC, Salk Institute for Biological Studies, La Jolla, CA.

What features of a neural circuit change during development to enable mature behavior? We study the development of well-defined chemosensory circuits to uncover general mechanisms of neural circuit maturation. Here we present data that examines circuit development at the cellular level; we assayed circuit function by examining chemotaxis behavior in response to attractive and repulsive odors. We expand on published work that characterized chemotaxis responses to various odors in adult worms by characterizing responses to those odors in L1 to L4 stage worms. In population assays, we found that, compared to L4 and adult animals, L1, L2 and L3 animals have reduced attraction to the odors benzaldehyde and diacetyl. Thus, we use increased attractive chemotaxis as a proxy for circuit maturity. Interestingly, repulsive chemotaxis responses to nonanone were similar for L3, L4 and adult animals, suggesting circuit-specific mechanisms for maturation. Since the greatest behavioral difference occurred between L3 and adult animals we restricted further experiments to those two stages.

Having characterized a difference in chemotaxis behavior between younger and older animals we next asked whether differences in the cellular circuit of L3 and adult animals may account for their behavioral responses. Since the sensory cells underlying chemotaxis responses in adults have been identified we asked whether AWA for diacetyl and AWC for benzaldehyde are required in L3 worms for attractive chemotaxis. By analyzing behavior of mutants lacking AWA and AWC, we found that those cells are also required for L3 attractive chemotaxis. Moreover, when we analyzed mutants lacking other food-sensing sensory neurons, we found that an additional cell, ASK, is also required in adult but not L3 worms for benzaldehyde chemotaxis. Collectively, these results suggest that a larger cellular network of sensory cells may underlie the increased attractive chemotaxis response in adult animals. In future experiments, we will image activity of individual neurons in L3 and adult worms during odor presentation to characterize the functional role of those cells

in younger and older chemotaxis circuits.

559C. The C2A domain of synaptotagmin-1 drives synaptic vesicle fusion and endocytosis. **Robert J. Hobson**, Eric G. Bend, Shigeki Watanabe, Erik M. Jorgensen. Dept Biol, HHMI, Univ Utah, Salt Lake City, UT, USA.

Synaptotagmin-1 is an integral synaptic vesicle protein and the major calcium sensor for synaptic vesicle fusion. Synaptotagmin-1 also plays a major role in the endocytosis of synaptic vesicles. Synaptotagmin-1 consists of two C2 domains, C2A and C2B. The C2B domain of synaptotagmin-1 is the critical regulator of calcium dependent fusion while the C2A domain is thought to play a minor role. The role the C2A domain of synaptotagmin-1 plays is unknown. Here we show that the C2 domains of synaptotagmin-1 can localize to the synapse and function in the absence of a transmembrane anchor and that the C2A domain is sufficient to drive synaptic vesicle fusion and endocytosis. *Caenorhabditis elegans* synaptotagmin-1 (*snt-1(md290)*) null mutants exhibit profound defects in synaptic vesicle fusion and membrane recycling. We find that individual C2 domains fail to rescue the synaptotagmin-1 null mutant while tandem C2A domains (C2AA), but not tandem C2B domains (C2BB), are capable of restoring synaptic vesicle fusion. Further, the C2AA, but not C2BB, is capable of rescuing the ssynaptotagmin-1 null mutant membrane recycling defects. Our results suggest that the C2A domain is required for localizing to synaptic regions and can function directly in synaptic vesicle exocytosis and endocytosis. While the C2B domain is required for export from the cell body, but does not function in the absence of the C2A domain. These results are in contrast to previous studies showing that the C2B domain was essential for synaptic transmission; it is likely these studies were unable to separate roles in transport and fusion for C2 domains.

560A. The UNC-73/Trio Sec14 Domain Localizes to a Subcellular Compartment in *C. elegans* Neurons. Daniel Hoffman, **Alyssa Hoop**, Robert Steven. Department of Biological Sciences, University of Toledo, Toledo, OH.

Rho family GTPase signaling regulates multiple fundamental cellular processes such as cytoskeleton organization, cell polarity and proliferation, cytokinesis and vesicle transport. In the nervous system, this signaling influences neuronal development and modulates neurotransmission. Human defects in Rho family GTPase pathway regulatory or effector molecules result in severe cognitive disorders including mental retardation. Like its mammalian homologs Kalirin and Trio, *C. elegans* UNC-73 is a RhoGEF activator of Rho family GTPases containing two RhoGEF domains vital for nervous system development and function. UNC-73 RhoGEF-1 activation of Rac GTPase pathways is required for the processes of axon guidance and cell migration. UNC-73 RhoGEF-2 isoforms play a physiological role in regulating locomotion likely through DCV-mediated mechanisms of neurotransmission modulation functioning upstream of or in parallel to a Gs heterotrimeric G protein pathway. In addition to the RhoGEF domains, *unc-73* encodes a Sec14 domain, which, in the *S. cerevisiae* Sec14 protein, functions to bind phospholipids. The role of the UNC-73 Sec14 domain is not clear and it is not known whether its role is required for UNC-73 isoform function. To examine Sec14 domain function, transgenic worms expressing a green fluorescent protein (GFP) tagged UNC-73 Sec14 domain in the nervous system were created. Interestingly, Sec14::GFP was observed in a subcellular location, appearing to coincide with the Golgi in cell bodies. Also, transgenic animals expressing Sec14::GFP display an uncoordinated movement phenotype similar to *unc-73* RhoGEF-1 mutants suggesting free Sec14 domain interferes with UNC-73 isoform function. The animals also develop more slowly than wildtype, which may indicate the domain is interfering with the functions of other Sec14 domain containing proteins. To determine if the Sec14 domain is essential for UNC-73 function a construct encoding UNC-73B delta Sec14 is being made for expression in *unc-73* RhoGEF-1 mutants. These data point to the importance of the Sec14 domain in nervous system function and protein subcellular localization.

561B. Combination of optogenetics and reverse genetics: novel behavior screening for regulators of neural differentiation. **S. Hori**[1], S. Oda[2,3], Y. Suehiro[1], Y. Iino[2], S. Mitani[1]. 1) Dept of Physiol, Tokyo Women's Med Univ Sch of Med, Tokyo; 2) Dept. Biophys & Biochem., Grad. Sch. of Sci., Univ. of Tokyo, Tokyo; 3) MRC Lab Molecular Biology, Cambridg.

Neural system processes information to guide behaviors. It is difficult to analyze its molecular basis, since it has complex circuits with compensation mechanisms. To overcome this difficulty, we have established a novel behavioral screening system based on optogenetics combined with reverse genetics in *C. elegans*. In this screening, blue light selectively activates ASH sensory neurons that express channelrhodopsin-2 (ChR2), and it induces avoidance behavior. In contrast to the natural aversive stimulus perceived by multi-modal sensory neurons, ChR2 enables us to selectively analyze the downstream circuit of the ASH, which is composed of 'primary' and 'secondary' circuits. We combined ChR2 with feeding-RNAi enhanced by transgenic pan-neuronal expression of *sid-1* (Calixto et al., 2010). First, *unc-42*, required for neurogenesis of ASH, was knocked down as a positive control. The resultant worm showed avoidance disorder, showing the system works well. To identify essential genes for neurogenesis, we then RNAi screened selected 217 neural transcriptional factors. As a result, 18 candidates caused partial avoidance disorder. Then, we focused on one candidate, *lin-32* (*abnormal cell LINeage protein 32*). *lin-32* is an orthologue of insect *atonal* and mammal *Math1*, but their function in avoidance behavior is unknown. *lin-32* null mutants also showed reduced responses to ChR2 and natural aversive stimulus, suggesting defects in local circuits. In fact, *lin-32* mutant had differentiation failure of inhibitory interneurons in the secondary circuit and induced prolonged calcium influx in their postsynaptic neurons. Double mutation of *lin-32* and *fax-1*, which regulates development of AVA, important interneurons of the primary circuit, completely lost avoidance behavior, suggesting that *lin-32* plays an important role in the development of the secondary circuit. *lin-32* and *fax-1* mutants are useful in focused analysis on more local circuit eliminating the compensation. Our screening system will clarify a wide range of essential genes for avoidance behavior.

562C. Worm migraines: Characterization of a Gain-of-Function Mutation in the Voltage-Gated Calcium Channel, UNC-2/Ca_V2. **Yung-Chi Huang**[1], Jennifer K. Pirri[1], Diego Rayes[1], Yasunori Saheki[2], Cornelia I. Bargmann[2], Michael M. Francis[1], Mark J. Alkema[1]. 1) Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA; 2) Howard Hughes Medical Institute, The Rockefeller University, New York, NY.

The fusion of synaptic vesicles with the cell membrane is initiated by the activation of presynaptic voltage-gated calcium channels (Ca_V2). *C. elegans* has a single predicted presynaptic Ca_V2a subunit, encoded by the *unc-2* gene. We have identified an *unc-2* gain-of-function (gf) mutant, which displays an increased reversal frequency, as well as hyperactive locomotion and egg-laying. In contrast, *unc-2* loss-of-function mutants are uncoordinated and sluggish. Transgenic animals that express the *unc-2(gf)* in the backward locomotion interneurons exhibit an increased reversal frequency, while expression in the hermaphrodite specific neurons (HSN) produces hyperactive egg-laying. This suggests that the *unc-2(gf)* transgene can be used to chronically hyper-

activate selective neurons within a neural circuit. *unc-2(gf)* mutants are hypersensitive to the acetylcholine esterase inhibitor, aldicarb and show an increased frequency of endogenous synaptic release events, indicating elevated levels of neurotransmitter release. Whole cell recordings from HEK cells expressing the human $Ca_V2.1a$ with the corresponding *unc-2(gf)* mutation revealed activation at a lower membrane potential and a higher current density than the wild type. Our findings are similar to those reported for mutations in the human $Ca_V2.1a$, CACNA1A, that cause Familial Hemiplegic Migraine (FHM1). Interestingly, transgenic animals that express *unc-2* carrying corresponding FHM1 mutations display a hyperactive phenotype similar to that of the *unc-2(gf)* mutants. Therefore, the *unc-2(gf)* mutants provide an invertebrate model to study mechanisms underlying FHM1. To understand mechanisms of Ca_V2 function *in vivo*, we performed a screen for mutants that suppress the hyperactive phenotype of *unc-2(gf)* mutants. Genes identified from this suppressor screen may ultimately provide novel targets for the treatments of calcium channelopathies.

563A. Regeneration of synaptic vesicles from large endocytic vesicles. **Edward J. Hujber**, Shigeki Watanabe, M. Wayne Davis, Erik M. Jorgensen. Department of Biology, HHMI, University of Utah, Salt Lake City, UT.

Sustained synaptic transmission requires endocytosis to retrieve exocytosed membrane and proteins and regenerate synaptic vesicles. Synaptic vesicles are thought to be regenerated directly from the plasma membrane via clathrin scaffolds or by closure of a transient fusion pore. Studies supporting these models typically use nonphysiological conditions, such as exhaustive stimulation and application of drugs, or optical assays that do not provide the resolution necessary to directly observe membrane dynamics. To address this, we have developed a technique coupling optogenetic neuronal stimulation of whole live worms with rapid freezing and electron microscopy. This technique allows us to observe physiological membrane dynamics with high spatial and temporal resolutions. By applying a single stimulus to live worms and freezing them at various time points after stimulation (20 ms - 10 s), we found a novel endocytic pathway. In this pathway, termed ultrafast endocytosis, uncoated endocytic pits form at the edges of the active zone, then pinch off to become large vesicles ~2-3 times the diameter of a synaptic vesicle. This process is complete in as little as 50 ms after stimulation. To test if these large vesicles are precursors to synaptic vesicles, we froze worms 30 s after 5 stimuli. With these stimulation conditions, we found large vesicles more frequently than with a single stimulus. We observed structures resembling large vesicles being pinched in the middle. We were unable to detect coats on these structures, suggesting clathrin is unlikely to be involved in the process. Furthermore, these structures appear to be randomly distributed within the synapse. To further characterize these events, we intend to identify involved proteins using a candidate approach and to more precisely determine the kinetics of the process.

564B. Mechanisms of C. elegans locomotion speed control during oxygen-chemotaxis. **Ingrid M. Hums**, Fanny Mende, Lisa Traunmüller, Michael Sonntag, Manuel Zimmer. IMP, Research Institute of Molecular Pathology, Vienna, Austria.

Chemotaxis behaviors are essential for survival, i.e. they are required to locate food sources and to avoid noxious conditions. In current chemotaxis models, locomotion along a gradient is adjusted according to temporal changes in stimulus strength. C. elegans regulates, for example, transitions between elementary locomotion states such as forward movement, reverse movement and omega turns in a stimulus-dependent manner, a strategy called klinokinesis. Another chemotaxis strategy, however, is orthokinesis during which temporal changes in stimulus strength control locomotion speed. While the interneuron circuits mediating klinokinesis behaviors in C. elegans are widely studied, we lack in-depth insights into the mechanisms and neural circuits that mediate orthokinesis. We set out to explore the neural circuit mechanisms mediating orthokinesis using oxygen-chemotaxis as an experimental paradigm. In particular, we study distinct components of adaptive speed modulation in response to changing ambient oxygen levels. The oxygen-chemosensory neurons BAG and URX elicit transient and sustained slowing periods in response to decreasing and increasing oxygen levels, respectively. In order to obtain behavioral data with high statistical power, we developed image analysis tools to measure detailed morphometric parameters simultaneously from multiple worms. This enabled us to delineate the contributions of different strategies to locomotion speed control, such as body curvature and bending frequency regulation. Furthermore, we identified peptidergic interneurons downstream of the oxygen-sensory system to play a major role in speed modulation. Currently, we are investigating the speed controlling circuits in more depth through genetic studies of implicated candidate genes in combination with cell ablation experiments and calcium imaging in freely moving worms. Finally, we show that accurate stimulus-controlled speed regulation is crucial for properly navigating oxygen gradients.

565C. VPS-39 promotes synaptic vesicle fusion in *C. elegans*. **Susan M. Klosterman**, Szi-chieh Yu, Anna O. Burdina, Janet E. Richmond. Dept Biol, Univ Illinois Chicago, Chicago, IL.

C. elegans tomosyn (TOM-1) negatively regulates synaptic transmission through interactions with syntaxin and SNAP-25, components of the core vesicle fusion machinery. In a yeast two-hybrid screen for TOM-1 binding partners, we identified an additional interactor, VPS-39, which is a highly conserved member of the HOPS (homotypic fusion and protein transport) complex. Since the HOPS complex has been implicated in the regulation of SNARE-dependent fusion, we obtained *vps-39* mutants from the CGC to examine the potential role of this protein in synaptic vesicle exocytosis. Homozygous *vps-39* mutants from a GFP balanced strain, grow to adulthood but lay dead embryos. A *vps-39* translational mCherry fusion construct, capable of rescuing this embryonic lethality, indicates that VPS-39 is expressed in many tissues, including neurons. Furthermore, the *vps-39* mutants exhibit pharmacological, electrophysiological and ultrastructural evidence of reduced neurotransmitter release. Both the lethality and synaptic defects of *vps-39* mutants can be reversed by expressing VPS-39 specifically in neurons, indicating that these functions are cell autonomous. Epistasis experiments suggest that VPS-39 functions upstream of the priming factor, UNC-13. Experiments are presently underway to explore the relationship between VPS-39 and TOM-1 function in the synaptic vesicle cycle and further define which stage of the release process is impacted.

566A. Single Cell Mass Spectrometry of Neuropeptides in Ascaris suum Motor Neurons. **Christopher J. Konop**[1], Jenny Knickelbine[1], Molly Seygulla[1], Martha Vestling[2], Antony O. W. Stretton[1]. 1) Zoology Dept, University of Wisconsin, Madison, WI; 2) Chemistry Dept, University of Wisconsin, Madison, WI.

Neuropeptides have been shown to play a critical role as signaling molecules in neuronal circuits of both free-living and parasitic nematodes. FMRF-like peptides (FLPs) and peptides processed from neuropeptide-like proteins (NLPs) are known to have potent activities and are expressed widely in the nervous system. In order to better understand the role of neuropeptides in the parasitic nematode Ascaris suum, we used MALDI TOF and TOF/TOF MS

analysis of single dissected motor neurons (MNs) from the ventral cord to identify and localize *A. suum* FLPs(AFs) and *A. suum* NLPs (As-NLPs). Of the seven classes of ventral cord MNs, three distinct profiles were observed. Cholinergic cell types VE1, DE1, and DE3, which are analogous to *C. elegans* VA, AS, and DA MNs respectively, contained peaks corresponding to the mass and sequence of the previously characterized peptide AF9 (*flp-21*), and six novel peptides, including four that share the N-terminal GGR- motif. These six peptides are all encoded by the same transcript (*As-nlp21*), which is orthologous to *Ce-nlp21*. The two other cholinergic ventral cord MNs, VE2 and DE2 (*C. elegans* VB and DB MNs) contain peaks for AF2 (*flp-14*) and the six peptides encoded by *afp-1* (*flp-18*); all shown to be potent effectors of *Ascaris* body wall muscle. The third peptide profile was that of the GABAergic VI and DI neurons (*C. elegans*-VD and DD MNs) which contained a large peak corresponding to the mass of As-NLP22 (Ce-NLP22). We have shown that As-NLP22 has strong and prolonged inhibitory effects on ACh-induced muscle contraction and that it abolishes all spontaneous activity in the muscle strip assay. Localization of peptide encoding transcripts was confirmed by *in situ* hybridization. In contrast to the expression patterns in *A. suum*, expression of orthologous *C. elegans* transcripts (determined by GFP reporter constructs; Kim and Lee, 2004; Nathoo *et al*., 2001) shows no overlap except in the case of *nlp-21*, which also localizes to the A-type MNs, possibly suggesting a common physiological role, which can be exploited by novel antiparasitic drugs.

567B. The Anaphase-Promoting Complex Regulates GABA Transmission at the *C. elegans* Neuromuscular Junction. **Jennifer R. Kowalski**[1], Hitesh Dube[1], Denis Touroutine[2], Patricia R. Goodwin[3], Marc Carozza[1], Zachary Didier[1], Michael M. Francis[2], Peter Juo[3]. 1) Biological Sciences, Butler University, Indianapolis, IN; 2) Neurobiology, University of Massachusetts, Worcester, MA; 3) Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, MA.

Regulation of excitatory and inhibitory synaptic transmission is critical for nervous system function. Aberrant synaptic signaling, including altered excitatory to inhibitory balance, is observed in neurological diseases. The ubiquitin system controls the abundance of many synaptic proteins and thus plays a critical role in regulating synaptic transmission. The anaphase-promoting complex (APC) is a multi-subunit ubiquitin ligase and well characterized cell cycle regulator that also functions in postmitotic neurons to control synapse development and glutamatergic transmission. However, specific roles for the APC in regulating transmission at other synapse types are unknown. Here, we show that the APC regulates GABA transmission at the *C. elegans* neuromuscular junction (NMJ), a specialized synapse where a balance of excitatory cholinergic (acetylcholine) and inhibitory GABA signaling from presynaptic motor neurons controls muscle activity. In behavioral assays for NMJ function, temperature-sensitive loss-of-function mutants in several APC subunits (*emb-30* APC4, *emb-27* CDC16, *mat-2* APC1, and *mat-3* CDC23) exhibit increased muscle contraction indicated by hypersensitivity to the acetylcholinesterase inhibitor aldicarb. This defect is rescued by expressing the missing APC subunit in GABA neurons. Increased muscle excitation also occurs following GABA neuron-specific APC inhibition by ectopic expression of the APC inhibitor hEmi1. In addition, APC mutants exhibit convulsions in a GABA-regulated seizure assay, suggesting the APC may be a broad regulator of GABA signaling. Quantitative imaging and electrophysiological analyses further indicate that APC mutants have decreased GABA release but normal cholinergic transmission. Finally, colocalization studies demonstrate that the APC can localize to presynaptic sites in GABA motor neurons. Together, these data suggest a model in which the APC acts at GABA presynapses to promote GABA release and inhibit muscle excitation.

568C. Ca^{2+} dynamics of a whole single neuron. **Sayuri Kuge**[1,2], Takayuki Teramoto[1,2], Takeshi Ishihara[1,2]. 1) Department of Biology, Kyushu University, Fukuoka, Fukuoka, Japan; 2) JST, CREST, Chiyoda, Tokyo, Japan.

The nematode *C.elegans* is an excellent model organism for studying how neuronal circuits activity transform sensory signals. Ca^{2+} imaging of neurons using Ca^{2+}sensitive fluorescent proteins including GCaMP and yellow cameleon has revealed functions of the neuronal circuits. However, the mechanism of circuit function at single-neuron resolution is still unclear.

To elucidate the functional characteristics of a single neuron, we analyzed Ca^{2+} dynamics of a whole single neuron by a 4-D imaging system based on a confocal microscope with a piezo lens positioner. The system enabled us to capture 3-D reconstructed images of a whole single neuron with more than 4 steric images per second.

To analyze the Ca^{2+} dynamics of a whole neuron, we used G-GECO series of Ca^{2+} sensors, which were developed from G-CAMP. To observe the various basal Ca^{2+} concentrations, we developed G-GECO derivatives with higher affinity to Ca^{2+}. We analyzed animals expressing Ca^{2+} indicators in AWC^{ON} neuron by *str*-2 promoter in the olfactory chip.

We hope that our 4-D imaging system enables us to observe Ca^{2+} dynamics at dendrites, a cell body, and an axon independently and to analyze precise mechanisms of sequential rapid neuronal activation and inactivation in a whole single neuron.

569A. Maintaining sensitivity: dissecting sensory adaptation using high-throughput *in vivo* calcium imaging. **Johannes Larsch**[1], Dirk R. Albrecht[2], Cori I. Bargmann[1]. 1) Rockefeller University, New York, NY; 2) WPI, Worcester, MA.

Sensory neurons face a dilemma: Biologically relevant changes in stimulus strength are often small compared to the possible range of stimulus intensities. One solution to this problem is sensory adaptation, in which the sensory neuron continuously adjusts its dynamic range based on stimulus history to maximize sensitivity while avoiding saturation. During chemotaxis, *C. elegans* relies on known chemosensory neurons to detect odors over at least five orders of magnitude in concentration, suggesting a sophisticated molecular machinery to keep ongoing sensory activity within dynamic range. To probe adaptation in *C. elegans*, we wanted to deliver a broad range of odor stimulus concentrations and patterns while monitoring neural responses. To this end, we developed a high throughput system for *in vivo* calcium imaging that allows us to record up to 20 animals simultaneously for hours, enabling quantitative mapping of sensory receptive fields and their adjustment to constant or changing stimulus levels. We observed odor-evoked calcium dynamics in AWA sensory neurons across a million-fold range of concentrations. Neurons adapted upon repeated stimulation with odor pulses on two different time scales: fast inactivation of the calcium transient during each odor pulse and slow adjustment of response magnitude and dynamics across repeated pulses. We have screened mutants and pharmacological interventions for effects on odor-evoked calcium transients and their adaptation, seeking to define mechanisms for odor sensing and fast vs. slow adaptation. Paradoxically, several chemotaxis mutants with structurally defective cilia (*che-2, che-3, osm-6*) had stronger and more slowly adapting calcium responses to odor than wild-type animals suggesting that IFT cilia genes are more important for rapid adjustment of sensory sensitivity than for primary transduction in AWA.

570B. Pattern generation in the locomotory system by optogenetic stimulation of command neurons and sensory neurons. **J. Liewald**[1], C. Schmitt[1], S. Wabnig[1], C. Glock[1], J. Akerboom[2], L. Looger[2], N. Pokala[3], C. Bargmann[3], E. Ardiel[4], C. Rankin[4], A. Gottschalk[1]. 1) BMLS, Goethe-University, Frankfurt, Germany; 2) HHMI Janelia Farm, Ashburn, USA; 3) Rockefeller University, New York, USA; 4) UBC, Vancouver, Canada.

The command interneuron AVA integrates sensory stimuli to relay such information to motor neuron (MN) circuits, for example to generate reversal behavior. How exactly command interneurons regulate motor circuits is only partially understood. Either the MN units, appearing in recurrent networks along the body, constitute local pattern generators that are coordinated by command neurons. Or a central pattern generator relays its activity via proprioceptive feedback to MNs, which may be switched to a "responsive state" by command neurons. Using the Cre/loxP system, we expressed ChR2 in AVA. In high throughput behavioral analysis (multi-worm-tracker) we observed withdrawal reactions in response to blue light; this was also seen in animals expressing ChR2 in touch receptor neurons (TRNs) and in the aversive sensory neuron ASH. However, in contrast to photostimulation of TRNs, the reversal frequency of ASH and AVA lines did not habituate during repeated stimulation. To correlate this behavior with possible pattern generation, we recorded electrical events from body-wall muscle cells (BWMs) of immobilized animals. In response to AVA photostimulation we observed increased amplitude and frequency of currents and an increased frequency of action potentials (APs). Sometimes stimulation of AVA or TRNs evoked rhythmic patterns, hinting at pattern generation in local motor circuits. In *unc-7* gap junction mutants activity was largely altered, apart from disappearance of low-frequency APs, indicating that gap junctions are not affecting AP firing in single muscle cells. To further investigate if activity patterns are induced in intact immobilized animals, we are currently conducting Ca^{2+} imaging (RCaMP) in BWMs following photostimulation of AVA and ASH. Complementing analyses by electrophysiology and Ca^{2+} imaging, following photostimulation of ASH and TRNs and of AVA in animals lacking chemical transmission from command neurons are also in progress.

571C. Postsynaptic current bursts instruct action potential firing at a graded synapse. **Ping Liu**, Bojun Chen, Zhao-Wen Wang. Department of Neuroscience, UConn Health Center, Farmington, CT.

Nematode neurons generally produce graded potentials instead of action potentials (APs). It is unclear how the graded potentials control postsynaptic cells under physiological conditions. Here we show that postsynaptic currents (PSCs) frequently occur in bursts at the neuromuscular junction of *C. elegans*. Cholinergic bursts concur with facilitated AP firing, elevated cytosolic [Ca^{2+}], and contraction of the muscle whereas GABAergic bursts suppress AP firing. The bursts, distinct from artificially evoked responses, are characterized by a persistent current (the primary component of burst-associated charge transfer) and increased frequency and mean amplitude of PSC events. The persistent current of cholinergic PSC bursts is mostly mediated by levamisole-sensitive acetylcholine receptors, which correlates well with locomotory phenotypes of receptor mutants. Eliminating command interneurons abolishes the bursts whereas mutating SLO-1 K^+ channel, a potent presynaptic inhibitor of exocytosis, greatly increases the mean burst duration. These observations suggest that motoneurons control muscle by producing PSC bursts.

572A. Multiple innexins contribute to electrical coupling of *C. elegans* body-wall muscle. **Ping Liu**[1], Bojun Chen[1], Zeynep Altun[2], Maegan Gross[1], Alan Shan[1], Benjamin Schuman[1], David Hall[2], Zhao-Wen Wang[1]. 1) Department of Neuroscience, UConn Health Center, Farmington, CT; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

The *C. elegans* genome contains 25 innexin (INX) genes, which encode proteins for gap junctions (GJs) and hemichannels. A single type of cells often expresses two or more INXs. Little is known why multiple INXs are expressed in the same cells. To gain insight into this question, we assessed potential body-wall muscle expression for all the INXs by expressing promoter::GFP transcriptional fusions, and analyzed potential contributions of all but three INXs (INX-3, -12, -13) to electrical coupling by recording junctional current (I_j) between neighboring muscle cells. The expression of three INXs is observed in body-wall muscle cells, including INX-11, -18 and UNC-9. Comparisons of I_j between wild type and innexin mutants, however, suggest that six INXs contribute to muscle electrical coupling, including UNC-9, INX-1, -10, -11, -16, and -18. These INXs appear to contribute to the coupling cell-autonomously because the I_j deficiency in each specific INX mutant is rescued completely by expressing a corresponding wild-type INX specifically in muscle. Loss-of-function (*lf*) mutation of *inx-1, -10, -11* or *-16* inhibits I_j to a comparable degree (50~65%), and the I_j deficiency does not become more severe in various double mutants, raising the possibility that all the four INXs may contribute to the function of a single population of GJs. *unc-9(lf)* inhibited coupling by ~65%, which is not worsened by *inx-18(lf)* although I_j is decreased by ~40% in *inx-18(lf)* single mutant, suggesting that INX-18 and UNC-9 might be involved in the function of a single population of GJs. In double or triple mutants affecting both populations of GJs, I_j is essentially undetectable. Consistent with their roles in electrical coupling, five of the six INXs show punctate localization at muscle intercellular junctions when expressed as GFP fusion proteins. The remaining one (INX-11) shows diffuse expression in the cell membrane. These analyses present an unexpectedly complex picture about potential functions of INXs in *C. elegans* body-wall muscle.

573B. Regulation of the nicotinic acetylcholine receptor ACR-16. **Ashley A. Martin**, Feyza Sancar, Janet E. Richmond. University of Illinois at Chicago, Chicago, IL, USA 60607.

At the *C. elegans* body wall neuromuscular junctions (NMJs) there are two cholinergic ionotropic receptor types, one that is heteromeric and activated by levamisole (LAChR) and one that is homomeric and activated by nicotine (NAChR). Screens designed to isolate levamisole-resistant mutants in *C. elegans* have identified subunits of the LAChR and genes that affect LAChR assembly, trafficking, and receptor clustering at the NMJ. Specifically LEV-9, LEV-10, and OIG-4 have been implicated in the clustering of LAChRs, but the expression of the colocalized NAChR appears completely normal when these genes are perturbed. The only receptor subunit known to be required for the *C. elegans* NAChR is ACR-16, which can form functional homo-pentameric receptors. Published data implicates CAM-1 as well as LIN-17, CWN-2, and DSH-1 as working in a pathway to help localize ACR-16 to the synapse. However, it is possible that other proteins also play a role in the regulation of this receptor.

A forward genetic screen was performed to isolate candidate genes involved in the regulation of ACR-16. The screen utilized a single-copy insertion of ACR-16::GFP in an *unc-63;acr-16* mutant background to isolate mutants that specifically impact the ACR-16::GFP expression pattern. From this screen, 3 mutants were identified. All mutants exhibited a significant reduction in ACR-16::GFP expression at NMJs. Electrophysiological recordings also

demonstrated a reduction in the evoked NMJ responses in the EMS mutants. Further characterization of these mutants demonstrated that the LAChRs may be unaffected as there was no change in response to pressure ejected-levamisole in the mutants and the fluorescence level of RFP-tagged LAChRs was also normal. Immunostaining for the cholinergic vesicular marker, UNC-17 appeared to be wild type, eliminating synaptogenesis defects. Responses to pressure-ejected nicotine revealed a reduction in amplitude for two of the mutants, while the amplitude remained wild type in the third. This may relate to different defects of ACR-16 in these mutants. Whole genome sequencing of these mutations and continued characterizations are underway.

574C. Chemical tuning of CO2-responsive BAG neurons. Ewan St. John Smith[2], **Luis Antonio Martinez-Velazquez[1]**, Niels Ringstad[1]. 1) Skirball Institute, Skirball Institute of Biomolecular Medicine, Molecular Neurobiology Program and Dept. of Cell Biology, NYUMC, New York, NY 10016NYU School of Medicine, New York, NY; 2) Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1PD, United Kingdom.

Animals from diverse phyla possess neurons that are activated by the product of aerobic respiration, carbon dioxide (CO2). It has long been thought that such neurons primarily detect the products of CO2 hydration, protons and bicarbonate. To identify the specific chemical cue that activates BAG neurons, we studied isolated BAG neurons in culture, using methods that allow both monitoring of cell physiology and control of the extracellular and intracellular environments. We show that the majority of isolated BAG neurons showed calcium responses to molecular CO2, although a fraction of these cells can also be activated by acid stimuli. These responses to acid stimuli were not seen when the BAG neurons were tested in situ. One component of the BAG transduction pathway, the receptor-type guanylate cyclase GCY-9, suffices to confer cellular sensitivity to both CO2 and acid, indicating that it is a bifunctional chemoreceptor. We speculate that in other animals, receptors similarly capable of detecting molecular CO2 might mediate effects of CO2 on neural circuits and behavior.

575A. The DAF-7/TGF-b signaling pathway regulates abundance of the glutamate receptor GLR-1. **Annette McGehee**, Benjamin Moss, Peter Juo. Molecular Physiology and Pharmacology, Tufts University, Boston, MA.

We study the regulation of the *C. elegans* glutamate receptor (GluR) GLR-1 using quantitative microscopy to analyze the localization and abundance of GFP-tagged GLR-1 (GLR-1::GFP) in various mutant backgrounds to identify genes involved in the regulation of GLR-1. In *C. elegans* the DAF-7/TGF-b signaling pathway senses environmental conditions and regulates entry into dauer. Here we show that multiple mutants in the DAF-7/TGF-b signaling pathway have increased abundance of GLR-1 at synapses in the ventral nerve cord (VNC). We found that GLR-1::GFP puncta fluorescence increased significantly in the VNC of multiple DAF-7/TGF-b pathway mutants as compared to wild type animals. Immunoblot analysis indicates that total levels of GLR-1::GFP are increased in DAF-7/TGF-b pathway mutants. Additionally, the rate of spontaneous reversals (a GLR-1-dependent behavior) is increased in both *daf-7* and *daf-8* mutants, suggesting that endogenous GLR-1 signaling is increased in these mutants. Together, these results suggest that the DAF-7/TGF-b pathway is required to regulate the proper abundance of GLR-1 in the VNC. The increase in GLR-1 observed in DAF-7/TGF-b pathway mutants could result from either increased synthesis or decreased degradation of GLR-1. Preliminary pulse-chase imaging experiments using the photoconvertible fluorescent protein Dendra fused to GLR-1 (GLR-1::Dendra) indicate that the rate of GLR-1 degradation in the VNC is unaltered in DAF-7/TGF-b pathway mutants. Thus, we hypothesize that GLR-1 synthesis is increased in DAF-7/TGF-b pathway mutants. Consistent with this idea, we found that *daf-7* mutants exhibit increased levels of GFP driven by a *glr-1* transcriptional reporter (*Pglr-1::NLS-GFP*). Taken together, these results suggest that the DAF-7/TGF-b signalling pathway negatively regulates the abundance of GLR-1 by controlling transcription of the receptor. These results identify a novel function for the DAF-7/TGF-b pathway in regulating GluRs, and provide an interesting potential connection between the sensing of environmental conditions and the regulation of GluRs and nervous system function.

576B. Locating synaptic calcium channels. **Sean Merrill**, S. Watanabe, J.R. Richards, C. Frøkjær-Jensen, E.M. Jorgensen. Dept Biol, HHMI, Univ Utah, Salt Lake City, UT, USA.

Calcium is the essential trigger for synaptic vesicle fusion. When open, calcium channels create steep calcium gradients that fuse nearby vesicles while sparing distal vesicles. The extent and duration of the gradients depends on the type and location of activated calcium channels. Similarly, vesicles must be primed and positioned to respond to the calcium influx. UNC-13 is required for priming vesicles in presynaptic active zones, while RIM (UNC-10) delivers primed vesicles specifically to dense projections where they can most rapidly respond to calcium. Mammalian calcium channels can be divided into four major classes, encoded by at least 10 genes and thousands of unique isoforms. C. elegans has just three calcium channels at the presynaptic neuromuscular junction: N-type (UNC-2), L-type (EGL-19), and the ryanodine receptor (UNC-68). Determining calcium channel organization has been difficult because of the small size of synapses. Nano-fEM is a new tool developed in our lab that correlates super-resolution fluorescence microscopy with scanning electron microscopy to localize proteins at nanometer resolution. We have transgenically tagged each channel with tdEos, a photoconvertible fluorophore used for fEM. We are determining the precise location of these calcium channels within neuromuscular synapses to build a model of their respective synaptic functions. Finally, we will investigate the effect of mutations in *unc-13* and *unc-10* on the location of both synaptic vesicles and their adjoining calcium channels.

577C. A Novel UNC-43 (CaM Kinase II) Dense Core Vesicle Trafficking Pathway Blocks UNC-31 (CAPS) - Dependent Secretion from Neuronal Cell Somas. Christopher Hoover[1], Stacey Edwards[1], Szi-chieh Yu[2], Maike Kittelmann[3], Stefan Eimer[3,4], Janet Richmond[2], **Kenneth Miller[1]**. 1) Genetic Models of Disease Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Dept of Biological Sciences, University of Illinois, Chicago, IL 60607; 3) European Neuroscience Institute, Center for Molecular Physiology of the Brain, Georg-August University Goettingen, Goettingen, Germany; 4) BIOSS Center for Biological Signaling Studies, Albert-Ludwigs-University, Freiburg, Germany.

Neurons rely on the controlled release of neuropeptides via dense core vesicle (DCV) exocytosis to evoke or modulate behaviors. We found that wild type *C. elegans* motor neurons send most of their DCVs to axons, leaving very few in the cell somas; however, the membrane trafficking pathway that mediates this skewed distribution and the extent to which the distribution can be altered by signaling pathways to control DCV numbers in axons or to drive release from cell somas for different behavioral impacts is unknown. Using a forward genetic screen we looked for mutations that inhibit the accumulation of DCVs in axons. Two mutations reduced the axonal levels of DCVs by ~90%, leaving small synaptic vesicles largely unaffected. Both

mutations disrupted UNC-43 (CaMKII). In *unc-43* null mutants, DCVs failed to enter the axon and instead were secreted at 133% of wild type levels from the cell soma and dendrites by an UNC-31 (CAPS) - dependent process. 96% of the neuropeptide secretion that occurs in *unc-43* null mutants is blocked in an *unc-43 unc-31* double mutant. Since *unc-31* single mutants only reduce neuropeptide secretion by ~50%, the results suggest that there are two cell soma neuropeptide release pathways: one UNC-31-dependent, and one UNC-31-independent, but CaMKII - dependent. Although cell soma exocytosis of DCVs accounts for the major loss neuropeptide cargos in *unc-43* mutants, our results also suggest that cell soma degradation of neuropeptide cargos contributes to the loss since neuropeptide cargo levels in *unc-43 unc-31* double null mutants are still reduced by ~30% and 58% in axons and cell somas, respectively, relative to *unc-31* single mutants. The results reveal an unexpected major new function for CaMKII.

578A. Does Cysteine String Protein contribute to *C. elegans* Nervous System Function? **Ben Mulcahy**, Paul Ibbett, Lindy Holden-Dye, Vincent O'Connor. University of Southampton, Southampton, United Kingdom.

Cysteine string protein (CSP) is a synaptic vesicle protein that uses its DnaJ domain to co-chaperone Hsc70 allowing it to refold SNAP-25 and other synaptic proteins. It appears to be a significant resilience factor at synapses as in *Drosophila* and mice, mutations in CSP result in synaptic dysfunction, neurodegeneration and early death. We have shown that both ageing and insulin signalling modulate synaptic function at the *C. elegans* body wall neuromuscular junction (Mulcahy et al., 2013), and wanted to see if we could model accelerated synaptic dysfunction with age using mutants deficient in the worm orthologue of CSP, *dnj-14*. Initial tests with these strains indicated that in contrast to the phenotypes seen in *Drosophila* and mice, there was not a decreased lifespan compared to wild-type. Therefore we repeated the lifespan assays after repeated backcrossing (6x) into our laboratory wild-type strain. There was no effect of CSP mutation on lifespan, even at elevated temperatures. Behavioural correlates of synaptic function are not defective when compared to wild-type, even across ageing. In addition, pharmacological assays argue against accelerated synaptic dysfunction across ageing in this genetic background. This is surprising considering the severity of the phenotypes seen in *Drosophila* and mouse CSP mutants. Electrophysiological recordings from the body wall muscle are currently underway and provide another route to identify altered neuronal signalling either at the neuromuscular junction itself or upstream in the neural network that regulates its activity. We are also extending our analysis into assays that require sensory integration in order to conclusively test if the worm CSP gene is required for the maintenance of intact behaviours across ageing in *C. elegans*.

579B. Age-dependent changes at the *C. elegans* neuromuscular junction. **Ben Mulcahy**, Lindy Holden-Dye, Vincent O'Connor. University of Southampton, Southampton, United Kingdom.

Age-dependent synaptic dysfunction is an established determinant of health span and plays a major role in neurodegenerative disease. However, mechanisms of synaptic dysfunction are poorly understood. We are using behavioural, pharmacological and electrophysiological techniques to probe the *C. elegans* neuromuscular junction across age. First we observe a progressive decline in motility as worms age. This could be due to altered neuromuscular transmission, muscle function, alterations upstream of the neuromuscular junction or a combination of these. To resolve this we designed pharmacological assays that can probe neuromuscular function in the absence of spontaneous movement, the latter being a confounding factor in aged worms. We used two cholinergic drugs, levamisole and aldicarb. Both cause contraction of the worms. Levamisole directly activates cholinergic receptors on muscle cells and aldicarb causes a build-up of acetylcholine (ACh) in the synaptic cleft that is first dependent on synaptic release from the motor neuron. Comparing the effects of these drugs evidenced increased release of ACh from the presynapse during early ageing, which declined during advanced age to levels similar to those seen in young worms. Using a *daf-2(e1370)* mutant worm we have shown that the increase in ACh release during early ageing is dependent on insulin signalling. These data also highlight that the ability of the muscle to contract is retained even in old age (Mulcahy et al., 2013). We have followed this up with patch clamp recording of mini-endplate currents from the body wall muscle to assess spontaneous neurotransmitter release across the same ageing time course. We show that the electrophysiological data correlate with the pharmacological data and substantiate an increase in neuromuscular signalling in early ageing followed by a gradual decline. It is interesting that the increased strength of neuromuscular transmission during early ageing is coincident with a period of behavioural decline. It is also interesting that in very old worms we see evidence of functional neuromuscular synapses yet the worms remain immobile. We are using patch clamp recording from the body wall muscles to follow this up further.

580C. A role for miRNA machinery at the neuromuscular junction? **Patrick J. O'Hern**, Anne Hart. Neuroscience Dept, Brown University, Providence, RI.

Synaptic signaling is strikingly similar across diverse species and studies of this process in *C. elegans* have broad significance. Ion channels, receptors, signaling molecules, and neurotransmitters coordinate rapid, high fidelity, trans-synaptic communication. Classically, Messenger RNAs (mRNAs) encoding synaptic proteins are likely regulated by microRNAs (miRNAs). Classically, miRNAs are a central part of the RNA-induced silencing complex (RISC) and bind to the complementary sequence of target mRNAs. Proteins in the miRNA pathway are also well conserved across species. Although individual miRNAs have been identified that regulate the NMJ, it is unknown whether perturbation of miRNA machinery proteins influences NMJ function. To study the function of miRNA pathway proteins at the neuromuscular junction, we used the drug aldicarb. Aldicarb inhibits synaptic acetycholinesterase and exposure leads to paralysis. The RISC components AIN-1, AIN-2, ALG-1, ALG-2, and CGH-1 as well as DCR-1, a well-characterized miRNA processing protein were examined. Loss-of-function of AIN-1;AIN-2 results in aldicarb hypersensitivity (Hic), whereas AIN-1, RDE-1, or CGH-1 loss-of-function resulted in aldicarb resistance (Ric). Surprisingly, single mutant strains for *alg-1*, *alg-2*, *ain-2*, or *dcr-1* were indistinguishable from wild-type animals. While these results seem contradictory, they suggest broad and diverse roles for miRNA machinery at the NMJ. In the future, we plan to identify specific miRNAs to help explain these opposing phenotypes at the NMJ.

581A. An unconventional role of a conserved sterol biosynthetic protein ERG-28 in SLO-1 function. **Kelly H. Oh**, Hongkyun Kim. Cell Biology & Anatomy, Chicago Medical School, Rosalind Franklin University, North Chicago, IL.

The calcium-activated potassium channel, SLO-1, reduces cellular excitability in response to high levels of calcium increases. This physiological property is essential for maintaining calcium homeostasis and proper excitability. To understand how the SLO-1 channel is regulated, we performed a genetic suppressor screen that takes advantage of sluggish, uncoordinated locomotory phenotype of gain-of-function *slo-1(ky399)* mutants. From this screen, we previously identified the alpha-catulin homologue, *ctn-1*, that encodes a cytoskeletal protein involved in localization of the SLO-1 channel at the

presynaptic terminals and near dense bodies of muscle. In the same genetic screen, we also identified a *cim16* mutation that suppresses the locomotory phenotype of *slo-1(ky399)*. However, *cim16* mutants do not show the head-bending phenotype, a hallmark phenotype of loss-of-function mutants in genes encoding *slo-1* and components of the dystrophin complex. To further understand the regulatory role of *cim16* for SLO-1, we cloned *cim16* by a combination of genetic mapping and transgenic rescue. *cim16* has a mutation in the *erg-28* gene, a conserved gene in eukaryotes. ERG-28 is originally identified in yeast as a protein that anchors ergosterol (sterol found in fungi) biosynthetic enzymes. However, our data indicate that ERG-28 is not involved in sterol synthesis. First, we found that other mutants defective in genes involved in sterol biosynthesis cannot suppress the locomotory defects of *slo-1(ky399)*. Second, our tissue specific rescue experiments show that neuronal, but not muscle, expression of *erg-28* reverts normal locomotion of *cim16;slo-1(ky399)* to the sluggish, uncoordinated locomotory phenotype of *slo-1(ky399)*, strongly suggesting that *erg-28* has a neuronal tissue specific role, as opposed to a role in the synthesis of diffusible sterol. One possibility is that ERG-28 is involved in neuronal trafficking of the SLO-1 channels. However, the localization of SLO-1 at presynaptic terminals is not obviously altered by *cim16* mutation. To verify this finding we are analyzing additional *erg-28* alleles. At the same time, we are exploring the possibility that *erg-28* mutations alter an accessory subunit of SLO-1.

582B. The molecular mechanisms of behavioral sexual dimorphism. **Meital Oren**[1], Oliver Hobert[1,2]. 1) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

Sexual dimorphism in various species does not only contribute to physical differences, but is also a major cause of the differences in behavior. At the molecular level, recent studies reveal extensive sex-biased gene expression changes in the nervous system. However, the mechanisms that regulate these differences are poorly understood. In *C. elegans*, the hermaphrodite and the male share 294 neurons that constitute the "core nervous system". The reconstruction of the posterior nervous system of the *C. elegans* adult male has shown that many of the shared neurons between the male and the hermaphrodite are strongly sexually dimorphic in their wiring, suggesting that these neurons may adopt different functions and fates in the male than those in the hermaphrodite (1). We focus on the shared neuron AVG, which is a ventral cord interneuron that is highly differentially wired in males versus hermaphrodites. AVG has been shown to act as a pioneer cell during embryonic development (2), but its post-embryonic roles haven't been characterized yet. We will analyze the behavior of animals in which AVG has been laser ablated, and use automated tracking methods to analyze the behavioral output. Several lines of evidence link the LIM homeodomain transcription factor LIN-11 to the AVG cell ((2, 3) and O. Hobert, unpublished results). LIN-11 is essential for AVG differentiation but since *lin-11* mutants fail to express cell-type specific markers further analysis of *lin-11* roles in AVG hasn't been performed. We will develop tools to visualize AVG dimorphic neuronal connections in wild-type and *lin-11* mutant animals. In addition, we will examine how *lin-11* and other AVG-expressed transcription factors affect AVG wiring. 1) T. A. Jarrell et al., Science 337, 437 (Jul 27, 2012). 2) H. Hutter, Development 130, 5307 (Nov, 2003). 3) R. Baumeister, Y. X. Liu, G. Ruvkun, Gene Dev 10, 1395 (Jun 1, 1996).

583C. Syntaxin Habc domain is required for synaptic function. **Leonardo Parra**, Jenna Whippen, Catherine Dy, Erik Jorgensen. Dept Biol, HHMI, Univ Utah, Salt Lake City, UT.

Intracellular membrane fusion is mediated by the assembly of specific SNARE complexes. These interactions are believed to provide the driving force for bilayer fusion. The neuronal SNARE protein syntaxin (UNC-64) contains a highly conserved three-helix bundle known as the Habc domain. The Habc domain folds back to interact with its own SNARE motif, rendering syntaxin “closed” in solution and preventing SNARE complex formation. However, it is not clear whether the Habc domain performs additional functions in synaptic transmission beside self-inhibition. I*n vitro* studies suggest that the Habc domain is dispensable for liposome fusion. However, to date, no one has tested the role of syntaxin’s Habc domain in regulating synaptic vesicle dynamics *in vivo*. To test the role of the Habc domain in vivo, we have performed rescue experiments in *C. elegans*. When the Habc domain of syntaxin is replaced with the homologous yeast Habc domain from Sso1, transgenes no longer rescue the *unc-64* null animals. Similarly, when the SNARE motif of syntaxin is replaced with the yeast SNARE motif of Sso1, the transgene is incapable of rescuing *unc-64*. However, when the two chimeric proteins are expressed together, they rescue the syntaxin null. We find that this paired chimera-rescue occurs independent of the N-peptide, which has previously been shown to be important for fusion. These preliminary observations suggest that the Habc domain may facilitate fusion by a mechanism independent of self-inhibition and transport. Since *C. elegans unc-64* nulls are lethal, we are currently generating a single copy insert expressing *unc-64(+)* in the acetylcholine neurons to restore viability of the *unc-64* null mutants. This mosaic strain will be used to express the yeast Habc domain chimera in GABA neurons to examine the role of the Habc domain in neurotransmission. Furthermore, in an effort to explore novel functions of the Habc domain, we propose to identify interacting proteins using proteomics.

584A. Sexually dimorphic synaptic connectivity in the *C. elegans* tail. Matthew Johnson[2], Deborah Ryan[1], **Douglas Portman**[1,2]. 1) Ctr Neural Dev & Disease, Univ Rochester Sch Med Dent, Rochester, NY; 2) Dept. of Biology, University of Rochester, Rochester, NY.

Sex-specific wiring of neural circuits is likely to have important roles in behavior. However, little is known about how sexual cues might influence synapse formation or stability. Interestingly, the recently described connectome of the *C. elegans* male tail (Jarrell et al., 2012, *Science* **337**:437) has revealed several instances of sex-specific synaptic connections between non-sex-specific (“shared”) neurons. In particular, the architecture of phasmid and posterior touch sensory circuitry appears to to be significantly different in the adult male compared to the adult hermaphrodite, perhaps to enable efficient male copulatory behavior. Using GRASP and other fluorescent markers, we are asking several questions about these sexually dimorphic connections. (1) Are sex differences in connectivity established in the embryo, or do these arise later, as a re-wiring process that occurs in parallel with male-specific tail neurogenesis and circuit formation? (2) What determines the sex-specificity of these connections? Is the genetic sex of the pre- and/or post-synaptic cells important, or do non-autonomous sexual cues have a role? (3) How do these sex differences in connectivity alter neural circuit function and behavior? Our preliminary results indicate that one putative hermaphrodite-specific connection, PHB-AVA, is indeed present in larval males. Unexpectedly, we can also often detect strong PHB-AVA GRASP signal in adult males. One possibility is that PHB-AVA synapses are removed as part of male tail re-wiring in L4, but that GRASP is unable to detect (or perhaps even disrupts) this removal. We are currently using GRASP to label other putative sex-specific connections to explore this further. The unique tools available in *C. elegans* should offer the opportunity to understand how genetic sex regulates modulators of synapse specification and maintenance to bring about sex differences in circuit connectivity. We are grateful to S. Emmons (Albert Einstein)

for sharing unpublished data on male tail connectivity and to M. VanHoven (San Jose State) for sharing the *wyIs157* (PHB-AVA GRASP) transgene.

585B. *unc-17* suppressors and subway crowding. **Jim Rand**, Ellie Mathews, Greg Mullen. Gen Models Disease Res Program, Oklahoma Med Res Foundation, Oklahoma City, OK.

The *unc-17* gene encodes the vesicular acetylcholine transporter (VAChT). Null mutations in *unc-17* result in lethality; hypomorphic missense mutations result in slow growth, uncoordinated movement, and resistance to aldicarb. The *unc-17(e245)* allele is associated with a glycine-to-arginine substitution (G347R) which adds a positive charge in the middle of the 9th transmembrane domain (TMD9) of VAChT. Genetic screens to identify mutations that improve the locomotion of *e245* homozygotes have identified both extragenic and intragenic suppressors; extragenic suppressors include dominant neomorphic alleles of several loci that specifically suppress G347R mutant phenotypes. The extragenic suppressors employ a common protein mechanism: a neutral amino acid in the middle of a TMD is replaced by a negatively-charged residue. Thus, the *sup-1(e995)* mutation leads to a glycine-to-glutamic acid substitution (G84E), and the *snb-1(e1563)* mutation (previously known as *sup-8*) is associated with an isoleucine-to-aspartic acid substitution (I97D). We conclude that electrostatic interactions between the positively-charged TMD9 of UNC-17(G347R) and a nearby negatively-charged transmembrane domain are able to restore VAChT function. Based on analysis of suppressing alleles of *sup-1* and *snb-1*, as well as suppressing transgenic constructs, we have identified a limited set of "requirements" for suppression. Obviously, the suppressing protein must be expressed in cholinergic neurons, and at least some of the protein should be localized to synaptic vesicles. However, orientation in the vesicle membrane (*i.e.*, Type I or Type II) is not important. The presence of a signal peptide is also not important. The number of TMDs in the protein is apparently not important, except that for multi-pass proteins, the relevant TMD must be "external" and therefore in a position to interact with TMD9 of UNC-17. Finally, although suppression is allele-specific and clearly requires physical interaction between two mutant proteins, one may not assume a functional interaction between the wild-type proteins. Rather, dense protein packing in synaptic vesicle membranes leads to involuntary proximity (the "subway rush-hour" effect). (Supported by NIGMS).

586C. Regulating Rho and neurotransmitter release. **K. R. Ryan**, S. J. Nurrish. MRC, University of London, London, United Kingdom.

Mutations in Rho GTPase effectors and regulators cause mental retardation in humans. It has been assumed that these defects are caused by developmental defects given the important role of Rho GTPases in regulating the cytoskeleton and thus cell migration. However, in at least one case, the RhoA GAP oligophrenin, restoration of gene function in adult neurons restores at least some of the observed synaptic defects. This suggests an important role for RhoA in control of adult neuronal activity. Previously we have demonstrated a role for the single C. elegans, RhoA ortholog, RHO-1, in control of actylcholine (ACh) release from cholinergic motorneurons. RHO-1 acts as part of a network of G alpha signaling pathways that regulate neuronal activity by regulating both production and destruction of the second messenger diacylglycerol (DAG), which is a regulator of synaptic vesicle release. RHO-1 can be activated by at least two G alpha pathways, resulting in increased ACh release. GPA-12 (Ga12) acts via the RHGF-1 RhoGEF which activates RHO-1. EGL-30 (Gaq) acts via the UNC-73 RhoGEF which activates RHO-1. Hyperactivation of RHO-1 in cholinergic motorneurons causes hypersensitivity to the acetylcholinesterase inhibitor aldicarb suggesting elevated levels of ACh release, whereas inhibition of RHO-1 results in resistance to aldicarb suggesting decreased ACh release. However, rhgf-1 mutants have no phenotype in the absence of activated GPA-12 and UNC-73 mutants, although very lethargic, have a normal response to aldicarb. We tested whether these two RhoGEFs were redundant by constructing an unc-73; rhgf-1 double mutant. However, these mutants also had a wildtype response to aldicarb. Thus, either another RhoGEF acts to regulate ACh release, or a decrease in ACh release in unc-73 mutants is compensated by an increase in muscle response to ACh. RhoGTPases are activated by RhoGEFs and inhibited by RhoGAPs. To identify the RhoGAPs that regulate RHO-1 function in adult neurons we tested viable RhoGAPs for response to aldicarb. Two mutants were both hypersensitive to aldicarb, rga-3 and rga-4. RGA-3 and -4 are RhoGAPs that have redundant roles in regulating C. elegan embryogenesis. Experiments are ongoing to test if rga-3 and rga-4 act within cholinergic motorneurons to negatively regulate RHO-1 and thus decrease levels of ACh release.

587A. A circuit for decision making in *C. elegans*: a computational approach. **Tom Sanders**[1], Gert Jansen[2], Netta Cohen[1]. 1) School of Computing, University of Leeds, LS2 9JT, Leeds, United Kingdom; 2) Department of Cell Biology, Erasmus MC, 3000 CA Rotterdam, The Netherlands.

The head navigation circuit in the nerve ring of *C. elegans* performs a wide variety of computations with a limited number of neurons. Indeed, overlapping neurons have been implicated in navigation, sensory integration and decision making. Here, we create a computational model capable of reproducing a wide variety of behaviors, as observed in various assays performed on wild type, mutant and other defective animals. To this end, we developed a modular computational framework, in which neuronal, circuit and motor output functions and properties, as well as the assay specification can all be easily modified. In our model, individual worms are represented by position, direction pairs controlled by a reduced nervous system consisting of several sensory neuron pairs, an interneuron layer and an abstract motor system. We focus on a decision making assay, in which animals are placed on a dish with quadrants containing a particular concentration of NaCl or no NaCl. Before the assay, the animals are washed for 15 minutes with a low salt buffer to test their naïve response, or a buffer containing 100 mM NaCl to test the response of animals that have associated NaCl with the absence of food (called gustatory plasticity). We show that the model is capable of reproducing the behavior of naïve and conditioned animals in decision making. Model analysis suggests specific neuronal roles and circuit mechanisms. Specifically, our model distinguishes the roles of ASEL and ASER in decision making and suggests that ASH sensory neurons are recruited into the functional circuit, switching the preferred decision of the animals. We are currently testing the performance of our model in other navigation assays, for example a NaCl chemotaxis assay where animals are exposed to a shallow gradient of NaCl and a copper-diacetyl integration assay. The ability to validate the model by testing it in a variety of assays, and under a variety of conditions is a first step towards building more complete models of ever richer behaviors in *C. elegans*.

588B. The computational role of the head navigation circuit in *C. elegans*: Exploring the interneuron layer *in silico*. **Tom Sanders**, Netta Cohen. School of Computing, University of Leeds, LS2 9JT, Leeds, United Kingdom.

In the nematode *C. elegans* the head circuit drives the worm's navigation. Interestingly, computational models of *C. elegans* navigation tend to focus on the sensory layer and motor outputs, completely neglecting the complex interneuron circuit midstream. What then are the computational roles of the ~30 interneurons in this circuit? Here, we propose a role in sensory integration. We created computational models of the worm situated in virtual

environments mimicking an integration assay. In this assay animals are presented with an attractive odorant (diacetyl) on the opposite side of an aversive barrier (copper). Animals have to integrate both signals and either cross the barrier to reach the attractant or stay on their side of the barrier. Behavior is measured by the number of worms crossing the barrier as a function of the barrier and attractant concentrations. We tested the ability of different models, with increasing biological detail and complexity, to perform effective sensory integration. Our final model is capable of reproducing the behavior of wild type worms and known mutants, for separate stimuli and combinations thereof. Interestingly, highly abstract models could already show complex integration through nonlinearities in their motor system alone. The model sheds light on the computational role of specific head interneurons, and makes a number of concrete predictions, including a new stimulus encoding strategy in sensory neurons and a previously undescribed motor program.

589C. *lgc-40* Encodes a Choline-Gated Chloride Channel Subunit Expressed in Neurons and Muscles. **Steve Sando**, Bob Horvitz. MIT, Cambridge, MA.

How neurons interact with the environment and each other to process information in the nervous system is a basic question in neurobiology. To understand these fundamental interactions, it is important to identify neurochemical signals and receptors and characterize their effects on neuronal physiology and animal behavior. Guided by a BLAST search of the *C. elegans* genome using the protein sequence of the serotonin-gated chloride channel *mod-1*, Ringstad et al. (*Science* 325, 96, 2009) identified LGC-40 as a putative cys-loop chloride channel subunit. Using electrophysiological recordings in *Xenopus* oocytes, they further showed that LGC-40 is gated at low affinity by serotonin (EC50=905 mM) and acetylcholine (EC50=87 mM) and at high affinity by choline (EC50=3.4 mM), raising the intriguing possibility that LGC-40 acts in vivo as a high-affinity ionotropic choline receptor. I am using expression studies and behavioral analyses to understand the role of *lgc-40* in *C. elegans* physiology. Translational fusions to GFP indicate that *lgc-40* is expressed in a subset of neurons in the head, tail, and body of the worm, including the PHA chemosensory neurons and likely the NSM neurons. Additionally, the anal depressor and vulval muscles as well as the distal tip cell of the gonad express *plgc-40::GFP*. Using the *lgc-40* expression pattern as a guide, I am now performing behavioral assays of *lgc-40* deletion mutants to identify behavioral defects.

590A. A gustatory neural circuit for salt concentration memory in *Caenorhabditis elegans*. **H. Sato[1]**, H. Kunitomo[1], S. Oda[2], Y. Iino[1]. 1) Dept Biophys Biochem, Grad Sch Sci, The University of Tokyo, Tokyo, Japan; 2) Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.

Caenorhabditis elegans is able to memorize a salt concentration. However, little is known about how worms form memory and how they modulate their behaviors on the basis of the memory. To answer the question, we first monitored the activity of the salt-sensing chemosensory neuron ASER by calcium imaging. We found that ASER changed the magnitude of its responses depending on previously exposed salt concentrations. We then investigated the response of ASER in two mutants, which are defective in the release of either synaptic vesicles or dense-core vesicles. In both mutants, the changes of the amplitude of ASER responses were similar to the wild type. The results suggested that the plasticity of ASER response is independent of inputs from other neurons. Next, we investigated the activity of three interneurons; AIA, AIB, and AIY. These neurons are postsynaptic neurons of ASER. We found that AIB and AIY changed their responses depending on the salt concentration at which the worms had been cultivated. On the other hand, the response of AIA did not significantly change depending on the past experience. To determine whether the sensory input to ASER is sufficient for the change of AIB responses, we used cell-specific rescue of sensory functions, and the results indicated that ASER can in fact drive the change of AIB responses. Furthermore, to assess the contribution of the three interneurons to the behavior, we investigated behavioral responses of worms whose interneurons were genetically ablated individually. Although the worms showed normal salt chemotaxis, their reversal frequency was different from that of the wild type. Therefore, each of these interneurons contributes to the regulation of reversal behavior, but there are redundancies in the neural circuit for salt responses. These results provide deeper understandings into the mechanisms for making the salt concentration memory and modulating the animal's behavior.

591B. Understanding the auto-receptor component of the DOP-2 signal transduction pathway in modulating dopamine release. **Jatinder Singh**, Roderick King, Crystal Clark, Ping Han, Singh Harbinder. Center for Neuroscience, Delaware State University, Dover, DE.

Modulating the levels of dopamine is important in a wide range of physiological and neural functions We are interested in understanding the auto-receptor functional component of the DOP-2 receptor in modulating dopamine release. Our results indicate that mutants in the *dop-2* pathway habituate faster and display deficits in associative learning. In order to dissect function(s) of individual splice variants we also present preliminary results on the differential levels of alternatively spliced forms of the *dop-2* transcript. Binding of dopamine to its G-protein coupled seven-transmembrane D1-like or D2-like dopamine receptors can trigger antagonistic signal transduction cascades. Four dopamine receptors have been identified in the C. elegans genome: DOP-1 is a D1-like receptor, DOP-2 and DOP-3 are D2-like receptors, and DOP-4 is an invertebrate specific receptor. DOP-2 is expressed in the 8 dopaminergic neurons in the hermaphrodite (plus additional neurons mainly in the male tail). The *dop-2* transcript is spliced into 4 alternate forms and its protein product may play a role in modulating levels of dopamine released by acting as an auto-receptor. Recent work in our lab has shown that DOP-2 physically interacts with GPA-14, an inhibitory G-alpha subunit$_i$, and that both *dop-2* and *gpa-14* deletion mutant habituate at a significantly faster rate as compared to wild-type. Furthermore, *gpa-14* deletion mutants also show associative learning deficits similar to those previously reported for *dop-2* deletion. In order to characterize the downstream molecular components of the DOP-2 auto-receptor function we are characterizing *trp-4* loss-of-function mutants. Key references: Suo et al. (2003) J Neurochem. 86: 869-878; Kindt et al. (2007) Neuron. 55(4):662-676; Voglis and Tavernarakis (2008). EMBO J. 27: 3288-3299; Pandey and Harbinder (2012) J. Molecular Signaling. 7: 1-10.

592C. Notch signaling regulates synaptic transmission at the *C. elegans* neuromuscular junction. **Altar Sorkac[1]**, Michael DiIorio[2], Hannah Graham[1], Komudi Singh[1], Anne Hart[1]. 1) Neuroscience Dept, Brown University, Providence, RI; 2) Dana Farber Cancer Institute, Boston, MA.

Notch is a signaling pathway that has been conserved across the animal kingdom. The role of Notch signaling during nervous system development has been extensively studied whereas its effects outside cell fate specification are less well described. Notch signaling is active in adult mice, *Drosophila* and *C. elegans*, regulating synaptic activity and other neurobiological processes. Notch receptors LIN-12 and GLP-1 are activated by DSL proteins with DOS-motif proteins acting as co-ligands. In *C. elegans*, Notch signaling affects avoidance of aversive chemicals and quiescence. Intriguingly, Notch DOS co-ligands OSM-7 and OSM-11 are regulated by environmental osmolarity; animals lacking either one of these co-ligands are preadapted to osmotic stress. Here, we show that Notch signaling impacts synaptic transmission at the neuromuscular junction of *C. elegans*, possibly in this neuroethological context. Complete

loss of the Notch receptor LIN-12 results in hypersensitivity to aldicarb (Hic), an inhibitor of acetylcholinesterase. Loss the DSL ligand DSL-3 or either DOS co-ligand, OSM-7 or OSM-11, also results in hypersensitivity; these ligands likely activate LIN-12 in this paradigm. Notch signaling in adults is sufficient as overexpression of OSM-11 exclusively in adult animals causes resistance to aldicarb (Ric). Moreover, knock-down by RNAi of *osm-11* in early larval stages does not cause adult aldicarb hypersensitivity. Also, *osm-11* knock-down after the L2 larval stage results in hypersensitivity. Loss of the transcription factor LAG-1 resulted in hypersensitivity to aldicarb suggesting that LIN-12 signals via the canonical pathway. Next, the site of action of LIN-12 will be elucidated and candidate downstream targets will be examined in order to further our understanding of how Notch signaling regulates synaptic transmission.

593A. Role of serotonin signaling in *C. elegans* fat metabolism. **T. Noble**, S. Srinivasan. The Scripps Research Institute, La Jolla, CA.

Serotonin is an ancient and evolutionarily conserved neurotransmitter that plays a key role in many biological processes. In humans serotonin is known to regulate mood, sleep, memory and learning, as well as appetite and weight loss. In *C. elegans* serotonin regulates diverse behaviors such as locomotion, egg-laying, food-associated learning, food intake by increasing pharyngeal pumping rate and fat loss. Some of the serotonin-regulated behaviors in *C. elegans* like egg-laying have been extensively studied. However, the serotonin signaling pathways that regulate fat are not well understood. We seek to unravel the mechanisms through which serotonin signaling in the nervous system leads to fat loss in the *C. elegans* intestine. In the process we also hope to identify key neurons that function in multiple fat metabolism pathways.

Using Oil-Red-O staining, transgenic rescue, antisense technology and GFP analysis we have discovered a neural circuit in which serotonin signaling working through the serotonergic chloride channel *mod-1* regulates body fat in *C. elegans*. In future studies we want to decipher the neuroendocrine mechanisms that facilitate signaling from the nervous system to distal tissues to regulate body fat.

594B. Integration of Sensory Perception, Lipid Metabolism and Food Intake in *C. elegans*. **J. Stieglitz**[1,2], S. Srinivasan[1,3]. 1) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA; 2) Kellogg School of Science and Technology, The Scripps Research Institute, La Jolla, CA; 3) Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA.

Obesity results from the dysfunction of energy balance circuits that integrate environmental signals from the nervous system, with internal cues from tissues where body fat is stored. An area of growing importance within the energy balance field is sensory perception - the way our brains process sensory cues from the external environment. In humans, mice and *C. elegans*, loss of sensory perception by elimination of sensory cilia in neurons leads to severe obesity. Despite its importance, the molecular mechanisms that connect discrete sensory modalities with energy balance and the maintenance of body fat remain largely unknown.

G protein coupled receptor (GPCR) mediated G protein signaling is a major mechanism by which sensory information is relayed in the nervous system. Our laboratory screened the 21 Ga proteins in the *C. elegans* genome and found that loss of GPA-3, a Ga protein expressed exclusively in eight sensory neurons, leads to a profound decrease in body fat and a significant decrease in food intake. We used a Gateway-compatible, *Mos1*-mediated single-copy transgene insertion system with antibiotic selection (MosSCI-biotic) to restore GPA-3 to subsets of the neurons GPA-3 is normally expressed in. Our transgenic strains revealed that a single sensory neuron is in large part responsible for the gpa-3 fat phenotype. We will use this information to determine how the GPA-3-mediated signal is propagated through the nervous system to the intestine.

Feeding is regulated by a separate and more disperse circuit of neurons. Restoration of GPA-3 to any one of the eight sensory neurons it is normally expressed in will rescue wild-type feeding rates. We find it interesting that the same protein is involved in different metabolic phenotypes that are mediated through dissimilar types of neural circuits.

595C. Dopamine regulates acetylcholine signaling and body size via octopamine and CREB signaling in *C. elegans*. **Satoshi Suo**, Eitaro Oami, Midori Yoshida, Shoichi Ishiura. Graduate School of Arts & Sciences, University of Tokyo, Tokyo, Japan.

cAMP response element binding protein (CREB) is a signal-activated transcription factor that enables the coupling of external stimuli and gene expression to induce adaptive changes. It has been shown that bioamine neurotransmitters regulate CREB and that such regulation is important for long-term changes in various nervous system functions. In *C. elegans*, a bioamine octopamine activates a CREB homolog CRH-1 in cholinergic SIA neurons, whereas dopamine suppresses CREB activation by inhibiting octopamine signaling in response to food stimuli.

In this study, the effect of dopamine, octopamine, and CREB on acetylcholine signaling was analyzed using the acetylcholine esterase inhibitor aldicarb. Mutants with decreased dopamine signaling exhibited reduced acetylcholine signaling, and octopamine and CREB functioned downstream of dopamine in this regulation. Furthermore, cell-specific rescue and knockdown experiments revealed that CREB works in SIA neurons to control the release of acetylcholine from these neurons upon regulation by bioamines.

We also demonstrate that the same bioamine signaling pathway contributes to the regulation of body size in *C. elegans*. Dopamine signaling mutants were larger than wild type animals, suggesting that dopamine negatively regulates body size. Genetic analyses revealed that octopamine and CREB function downstream of dopamine in the body size regulation. We also show that dopamine functions independently of DBL-1, which is known to control body size, but requires a PR-protein LON-1, the downstream factor of the DBL-1 signaling pathway. These results suggest that the dopamine-dependent pathway converges with the DBL-1 pathway in the regulation of body size of *C. elegans*.

596A. Neuropeptides Function in a Homeostatic Manner to Modulate Excitation-Inhibition Imbalance in *C. elegans*. **Seika Takayanagi-Kiya**, Tamara M. Stawicki, Keming Zhou, Yishi Jin. Division of Biological Sciences, Section of Neurobiology, Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA, USA.

Neuropeptides play crucial roles in modulating neuronal networks, including changing intrinsic properties of neurons and synaptic efficacy. We previously reported a *C. elegans* mutant, *acr-2(gf)* that displays spontaneous convulsions as the result of a gain-of-function mutation in a neuronal nicotinic acetylcholine receptor subunit (Jospin et al. 2009). The ACR-2 channel is expressed in the cholinergic motor neurons, and *acr-2(gf)* causes cholinergic overexcitation accompanied with reduced GABAergic inhibition in the locomotor circuit. Here we show that neuropeptides play a homeostatic role that compensates for this excitation-inhibition imbalance in the locomotor circuit. Loss of function in genes required for neuropeptide processing or

release of dense core vesicles specifically modulate the convulsion frequency of *acr-2(gf)*. The proprotein convertase EGL-3 is required in the cholinergic motor neurons to restrain convulsions. Electrophysiological recordings of neuromuscular junctions show that loss of *egl-3* in *acr-2(gf)* causes a further reduction of GABAergic inhibition. We identify two neuropeptide encoding genes, *flp-1* and *flp-18*, that together counteract the excitation-inhibition imbalance in *acr-2(gf)* mutants. We further find that *acr-2(gf)* causes an increased expression of *flp-18* in the ventral cord cholinergic motor neurons and that overexpression of *flp-18* reduces the convulsion of *acr-2(gf)* mutants. The effects of these peptides are in part mediated by two G-protein coupled receptors, NPR-1 and NPR-5. Our data suggest that the chronic overexcitation of the cholinergic motor neurons imposed by *acr-2(gf)* leads to an increased production of FMRFamide neuropeptides, which act to decrease the activity level of the locomotor circuit, thereby homeostatically modulating the excitation and inhibition imbalance.

597B. 4-D Ca^{2+} imaging of the multiple neurons in a local circuit regulating behavioral choice. **Takayuki Teramoto[1,2]**, Yuta Yamamoto[1], Takeshi Ishihara[1,2]. 1) Dept. of Biology, Kyushu Univ, Faculty of Sci, Fukuoka, Japan; 2) JST, CREST, Chiyoda, Tokyo, Japan.

Animals receive multiple environmental signals simultaneously, and then they integrate these signals to execute a proper behavior. Although this sensory integration process is important for their behavior, its neural mechanisms remain unclear. In *C. elegans*, sensory integration between a repellent Cu^{2+} and an attractive odorant diacetyl is regulated by a local circuit including sensory neurons AWA and ASH, and interneurons AIA and AIB. AIA interneurons receive chemical synapses from ASH, which are activated by Cu^{2+}, and connect with gap-junctions to AWA, which are activated by diacetyl. This diagram suggests that the signals integrated at AIA may regulate the downstream interneurons AIB, of which activation leads to backward movements. This model is consistent with results from our behavioral analyses; however, simultaneous measurements of the multiple neurons have not been succeeded because they are arranged in three-dimensional space. To visualize and measure the activities of these neurons, we designed a 4-D imaging system based on a confocal microscope with a piezo lens positioner. Combining this system and the Ca^{2+} indicator Yellow Cameleon, we carried out Ca^{2+} imaging of multiple neurons: AWA, AIA, and AIB. We observed the following: 1) AWA and AIA were simultaneously activated when AWA were stimulated; 2) AIB interneurons, which receive chemical synapses from AIA, exhibit reciprocal responses to AWA/AIA. These imaging results are consistent with the model that we proposed. Therefore this 4-D imaging system enables us to visualize and measure the multi-neuronal activities, and it may contribute to understanding mechanisms not only for the sensory integration but also for other information processing. On the other hand, 4-D imaging of multiple neurons still has technical difficulties such as indistingishability of each neuronal cells and inconsistent expression of Ca^{2+} indicators. We are now performing 4-D imaging of many neurons using integrated transgenic lines expressing Ca^{2+} indicators in the nuclei that makes us able to distinguish individual neurons.

598C. Neurexin and Neuroligin Mediate Retrograde Synaptic Inhibition in C. elegans. Zhitao Hu[1,2], Sabrina Hom[1,2,3], Tambudzai Kudze[1], **Xiajing Tong[1,2]**, Seungwon Choi[1,2,3], Gayane Aramuni[4], Weiqi Zhang[5], Joshua Kaplan[1,2,3]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, 02114 MA, USA; 2) Department of Neurobiology, Harvard Medical School, Boston, 02115 MA, USA; 3) Program in Neuroscience, Harvard Medical School, Boston, 02115 MA, USA; 4) Max Planck Institute of Neurobiology,D-82152 Martinsried, Germany; 5) Department of Psychiatry, University of Münster, D-48149 Münster, Germany.

The synaptic adhesion molecules neurexin and neuroligin alter the development and function of synapses and are linked to autism in humans. Here, we found that Caenorhabditis elegans neurexin (NRX-1) and neuroligin (NLG-1) mediated a retrograde synaptic signal that inhibited neurotransmitter release at neuromuscular junctions. Retrograde signaling was induced in mutants lacking a muscle microRNA (miR-1) and was blocked in mutants lacking NLG-1 or NRX-1. Release was rapid and abbreviated when the retrograde signal was on, whereas release was slow and prolonged when retrograde signaling was blocked. The retrograde signal adjusted release kinetics by inhibiting exocytosis of synaptic vesicles (SVs) that are distal to the site of calcium entry. Inhibition of release was mediated by increased presynaptic levels of tomosyn, an inhibitor of SV fusion.

599A. Identifying molecules involved in dense-core vesicle biology. **Irini Topalidou**, Brooke Jarvie, Jill Hoyt, Michelle Giarmarco, Angela L. Barr, Michael Ailion. Biochemistry Department, University of Washington, Seattle, WA.

Neuromodulation is a form of chemical signaling mediated by the release of neuropeptides and monoamines that activate G protein coupled receptors. The regulated release of neuromodulators is mediated by a unique organelle, the dense-core vesicle (DCV). The biogenesis, trafficking, and release of DCVs are not well understood, mainly because few proteins specific to DCV function have been identified. We previously identified at least six molecules involved in DCV function by screening for genetic suppressors of the activating Gq mutation, *egl-30*(*tg26*). Two of the new proteins, RUND-1 (a RUN domain protein) and CCCP-1 (a coiled-coil protein), act as effectors of the small GTPase RAB-2 a protein previously implicated in DCV maturation. Here we report the identification of two independent missense mutations in the same region of the coiled-coil domain of RUND-1 that give stronger phenotypes than the null mutant, implying important roles for the RUND-1 coiled-coil domain. We also characterized the function of another Gq suppressor, which encodes a conserved protein containing WD40 repeats. We show that mutants of this gene have the “unmotivated” phenotype typical of DCV mutants, characterized by little spontaneous movement on food but capable of coordinated locomotion when stimulated. The WD40 protein is required for trafficking of DCV cargos and its expression in the head cholinergic neurons partially rescues the unmotivated phenotype. We are currently investigating whether the WD40 protein interacts with the RUND-1 effector complex. To identify molecules that interact with the WD40 protein, we screened for suppressors of the WD40 unmotivated phenotype. We identified seven suppressors, at least one of which partially rescues the DCV cargo trafficking defect. By screening for additional suppressors of activated Gq to saturate the original screen, we identified three new genes that seem to be involved in DCV biology. We are currently sequencing two of our identified mutants and characterizing their DCV trafficking phenotype.

600B. A computational model of the intracellular signaling pathway for odor receptor neuron in *C. elegans*. **Mamoru Usuyama[1]**, Yuishi Iwasaki[2], Chisato Ushida[3], Ryuzo Shingai[1]. 1) Department of Chemistry and Bioengineering, Iwate University, Morioka, Iwate, Japan; 2) Department of Intelligent System Engineering, Ibaraki University, Hitachi, Ibaraki, Japan; 3) Department of Biochemistry and Biotechnology, Hirosaki University., Hirosaki, Aomori, Japan.

Caenorhabditis elegans has only three pairs of olfactory receptor neurons, and AWC neurons are known to a pair of them. AWC neurons can respond to odor stimulus, and these responses are reported. In AWC neurons, intracellular [Ca^{2+}] decrease is induced by attractive odor application, and [Ca^{2+}]

transiently increase by odor stimulus removal. The magnitude of this increase is positively corrected with time length of stimulation. AWCs can sense attractive odor, and using cGMP as a second messenger for intracellular signaling. But, how odor stimulus controls cGMP synthesis is unclear. In this study, we developed hypothetical model of olfactory signal transduction pathway to better understanding for olfaction in C. elegans. We indentified likely candidates of components for signal transduction, using available gene expression and physiological data from AWCs. We assume that, odor stimulus inhibit cGMP synthesis, owing to suppression of guanylate cyclase by G-protein signaling. In addition, we assumed Ca^{2+} dependant negative feedback loop to enhance the cGMP synthesis. These assumptions were necessary to replicate major features of the calcium dynamics in AWCs. AWC neurons indicated [Ca^{2+}] fluctuation in decreasing phase of transient excitation. But, our model could not generate these fluctuations. So, to examine which component was related with generating of these fluctuations, adding random signal into the model pathway. Adding noise into model components, fluctuations were closely related with activity of phosphodiestelase and inflow/outflow of calcium ion and its buffering. Kinetic parameter for activity of guanylate cyclase was not effective to fluctuations in our model.

601C. A Genetic Resource for Assaying Neuropeptide Function *in vivo*. **Amy B. Vashlishan Murray[1,2]**, Edward Pym[1], Joshua Kaplan[1]. 1) Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Emerson College, Boston MA.

Neuropeptides represent a large family of small peptide neurotransmitters that are broadly expressed in the nervous system and are involved in the modulation of a diverse set of vital processes and behaviors. In *C. elegans*, there are 115 pro-neuropeptide genes that encode over 250 distinct neuropeptides and elicit effects on metabolism, lifespan, chemotaxis, egg-laying, and synaptic transmission. Loss-of-function mutations have helped identify roles for neuropeptides in the regulation of this diverse set of nervous system functions. However, no resource currently exists for systematically assaying neuropeptide function *in vivo*. We have showed that a stretch-sensitive neuron (DVA) secretes two neuropeptides (NLP-12 and NLP-21) following exposure to the acetylcholinesterase inhibitor aldicarb. Aldicarb-induced NLP-12 secretion in turn stimulates acetylcholine secretion at neuromuscular junctions (NMJs). Our current work is designed to exploit the inducible and rapid release of neuropeptides from DVA to create a resource for assaying neuropeptide function *in vivo*. This presentation describes the strategy used to create and validate a genetic resource for inducible neuropeptide secretion *in vivo* and discusses an application of this tool for identifying novel neuropeptides that regulate cholinergic and GABAergic transmission.

602A. Does local protein synthesis in the sensory dendrites of the AFD thermosensory neuron play a role in long-term memory? **V. Venkatachalam[1]**, S. Yogev[2], J. Calarco[3], A. Calvo[4], J. Hawk[4], M. Klein[1], D. Colon-Ramos[4], K. Shen[2], A. Samuel[1]. 1) Physics, Harvard University, Cambridge, MA; 2) Biology, Stanford University, Palo Alto, CA; 3) Systems Biology, Harvard University, Cambridge, MA; 4) Biological and biomedical sciences, Yale University, New Haven, CT.

Long-term exposure to new temperatures for several hours will reset the thermotactic setpoint of C. elegans, the temperature towards which animals will navigate on spatial temperature gradients. The principal thermosensory neuron that drives thermotactic behavior is AFD. Earlier, we and others showed that the physiological properties of the AFD neuron can be used to infer the thermotactic setpoint. AFD responds to temperature gradients only at temperatures near and above the setpoint. Thus, the primary sensory neuron also stores the long-term memory of the animal's thermotactic setpoint. However, the molecular determinants of memory storage and memory dynamics in AFD are poorly understood. To study memory dynamics in this sensory neuron, we have developed a chronic imaging assay that allows us to monitor the calcium dynamics of single AFD neurons in animals monitored for several hours as they are subjected to defined temperature waveforms. Rapid changes in temperature on the time scale of seconds allow us to read out the memory of the thermotactic setpoint. Sustained changes in temperature on the time scale of hours allow us to shift the memory. An unusual property of the AFD neuron is that ribosomes and RNA binding proteins can be found near the base of the sensory cilia near the nose of the animal. Might local protein synthesis play a role in memory dynamics by shifting the sensitivity of thermoreceptors in the sensory cilia? To test this possibility, we used laser ablation to snip the sensory dendrites of individual AFD neurons, and confirmed that the isolated sensory cilia were capable of learning new temperatures. We predict that mutations that disrupt ribosome or mRNA translocation to the sensory dendrites would have a profound effect on memory dynamics in the sensory cilia of the AFD neuron.

603B. *Track-A-Worm*, an open-source system for quantitative assessment of C. elegans locomotory and bending behaviors. Sijie Wang[1], **Zhao-Wen Wang[2]**. 1) Medical School, UConn Health Center, Farmington, CT; 2) Department of Neuroscience, UConn Health Center, Farmington, CT.

Automated single-worm trackers allow the extraction of detailed locomotory and bending information for quantitative comparisons. Here we described *Track-A-Worm*, which is a single-worm tracking system that is rich in functionalities, open in source codes, easy to setup, and easy to use. The system includes a stereomicroscope, a motorized stage, a digital camera, a PC computer with Windows 7 operating system, and custom software written to run with Matlab. A freely moving worm is imaged at a chosen frame rate while it is kept near the center of the imaging field. The acquired images are automatically converted to binary images followed by head identification and placement of 13 markers along a deduced spline. The software can extract and quantify a variety of locomotory and bending parameters, including total distance traveled, average speed, distance/time/speed of forward and backward locomotion, frequency and amplitude of dominant bends, overall bending activities measured as root mean square, and sum of all bends. The software can also plot worm traveling path, bend trace, and bend frequency spectrum. All functionalities of the system are performed through graphic user interfaces. These features make *Track-A-Worm* a good candidate for implementation in other research labs.

604C. DAF-19 acts as a negative regulator to modulate environment-dependent GABA phenotypes. **Yusu Xie**, Mustapha Moussaif, Ji Ying Sze. Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

Our lab is interested in the regulation of neurotransmitter biosynthesis under aversive conditions. Using GFP reporters, we found that the expression of GABA synthesis gene unc-25/glutamic acid decarboxylase was increased, while acetylcholine (ACh) synthesis gene cha-1/choline acetyltransferase was reduced in dauer animals. By contrast, we observed that in starved L1 animals, the expression of both unc-25::gfp and cha-1::gfp was decreased, suggesting that GABA biosynthesis is differentially regulated under distinct aversive conditions. A previous report indicated that the RFX transcription factor DAF-19 is expressed in the locomotory neurons and daf-19 mutants exhibited enhanced resistance to aldicarb (Senti and Swoboda, 2008). We found

that daf-19::gfp levels in the locomotory neurons was reduced in wild-type dauers. Since either reduced ACh neurotransmission or increased GABA neurotransmission could lead to enhanced resistance to aldicarb (Loria et al., 2004; Vashlishan et al., 2008), we analyzed unc-25::gfp and cha-1::gfp in daf-19 mutants. We observed that cha-1::gfp was reduced in daf-19 mutants. Furthermore, we found that the expression of unc-25 and unc-47/vesicular GABA transporter was significantly increased in both daf-19(m86) and daf-19(yz70) mutants. While several previous studies have shown that DAF-19 acts as a transcriptional activator (Swoboda et al., 2000; Wang et al., 2010; Xie et al., 2013), our results suggest that DAF-19 may also act as a negative regulator of GABA neurotransmission under optimal growth conditions. Enhanced GABA neurotransmission could represent a genetic program that inhibit un-necessary locomotion under aversive growth conditions and may contribute to the characteristic relax appearance of dauers. References: Loria PM, Hodgkin J and Hobert O, 2004. J. Neurosci. 24:2191-2201.Senti G and Swoboda P, 2008. Mol Biol Cell 19(12):5517-28.Swoboda P, Haskell T, Adler HT and Thomas JH, 2000. Mol Cell 5:411-421.Vashlishan AB, Madison JM, Dybbs M, Bai J, Sieburth D, Ch'ng Q, Tavazoie M and Kaplan JM, 2008. Neuron 58:346-361.Wang J, Schwartz HT, Barr MM, 2010. Genetics 186:1295-1307.Xie Y, Moussaif M, Choi S, Xu L and Sze JY, 2013. PLoS Genet 9(3): e1003324.

605A. A role for T-type calcium channels in serotonin signaling. **Kara Zang**, Niels Ringstad. Skirball Institute, New York University, New York, NY.

Serotonin is a neuromodulator that modulates various neurological processes, e.g. mood and metabolism. In the nematode worm C. elegans, its release by the hermaphrodite specific neurons (HSNs) is required for egg-laying. The HSNs express the G-protein coupled receptor (GPCR) EGL-6, which inhibits serotonin release. egl-g(gf) mutants become bloated with unlaid eggs. We performed a screen for suppressors of egl-6(gf) to identify novel regulators of serotonin signaling and recovered an allele of cca-1, which encodes a T-type Ca2+ channel. cca-1(n5209) potently suppresses the egg-laying defect of eg-6(gf) mutants but on its own has no overt effect on egg-laying behavior. We have created a cca-1::gfp reporter transgene, which shows that cca-1 is expressed in neurons of the C. elegans egg-laying system. A role for cca-1 in muscle electrophysiology has been shown (Shtonda and Avery 2005; Steger et al. 2005), but its function in the C. elegans nervous system is unclear. In other organisms T-type channels participate in the formation of plateau potentials and promote burst-like modes of neurotransmission (Huguenard 1996; Constantin 2011). We will describe progress towards determining the effect of the n5209 mutation on cca-1 function and identifying where in the egg-laying circuit cca-1 functions.

606B. The Pristionchus pacificus obi-3 mutant lacks attraction to beetle host pheromone and shows increased turning frequency. **Georgina Aguilar-Portillo**, Jimmy Escobedo, Neomal Muthumala, Ray Hong. Biology, CSU, Northridge, Northridge, CA.

Understanding chemosensation and neurophysiology from varying nematode species is crucial for investigating gene function evolution. The soil-dwelling, free-living C. elegans has elucidated key neurons and proteins required for chemosensation of volatile odors, pheromones, and food. A more recently discovered nematode species, Pristionchus pacificus has been found to associate with specific beetle hosts, including the oriental beetle found in Japan and northeastern United States, likely through attraction to its sex pheromone, ZTDO. To determine the genes responsible for insect pheromone sensing, two chemosensory mutants were identified in a genetic screen for lack of attraction towards ZTDO after cGMP treatment and were named obi-1 and obi-3 (oriental beetle pheromone insensitive). This study focuses on obi-3, which also has a short body length and locomotion phenotype. Wild-type P. pacificus reverses 3x more frequently as C. elegans and reversal rates are known to affect approach behavior in chemotaxis. Obi-3's higher reversal rate was quantified by counting omega turns on and off food. An omega turn is defined by a worm turning its head 180°, touching the tip of its tail, and proceeding in a forward motion in a new direction. Interestingly, C. elegans egl-30 mutants show a similar higher reversal rate. Defects in EGL-30 affects the Gqa pathway that mediates the release of acetylcholine from motor neurons in order for normal muscle movement to occur. We also found that the associated coiling behavior is further accentuated in liquid medium. Wild-type shows a thrashing motion whereas obi-3 coils into a superimposed circle. This coiling-swimming phenotype was utilized for positional mapping in obi-3 x Japan recombinant inbred lines and rough mapping the obi-3 mutation to 38 cM interval of Chromosome I using SSLP markers. The long-term goal is to determine if obi-3 and obi-1 constitute components of an unknown signaling pathway, part of the conserved cGMP-dependent G protein signaling pathway, or TRPV channels lipid signaling pathway involved in sensing insect hosts. [This study was supported by NIH SCORE SC2GM089602 and NSF HRD-1139803 CSU-LSAMP Bridge to the Doctorate].

607C. Insights into the molecular mechanisms of memory rewriting in Caenorhabditis elegans. **Ichiro AOKI**, Ikue MORI. Graduate School of Science, Nagoya University, Nagoya, Aichi, Japan.

The ways of information processing and memory storage by neuronal circuits remain enigmas. C. elegans can memorize ambient temperature in association with the presence of food and migrate to that temperature on a temperature gradient. This behavior, called thermotaxis, is achieved with a simple neuronal circuit consisting of a small number of neurons and has provided opportunities to dissect the molecular mechanisms of sensory perception, behavioral regulation, neural plasticity such as learning and memory. Interestingly, thermotaxis behavior itself is plastic; worms cultivated at 23°C and then transferred to 17°C merely for 3 hours already prefer 17°C. We are performing a screen for genes involved in this replacement of the old memory by the new one. Identification of genes important for rewriting memory and subsequent elucidation of neurons, in which these genes act during the memory rewriting, will pave the way for understanding the entity of the memory and how the nervous system process the input and output the signals to regulate behavior.

608A. Response to repeated activation of ASH requires glutamate, dopamine, and neuropeptide signaling. **Evan L. Ardiel**[1], Andrew C. Giles[1], Theodore Lindsay[3], Ithai Rabinowitch[2], William Schafer[2], Shawn Lockery[3], Catharine H. Rankin[1]. 1) University of British Columbia; 2) MRC Laboratory of Molecular Biology; 3) University of Oregon.

The objective of this research was a comparative analysis of habituation in two neural circuits that synapse onto the same interneurons. Previous work in the lab has focused on habituation to a non-localized mechanical stimulus - a plate tap. Here we studied the response to repeated activation of the ASH sensory neurons, which detect a variety of aversive stimuli. A strain expressing ChR2 exclusively in ASH (Ezcurra et al., 2011) was used for consistent and discrete delivery of simulated aversive stimuli to worm populations being tracked by real-time computer vision software (Swierczek et al., 2011). In addition to increasing throughput, this optogenetic approach allowed us to prevent sensory adaptation and specifically activate ASH. Whole-plate blue light illumination elicited backward crawling (reversal) in the majority of animals. As with the tap-withdrawal response, the magnitude of ASH-mediated

reversals decremented with repeated stimulation in a manner dependent on the stimulus intensity and frequency and recovered to baseline in minutes. Electrophysiological recordings of repeatedly activated photocurrents in ASH demonstrated that the decrement was not caused by ChR2 desensitization. Furthermore, a tap could dishabituate decremented responding. Cross-modal habituation was evident in only one-direction, ie the tap-withdrawal response was decremented by repeated activation of ASH, thereby localizing the site of plasticity to the shared circuitry. Although their magnitude decremented over the trial, ASH reversals persisted far longer than tap-induced reversals. This response maintenance was dependent on glutamate and dopamine signaling. Repeated ASH activation also led to increased speed of forward locomotion and a greater suppression of spontaneous reversals in the periods between stimuli, as compared to tap. This change was dependent on neuropeptide signaling. These distinct behavioural strategies are hypothesized to facilitate escape from a hazardous area.

609B. NLP-7 peptide modulation of the egg-laying circuit. **Navonil Banerjee**, Raja Bhattacharya, Michael Francis. Neurobiology, UMass Medical School, Worcester, MA.

Fast synaptic transmission mediated through ionotropic receptors underlies rapid communication between neurons. However, the strength of fast synaptic signaling and its effects on the activity of neural circuits can be potently modulated by a host of signaling molecules, including neuropeptides. Neuromodulation can have long-lasting effects and is often responsive to changing environmental conditions, as well as intrinsic physiological states of the body. Neuromodulators have been implicated in the regulation of a variety of behaviors but much less is known about functions for specific neuromodulators in the context of the neural circuits in which they act. The simple genetics and well-described connectivity of the Caenorhabditis elegans nervous system permit detailed functional analysis of neuromodulators and their effects on neural circuit activity. We are working to characterize the role of the neuropeptide NLP-7 in C. elegans egg-laying. We found that overexpression of NLP-7 produced severe defects in egg laying. Animals overexpressing NLP-7 (NLP-7 OX) retained more eggs in their uterus and also laid late stage embryos compared to wild type animals. nlp-7 is expressed in several components of the egg-laying circuit, including the VC4 and VC5 neurons, as well as the uv1 cells. NLP-7 OX animals were normally responsive to exogenous serotonin but were resistant to fluoxetine (serotonin reuptake inhibitor), suggesting NLP-7 may act by reducing serotonin release from the HSNs. We have found that a loss-of-function mutation in egl-47, a putative seven transmembrane receptor expressed in the HSNs (Moresco & Koelle, 2004), partially suppressed the effects of nlp-7 overexpression. In addition, mutation of the potassium chloride cotransporter kcc-2 (Tanis JE et. al. 2009) completely suppressed the egg-laying defects of NLP-7 OX animals. We are continuing to investigate how NLP-7 is acting to mediate its inhibitory effects as well as identification of NLP-7 receptor(s). Together, our results indicate that a neuropeptide signaling pathway involving NLP-7 operates to modulate egg-laying behavior in C. elegans.

610C. Molecular regulators of male sex-drive. Scott W. Emmons[1], **Arantza Barrios[2]**. 1) Dept of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Dept Cell and Developmental Biology, University College London, London, United Kingdom.

Well-fed, mate-deprived adult males make the risky choice to leave a plentiful source of food to explore their environment in search of mates. In contrast, recent experience with a mate while exposed to food produces a durable behavioral switch that restricts exploration within the limits of the food source. From a forward genetic screen for males that do not leave food (*leaving assay defective -las*), we isolated several mutants. We have recently shown that *las-1(bx142)* encodes a secretin-like G protein-coupled receptor for the neuropeptide *pigment dispersing factor (pdf-1*). Male exploratory behavior results from the balance of two physiological needs -feeding and reproduction- that compete for the control of a distributed network for navigation. The phenotype of *pdfr-1* males reflects an imbalance in the relative contribution of the circuits that control exploration. *pdfr-1* is required in gender-shared sensory neurons PHA, PQR and URY to generate the state of arousal to leave food in search of mates. Thus, *pdfr-1* modulates a circuit that senses the internal environment of the animal and antagonizes the food-sensing circuit (Barrios *et al.*, 2012, DOI 10.1038/nn.3253). We are currently identifying the molecular lesion responsible for the phenotype of *las-2(bx143)*. We have mapped *bx143* to the distal left arm of chromosome I and through whole genome sequencing we have identified missense mutations in two candidate genes. Rescue experiments and complementation tests with these two candidates are under way. *bx143* males display normal locomotion on food but are strongly Las and mate response defective with no apparent morphological defects. These phenotypes suggest a role for *bx143* in the regulation of the neural circuits that convey male sex drive.

611A. Conserved neuropeptidergic regulation of associative learning by vasopressin/oxytocin-related peptides. **I. Beets[1]**, L. Temmerman[1], T. Janssen[1], E. Meelkop[1], L. Frooninckx[1], G. Jansen[2], L. Schoofs[1]. 1) Biology, KU Leuven, Leuven, Belgium; 2) Cell Biology, Erasmus MC, Rotterdam, Netherlands.

Neuropeptides play an important role in modulating the behavioral output of neuronal circuits according to environmental and internal state cues. Their mode of action especially in learning and memory processes however remains elusive. Using bioinformatics and reverse pharmacology, we have identified a neuropeptidergic signaling system in *C. elegans* that is related to hypothalamic vasopressin and oxytocin signaling in mammals. Candidate vasopressin/oxytocin-like receptors were challenged in a cellular system with a synthetic library of over 250 *C. elegans* peptides. A vasopressin/oxytocin-like peptide originating from the nematocin precursor NTC-1 activated the *C. elegans* nematocin receptor NTR-1 dose-dependently. Expression analyses of nematocin and its receptor indicate a modulatory function in the sensory circuit for salt chemotaxis. Worms lacking nematocin signaling show defects in modifying salt chemotaxis behavior in light of previous experience, a type of associative learning termed gustatory plasticity. We found that nematocin targets the salt-sensing ASEL neuron of the gustatory plasticity circuit, and that this peptidergic signal originates at least partially from the AVK interneurons. Genetic and supplementation studies showed that serotonin and dopamine signaling interacts with the nematocin pathway in gustatory associative learning.

Vasopressin/oxytocin-related peptides influence cognitive processes in mammals that often translate to the experience-based association of stimuli. Our results indicate an ancient neuromodulatory function of vasopressin/oxytocin-like signaling in associative learning circuits, and shed light on the cellular and molecular mechanisms underlying these effects. Further research focuses on the neuronal activity dynamics in the nematocinergic circuit for gustatory plasticity and on other neuropeptidergic systems that might be involved in *C. elegans'* learning, including insect-related myoinhibiting peptide and tachykinin signaling.

612B. Notch DSL ligand *lag-2* is required for *C. elegans* lethargus quiescence. **Heather L. Bennett**[1], Huiyan Huang[2], Komudi Singh[2], Anne C. Hart[2]. 1) Department of Molecular Biology, Cellular Biology & Biochemistry, Brown University, Providence, RI; 2) Department of Neuroscience, Brown University, Providence, RI.

Sleep is an evolutionarily conserved behavior defined as a rapidly reversible period of inactivity with decreased sensory responsiveness. Despite this knowledge, the genetic underpinnings, mechanisms of sleep regulation, and the specific tissues involved in this behavior remain elusive. *C. elegans* undergoes sleep-like quiescence during lethargus in coordination with each larval cuticle molt (Raizen *et al.,* 2008). Our laboratory has shown that Notch signaling regulates quiescence, as perturbations in the Notch pathway change the arousal thresholds and amount of quiescence during the last larval lethargus during transition from L4 stage to adult (Singh *et al.,* 2011). We find that decreased function of *lag-2*, which encodes a Notch DSL ligand, results in increased quiescence and reduced arousal thresholds during the L4 to adult lethargus. However, it remains unclear how specific changes in *lag-2* gene function disrupt aspects of this behavior. We are now characterizing the role of *lag-2* in regulating behavioral quiescence. The specific cells where LAG-2 functions in this behavior and when *lag-2* is required to regulate quiescence is being determined. The knowledge gained from addressing these aims will help us understand the role of Notch signaling, particularly the role of *lag-2* in regulating *C. elegans* quiescence and may illuminate evolutionary conserved mechanisms required to regulate sleep across species.

613C. A Mutant in Another cGMP-dependent Protein Kinase, PKG-2, is defective in short and long-term odor adaptation. **Eduardo Bernal**, Neomal Muthumala, Ray L Hong. Biology Department, Cal State Northridge, Northridge, CA.

The cGMP-dependent protein kinase EGL-4, also known as PKG-1, plays an important role in body size, locomotion, longevity, chemotaxis, and odor adaptation. Although multiple alleles of *egl-4* has been isolated in various unrelated forward genetic screens, the role of the closest paralog, *pkg-2*, has remained curiously enigmatic due to its glaring absence from similar screens. To address this deficit directly, we have begun analyzing a deletion mutation *pkg-2(tm3878)*. *Pkg-2(tm3878)* exhibits a similar odor adaptation defect as *egl-4(n479)* in diacetyl sensing and is defective in both short and long-term adaptation following 5, 30, and 60 minute odor exposures prior to chemotaxis assays. To determine if these two closely linked genes share redundant or non-over lapping functions, we also analyzed the near-complete loss of both PKG activity in the *egl-4(n479);pkg-2(tm3878)* double mutant.

614A. Effects of mutations in the C. elegans presenilin homologue, sel-12, on tap habituation. **T. Bozorgmehr**[1], C. Rankin[1,2]. 1) Brain Research Center, University of British Columbia,Vancouver, BC, Canada; 2) Department of Psychology, University of British Columbia,Vancouver, BC, Canada.

Alzheimer's disease (AD) is the most common form of age-related dementia in the elderly. Mutations in presenilins are responsible for the vast majority of early-onset familial Alzheimer's disease cases. The presenilin gene is highly conserved through animal kingdom; the C.elegans homologue of presenilin is known as sel-12 and the gene products of sel-12 share 50 to 52% ; amino acid identity with human presenilin. The main purpose of this experiment was to show whether or not sel-12 plays a role in a non-associative learning task in C.elegans: short-term tap habituation. Habituation refers to a decrease in responding to a stimulus that is presented repeatedly, and it is considered to be the simplest form of learning. Habituation in C. elegans was first observed for a reversal response (moving backwards for a short distance) elicited by a mechanical tap to the Petri plate in which the worms live in the laboratory; this response was named the tap withdrawal response. To run this experiment I used a newly developed high-throughput worm tracking system, the Multi Worm Tracker, to screen mutant strains of nematodes for habituation defects. For these studies I measured habituation of reversal probability and reversal distance. I found that a C. elegans strain carrying a loss-of-function mutant allele of sel-12 showed abnormal habituation for response probability. I assessed habituation in several sel-12 loss-of-function alleles and found that the most robust deficit in habituation was found in the strain RB1672 with a big deletion in sel-12; the other strains, characterized as point mutations, showed variations in the habituation deficit. Plasmids for expressing the sel-12 gene under control of the sel-12 promoter and for expressing the wile-type and mutant human presenilin genes with the sel-12 promoter are currently being constructed to rescue the habituation deficit of sel-12. We will be determining which mutations in human presenilin rescue the notch mediated egg-laying deficit and which rescue the habituation phenotype we have observed.

615B. The voltage-gated chloride channels encoded by *clh-3* regulate the excitability of the HSN neurons. **Robyn Branicky**[1], Hiroaki Miyazaki[2], Kevin Strange[2], William R. Schafer[1]. 1) MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge Biomedical Campus, Cambridge CB2 0QH, UK; 2) Boylan Center for Cellular and Molecular Physiology, Mount Desert Island Biological Laboratory, P.O. Box 35 Salisbury Cove, ME 04672, USA.

clh-3 encodes two hyperpolarisation-activated, inwardly-rectifying chloride channel variants, CLH-3a and CLH-3b. Although the properties of these channels as well as those of their mammalian homologue CLC-2 have been well characterised, their function in neurons has not been elucidated. CLC-2 has been proposed to regulate neuronal excitability indirectly by providing a chloride efflux pathway that is necessary for $GABA_A$-receptor mediated inhibition, or alternatively to regulate excitability directly, via a chloride influx that inhibits depolarisation. We have identified a role for *clh-3*-encoded channels in regulating the excitability of the HSN neurons. A gain-of-function mutation that alters the voltage sensitivity of the channels inhibits the egg-laying behaviour of *C. elegans* by suppressing HSN activity. Loss-of-function mutations lead to hyperactive egg-laying, including inappropriate egg-laying in the absence of food, indicating that CLH-3 channels are required for modulating the activity of the HSNs. CLH-3 channels are not required for $GABA_A$-receptor mediated inhibition of the HSN, and do not appear to mediate chloride efflux. Rather, optogenetic and genetic epistasis experiments indicate that these channels inhibit the excitability of the HSN directly by promoting chloride influx. Thus, this family of channels could act generally to inhibit neuronal excitability by providing a pathway for chloride influx.

616C. A quantifiably complete repertoire of *C. elegans* locomotion. **Andre E.X. Brown**[1], Roland Schwarz[2], Robyn Branicky[1], William Schafer[1]. 1) MRC Lab of Molecular Biology, Cambridge, United Kingdom; 2) European Bioinformatics Institute, Hinxton, United Kingdom.

Visible phenotypes have played a critical role in understanding the molecular basis of behaviour in model organisms. However, most current descriptions of behaviour are based on manually identified events or a limited set of quantitative parameters. Here we report an extension of the concept of behavioural motifs to exhaustively catalogue *C. elegans* locomotion and derive a repertoire that is quantifiably complete. A repertoire learned for spontaneous behaviour in wild-type worms can be used to fit data from mutants or worms in different environmental conditions and provides a sensitive

measure of phenotypic similarity. Repertoire comparison can also be used to assess inter-individual variation and the compositionality of behaviour, that is, the extent to which behavioural adaptation involves the creation of novel repertoire elements or the reuse of existing elements in novel sequences. Repertoire derivation is general, so that given a representation of posture, our approach will apply to other organisms.

617A. Acute odor recognition in AWC neuron of *C. elegans* after adaptation. **Chantal Brueggemann**[1], Damien O'Halloran[2], Noelle L'Etoile[1]. 1) Cell and Tissue Biology, University of California, San Francisco, CA; 2) Department of Biological Sciences, George Washington University, Washington, DC.

Odor sensation and chemotaxis are essential responses that allow an organism to locate and move towards food. The paired AWCs neurons sense innately attractive odors such as benzaldehyde, butanone or isoamyl alcohol. However, the organism must also be able to ignore profitless odors. Thus, prolonged exposure of the AWC neurons to benzaldehyde (BA) in the absence of food leads to adaptation of the odor-seeking response. This allows the worm to ignore odors that are not associated with nutrition. Continuous exposure to BA in the absence of food, leads to a relocalization of the cGMP-dependent protein kinase EGL-4 from the cytoplasm into the nucleus. Nuclear EGL-4 is necessary and sufficient to adapt the AWC-mediated response to the odor. We examine the effects of both short- and long-term odor exposure on the acute odor response in AWC and its downstream interneurons by using the GFP-based calcium reporter GCaMP3. We hope to correlate the changes in intercellular calcium flux in response to odor with the subcellular localization of EGL-4 and in parallel with the behavior of the worms.

618B. The AIB interneuron is required for thermotaxis. **Ana C. Calvo**[1], Josh Hawk[1], Nathan Cook[1], Vivek Venkatachalam[2], Aravinthan D.T. Samuel[2], Daniel A. Colon-Ramos[1]. 1) Department of Cell Biology, Yale School of Medicine, New Haven, CT; 2) Dept of Physics & Center for Brain Science, Cambridge MA 02138.

The nematode Caenorhabditis elegans moves towards the cultivation temperature when placed in a thermal gradient, tracking isotherms once this temperature is reached. AFD is the principal thermosensory neuron and is required to perform all modes of thermotactic behavior. But how one neuron can drive the distinct sensorimotor transformations that underlie movement down or up gradients towards the cultivation temperature vs. isothermal tracking near the cultivation temperature is not understood. To study this question, we explored the downstream synaptic pathways from AFD. AFD has direct synaptic output to only two interneurons, chemical synapses to AIY and electrical synapses to AIB. While AIY role in thermotaxis has been widely studied and its inactivation is known to cause a constitutive cryophilic movement (movement towards colder temperatures), little is know of the AIB role. Using a genetic approach based on reconstituted caspases, we have ablated the AIB interneuron and found that these animals display no preference to move up or down the gradient. Moreover, AIB ablated worms show an increased isothermal tracking behavior: they track isotherms at temperatures where wild type animals show cryophilic or thermophilic behavior. Mutants that lack the gap junction between the AFD and AIB neurons (inx-1) exhibit a similar tracking behavior as the one seen in AIB ablated animals. Taken together, these results suggest that electrical and chemical synaptic pathways from the AFD neuron are differentially used to perform distinct modes of thermotaxis.

619C. Oxygen sensing neurons control carbon dioxide response in C. elegans. **M.A. Carrillo**, M.L. Guillermin, S. Rengarajan, R. Okubo, E.A. Hallem. Microbiology, Immunology, and Molecular Genetics, UCLA, Los Angeles , CA.

Many sensory behaviors are flexible, allowing animals to generate context-appropriate responses to changing environmental conditions. To investigate the neural basis of behavioral flexibility, we are examining the regulation of carbon dioxide (CO2) response in C. elegans. CO2 is a critical sensory cue for many animals, mediating responses to food, conspecifics, predators, and hosts. In C. elegans, CO2 response is regulated by the polymorphic neuropeptide receptor NPR-1: animals with the N2 variant of npr-1 avoid CO2, while animals with the Hawaiian (HW) variant or an npr-1 loss-of-function (lf) mutation appear virtually insensitive to CO2. We examined the mechanism by which NPR-1 regulates CO2 avoidance behavior. We found that ablation of URX neurons in npr-1(lf) mutants restores CO2 avoidance, suggesting that NPR-1 enables CO2 avoidance by inhibiting URX. In npr-1(lf) mutants, O2-induced activation of URX inhibits CO2 avoidance. Mutation of the URX-expressed neuropeptide genes flp-19 and flp-8 also restores CO2 avoidance to npr-1(lf) mutants, consistent with the possibility that neuropeptide release by URX regulates CO2 response. In addition, we found that both HW and npr-1(lf) animals avoid CO2 under low O2 conditions, when URX neurons are inactive. Our results suggest that in HW and npr-1(lf) animals, URX neurons control CO2 response by coordinating the response to CO2 with the response to ambient O2 such that CO2 is repulsive at low ambient O2 but neutral at high ambient O2. The fact that wild C. elegans strains contain the HW allele of npr-1 suggests that O2-dependent regulation of CO2 avoidance is likely to be an ecologically relevant mechanism by which nematodes navigate gas gradients.

620A. Natural polymorphisms in HECW-1 E3 ubiquitin ligase affect C. elegans pathogen avoidance behavior. **Howard Chang**[1,2], Dennis Kim[1]. 1) Department of Biology, MIT, Cambridge, MA; 2) Department of Biological Sciences, Binghamton University, Binghamton, NY.

C. elegans elicits aversive behavior when encountering toxic microbes. This avoidance behavior is especially advantageous to *C. elegans* survival when high levels of pathogens are present. Our lab uses *C. elegans* and *P. aeruginosa* as a host and pathogen model to study pathogen avoidance behavior. Previously, we showed that neuropeptide receptor NPR-1 promotes *P. aeruginosa* avoidance and consequently improves *C. elegans* resistance to *P. aeruginosa*. Here, we report that HECW-1, a previously uncharacterized E3 ubiquitin ligase, inhibits *C. elegans* pathogen avoidance and makes *C. elegans* more susceptible to *P. aeruginosa* infection. We also discovered that HECW-1 functions in sensory neurons OLLR and OLLL. Both *hecw-1* deletion mutant animals and animals lacking OLL neurons have nose touch defects. This suggests *C. elegans* may use mechanosensory circuits to detect the presence of *P. aeruginosa*. Finally, we found *hecw-1* acts upstream of *npr-1*, since animals carrying both *hecw-1* and *npr-1* null mutations elicit similar phenotypes as animals carrying *npr-1* alone. Intriguingly, both *hecw-1* and *npr-1* were identified via phenotypic variances contributed by polymorphisms among *C. elegans* wild strains. These naturally occurring polymorphisms may provide survival benefits for *C. elegans* in nature.

621B. Pleiotropic genes affecting touch sensitivity in *C. elegans*. **Xiaoyin Chen**, Martin Chalfie. Biological Sciences, Columbia Univ, New York, NY.

Six touch receptor neurons (TRNs) sense gentle touch along the body in *C. elegans*. Although saturated mutageneses have identified many genes needed for mechanosensation in the TRNs, these screens were ineffective in identifying several groups of genes, including pleiotropic genes. To circumvent this

problem, we used neuronally-enhanced feeding RNAi to screen 1005 pleiotropic genes for effects on mechanosensation and identified 61 genes affecting touch sensitivity. In addition to 11 genes involved in general transcription and translation, which likely play housekeeping functions, the remaining 50 genes are involved in protein degradation, calcium signaling, cell adhesion and cytoskeleton, mitochondrial function, endocytosis and exocytosis, and classical signaling pathways such as wnt, hedgehog, small GTPase and MAP kinase. We further confirmed six genes (*cdk-1*, *tag-170*, *wmr-1*, *ifb-1*, *tom-1*, and *mca-3*) that affect anterior touch sensitivity using available viable alleles. *tag-170* encodes a thioredoxin domain-containing protein required for cell division and microtubule growth. Loss of *tag-170* eliminated acetylated tubulin and disrupted microtubule organization in the ALM and PLM neurons, but less so in the AVM and PVM neurons. Consistent with previous findings of Bounoutas et al. (2011), the disruption of microtubules further led to reduced protein production from genes needed for mechanosensation, including *mec-2* and *mec-18*. *mca-3* encodes a plasma membrane calcium ATPase (PMCA) that should pump cytosolic calcium out of the cell. Mutations in mammalian orthologs of *mca-3* cause deafness and balance defects in rats and exacerbate hearing loss in human. A partial loss-of-function allele of *mca-3* reduced the touch-induced calcium response in the TRNs by four fold, but not the response to potassium depolarization, suggesting that mechanotransduction may be regulated by cytosolic calcium level. In conclusion, reverse genetic screens using neuronally-enhanced feeding RNAi complement forward mutagenesis screens to reveal additional genes needed for TRN development and function.

622C. Neuropeptides can regulate feeding behavior in the absence of MC activity. **Mi Cheong Cheong**[1], Young-Jai You[2], Leon Avery[1]. 1) Department of Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA; 2) Department of Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA.

Food stimulates pumping through MC cholinergic motor neurons. However fasted worms still pump even without MC. Here we found that neuropeptides can regulate feeding in *eat-2* mutants, which lack MC neurotransmission. *eat-2* mutants are functionally MC-minus and become starved even in the presence of food. *egl-3* encodes a proprotein convertase necessary for the maturation of neuropeptides, and *egl-3* mutants lack almost all neuropeptides.*eat-2; egl-3* mutants have a decreased pumping rate compared to *eat-2* mutants. To identify the specific neuropeptides, we did an RNAi screen of 113 neuropeptide genes, testing whether they affect pumping and growth in an *eat-2* mutant background. We found that *nlp-34* RNAi decreased *eat-2* growth rate and *nlp-3* RNAi increased it. *nlp-34*-encoded peptides have a conserved YGGXX sequence, similar to mammalian opioid neuropeptides. The Komuniecki Lab has shown that *nlp-3* signaling is mediated by *npr-17*, which has sequence similarity to opioid receptors. We found that 10 mM naloxone inhibits the pumping rate of wild-type worms, but does not affect *npr-17* mutants. Thus, we suggest that *C. elegans* has an endogenous opioid system that acts through *npr-17*, and that opioids regulate feeding behavior.

623A. Elucidation of a neuronal mechanism of nictation, a dispersal behavior, in *C. elegans*. **Myung-kyu Choi**[1], Harksun Lee[1], Daehan Lee[1], Dongjun Park[1], Junho Lee[1,2]. 1) Institute of Molecular Biology and Genetics, Seoul National University, Seoul, Korea; 2) World Class University Department of Biophysics and Chemical Biology, Seoul National University, Seoul, Korea.

Charles Darwin described that invertebrates can be dispersed by hitch-hiking on other animals. However, a neuronal and molecular mechanism of such dispersal behaviors is not studied well. As a means to dispersal, *C. elegans* exhibits dauer-specific behavior called nictation: standing and waving at the tip of three-dimensional objects. We have shown that nictation is carried out to facilitate relocation to a favorable environment. We also demonstrated that acetylcholine in IL2 ciliated neurons is important for nictation. According to the observation that dauers only nictate in three-dimensional objects such as micro-dirt chips, we have speculated that mechanical stimuli rather than chemical ones provide initial clues to nictation. Now we are searching for external signals that can activate IL2 neurons. For searching better habitats, individuals must make a decision between exploration and exploitation. Nictation is a decision making behavior because nictation is the outcome of the decision of dauers on whether they ambush or cruise. It is known that a neuromodulatory system can offer flexibility to the output of neural circuits. So, we have also established mutant screening about genes involved in neuromodulation. The results will show that stage-specific alteration in neural substrate can affect stage-specific, dispersal behavior.

624B. Single-cell transcriptomic analysis identifies quiescence-inducing neuropeptides. **Elly S. Chow**[1], Erich M. Schwarz[1,2], Paul W. Sternberg[1]. 1) Division of Biology and Howard Hughes Medical Institute, 156-29,California Institute of Technology, Pasadena, CA, 91125, U.S.A; 2) Department of Molecular Biology and Genetics, Biotechnology 351, Cornell University, Ithaca, NY, 14853-2703, U.S.A.

Neuropeptides are found throughout animal nervous systems and are important in regulating many behaviors and physiological functions. We used the nematode Caenorhabditis elegans to explore the interaction of neuropeptides and a conserved sleep regulator, Epidermal Growth Factor Receptor (EGFR) signaling, in a prolonged and reversible sleep-like state in young adults, when animals are normally active. RNA-seq reveals that 12 neuropeptide-encoding genes in C. elegans are enriched in the ALA neuron. Using conditional activation of neuropeptides and genetic screening, we identified a behavioral quiescence regulatory system consisting of two FMRFamide (Phe-Met-Arg-Phe-NH2)-like neuropeptide encoding genes, flp-13 and flp-24, and a neuropeptide-related receptor, npr-22, orthologous to human neuropeptide Y receptor (24.7% identity) and human opiate receptor-like 1 (19% identity). Overexpression of flp-13 and flp-24 reduces locomotion, feeding, and sensory responsiveness. In particular, hs:FLP-13 animals are inert to tactile and chemosensory stimuli while hs:FLP-24 animals exhibit delayed responses to them; and joint induction of hs:FLP-13 and hs:FLP-24 is stronger than either alone, suggesting functional redundancy. Loss of npr-22 function blocks the effects of neuropeptide-induced quiescence behavior. Our results indicate that nematodes and vertebrates share conserved neural signals that regulate sleep.

625C. Investigating the role of polycystins in sex pheromone chemotaxis of male *C. elegans*. Ching-Ki Li, **King-Lau Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

Like many animals, nematodes rely on sensory modalities to locate their mating partners. Although *Caenorhabditis elegans* is hermaphroditic, the males can sense the sex pheromone produced by the females of a dioecious *C. remanei*. We use *C. elegans* to study how animals perceive chemical signal that elicits sexual behavior. Four male-specific CEM neurons are required for sex pheromone chemotaxis relying on the transient receptor potential polycystin (TRPP) channel encoded by LOV-1 and PKD-2. These two molecules are localized at the cilia of CEMs and have been speculated to act in these cells to modulate chemotactic response. They are indispensable for males to display a normal sex pheromone response. Interestingly, polycystins were only

implicated in mammalian system for mechanosensory function but not chemosensory activities. With the use of optical imaging, we were able to document a calcium transient elicited by sex pheromone in CEMs. To validate this novel function, we monitor cellular responses of CEMs in *pkd-2* and *lov-1* sensitized backgrounds. Results from this experiment would yield definitively the functional activity of polycystins as chemosensory components. In addition, we are interested in probing into the resemblance of LOV-1/PKD-2 and their mammalian counterparts in regulating G protein signaling. In *C. elegans*, G protein signaling is required for transducing the sex pheromone signal in AWAs. However, its involvement in CEMs signal transduction remains unknown. The *C. elegans* genome encodes 21 G-alpha, 2 G-beta and 2 G-gamma subunits. We systematically knock down G proteins in a cell-specific manner to determine whether G proteins act in CEMs and hence whether they act downstream of LOV-1/PKD-2. The result of this characterization would be presented. The outcome would shed light on the mechanism of polycystin channel signaling. (This study is supported by Research Grants Council, Hong Kong.).

626A. SRD-1 is required for *C. elegans* males to respond to the *Caenorhabditis remanei* female sex-pheromone. Hainan Yang, Yuan Zhou, **King-Lau Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

Dioecious *Caenorhabditis remanei* and androdioecious *C. elegans* are two closely related species. Despite their distinct mating systems and evolutionary divergence, our lab has demonstrated that virgin *C. remanei* females secrete a potent sex-specific pheromone that attracts young adult males of both species from afar. Intriguingly, this chemotaxic behavior requires AWA and CEM neurons in male *C. elegans*, probably via different receptors expressed on these neurons. Here I will present our experiment data suggesting the requirement of the G-protein coupled receptor SRD-1 in AWA neurons in male *C. elegans* for sensing the pheromone secreted by *C. remanei* females. The *srd-1* was found expressed in both AWA and ASI neurons. The sub-cellular localization of SRD-1 receptor in male AWA neuron was found in the tip of cilia. Using the chemotaxis assay, we found that the *srd-1* mutant males lose their pheromone responsiveness. This defect can be rescued using *srd-1* cDNA driven by AWA specific promoter of gene *odr-7*. Ectopic expression of *srd-1* in AWB neuron in *srd-1* mutant male elicits a mild repulsive behavior against the pheromone. We visualized the excitation of AWA neurons upon treatments of multiple odors and found that AWA neurons of the *srd-1* mutant males cannot be stimulated by the pheromone extract, but is responsive to other chemoattractants. Consistent with this finding, heterologously expressing *srd-1* cDNA in HEK293 FT mammalian cell line can mildly confer the host sensitivity to concentrated pheromone extract. Moreover, we employed the *C. elegans* ARR-1 protein from the desensitization pathway and constructed a protein complementation system with two split beta-lactamase fragments separately tagged onto ARR-1 and GPCRs (ODR-10 and SRD-1). When expressing the system in HEK293 FT cell line, we visualized the desensitization of both ODR-10 and SRD-1 upon the stimuli of diacetyl and concentrated pheromone extract, respectively, using an auto FRET substrate of beta-lactamase, CCF-2 AM. With the above evidence, we conclude that SRD-1 is one receptor responsible for sensing active pheromone extract component derived from virgin females of *C. remanei*. (This research is supported by Research Grants Council, Hong Kong).

627B. AWC Neurons Mediate Navigation in a DC Electric Field. **Steven D. Chrisman**, Christopher B. Waite, Eric P. Foss, Lucinda Carnell. Biological Sciences, Central Washington University, Ellensburg, WA.

C. elegans will orient and travel in a straight uninterrupted path directly towards the negative pole of a DC electric field, a behavior referred to as electrotaxis[1]. Additionally, animals widen their approach angles proportionally to increasing field strength[2]. To elucidate the neural basis for this behavior, we examined populations of animals in a uniform field that is fixed in direction and magnitude. We have determined that *C. elegans* navigate in order to remain within a specific range of field strengths, which is the reason for the changes in their approach angles towards the negative pole. *eat-4* mutants are severely electrotaxis defective and addition of the wild-type *eat-4* gene in AWC neurons recovers the behavior. The pair of AWC neurons are functionally asymmetric in regard to chemotaxis and have been shown to express different genes. In particular, one of the AWC neurons expresses STR-2, a G-protein coupled receptor, and is referred to as AWC^{ON}. To test the role of the AWC neurons in electrotaxis behavior we examined the following mutant animals: *ceh-36*, which are defective in the terminal differentiation of the AWC neurons, *inx-19*, which have two $AWC^{OFF/OFF}$ neurons, and *nsy-1*, which have two $AWC^{ON/ON}$ neurons. We found that only *nsy-1* mutant animals are able to sense field gradients, suggesting AWC^{ON} is required for electrotaxis behavior. Although STR-2 is expressed only in AWC^{ON}, *str-2* mutants exhibit wild-type behavior indicating STR-2 is not an electroreceptor. Here, we demonstrated that AWC^{ON} functions as an electrosensory neuron allowing animals to sense and adjust approach trajectories angles to match the preferred field strength.

1. Sukul NC, Croll NA. 1978. Influence of Potential Difference and Current on the Electrotaxis of *Caenorhabditis elegans*. J Nematol 10:314-317.
2. Gabel CV, Gabel H, Pavlichin D, Kao A, Clark DA, Samuel AD. 2007. Neural circuits mediate electrosensory behavior in *Caenorhabditis elegans*. J Neurosci 27:7586-7596.

628C. Dopamine down-modulates the activity of a re-current circuit via D2-like signaling during male mating. **Paola Correa**[1], Luis Rene Garcia[2]. 1) Texas A&M, College Station, TX; 2) Howard Hughes Medical Institute, Chevy Chase, MD.

Neuro-modulation of self-amplifying circuits is required to drive the execution of behaviors to their appropriate context. Even though recurrent neuronal networks are found throughout the C. elegans connectome, the mechanism that fine-tunes the activity of these reciprocal synapses is unknown. In our work we dissect the cellular and molecular components involved in male copulation, a goal oriented behavior that encompasses multiple sub-steps that entail initiation and termination under appropriate circumstances. The C. elegans male mating circuit integrates sensory-motor cues that result in successful insertion of a pair of copulatory spicules into the hermaphrodite vulva. During mating, the post-cloaca-sensila (p.c.s.) and their post-synaptic sex muscles maintain the male's position over the vulva. Simultaneously, the cholinergic p.c.s. neurons and the reciprocally synapsed glutamatergic PCA neuron trigger rhythmic spicule thrusts. However, distinct signaling mechanisms that restrict repetitive spicule movements to vulva cues are unclear. We found that dopamine (DA) signaling directs spicule insertion attempts to the hermaphrodite vulva by dampening stimulus-independent activity of the spicule circuit. Our pharmacogenetic analyses indicate that DA antagonizes stimulatory ACh signaling via the D2-like receptors, DOP2 and DOP3 and Gao/i proteins, GOA-1and GPA-7. Live calcium imaging results suggest that DA ray neurons are coincidentally active with the cholinergic and glutamatergic cloacal ganglia when the male thrusts his spicules against the vulva. Via optogenetics and targeted illumination we find that activity in a pair of DA neurons can be triggered by stimulation of the p.c.s., and that in a hypodopaminergic state spurious spicule thrusts last even after artificial PCA stimulation is

removed. Furthermore, D2-like receptor signaling attenuates the excitability of additional mating circuits to reduce the duration of unproductive mating attempts. Therefore, during a looped behavioral routine, such as spicule insertion attempts, DA down-modulates the activity threshold of a recurrent circuit, confining the behavior to a proper context.

629A. Uncovering the molecular basis for ethanol action on the BK channel using genetic screens. **Scott Davis**, Kevin Hu, Jon Pierce-Shimomura. The Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX.

Development of alcohol abuse has a strong genetic component. People who are innately resistant to intoxication are at high risk to abuse alcohol and vice versa. This genetic predisposition is linked to variation in genes that encode targets of alcohol or their downstream effectors involved in intoxication. Genetic screens in *C. elegans* previously determined that the gene *slo-1* represents a central target for alcohol intoxication in the worm. *slo-1* encodes the highly conserved large-conductance, calcium-activated potassium (BK) channel. The BK channel has emerged as a key mediator for intoxication and tolerance across species from worm, fly, mouse to human. At the physiological level, BK channel activity is modulated by clinically relevant concentrations of ethanol (~20mM). To reveal specific residues in the BK channel that are required for activation by alcohol, and thus may influence sensitivity to behavioral intoxication, we are performing multiple genetic screens. First, we are screening *slo-1* mutants from the million mutation project. Second, we are performing site-directed mutagenesis on the worm and human BK channels. We are able to study the human BK channel in the worm because we rescued ethanol sensitivity in a *slo-1* null worm by expression of *hslo*. Novel mutants that are resistant to intoxication will either contain 1) a null mutation in *slo-1* that provides no new information, or 2) a novel non-null mutation that provides insight into how specific residues on the BK channel interact with alcohol, and how this interaction alters intoxication. To distinguish non-null candidates, the locomotor posture of mutants recovered from the genetic screens will be compared against wild-type and a known *slo-1* null mutant. Candidate non-null mutants will display different locomotor posture than the known *slo-1* null mutant. Currently, we have isolated multiple candidate non-null mutants with resistance to intoxication. Further analysis using *in vivo* patch-clamp recordings will assess how specific mutations alter basal BK channel function and the response to alcohol.

630B. Genetic sex alters the logic of sensory behavior. **Kelli A. Fagan**[1], Jessica R. Bennett[1], Frank C. Schroeder[2], Douglas S. Portman[1]. 1) Center for Neural Development & Disease, University of Rochester, Rochester, NY 14642; 2) Boyce Thompson Institute at Cornell University, Ithaca, NY 14853.

Hard-wired neural circuits can be regulated to generate variations in behavior. This behavioral flexibility allows animals to respond appropriately to changing environmental conditions and increases the likelihood of survival and reproductive success. In many animals, behavior is modulated by the sex chromosomal content, or genetic sex, of the nervous system. However, the mechanisms by which genetic sex regulates neural circuit activity are largely unknown. In order to better understand these, we are investigating the sexually dimorphic attraction behavior displayed by *C. elegans* males in response to ascaroside pheromones. By genetically reprogramming the sexual state of the nervous system, we found that the circuitry required for ascaroside attraction is present in both sexes. However, this circuitry is differentially regulated by genetic sex to generate differences in ascaroside attraction behavior. Next, we asked where specific sites of sexual regulation are located in the ascaroside attraction circuit. We found that genetic sex-reversal of shared sensory neurons and interneurons can disrupt attraction behavior. This suggests that genetic sex has distributed effects on the ascaroside circuit, consistent with previous work on non-ascaroside pheromones (White & Jorgensen, 2012, Neuron 75:593). Additionally, we found that serotonergic and neuropeptidergic signaling is required for male ascaroside attraction. Both of these signals appear to also be necessary for attraction in pan-neural sex-reversed hermaphrodites, suggesting that signaling through these pathways occurs in shared circuitry. Finally, we have found that the DM domain transcription factor *mab-3* acts in males to promote ascaroside attraction. Together, these data raise the possibility that *mab-3* may bring about sex-specific differences in neuromodulatory signaling to alter circuit activity and thereby promote male-specific ascaroside attraction.

631C. HLH-17 Dependent Regulation of the Dopamine Transporter Gene, *dat-1* and the Dopamine Receptor Gene, *dop-3* Can Be Tied to the Oxidative Stress Response in *C. elegans*. **Chaquettea M Felton**, Casonya Johnson. Biology, Georgia State University, Atlanta , GA.

In *Caenorhabditis elegans*, the dopamine transporter DAT-1 regulates synaptic DA signaling by controlling extracellular DA levels. In *dat-1* (ok157) animals, DA is not taken back up presynaptically but instead reaches extrasynpatic sites where it activates the dopamine receptor, DOP-3 on choligeneric motor neurons and causes animals to become paralyzed in water. This phenotype is called Swimming Induced Paralysis (SWIP) and is dependent on *dat-1* and *dop-3*. Upstream regulators of *dat-1* and *dop-3* have yet to be identified. In our previous studies, we defined a role for HLH-17 during dopamine response. To further characterize HLH-17, we wanted to determine if the loss-of *hlh-1*7 would also affect dopamine reuptake. Here, through qPCR we show that HLH-17 can regulate the expression of both *dop-3* and *dat-1*. Using the SWIP assay we show that animals lacking functional HLH-17 have defects in dopamine transport. Since DAT-1 has also been associated with the oxidative stress response, we wanted to know if *hlh-17* (ns204) animals would also have a defect in their ability to respond to oxidative stress. We determined that *hlh-17*(ns204) animals are less sensitive to oxidative stress. This work suggests that HLH-17 is required to maintain normal levels of dopamine in the presynaptic cleft through its regulation of *dop-3* and *dat-1*. This work will help us to better understand the relationship between dopamine signaling and the oxidative stress response.

632A. Caenorhabditis-in-Drop (CiD) method to measure worm behavior and longevity. **Benjamin L Freedman**[1], Samy Belfer[1], Han-Sheng Chuan[2], Jinzhou Yuan[2], Michael Norton[2], Haim Bau[2], David Raizen[1]. 1) Department of Neurology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; 2) Department of Mechanical Engineering and Applied Mechanics, University of Pennsylvania, Philadelphia, PA.

Lethargus—a sleep-like stage occurring during C. elegans larval development—is regulated by some of the same mechanisms that regulate sleep in mammals and fruit flies, suggesting that lethargus can be used to study sleep. To monitor the behavioral quiescence of 24 nematodes simultaneously, we have developed the Caenorhabditis-in-Drop (CiD) method, which entails automated videography of worms housed individually in 125 nL aqueous droplets on a polydimethylsiloxane (PDMS) surface and covered with mineral oil. We used the CiD method to demonstrate reduced quiescence in kin-2 mutants, and to reproduce previous observations of increased quiescence in animals over-expressing LIN-3C (Van Buskirk and Sternberg 07). We found that egl-4 mutants, which have reduced quiescence when housed on an agar surface (Raizen et al, 08), had no change in lethargus quiescence in CiD. This indicates that the chamber used to house the animal can affect behavior and raises a note of caution in the interpretation of both positive and negative results. In

comparison to behavior on an agar surface, animals in CiD were more active and showed reduced survival, with most animals dead after one week. Housing the worms in a 10-fold higher volume (1250 nL) of aqueous solution on a PDMS surface increased survival in comparison to survival in 125-nL droplets, indicating that reduced longevity is not explained by the liquid habitat, by exposure to mineral oil, or by exposure to the PDMS surface. Surprisingly, animals survived longer in 125-nL droplets that previously housed a worm for a week.

633B. ASI regulates satiety quiescence. **Thomas L. Gallagher**[1], Leon Avery[2], Young-jai You[1]. 1) Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA; 2) Physiology and Biophysics, Virginia Commonwealth University, Richmond, VA.

Satiety quiescence is a worm behavior that mimics mammalian satiety. We have developed an automated and quantitative worm locomotion tracking system and Hidden Markov Model analysis to better study this behavior. This identifies worm locomotive behavioral states (quiescence, roaming, and dwelling) over time, quantifies the percent time in each state, and finds the frequency of switching between states. This allows us to study the genes and neuronal circuitry that affect these behavioral characteristics. Our data has validated previous results establishing that quiescence depends on feeding history and food quality and is conveyed by *egl-4* (cyclic GMP dependent protein kinase) and *daf-7* (TGF-b). Restoring *egl-4* expression in a dozen head neurons under the *tax-4* promoter rescues the quiescence defect in *egl-4* worms. Restoring either *egl-4* or constitutively active *egl-4* in the ASI neuron pair alone (included in the *tax-4* set) partially rescues quiescence in *egl-4* worms. Additionally we found that the ASI neuron promotes quiescence by inhibiting the switch from quiescence to dwelling and accelerating the switch from dwelling to quiescence; genetic ablation of ASI results decreases quiescence in both fasted-refed and nonfasted worms. Using calcium imaging, we found that ASI is activated by nutritional content of its environment, suggesting that ASI connects sensing nutritional status to promotion of satiety quiescence.

634C. Neuropeptide modulation of C. elegans light avoidance circuitry. **D. Dipon Ghosh**[1], Michael R. Koelle[2], Michael N. Nitabach[1,3,4]. 1) Dept. of Cellular and Molecular Physiology, Yale University, New Haven, CT; 2) Dept. of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT; 3) Dept. of Genetics, Yale University, New Haven, CT; 4) Program in Cellular Neuroscience, Neurodegeneration, and Repair, Yale University, New Haven, CT.

Neuropeptides modulate neuronal circuit activity to regulate animal behavior. We used Caenorhabditis elegans as a model system to investigate the mechanism by which these neuromodulators fine tune cellular circuit outputs to optimize behavioral responses. Recently, homologs of an arthropod peptide Pigment Dispersing Factor (PDF) and its cognate receptor, PDF Receptor (PDFR), were identified in C. elegans. Secretion of PDF and its mammalian relative, Vasoactive Intestinal Peptide (VIP), is triggered by activation of light sensors and can modulate locomotor rhythms in insects and mammals, respectively. We found that null mutant worms lacking a PDF homolog exhibit aberrant light avoidance responses. Further analysis of these deficits indicates that this peptide could function in sensorimotor circuitry to modulate navigation in the presence of light. Our results suggest a link between circadian light entrainment in mammals and photophobic responses in the simpler worm, and a physiological mechanism by which neuromodulators regulate behaviors.

635A. Modular genetic architecture shapes individual variation in innate avoidance behavior in *C. elegans*. **Rajarshi Ghosh**[1], Aylia Mohammadi[2], William Ryu[2], Leonid Kruglyak[1]. 1) Lewis Sigler Institute for Integrative Genomics, Department of Ecology and Evolutionary biology, Princeton University, Princeton, NJ; 2) Department of Physics & Cell and Systems Biology Banting and Best Department of Medical Research University of Toronto 60 St. George St., Toronto, Canada.

Individuals behave differently in response to a given stimulus. The origins of behavioral variation among individuals continue to be a grand challenge. These differences could be innate i.e. attributed to genetic causes and/or due to differences in experience or environment. We are interested in identifying the genetic changes that manifest in individual differences in behavior.

To address this we developed a novel assay where we transiently raised the local temperature around a worm to noxious levels and simultaneously quantified several aspects of the resulting escape behavior. We quantified multiple aspects of this thermal-pulse-induced escape response for N2 and a Hawaiian wild isolate. We found that various aspects of the thermal avoidance response differed between these two strains. To identify the genetic basis of these differences we took a quantitative trait loci (QTL) mapping approach. QTL analysis of different aspects thermal avoidance behavior identified six distinct loci underlying different metrics of escape behavior. These data suggest that different features of the escape response are under discrete genetic control. Using a panel of introgression lines and transgenic rescue we are currently fine mapping the QTL to identify causative gene(s). We also conducted association mapping with wild isolates of *C. elegans* that capture most of the natural genetic diversity of the species. The pattern of detected loci suggests that, as with the QTL mapping, discrete genetic loci underlie different aspects of the escape behavior. Through QTL and association mapping we identified genetic constraints that may shape the evolution of avoidance response in *C. elegans*.

636B. Toward the identification of behavioral strategies underlying *C. elegans* thermotaxis using the Multi-Worm Tracker. **Andrew C. Giles**, Yuki Tsukada, Shunji Nakano, Ikue Mori. Division of Biological Science, Nagoya University, Nagoya, Japan.

C. elegans navigate thermal gradients to find their cultivation temperature. Studying mechanisms of thermotaxis will provide insight into neural functions including thermosensation, memory and the computations that neurons use to integrate information and decide on motor output. Assays that measure the accumulation of worms on thermal gradients rapidly quantify thermotaxis but are not capable of determining the behavioral strategies that worms utilize while navigating. Analyses of locomotion on thermal gradients and in response to temperature ramps have revealed some of the underlying strategies, such as biased random walk during cryophilic movement, but have not yet identified components needed for other aspects of the behavior, including thermophilic movement. To develop a high-throughput assay that captures components of locomotion in environments where thermophilic movement has been observed, we adapted the Multi-Worm Tracker (MWT), a software-hardware package that measures locomotion-based behavior for dozens of worms simultaneously, to work with our accumulation assay. There were two main obstacles: lighting and the size of the field of view. We found that using black anodized aluminum under the assay plate to transmit the thermal gradient in combination with low angle lighting above provided good contrast between worms and background. To image a 13.5 x 10 cm thermotaxis assay plate, we found the Dalsa Falcon 4 MP camera previously used by the MWT did not provide enough resolution to adequately visualize body shape, which impaired automated identification of certain components of

locomotion. We found the Toshiba-Teli CleverDragon 12 MP camera was compatible and improved the imaging of body shape and locomotion while maintaining adequate temporal resolution (~13 Hz). We conclude that this setup can be used to track locomotion components during multi-worm thermotaxis assays and can likely be applied to analyze other behaviors that require a large assay area, such as chemotaxis. We hope that high-throughput analysis with this system will reveal more of the behavioral strategies underlying *C. elegans* thermotaxis behavior.

637C. The neuronal basis of food choice behavior in *Caenorhabditis elegans*. Brian Conroy, Maria Morabe, Lillian Haynes, Melissa Chambers, Rachel Macfarlane, **Elizabeth Glater**. Department of Biology, Harvey Mudd College, Claremont, CA.

Caenorhabditis elegans uses chemosensation to distinguish among various species of bacteria, their major food source (Ha et al., 2010; Shtonda and Avery, 2006). Although the neurons required for the detection of specific food-odors have been well-defined (Bargmann, 2006), less is known about the sensory circuits underlying the discrimination among the mixtures of odors released by bacteria. We plan to examine the neural machinery underlying bacterial preference among a diverse set of bacterial species. Does bacterial choice use one common neuronal mechanism or a diversity of mechanisms depending on the bacteria? Do some bacterial choices involve a single sensory neuron and others involve multiple sensory neurons? To address these questions, we are testing the food preferences of *C. elegans* for bacteria found in their natural habitats (kindly provided by Marie-Anne Felix, Institut Jacques Monod, Paris, France). We have found that *C. elegans* strongly prefers the odors of *Providencia sp.*, *Alcaligenes sp.*, and *Flavobacteria sp.*, to *Escherichia coli* HB101, a commonly used food source for *C. elegans*. We have identified that the olfactory neuron AWC is necessary for this preference. We intend to test whether other amphid sensory neurons are also necessary for bacterial preference. In the future we will extend our analysis to other bacterial species to determine the diversity of the underlying neuronal mechanisms.

Bargmann, C.I. (2006). http://www.wormbook.org.
Ha, H.I., Hendricks, M., Shen, Y., Gabel, C.V., Fang-Yen, C., Qin, Y., Colon-Ramos, D.,
Shen, K., Samuel, A.D., and Zhang, Y. (2010). Neuron 68, 1173-1186.
Shtonda, B.B., and Avery, L. (2006). J Exp Biol 209, 89-102.

638A. Unbiased optogenetic circuit mapping: AVK interneurons, a case study. C. Schultheis, K. Erbguth, S. Wabnig, M. Brauner, **A. Gottschalk**. Buchmann Institute, Goethe University, Frankfurt, Germany.

C. elegans has only 302 neurons, for many of which still no function is known. Optogenetic tools offer the possibility to map the function of each neuron in behavior, provided they are specifically activated in these neurons. We began to map the function of previously uncharacterized neurons. As a first example, we analyzed AVK interneurons, taking advantage of the availability of an AVK-specific promoter. Photoinhibiting AVK interneurons using halorhodopsin strongly affected behavior: Animals reduced swimming cycles by »50%, and showed deeper body bends on solid. In contrast, photostimulation using ChR2 had no obvious effects. Genetic ablation of AVK by caspase expression or acute ablation using miniSOG (minimal singlet O_2 generator), as well as specific expression of tetanus toxin (abolishing chemical transmission), recapitulated the hyperpolarization phenotypes. This indicates that AVK tonically releases a transmitter to mediate normal locomotion. This transmitter may be FLP-1 neuropeptides, as *flp-1* knockdown specifically in AVK had similar effects on swimming behavior, AVK::FLP-1 expression in *flp-1* mutants recued the phenotypes, and AVK::FLP-1::mCherry was detectable in coelomocytes, verifying that AVK releases FLP-1 peptides. Also, lack of FLP-1 or of AVK led to increased local dwelling of the animals. AVK neurons span the length of the animal and may be proprioceptive, as body bending during semi-restrained locomotion caused Ca^{2+} fluctuations in the AVK nervering process. AVK may act antagonistically to the proprioceptive DVA neuron, photoactivation of which increases body bends, while photoinhibition of DVA causes decreased bending. Both AVK and DVA are likely modulated by dopamine (DA), released from PDE neurons, which are the most prominent presynaptic partners of both AVK and DVA. Importantly, photostimulated DA transmission leads to reduced bending angles and a slowing of locomotion. We analyzed which DA receptors mediate this modulation in AVK and DVA. Furthermore, we currently analyze neurons acting downstream of AVK for their roles in this novel pathway impacting on locomotion control in *C. elegans*. Our approach should contribute to a holistic understanding of the *C. elegans* nervous system.

639B. Monoamines amplify and focus global peptidergic signaling cascades to modulate nociceptive responses in *Caenorhabditis elegans*. **V. Hapiak[1,2]**, A. Stein[1], W.J. Law[1], A. Ortega[1], R.W. Komuniecki[1]. 1) Department of Biology, Brandeis University, Waltham, MA; 2) Department of Biological Sciences, University of Toledo, Toledo, OH.

Most key behaviors in *C. elegans* are modulated by monoamines through 14 distinct monoamine receptors, many with clear homologues in vertebrates. For example, aversive responses mediated by the two nociceptive ASH sensory neurons are inhibited by both tyramine (TA) and octopamine (OA) that appear to function similarly to epinephrine and norepinephrine in vertebrates. The present study demonstrates that the TA inhibition of food or serotonin-stimulated aversive responses to dilute 1-octanol requires the Ga_q-coupled TA receptor, TYRA-3 and the activation of a global “inhibitory” signaling cascade involving a complex mix of additional monoamines, including OA and dopamine, and neuropeptides. Importantly, the neuropeptides released from the ASI sensory neurons required for either tyraminergic or octopaminergic inhibition of aversive responses are distinct, suggesting that individual monoamines can stimulate the release of different subsets of ASI neuropeptides. Together, these studies highlight the complexity of TA inhibition, with TA activating a widespread signaling cascade, and suggest that signaling from a complex “humoral soup” of monoamines is amplified and focused by the localized synaptic (perisynaptic) release of neuropeptides to define nutritional status in the modulation of a range of sensory-mediated behaviors. In addition, they suggest that the TA and OA inhibition of aversive responses in *C. elegans* parallels the noradrenergic modulation of pain in mammals where noradrenergic inhibition also activates global, inhibitory, peptidergic signaling cascades. These studies are continuing to define the relationship between monoaminergic and peptidergic signaling in the modulation of sensory-mediated locomotory decision-making. These studies were supported by NIH Grant AI072644 to RWK.

640C. How do worms choose the right food? - Dissecting the signaling mechanisms underlying preference of food odors. **Gareth Harris**[1], Yu Shen[1], Heon-ick Ha[1], Alessandra Donato[2], Xiaodong Zhang[1], Yun Zhang[1]. 1) Department of Organismic and Evolutionary Biology, Centre for Brain Sciences, Harvard University, Cambridge, MA; 2) Institute of Genetics and Biophysics, Via Pietro Castellino 111 - 80131 Napoli, Italy.

Food is an essential environmental cue. Many organisms, including *Caenorhabditis elegans*, utilize chemosensory systems and locomotory strategies to sense food availability and to locate preferred food sources that are essential for survival. However, the signaling mechanisms underlying food-seeking decisions are not well understood. Previously, we have mapped an olfactory circuit required for animals to generate olfactory preference between two bacterial foods (Ha et al., 2010). Here, we characterize the molecular and cellular events that act in this neuronal network to regulate sensory-motor behavior during food odor preference. Using a combination of behavior, reverse genetics and physiological analysis, we show that both of the major olfactory sensory neurons, AWB and AWC, are required for food-odor preferences via sensory-motor control of locomotion. A canonical signaling pathway of Ga protein signaling, together with two guanylate cyclases and a cGMP-gated channel regulate behavioral responses to food odors. Further, the Ga protein signaling also regulates neuronal calcium response of AWB and AWC sensory neurons during food-odor perception. These food-odor evoked signals are transmitted from AWC via co-transmission of glutamate and neuropeptides and from AWB via neuropeptidergic signals and predicted downstream neuropeptide receptors. Furthermore, sensory dependent glutamate signaling requires a combinational function of the AMPA and Kainate-like glutamate receptor subunits to generate food odor preference. These mechanistic logics together with a previously mapped neural circuit provides a functional network that links sensory transduction, sensory signaling output and downstream receptor targets during the perception and processing of complex odors to generate the appropriate behavioral decision essential for survival.

641A. Exploring the low-dose activating effects of ethanol in *C. elegans*. **E. G. Hawkins**, J. C. Bettinger, A. G. Davies. Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

We use *C. elegans* to study the molecular mechanisms behind the intoxicating effects of ethanol, which are not well understood. Ethanol produces a biphasic behavioral response (activation at lower concentrations, depression at higher concentrations) in both vertebrates and invertebrates. The purpose of this study is to determine the molecular mechanisms that mediate an activation response that alters locomotion speed. We have observed that ethanol causes a prolonged maintenance of speed over time under conditions where the speed of wild-type worms would normally decline. Wild-type worms are first deprived of food for 30 minutes and then are moved to seeded plates that contain either no ethanol (control) or 200mM ethanol (treated). The ethanol-treated worms maintain a constant speed or slightly increase their speed over the course of 50 minutes in contrast to control worms whose speed declines by 62%. We calculate a % relative speed (treated speed/untreated speed x100) to take into account the basal speed at any particular time point. Ethanol dose-dependently inhibits the speed decline; this results in an activated phenotype in the presence of ethanol when on food (N2 at 30 min=168.87±14.10% of untreated). The basal slowing response on food is dopamine-mediated, but we have observed no effect on the ethanol-induced activation response of animals that are defective in dopamine synthesis; *cat-1(e1111)* and *cat-2(e1112)* mutants have reduced levels of dopamine and normal activation (189.46±21.95%, 162.58±26.39% respectively) compared to N2 (168.87±14.10%). We observed a similar outcome with mutants of the dopamine receptors DOP-1, -2, -3 and -4. Serotonin plays a role in the enhanced slowing response when worms are starved before reintroduction to food, however, neither of the serotonin deficient mutants we tested had defects in the ethanol activation effect; *cat-1(e1111)* (see above), and *bas-1(ad446)* (158.34±21.43%), were not different from N2. These data suggest that neither dopamine nor serotonin is responsible for ethanol-induced locomotor activation on food. Future studies will investigate the role of other neurotransmitters, neuropeptides and signaling molecules in this behavioral response.

642B. A Common Behavioral Model Underlies the Motility of a Diverse Set of Nematodes. **Stephen J Helms**[1], Leon Avery[2], Greg J Stephens[3], Thomas S Shimizu[1]. 1) Systems Biology Group, FOM Institute AMOLF, Amsterdam, Noord-Holland, Netherlands; 2) Dept. of Physiology and Biophysics, Virginia Commonwealth Univ., Richmond, VA; 3) Dept. of Physics, Vrije Universiteit Amsterdam, Noord-Holland, Netherlands.

Animal behavior emerges from many layers of biological organization—from molecular signaling pathways and neuronal networks to mechanical outputs of muscles. Naively, the large number of interconnected variables at each of these layers would seem to imply dynamics that are complex and hard to control or even tinker with. Yet, for organisms to survive in a competitive, ever-changing environment, behavior must readily adapt. We have quantitatively characterized motile behavior in a diverse set of nematodes spanning the lab strain *C. elegans* N2 to wild strains and distant species. Using high-resolution imaging, we measured the centroid trajectory and body shape dynamics of individual worms moving on agar plates surrounded by a repellant boundary. We found that a simple physical model accounting for variable speed, stochastic reversal events, and an anomalously diffusing bearing can describe all the tested species. Furthermore, these behaviors are driven by conserved patterns in the postures adopted by the worms. Finally, we found that the behavioral parameters varied over time, among individuals, and across strains and species in a correlated way that is consistent with a trade-off between exploratory and exploitative behavior. This suggests a surprisingly simple underlying mechanism controlling variation in motile behavior that matches likely relevant ecological constraints.

643C. A genetic screen for Notch downstream targets regulating *C. elegans* sleep. **Huiyan Huang**[1], Chen-Tsen Zhu[2], Anne Hart[1]. 1) Department of Neuroscience, Brown University, Providence, RI; 2) Department of Ecology and Evolution, Brown University, Providence, RI.

The conserved Notch signaling pathway plays well-defined roles in cell fate determination during development. Recently, Notch has also been implicated in non-developmental neuronal processes across species, including invertebrate sleep. In *C. elegans*, the level of Notch activity affects both quality and quantity of lethargus quiescence, a sleep-like behavior coinciding with larval molts. Also, transiently overexpressing the Notch co-ligand, OSM-11, is sufficient to drive inappropriate quiescence in adult animals, which can be suppressed by loss of either Notch receptor or downstream players in Notch signaling (Singh *et al.*, 2011). To find pertinent transcriptional targets of the Notch pathway and to gain insight into regulation of sleep, we undertook a classical suppressor screen to identify suppressors of inappropriate quiescence in adult *hsp::osm-11* animals. In this screen, 2122 mutant lines were assessed; 79 independent isolates suppressed the OSM-11-induced inappropriate quiescence. To exclude mutations with pervasive effects on locomotion, we examined endogenous L4/adult lethargus quiescence in these 79 strains using both an adaption of the Multi-Worm Tracker (Swierczek *et al.*, 2011) and the microfluidic chamber-based assay (Singh *et al.*, 2011). Twenty-seven strains had defects in endogenous lethargus quiescence. We are in the process of

identifying the corresponding genes by whole genome sequencing. As Notch signaling also modulates sleep behavior in *Drosophila* (Seugnet *et al.*, 2011), we are confident that the genes identified in this screen will be conserved regulators of arousal and sleep across species.

644A. A calcium-rich breakfast: physiological activity in C. elegans serotonergic neurons during the enhanced slowing response and upon emergence from lethargus. **Shachar Iwanir**, Adam Brown, Dana Najjar, Meagan Palmer, Ivy Fitzgerald, David Biron. The Department of Physics, JFI /IBD, The University of Chicago, Chicago, IL 60637.

Serotonin has been implicated in the assessment of resource availability and in the modulation of sleep in vertebrates. Extracellular serotonin levels in the hypothalamus are increased by the presence of food. Additionally, the firing of serotonergic cells in the brainstem raphe nuclei is tonic during wakefulness and significantly reduced during sleep states. In the nematode Caenorhabditis elegans, serotonin signaling has been shown to mediate the enhancing effect of starvation on the slowing response to encountering a food source. However, the understanding of the cellular mechanisms and physiological dynamics underlying this response is incomplete. We combined behavioral studies with physiological imaging of calcium dynamics in the serotonergic NSM and ADF neurons of freely-behaving animals in order to compare the responses to reintroduction of food in two situations: following a period of food deprivation and at the emergence from lethargus, a sleep-like state that involves a cessation of feeding. We identified similarities in the patterns of locomotion and physiological activity of NSM between these two circumstances. The enhanced slowing response was characterized by two phases: (i) preceding a spatial encounter with food, physiological activity in ADF neurons increased (likely via a chemosensory mechanism), concomitant with a gradual decrease in the rate of locomotion, and (ii) upon encountering food, NSM exhibited calcium transients lasting 1-2 minutes, accompanied by a sharp decrease in the rate of locomotion. Emergence from lethargus was associated with a period of extensive feeding and reduced locomotion, accompanied by a transient increased activity in NSM (but not ADF). Our results may point to a common origin of the modulation of serotonin signaling by arousal and feeding states, and suggest roles for both chemosensation and metabolic cues in the regulation of the enhanced slowing response.

645B. ASE and ASH neuron sensitivities determine NaCl attraction or avoidance behaviour. Oluwatoroti Umuerri, Martijn Dekkers, **Gert Jansen**. Cell Biology, Erasmus MC, Rotterdam, Netherlands.

Behavioral analyses in the quadrant assay has shown that *C. elegans* is attracted to 0.1 to 200 mM NaCl and avoids higher NaCl concentrations. In addition, *C. elegans* shows NaCl-taste related learning behavior: after 15 minutes exposure to 100 mM NaCl, in the absence of food, the animals avoid all concentrations of NaCl. We call this behavior gustatory plasticity. Previous analyses have shown that NaCl attraction is mainly mediated by the ASE neurons, while avoidance is mainly mediated by the ASH neurons. The ASE and ASH neurons are also essential for gustatory plasticity. We used Ca^{2+} imaging to visualize the responses of the ASE and ASH neurons both in naive animals and after pre-exposure to NaCl. Ca^{2+} imaging in naive animals confirmed that the left ASE neuron responds to an increase in NaCl (upstep), both at low and high NaCl concentrations. The ASER neuron has been reported to respond to a decrease in NaCl concentration (downstep). However, we found no downstep response of ASER after only 3 or 30 sec exposure. ASH neurons responded to NaCl concentrations above 100 mM. Interestingly, prolonged exposure to NaCl desensitized ASEL and completely abolished its responsiveness to NaCl after 5 or 10 minutes pre-exposure. In addition, prolonged exposure to NaCl sensitized ASER, resulting in robust downstep responses after 1 to 10 minutes exposure to NaCl. These processes seem mostly cell-autonomous, although serotonin and glutamate play a role. Prolonged exposure to NaCl also sensitized ASH. In agreement with the results of our behavioral assays sensitization of ASH required functional ASE neurons. In addition, we found that sensitization of ASH involves serotonin, dopamine, glutamate and neuropeptide signalling. Our results suggest that naive *C. elegans* are attracted to NaCl, predominantly mediated by ASE, and that at high NaCl concentrations this attraction is overruled by osmotic avoidance, mediated by ASH. Pre-exposure to 100 mM NaCl in the absence of food, sensed by ASE and other neurons, changes this circuit, resulting in desensitization of attraction and sensitization of avoidance.

646C. Regulation of motivational states in *C. elegans*. **Changhoon Jee**, L. René Garcia. Howard Hughes Medical Institute, Texas A&M Univers, college station, TX.

Motivation is a goal directed driving force based on physiological needs such as hunger, thirst, and sexual arousal. Pathological neuroadaptive changes in brain reward and stress system direct normal motivated behaviors into compulsive behaviors such as binge eating, obsessive drug seeking, and sexual behavior. To further understand how stress molecularly modulates the motivated behavioral state, we studied sex-specific mating behavior of *C. elegans*. The male mating behavior of *C. elegans* is a motivated behavior and is modulated by stress. For example, mating potency declines in aging wild-type old males and food deprivation stress during early adulthood leads to neuroadaptive changes, resulting in maintaining sexual potency in older animals. We developed the mating interference assay to quantify the motivational states. The wild-type male mating behavior is interfered by blue light, which is noxious stimulus to worm in dose-dependent manner. We also quantified the motivational states of *seb-3* mutant animals, which is CRF receptor-like GPCR, in C. elegans that is a key mediator in stress response. Interestingly, *seb-3* (*gf*) mutants exhibited enhanced motivational states whereas *seb-3* (*lf*) mutant animals showed decreased motivational states, suggesting activation of SEB-3 induces behavioral arousal and leads to powerful motivation. We are currently pursuing an answer of where and how SEB-3 regulates cellular excitability of behavioral circuit components.

647A. Studying the neural circuits of food choice imprinting in C. elegans. **Xin Jin**, Navin Pokala, Cori Bargmann. Rockefeller University, New York, NY.

Imprinting, a special form of learning during an early developmental stage, can generate a long-lasting memory and change animal behavior. We discovered a novel behavior in which early exposure of larval C. elegans to pathogen elicits a long-term aversion that is maintained for days, which we call food choice imprinting. It has been previously reported that adult C. elegans can learn to avoid pathogen Pseudomonas aeruginosa (PA14) after a single 6-hour exposure, but will lose the memory after 12 hours and return to the same preference as naïve worms (Zhang et al., 2005). We modified this learning assay by performing pathogen training of newly hatched C. elegans larvae. Remarkably, larval-trained (henceforth, imprinted) animals retain this food choice memory days later, showing aversion to pathogen even as aged adults. To dissect the neural circuits of food choice imprinting, we used an inhibitory chloride channel to disrupt neuronal activity at different developmental stages, asking whether candidate neurons are required for memory formation during training, memory consolidation, or memory retrieval. In initial experiments, we have found that inhibiting the AIB interneuron during

larval training disrupts performance, whereas inhibiting AIB during adult training does not affect adult learning. This result suggests that AIB is required to form a memory of the imprinted food choice, but not required for avoidance learning in adults. Through a combination of genetic and functional methods, we hope to elucidate the molecular basis of food choice imprinting in C. elegans. Reference: Zhang, Y.; Lu, H.; Bargmann, C. I., Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. Nature 2005, 438 (7065), 179-84.

648B. Identification of novel roles of the ETS-5 transcription factor in sensory neuron specification. **Vaida Juozaityte**, Roger Pocock. BRIC, University of Copenhagen, Ole Maaløes vej 5, Copenhagen 2200, Denmark.

In the laboratory, we are searching for factors required for differentiation of the oxygen and carbon dioxide sensing neurons: BAG, URX, AQR, PQR. By dissecting the promoters of BAG terminal differentiation genes we noticed that all the promoters contain multiple conserved ETS-family transcription factor binding sites. This led us to further investigate whether any of the 10 ETS transcription factors in *C. elegans* function in BAG neuron differentiation. Subsequently, we identified that ETS-5, an ETS-family transcription factor, is required for proper BAG neuron differentiation (Brandt *et al*., 2012). Interestingly, we also found that *ets-5* mutant animals fail to express *flp-19::gfp* reporter in the AWA odorsensory neurons, suggesting a potential role for *ets-5* in specifying the AWA neurons. Therefore, we have recently focused our work on further characterizing the neuronal role of ETS-5 in AWA specification and we will present our most recent findings.

649C. Pheromones regulate nematode dispersal. **F. Kaplan**[1,2], H. Alborn[3], S. von Reuss[4], F. Schroeder[4]. 1) Kaplanschiller Research LLC, Gainesville, FL; 2) University of Florida, Gainesville, FL; 3) USDA-ARS, Gainesville, FL; 4) BTI/Cornell University, Ithaca, NY.

Dispersal is an important nematode behavior for survival. Upon crowding or food depletion, the free living bacteriovorus nematode *Caenorhabditis elegans* produces stress resistant dispersal larvae, known as dauer. Other nematodes also have dispersal larvae. In plant parasitic *Meloidogyne* spp., it is called J2 and in insect parasites (entomopathogenic nematodes, EPN), it is known as infective juveniles (IJs). Even though pheromones regulate entry into dispersal larvae in *C. elegans* and insect parasites, it is not known whether pheromones regulate dispersal. We hypothesized that pheromones may regulate dispersal behavior in *C. elegans* and in other nematodes. Liquid chromatography-mass spectrometry analysis of *C. elegans* dauer/dispersal supernatant with dispersal activity revealed four known ascarosides (ascr#2, ascr#3, ascr#8, icas#9). A synthetic pheromone blend at physiologically relevant concentrations dispersed *C. elegans* in the presence of food and also caused dispersion in insect parasite (*Steinernema feltiae*) and plant parasitic (*Meloidogyne* spp). Assay guided fractionation revealed structural analogs as major active components of the *S. feltiae* (ascr#9) and *C. elegans* (ascr#2) dispersal blends. Further analysis revealed that all *Steinernema* spp. and *Heterorhabditis* spp. infected insect host cadavers share a common pheromone, ascr#9, suggesting one species can recognize another's blend. Pheromones are fundamentally important for nematode communication across diverse habitats, and thus may provide sustainable means for control of parasitic nematodes.

650A. Chemotaxis follows the nose. **Rex A. Kerr**. Janelia Farm Research Campus, Asburn, VA.

Worms can reliably chemotax: that is, follow a gradient of an attractive chemical (soluble or volatile) to its peak. In addition to the well-known pirouette and weathervane mechanisms, high throughput behavioral analysis identified at least nine other apparently distinct behavioral modifications which, at least in theory, aid chemotaxis behavior. This suggests that the control of behavior by chemotaxis is particularly broad-ranging. Recent imaging results from a number of labs have implicated individual sensory and inter-neurons involved in chemotaxis in driving head swings. I therefore asked: is the profusion of different strategies actually just a reflection of simple regulation of head swings that is not apparent when performing traditional center-of-mass analysis of chemotaxis? To a large extent, at least, the answer is yes; there seem to be only four fundamental behaviors which are regulated in simple ways by concentration changes at the nose, namely head swing size, reversal probability, reversal size (a novel finding), and movement speed. Other behaviors are in large part and perhaps completely a consequence of these. That reversal size and probability are at least partially independently regulated is confirmed by multi-sensory integration experiments. In response to tap, worms execute a reversal with characteristic size and probability. When tap is delivered during a chemotaxis assay, the probability of reversal is essentially unaffected by whether the animal is traveling in the right or wrong direction in the gradient. However, the reversal distance is strongly modulated, and both results hold during habituation to tap. Thus, chemotaxis can be viewed as a small set of coordinately regulated simple behaviors that nonetheless can sensibly integrate with other sensory cues.

651B. The regulation of nictation, a dispersal behavior in *C. elegans*, by insulin-like molecules. **Nari Kim**[1], Harksun Lee[1], Myung-kyu Choi[1], Daehan Lee[1], Junho Lee[1,2]. 1) Research Center for Functional Cellulomics, Institute of Molecular Biology and Genetics, Seoul National University School of Biological Sciences, Seoul, Korea; 2) World Class University, Department of Biophysics and Chemical Biology, Seoul National University, Seoul, Korea.

Nictation is a dauer-specific behavior in which a dauer climbs up pointed places, then stands on its tail and waves its head. We previously reported that nictation is a dispersal behavior performed by dauers for the purpose of transmission to a new environment for their survival and reproduction. Dauer formation is regulated by guanylyl cyclase, TGF-b-like, insulin-like pathways, which are evolutionarily conserved. As reported in many studies, Insulin-like signaling (ILS) is involved in various behaviors such as pathogen avoidance, food-associated learning and mate searching. However, the involvement of ILS genes on the nictation behavior has not been reported. A recent report that ILS mediates synaptic plasticity led to a hypothesis that ILS can affect synaptic plasticity of neurons regulating nictation. To test this hypothesis, we are trying to examine ILS mutants with micro-dirt chips assay and clarify roles of ILS component genes on nictation behavior.

652C. Identification of regulatory factors for forgetting in *C. elegans*. **Tomohiro Kitazono**[1], Akitoshi Inoue[1], Takeshi Ishihara[1,2,3]. 1) Graduate School of System Life Sciences, Kyushu University, Japan; 2) Faculty of Sciences, the Department of Biology, Kyushu University, Japan; 3) JST CREST.

To properly respond to continuously changing environment, animals have to forget past memories that are inconsistent with present circumstances. To elucidate the regulatory mechanisms of forgetting, we use the olfactory adaptation in *C. elegans* as a simple model. By forward genetic analyses, we found that TIR-1, a p38 MAPK adaptor protein, and its downstream signaling pathway (TIR-1/JNK-1 pathway) mutants exhibit prolonged retention of the olfactory adaptation to diacetyl and isoamylalchol, which are the odorants sensed by AWA and AWC, respectively. We also demonstrated that the TIR-

1/JNK-1 pathway accelerates forgetting by releasing forgetting signals from AWC neurons to other neurons. Our study suggested that the neuronal circuit including AWC neurons regulates forgetting. However, the molecular mechanisms underlying this forgetting remain largely unknown. Therefore, to identify downstream components, we carried out a suppressor screening of the gain of function mutants of *tir-1*, which shows a weak adaptation phenotype probably because forgetting signals are always released. By the screening, we isolated 71 independent lines, which showed the prolonged retention of olfactory adaptation to diacetyl. These mutants are classified into 2 classes. One class shows the prolonged retention of olfactory adaptation to both diacetyl and isoamylalcohol. The other class shows the prolonged retention of olfactory adaptation to diacetyl, but not to isoamylalcohol. This result suggests that the distinct downstream signaling pathways regulate forgetting of olfactory adaptation in the distinct neurons. To identify downstream components of TIR-1/JNK-1 pathway, we analyzed the isolated mutants with the whole genome sequencing and genetic mapping. Among those mutants, we determined the complete DNA sequences of 20 mutants, which showed strong phenotypes. We identified a gene responsible for one of these mutants, *qj143* as *maco-1*, which is conserved across the species and is orthologous to human macoilin. This study enables us to provide new insights on the regulatory mechanisms of forgetting.

653A. The *C. elegans* cGMP-dependent Protein Kinase EGL-4 Regulates Nociceptive Behavioral Sensitivity. **Michelle C Krzyzanowski**[1], Chantal Brueggemann[2], Meredith J Ezak[1], Jordan F Wood[1], Kerry L Michaels[1], Christopher A Jackson[1], Bi-Tzen Juang[2], Kimberly D Collins[2], Michael C Yu[1], Noelle D L'Etoile[2], Denise M Ferkey[1]. 1) Department of Biological Sciences, State University of New York at Buffalo, Buffalo, NY 14260, USA; 2) Department of Cell and Tissue Biology, University of California, San Francisco, CA 94143, USA.

The ability of an animal to detect and avoid noxious compounds in the environment is critical to its survival, and signaling levels within sensory neurons must be tightly regulated to allow cells to integrate information from multiple signaling inputs and to respond to new stimuli. G protein-coupled signaling pathways play a major role in mediating *C. elegans* avoidance of several ASH-detected nociceptive stimuli, including the bitter tastant quinine. Herein, we report a new role for the cGMP-dependent protein kinase EGL-4 in the negative regulation of G protein-coupled nociceptive chemosensory signaling. We have found that *C. elegans* lacking the cGMP-dependent protein kinase EGL-4 function are hypersensitive in their behavioral response to low concentrations of quinine and exhibit an elevated calcium flux in the ASH sensory neurons in response to quinine. We provide the first direct evidence for cGMP/PKG function in ASH and our data suggest that activated EGL-4 dampens quinine sensitivity via phosphorylation and activation of the regulator of G protein signaling (RGS) proteins RGS-2 and RGS-3, which in turn downregulate Ga signaling in ASH and, as a result, behavioral sensitivity. Moreover, animals lacking the function of the transmembrane quanylyl cyclases ODR-1 and GCY-27, or the soluble guanylyl cyclases GCY-33 and GCY-34, are also hypersensitive in their response to dilute quinine. However, these GCYs do not appear to function directly in the ASHs, suggesting that they act in a non-cell-autonomous manner to regulate ASH function, and that other neurons in the circuit may provide the cGMP that regulates EGL-4 and ASH function.

654B. Thermoreceptor neurons regulate the temperature-dependence of motor programs. **S. Lasse**, V. Y. Wang, M. B. Goodman. Molecular & Cellular Physiology, Stanford University, Stanford, CA.

In small ectotherms like *C. elegans*, temperature can influence behavioral performance directly or indirectly through signaling from specialized thermoreceptor neurons. Such temperature-dependence is also found in endotherms like mammals and birds. For instance, cooling decreases rhythmic movements associated with essential tremor and can also reduce pain and itch in humans. Essentially nothing is known in either ectotherms or endotherms about the potential role for thermoreceptor neurons in regulating the temperature-dependence of behavioral performance. We are working to fill this gap in understanding, focusing on two simple *C. elegans* behaviors: egg laying and locomotion. In wild-type N2 worms both behaviors display a similar dependence on temperature: rates increase with temperature and reach a maximum at 22-25°C then sharply decline above 26°C (egg laying) or 30°C (locomotion). If thermoreceptor neurons contribute to the temperature dependence of behavioral performance, then the *rate-temperature* (*R-T*) relationship should differ between wild-type worms and mutants with defects in thermosensation. Consistent with this idea, *tax-4* mutants which lack the ability to respond to thermal gradients (Mori and Ohshima 1995) laid fewer eggs and crawled more slowly at 25-30°C. However, R-T curves had similar shapes in wild type and *tax-4* worms. This suggests that egg laying and locomotion motor programs are intrinsically dependent on temperature, but input from the thermoreceptor neurons is required for wild type performance. This relationship may represent an evolutionary optimization of behavioral performance over a physiological range of temperatures. The neural circuits that regulate egg laying and locomotion do not overlap, indicating this as a general strategy that worms use to modulate behavioral performance. Mori I, Ohshima Y (1995) Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature 376:344 -348.

655C. Food signals modulate sensory integration behavior. **Hiu Lau**[1,2], Sreekanth Chalasani[1,2]. 1) Biological Sciences Graduate Program, University of California San Diego, La Jolla, CA; 2) Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA.

Neural circuits integrate information to generate behavior outputs. One challenge in neuroscience is to understand how neural circuits generate flexible behaviors. We use a sensory integration assay[1] to ask how exposure to various food signals influences behavior. Here, *C. elegans* crosses a repellent barrier (copper) and chemotax towards a spot of an attractant (diacetyl)[1]. After *C. elegans* are exposed to the bacteria *P. aeruginosa* for 3 hours, we observe a two-fold increase in the number of animals that reach the attractant compared to those fed *E. coli*. We find that exposure to multiple strains of bacteria (including non-pathogenic ones) causes a similar behavioral change. To test the persistence of this behavior modulation, we transferred animals back to a diet of *E. coli* after *P. aeruginosa* exposure. We find that behavioral modulation by *P. aeruginosa* persists for two hours. Together these results present a form of neural circuit flexibility, where food signals modify behavioral outputs.

From a pilot screen of signaling molecules, we found that knocking down neuropeptides impairs integration behavior. Neuropeptides are cleaved by specific proprotein convertase enzymes to form mature neuropeptides. To evaluate the role of individual subsets of neuropeptides, we tested proprotein convertase mutants *bli-4*, *kpc-1*, *aex-5* and *egl-3* [2]. We found that *aex-5* mutants do not change behavioral response even after exposure to *P. aeruginosa*. This result suggests that neuropeptide(s) processed by AEX-5 is required for behavior modulation in response to food changes. We are in the process of identifying peptides involved in modulation of sensory integration behavior. Using this model, we aim to reveal the mechanisms regulating the dynamics of neural circuit functions in response to changes in prior food experience.

1. Ishihara T et al. *Cell*, 109: 639-649 (2002). 2. Li, C. and Kim, K. Neuropeptides (September 25, 2008), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.142.1, http://www.wormbook.org.

656A. Analyses of *C. elegans* male ray neuron activity during mating. Olivia Philpot[1], May Boggess[2], Rene Garcia[1], **Robyn Lints[1]**. 1) Dept Biol, Texas A & M Univ, College Station, TX; 2) School of Mathematical and Statistical Sciences, Arizona State University, Tempe, AZ.

C. elegans male mating behavior is a sequence of goal-oriented, sex-specific sensorimotor behaviors, culminating in insemination of the hermaphrodite. The sensory rays, external sensilla associated with the male genitalia, are responsible for sensing contact with a mate and guiding a systematic, contact-based search for the vulva. The rays do this by inducing the male tail to press against the hermaphrodite surface and move over her body with backward locomotion. During the search, the male modulates tail posture, movement speed and direction to accommodate changes in hermaphrodite position and body contours. Our long-term objective is to understand how ray activity alters in response to detection of hermaphrodite surface cues and how ray activity states appropriately transform tail posture and movement. We are using a combination of cell ablation and optogenetic approaches to address this question. Our preliminary studies using GCaMP reveal that ray neurons exhibit unusual and complex activity patterns. For example, initial ray-to-mate contact triggers a rebound depolarization event in many ray neurons i.e., a transient hyperpolarization followed by immediate depolarization of the cells. In support of this, solitary males exhibit vulva search-like motor behaviors after transient artificial hyperpolarization of ray neurons, using light-inducible anion channel NpHR, as do solitary males in which the same neuron population is transiently depolarized using Channelrhodopsin-2. Additionally, artificial activation and GCaMP imaging of A-neurons, the key effectors of search behavior, argue that A-neuron activity is graded (rather than bi-stable) and that the degree of activation directly correlates with the intensity of tail bending. These results and those of ongoing analyses will be presented.

657B. A neuronal flip-flop generates random search behavior in the nematode *C. elegans*. W. Roberts[1], S. Augustine[2], T. Lindsay[3], K. Lawton[4], T. Thiele[5], N. Pokala[6], R. Anderson[1], M. Britton[7], C. Bargmann[6], **S. Lockery[1]**. 1) University of Oregon, Eugene, OR USA; 2) University of California, Los Angeles, CA USA; 3) University of Washington, Seattle, WA USA; 4) Cornell University, Ithaca, NY USA; 5) Max Planck Institute for Neurobiology, Martinsried, Germany; 6) Rockefeller University, New York, NY USA; 7) HHMI Janelia Farm Campus, Ashburn, VA USA.

Foraging is an evolutionarily conserved set of behavioral strategies by which organisms from bacteria to humans successfully locate food when searching widely in an unpredictable environment. Prey items in many environments are randomly distributed, sparse, and nearly undetectable until contact is made. Under these conditions random search is a common strategy, but predictive neuronal models of this behavior are lacking. We therefore investigated foraging in *Ceanorhabditis elegans*, an organism with a simple, well-described nervous system that seeks and consumes bacteria in rotting plant material. Here we formulate and experimentally test a neuronal network model of random search based on the *C. elegans* connectome, whole-cell patch clamp recordings from locomotory command neurons, and the first large-scale kinetic analysis of *C. elegans* locomotory behavior at high spatiotemporal resolution. The model correctly predicts the signs of key synaptic connections in the nematode brain, assessed here opotogenetically. Further, the model explains the unexpected effects on search behavior of neuronal ablations and genetic perturbations of the network's overall electrophysiological state. The close agreement between model and data suggests that random search in *C. elegans* is generated by a neuronal flip-flop circuit involving reciprocal inhibition between two populations of binary, stochastic neurons in which self-excitation stabilizes locomotory states. Commonalities between this circuit in *C. elegans* and flip-flops in other organisms suggest that this may be an evolutionarily conserved motif for coordination of opposing states to generate a variety of adaptive behaviors.

658C. Different Stressors, Same Sleep: heat, cold, salt, alcohol all trigger ALA-dependent behavioral quiescence. **Jessie M. Lopez**, Cheryl Van Buskirk. California State University Northridge, Northridge, CA.

It has previously been shown that overexpression of the Epidermal Growth Factor (EGF) ligand LIN-3 can induce sleep-like behavior in *C. elegans* at any stage of development or during adulthood, an effect that is dependent on activation of EGF receptors on a single interneuron, ALA. The physiological significance of ALA-dependent sleep has not been clear: mutants lacking components of EGF signaling or the ALA neuron show very mild lethargus defects, pointing to a minor or partially redundant role in effecting these periods of developmental quiescence. Recently, our lab has uncovered a novel role for this pathway. We have found that EGF signaling and the ALA neuron mediate the cessation of pharyngeal pumping and locomotion that normally occurs immediately after exposure to heat stress.

We wished to address whether any other stressors that are known to inhibit *C. elegans* behavior, such as cold, high salt, and alcohol, might exert their effects via the ALA neuron. To this end, we compared the behavioral responses of wild-type young adult animals to those of *ceh-17(np1)* mutants, which are highly defective in ALA neuron function. We found, surprisingly, that the inhibition of activity induced by each of these stressors is ALA-dependent. This suggests that ALA-dependent sleep may serve to attenuate organismal activity in response to a wide variety of stressors. Our testable hypothesis is that this stress-induced sleep may allow for resources to be diverted toward metabolic changes aimed at coping with the new challenge. Other efforts are aimed at testing additional stressors for ALA-dependence, and assaying for involvement of components of EGF signaling. Our results may have broad implications for the understanding of the function of sleep, and may further our emerging understanding of the relationship between cellular stress and sleep in mammals.

659A. Characterizing the *hsf-1*-Independent Behavioral Response to Heat Shock. **Richard Mansfield**, Cheryl Van Buskirk. Biology, CSUN, Northridge, CA.

Inducible gene expression is a valuable tool in determining gene function, and the heat-shock inducible gene expression system is used extensively in studies of *C. elegans* development and behavior. However, we and others[1] have noted that heat itself can affect behavior, highlighting the importance of heat-shocked controls in behavioral studies. To guide the evaluation of such studies we wished to characterize the behavioral effects of heat. Here we show that young adult animals subjected to heat shock display suppressed pharyngeal pumping and locomotion that persists for up to an hour. These effects are seen immediately after heat shock and are, not surprisingly, *hsf-1*-independent. Unexpectedly, however, we find that this behavioral suppression is dependent on UNC-13, UNC-31, and PKC-1, known regulators of neuropeptide release, indicating that it is not due to cell-autonomous effects of protein misfolding. Additionally the effect is dependent on AFD thermosensory neurons, but not their postsynaptic AIY partners. This suggests

that AFD participates in a previously undescribed neuroendocrine pathway that inhibits active behavior in response to heat. As well as the characterization of recovery quiescence, we have also analyzed the behavior of animals during heat shock. Using a heated microscope stage, we have found that young adult animals cease feeding at approximately 32°C, consistent with previously reported results[1], and we have found this effect to be AFD-independent, consistent with a cell-autonomous effect of heat on pharyngeal pumping. Animals that are immediately removed from heat resume feeding within 1-2 minutes. Thus in addition to previously described thermotaxis, there appears to be two additional behavioral responses to heat. First, a transient cessation of pharyngeal muscle contraction in direct response to heat stress, which may be due to cell-autonomous effects of heat on protein activity. And second, and a prolonged suppression of feeding and locomotion that is mediated by neuroendocrine signaling. We speculate that this may function to conserve resources for metabolic changes associated with the adaptive transcriptional response to heat. 1. Jones D, Candido EP. 1999. J Exp Zool 24:147-157.

660B. The SWI/SNF chromatin remodeling complex modifies ethanol-responsive behaviors. **L. Mathies[1]**, G. Blackwell[1], L. Hack[3], A. Adkins[3], T. Webb[2], K. Kendler[2,3], B. Riley[2,3], A. Davies[1,2], J. Bettinger[1,2]. 1) Dept. of Pharmacology & Toxicology, VCU, Richmond, VA; 2) Dept. of Psychiatry, VCU, Richmond, VA; 3) Dept. of Human Genetics, VCU, Richmond, VA.

Alcohol abuse is a significant social problem for which there are few treatments. One reason for the difficulty in generating effective pharmacological interventions is the lack of understanding of the important direct and indirect physiological targets of ethanol. We use *C. elegans* to study the mechanisms involved in acute responses to ethanol. We measure two different behavioral responses: initial sensitivity is the reduction in locomotion speed upon initial exposure to ethanol and acute functional tolerance (AFT) is the increase in locomotion speed over the course of an exposure, despite the internal ethanol concentrations increasing slightly. Together, initial sensitivity and development of AFT contribute to the initial “level of response” that in humans predicts susceptibility to alcohol use disorders.

Recently, a human genome wide association study for loci that are associated with alcohol dependence has been completed at VCU. Among the top 50 genes that emerged from this analysis were two genes, Smarca2 and Brd7, which encode components of the SWI/SNF chromatin remodeling complex. SWI/SNF is a multi-protein complex that alters the interaction between nucleosomes and DNA and thereby influences gene expression. The *C. elegans* SWI/SNF complex is required for embryonic and larval development. We have found that it is also important for ethanol responses in adults. We tested the *C. elegans* homologs of Smarca2 and Brd7, *swsn-4* and *swsn-9*, for roles in acute ethanol responses and found that while loss of neither gene affected initial sensitivity, both were required for the development of AFT. There are existing alleles for ten of the twelve *C. elegans* SWI/SNF genes, including temperature-sensitive alleles of *swsn-1* and *swsn-4*. We are currently testing other *swsn* alleles for altered ethanol responses and are using temperature-sensitive alleles to define the temporal requirement for SWI/SNF in AFT. Our ultimate goal is to determine how SWI/SNF regulates chromatin structure to affect acute behavioral responses to ethanol.

661C. The Role of Heterotrimeric G-protein Signaling Pathways in Habituation. **Andrea McEwan[1]**, Andrew Giles[1], Catharine Rankin[1,2]. 1) Brain Research Centre, 2211 Wesbrook mall, Vancouver, B.C., V6T2B5; 2) Dept. of Psychology, 2136 West Mall, Vancouver, B.C., V6T 1Z4.

Despite its apparent simplicity, the soil-dwelling nematode *Caenorhabditis elegans* has a surprisingly large capacity to learn and remember. Previous characterization of *C. elegans* genome and neuronal circuit makes this worm an ideal choice for studying behavior and the mechanisms that underlie it. Through careful behavioral and genetic studies, nematodes have been shown to form many different types of memory, including short-term non-associative memory called habituation. Habituation is the defined as the decrement in response after repeated stimuli. Detailed analyses of all behaviours that occur in response to tap showed that as one response type, reversals, decreased other responses, accelerations, decelerations and pauses became more prevalent. An earlier large-scale screen of mutant strains of *C.elegans* showed that several genes (*goa-1* and *eat-16*) related to heterotrimeric G-protein family of signaling pathways exhibited striking defects in habituation. To follow-up on those findings, I investigated the role of heterotrimeric G-protein signaling pathway and showed that Gai and Gaq signaling pathways share a broad role regulating habituation whereas the Gas pathway modulates the rate of habituation. The analyses of all the behaviours nematodes preform in response to habituation training showed that heterotrimeric G-protein signaling pathways play a role in regulating the shift in behaviour during habituation training. Together these data add to our understanding of the mechanisms underlying habituation of the tap response in *C. elegans*.

662A. Acute Laser Dissection of Mechanosensory Circuitry in *C. elegans*. **Pavan Mehat[1]**, Lin Sun[1,2], Samuel Chung[1,2], Christopher Gabel[1,2]. 1) Physiology and Biophysics, Boston Univ. School of Medicine, Boston, MA; 2) Photonics Center, Boston Univ.

Employing high-resolution laser surgery techniques, we are studying how the *C. elegans* mechanosensory reflex circuitry recovers from specific neuronal lesion. We find that *in vivo* laser severing of the lateral synaptic branch of the posterior lateral microtubule neurons (PLM, the prominent posterior mechanosenory neurons) results in an initial hyperactivation of the downstream circuitry and elevated execution of posterior touch avoidance behaviors (*i.e.* increased forward movement as well as suppression of both spontaneous and anterior touch induced reversals). Over time the animal recovers and its behavior returns to its original baseline level within 24 h. Interestingly, this effect is seen only in surgeries that eliminate all sensory input from the PLM neurons, as single neuron surgery or severing the axon near the cell body have no effect. In addition, post-surgery hyperactivity is eliminated in glutamate receptor mutants suggesting that the effect maybe the results of neuronal modulation following sensory deprivation. Neuronal damage and sensory deprivation are known to elicit hyper-excitability and neuronal remodeling within the mammalian central nervous system. Prominent examples include phantom pain of amputated limbs, acute seizures and epilepsy resulting from traumatic brain damage and cortical rewiring after spinal cord injury or sensory deprivation. Here we are studying similar effects within a well defined, simple and tractable sensory transduction pathway in *C. elegans*.

663B. Serotonergic and peptidergic signaling interact to modulate aversive behavior. **Holly Mills**, Amanda Ortega, Richard Komuniecki. University of Toledo, Biological Sciences, Toledo, OH.

In *C. elegans*, nutritional status is translated by both monoaminergic and peptidergic signaling in the modulation of behavior. To characterize these interactions we have examined aversive responses to dilute octanol mediated by glutamatergeric ASH sensory neurons. Previously we demonstrated that food or 5-HT dramatically decreased the time taken to initiate aversive responses and this stimulation required the expression of the neuropeptide

encoding gene, *nlp-3*, in the ASHs (Harris et al., 2009). Surprisingly, the selective overexpression of *nlp-3* not only in the ASHs, but also a number of other *nlp-3* expressing neurons also increased aversive responses off food. Conversely, *nlp-3* RNAi knockdown in the ASHs or other *nlp-3* expressing neurons abolished the food or 5-HT sensitization of aversive responses, suggesting that *nlp-3* encoded peptides may be humorally released to sensitize the aversive circuit. *nlp-3* encodes three distinct neuropeptides (NLP-$3_{A,B,C}$) and the injection of NLP-3_C directly into wild type animals rapidly and transiently stimulated aversive responses, suggesting that the responses to NLP-3_C were not developmentally mediated. In contrast, the injection of NLP-3_A or NLP-3_B alone had no effect, and, more importantly, the injection of NLP-3_A with NLP-3_C abolished NLP-3_C mediated stimulation, suggesting that neuropeptides encoded by the same gene might play different and potentially antagonistic roles in signaling. As predicted, the overexpression of *nlp-3* or the injection of NLP-3_C into *npr-17* null animals had no effect on ASH-mediated aversive responses, supporting the identification of NPR-17 as the NLP-3_C receptor. Together, these data suggest that a humoral pool of both tonically and acutely released *nlp-3* encoded peptides sensitize the ASH-mediated locomotory circuit. These studies are continuing to functionally characterize the neuron-specific roles of NPR-17 signaling and how the tonic release of *nlp-3* encoded peptides modulates the 5-HT sensitization of the ASH-mediated aversive circuit. Given the similarities between nematode and mammalian receptors, these studies may provide useful insights into serotonergic/peptidergic interactions in mammals. Supported by NIH grant AI-145147.

664C. Alkaline pH sensation mediated by GCY-14, a transmembrane guanylyl cyclase. **T. Murayama[1]**, M. Fujiwara[1], J. Takayama[2], I. Maruyama[1]. 1) Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan; 2) Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Japan.

Animals can survive in a narrow pH range by monitoring the pH in their environment and body fluids. While animals sense acidic pH using ion channels and GPCRs, however, little is known about how animals including humans detect extracellular alkaline pH. Since *C. elegans* is attracted to alkaline pH up to 10.4, we have been trying to understand mechanisms underlying the alkaline-pH chemotaxis in order to find alkaline-pH sensor neurons and molecules. Wild-type worms were attracted to higher alkaline pH along a pH gradient ranging from 6.8 to 8.5, whereas wild-type animals with ASEL ablated or mutants defective in ASE failed to do so. We have also found by in vivo calcium imaging that ASEL is activated by pH upshifts and functions as a primary alkaline pH sensor. On the other hand, ASER is not a direct sensor since ASER in *unc-13* did not respond to the alkaline pH. We also found that a transmembrane guanylyl cyclase, GCY-14, and TAX-2/TAX-4 cGMP-gated cation channel are required for the alkaline pH sensation in ASEL. Furthermore, domain swapping and site-directed mutagenesis of *gcy-14* revealed that GCY-14 functions as a homodimer, and that histidine of the extracellular domain of GCY-14 play an essential role in the activation of ASEL upon environmental alkalinization. When GCY-14 was ectopically expressed in other alkaline-pH-insensitive neurons such as ASG, calcium transients were clearly observed in response to pH up-shifts from 6.8 to 10.0, indicating that GCY-14 confers alkalinity sensing capability. These results argue that in addition to ion channels and GPCRs, transmembrane receptor-type guanylyl cyclases also play a role in pH sensation.

665A. A longitudinal study of *C. elegans* larvae reveals a novel locomotion switch, regulated by G_{as} signaling. **Stanislav Nagy[1]**, Charles Wright[1], Nora Tramm[2], Nicholas Labello[3], Stanislav Burov[2], David Biron[1,2]. 1) Institute for Biophysical Dynamics, University of Chicago, Chicago, IL; 2) Department of Physics and the James Franck Institute, University of Chicago, Chicago, IL; 3) Research Computing Center, University of Chicago, Chicago, IL.

Despite the simplicity of invertebrate models, longitudinal studies designed to follow behavioral trends across development are rare. In particular, neither locomotion dynamics of *Caenorhabditis elegans* larvae, nor how they might be modulated during development, have been previously described. We sought to characterize these dynamics as well as their regulation by the activity of Protein Kinase A (PKA), which has been shown to promote hyper-kinetic behavior. To study how the patterns of *C. elegans* locomotion are modulated on a developmental timescale, we developed a suite of tools capable of leveraging high performance parallel computing resources. We obtained the first detailed record of locomotion from the mid fourth larval stage through the mid young adult stage, which includes the fourth lethargus period. Throughout this 14-hour period, we analyzed the initiation, propagation and demise of all individual dorsoventral body bends. While some locomotion patterns were gradually modulated, others displayed abrupt switching. We discovered a novel form of behavioral modulation, occurring on the timescale of hours: the late fourth larval stage could be divided into epochs that were dominated by either forward locomotion or dwelling, and animals were able to abruptly switch between these states. The duration of the forward-dominated epochs exhibited non-Poisson statistics, indicating that this state was actively stabilized. Increased G_{as} signaling stabilized forward locomotion before, during and after lethargus. In contrast, decreased G_{as} signaling, decreased neuropeptide release or decreased CREB activity destabilized the forward state outside of, but not during lethargus. Our findings support a model in which PKA activity stabilizes forward locomotion, through two of its downstream targets: neuropeptide release and CREB. However, during lethargus, when forward locomotion is suppressed, the role of PKA signaling in modulating locomotion and quiescence may be minor.

666B. Identification of New Genes Involved in *C. elegans* Thermotaxis Behavior. **Shunji Nakano[1]**, Jiang Tianyu[1], Takamasa Suzuki[2], Tetsuya Higashiyama[2,3], Ikue Mori[1]. 1) Division of Biological Science, Nagoya University; 2) JST ERATO, Nagoya University; 3) Institute of Transformative Bio-Molecules, Nagoya University.

C. elegans can sense and remember the environmental temperature and navigate themselves toward the cultivation temperature when placed on a thermal gradient. Although studies of the past decades have identified genes and neural circuits involved in thermotaxis, how *C. elegans* achieves this complex behavior still remains elusive.

To further reveal the neural mechanisms that underlie thermotaxis, we undertook genetic screens to look for mutants defective in thermotaxis. We screened approximately 70,000 F2 progeny of mutagenized wild-type animals for mutants that displayed abnormal thermotaxis behavior and recovered 24 isolates. These 24 mutants can be classified into three phenotypic classes: 12 mutants are thermophilic, 4 mutants cryophilic and 8 mutants athermotactic.

We began our analysis with the 12 thermophilic mutants. Based on complementation tests, we found that three of the 12 mutations are alleles of genes previously shown to be important for thermotaxis. *nj98* and *nj111* are alleles of *ttx-4/pkc-1*, and *nj113* is an allele of *tax-6*. The remaining 9 mutations likely represent new genes involved in thermotaxis. We thus far identified that 5 mutations mapped to regions in which no gene previously shown to regulate thermotaxis is present. *nj104* and *nj108* failed to complement each other and mapped to a 178 kb region of LG X. *nj97* mapped to a 1.9 Mb region

of LG X that excludes the *nj104* and *nj108* loci. *nj100* mapped to a 600 kb region of LG II. *nj102* mapped to a 308 kb region of LG IV. In addition, two mutations, *nj89* and *nj85*, at least semi-dominantly cause thermophilic defects and mapped to LG X. In short, our screens recovered mutations that represent at least 4 novel genes important for thermotaxis. We are currently performing whole-genome sequencing of these mutants to identify the genes mutated in these isolates. Identification and characterization of these new genes will further reveal the neural mechanisms that generate thermotaxis behavior.

667C. The CMK-1 CaMKI protein integrates food signals to regulate sensory neuron state. **Scott J. Neal**[1], Kyuhyung Kim[2], Piali Sengupta[1]. 1) Dept Biol, Brandeis Univ, Waltham, MA, USA; 2) Dept Brain Science, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu, Korea.

Food availability modulates multiple behavioral responses and developmental decisions in *C. elegans*. In particular, food has been shown to alter the responses of sensory neurons to many environmental stimuli. However, whether food availability or internal metabolic state alters the overall state of the sensory system is unclear. We find that adult worms with null mutations in the *cmk-1* CaMKI gene exhibit behaviors characteristic of starved animals even in the presence of abundant food. These behaviors include atypical food leaving, diminished area-restricted searching, decreased egg-laying and altered adaptation to food-related odorants. Moreover, we observe dynamic changes in CMK-1 subcellular localization in wild-type animals upon food deprivation, perhaps providing a basis for food-adaptive behaviors. Interestingly, *cmk-1* mutant larvae also display characteristics of food deprivation such that pheromone-induced dauer entry is not suppressed by abundant food. These behaviors are rescued by CMK-1 activity in different subsets of sensory neurons. Calcium imaging experiments indicate that the basal activity states of sensory neurons such as AWC are altered in *cmk-1* mutants in a manner reminiscent of wildtype animals deprived of food. These observations suggest that food signals are assessed via CMK-1 to set the appropriate activity states of sensory neurons, which in turn allows integration of food signals to appropriately modulate behavior and development.

668A. A new imaging system for high-throughput C. elegans analysis of temperature-entrained rhythmic gene expression. **Dru Charles Nelson**, Ari Winbush, Alexander van der Linden. Department of Biology, University of Nevada, Reno, NV 89557.

Temperature is a universal entrainment signal for the circadian clock, but relatively little is known about temperature entrainment, even though temperature plays a central role in the synchronization of clocks in many species including mammals and flies. The complex architecture of the fly and mammalian nervous system makes it difficult to understand how temperature signals reach the clock to fine tune circadian rhythms in behavior and physiology at an organismal level. Studying these processes in C. elegans, an extensively used animal model with a small and completely mapped nervous system, has the potential to functionally dissect the pathways from the temperature input to the circadian-driven behavioral output. These properties combined with our recent discovery of temperature entrained circadian molecular rhythms in C. elegans could provide fundamental information for how temperature signals entrain the clock(s). To examine how temperature input entrains the clock, we have developed a new in vivo automated imaging system for long-term recording and quantification of rhythmic gene expression in C. elegans. Using this system, we can robustly measure and quantify fluorescence levels emanating from a circadian reporter during temperature cycles. This imaging system gives us the opportunity to identify genes and neurons that function in the temperature entrained clock of C. elegans. We show that the tax-2 cGMP-gated ion channel, previously implicated in responses to temperature signals and thermotaxic behavior in C. elegans, is required for temperature entrainment of rhythmic gene expression. These results suggest that sensory signals are necessary to transmit temperature information to the C. elegans clock(s), a pathway that appears to be conserved in Drosophila. Our data establishes C. elegans as a new and experimentally amenable model system for understanding the temperature-entrained clock(s). This work was made possible by NIH 1P20GM103650 and 1R21NS078617.

669B. NLP-22 is a Neuromedin S-like neuropeptide which regulates behavioral quiescence. **Matthew Nelson**, David Raizen. Dept Neurology, University of Pennsylvania, Philadelphia, PA.

Sleep behavior in *C. elegans* occurs in association with larval molts during the four lethargus stages. Neuropeptides are small centrally secreted signaling molecules which play a central role during the regulation of sleep in mammals and flies (Crocker and Sehgal, 2010). However, roles for neuropeptide signaling during *C. elegans* sleep regulation are just beginning to be identified. We have found that *nlp-22* regulates sleep during lethargus and has both structural and functional similarities with the human neuropeptide neuromedin S (NMS). NMS is an anorexigenic neuropeptide, which shows a circadian pattern of expression and is expressed in a small subset of neurons in the brain, specifically in the superchiasmatic nucleus, a master circadian regulatory region (Ida et al, 2005; Mori et al, 2005). Both NMS and *nlp-22* have a FRP motif at or near their C-terminus. Simliar to NMS, *nlp-22* over-expression during normally active stages causes locomotion and feeding cessation. *nlp-22* mRNA shows cyclical expression in synchrony with the molting/lethargus cycle. NLP-22 signals downstream of LIN-42/PER. We find that *nlp-22* is expressed in a single pair of head interneurons, the RIAs. In addition, we find that reduction of *nlp-22* function results in reduced sleep during lethargus. The similar structure, relationship to a PER/LIN-42 based clock, restricted nature of expression and anorexigenic effects of NLP-22 and NMS, suggest that FRP neuropeptides serve an ancient role in the regulation of feeding and sleep.

670C. The effect of sex difference on olfactory learning in *Caenorhabditis elegans*. **Julia Nguyen**, Shane Smith*, Gareth Harris, Yun Zhang. Department of Organismic and Evolutionary Biology, Center for Brain Science, Harvard University, Cambridge, MA.

**Authors contributed equally.* Olfaction is an important means for animals to communicate with the environment, and experience can profoundly shape the representation of olfactory cues to an animal. Consistent findings from olfactory literature suggest that males and females detect, identify, and discriminate odors differently. However, little is known whether sex difference also influences olfactory learning. The nematode worm, *Caenorhabditis elegans*, feeds on bacteria in its natural environment but is susceptible to infection after the ingestion of harmful pathogenic bacteria. Because learning to avoid harmful food is essential for *C. elegans* survival, conditioned avoidance of odors associated with toxicity or infection is a robust form of olfactory learning. Previously, we have shown that naive hermaphrodites prefer the smell of the pathogenic bacteria *P. aeruginosa* (PA14) in comparison with the smell of the common lab food *E. coli* (OP50), and brief training with PA14 induces a learned olfactory aversion to the PA14 smell (Zhang et al., 2005, Ha et al., 2010). Here, we ask whether sex difference regulates olfactory learning. To this end, we used a high throughput micro-droplet assay system to measure the ability to learn to avoid the smell of PA14 in individual hermaphrodites or males. Preliminary data from our behavioral analysis has revealed

that males exhibit defects in naive food odor preference as well as in learning, suggesting sex difference in olfactory plasticity. Previously, we have mapped a neural network that underlies the aversive olfactory learning (Ha et al., 2010). We plan to interrogate the effect of sex difference in this circuit that leads to the behavioral difference in olfactory learning. We hope that these studies will help further the understanding of how gender differences may influence olfactory behavior and plasticity in organisms such as humans.

671A. Isoform-specific axonal translocation of a novel DAF-2 isoform regulates synaptic and behavioral plasticity. **Hayao Ohno**[1], Shinya Kato[1], Yasuki Naito[1], Hirofumi Kunitomo[1], Masahiro Tomioka[2], Yuichi Iino[1,2]. 1) Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan; 2) Molecular Genetics Research Laboratory, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

C. elegans memorizes external salt concentrations and switches the preference for them according to food availability (Kunitomo et al., submitted). The insulin/PI3K pathway and CASY-1 acting in the salt-sensing ASER sensory neuron play essential roles in the reversal of salt concentration preferences caused by starvation.

CASY-1 is the *C. elegans* homolog of calsyntenins/alcadeins, which are cadherin-like type I transmembrane proteins highly expressed in the nervous system. In *casy-1* mutants, the axonal localization of a novel isoform of the DAF-2 insulin/IGF-1 receptor, DAF-2c, was completely abolished. Further experiments suggested that CASY-1 is involved in the axonal transport of DAF-2c by acting as a molecular linker between DAF-2c and the kinesin-1 complex. The axonal localization of DAF-2c increases in response to starvation, and this DAF-2c translocation is pivotal for the regulation of synaptic transmission from ASER and the reversal of salt concentration preferences. We further found that the Ras-MAPK pathway negatively controls the CASY-1-dependent axonal transport of DAF-2c by targeting kinesin light chain.

These results suggest that the CASY-1 and kinesin-1 complex regulated by the Ras-MAPK pathway confers the functional diversity to the insulin/IGF-1 receptor gene through the isoform-specific axonal transport.

672B. Genetically imposed dietary restriction makes normally appetitive food repulsive by altering odor-sensing circuits. **Birgitta Olofsson**. MRC-Laboratory of Molecular Biology, Cambridge, United Kingdom.

It is well known that in many animals internal metabolic state can alter food preference. However the molecular mechanisms underlying this behavioral plasticity are poorly understood. I have set up a paradigm to explore how post-ingestive feedback modifies food perception in C. elegans. I show that normally appetitive food becomes repulsive if it is prevented from nourishing the animal. Using this paradigm I dissect how this switch in perceived food quality is achieved. I show that the AWC olfactory neurons, which mediate attraction to odors in well-fed animals, promote food avoidance in underfed C. elegans. AWC neurons act in concert with the AWB olfactory neurons, which have previously been implicated in avoidance of noxious odors. I also show that the ASJ and ASK gustatory neurons play a parallel role in promoting food avoidance. In well-fed animals these neurons promote local search behavior to locate food, suggesting that like for AWC, their role is switched in chronically underfed animals. I then investigate how animals monitor their nutrient state to switch their evaluation of food. I find that disrupting AMP kinase or ribosomal S6 kinase attenuates food avoidance. These data provide an entry point to explore how cellular nutrient state sensors alter perception of food. I also show that a TGF-b pathway mediated by DBL-1/ TGF-b inhibits food avoidance, and that HEN-1, a secreted LDL-motif containing protein implicated in integration of attractive and aversive cues, promotes food avoidance in my paradigm.

673C. The Role of Post-Translational Modifications in the Regulation of Serotonin Signalling. **Andrew C Olson**, Michael R Koelle. Yale University, New Haven, CT.

C. *elegans* uses serotonin as a neurotransmitter to slow locomotion, and we have used this model system to discover that post-translational modifications seem to regulate serotonin signaling. First, through large-scale genetic screens for mutants that fail to respond properly to serotonin (Gurel et al., 2012), we found that C. *elegans* strains carrying mutations in either of two subunits of the ELPC ELongator" Protein Complex are defective for response to serotonin. ELPC is highly conserved from C. *elegans* to humans and functions as a lysine acetylase to reversibly modify other proteins. This is first time that ELPC or lysine acetylation has been implicated in regulating serotonin signaling. Second, our lab has also shown that Ga_o, a G protein through which serotonin signals to slow locomotion, is post-translationally modified in a manner that alters its charge, which is a hallmark of lysine acetylation. We hypothesize that ELPC may reversibly acetylate Ga_o (and/or one of the other proteins that mediate serotonin signaling) to regulate serotonin response. Serotonin regulates worm movement in C. *elegans* by redundantly signaling through the MOD-1 serotonin-gated ion channel and the SER-4 G Protein Coupled serotonin Receptor (GPCR), the GPCR through which Ga_o signals (Gurel et al. 2012). It is likely that ELPC modifies signaling through one or both of these receptors. To test this genetically, we have developed an assay that optogenetically stimulates the release of serotonin from the NSM and ADF serotonergic neurons. This strongly decreases the speed of worms that have intact MOD-1 and SER-4 signaling pathways, but this serotonin response is defective in animals missing one or both of the receptors. With WormLab worm-tracking software we are able to monitor the speed of worms throughout the course of the experiment. Using this assay we will be able to genetically determine if ELPC affects signaling through the MOD-1 or SER-4 receptor. We are also working to identify the post-translational modifications of Ga_o using mass spectrometry. Analysis of Ga_o immunoprecipitated from both C. *elegans* lysates as well as mouse brain lysates will allow us to determine if worm and mammalian Ga_o are equivalently modified.

674A. Remote Control and Observation for more meaningful Behavioral Experiments. **Andy Papp**, John Biondo. Tritech Research, Los Angeles, CA.

C. elegans is an excellent organism for the study of the molecular genetics of a variety of neurologically-based phenomena such as mechanosensation, chemosensation, thermosensation, photosensation, behavior, learning, and memory because it manifests all of these via its simple, well characterized 302-cell nervous system (Murakami 2007). The sensitivity and interactions of these different systems necessitate carefully controlled experiments so that a single variable can be examined and meaningful conclusions can be drawn. For example, one would not want sensitive chemotaxis-based learning experiments to be interfered with due to thermotaxis, phototaxis, or mechanical stimulation caused by an inadequately controlled environment.

To create a more stable environment for these experiments, we have developed a small incubator with an integrated web-cam microscope that we call the "Telebator". It can be remotely controlled, and its contents observed, via a local or Internet computer network connection (US Pat. 7618808). This

system allows behavior to be observed and recorded in an environment that is isothermal with constant lighting and no changes in mechanical stimulation.

The Telebator's remote-control and monitoring features also make it ideal for various other applications, including: heat shocks, temperature shifts, knowing/controlling when a plate grown for DNA extraction is just about to starve, knowing/controlling when worms of a specific developmental stage are plentiful, knowing if/when a fluorescently marked transgene is being expressed, etc. In the event of a temperature or other alarm, the system is able to notify users by e-mail, SMS text message, and YahooIM. The incubator contents and temperature log data can be observed and the incubator temperature and other parameters can be controlled via any web browser, including a "smart phone".

Murakami (2007) Caenorhabditis elegans as a model system to study aging of learning and memory. Mol Neurobiol. 35(1):85-94.

675B. Genetic screens for IL2 lineage-specific regulators in *Caenorhabditis elegans*. **Dongjun Park**[1], Peter Swoboda[2], Junho Lee[1,3]. 1) IMBG, Seoul National University, SEOUL, South Korea; 2) Department of Biosciences and Nutrition, Karolinska Institute, Sweden; 3) World Class University, Department of Biophysics and Chemical Biology, Seoul National University, Seoul, Korea.

Ciliated IL2 sensory neuron is important for nictation, a dauer specific behavior. We previously reported that dauers in which IL2 neurons were genetically ablated could not nictate(1). IL2 sensory neurons consist of six cell bodies. Each cell body has a unique embryonic lineage. Neural progenitors divide asymmetrically into two lineages; One lineage becomes IL2 neurons and the other lineage divides into two sublineages, again. Two divided sublineages generate IL1 sensory neurons and apoptotic cells. However, any lineage-specific regulator has not been identified in neurogenesis of IL2 yet. In this study, we tried to find the genes that regulate these asymmetrical cell divisions. At first, we labeled IL1 and IL2 neurons with fluorescent markers dsRED and GFP, respectively. We treated worms with EMS for random mutagenesis. Then we sorted out candidate mutant worms with COPAS biosort. which optically analyzes and sorts living multicellular organisms on the basis of fluorescent protein expression patterns and other optical signatures, at rates up to about 100 organisms per second(2). We selected two mutant lines. One mutant line has only dsRED signals and the other lines has ectopic GFP signals that are positioned posterior to the normal positions. We try to find out which genes regulate these mutational phenomenon. Our study will contribute to elucidating the mechanism of IL2 development and/or differentiation. (1) H. Lee, M. Choi, et al., (2012) Nictation, a dispersal behavior of the nematode *Caenorhabditis elegans*, is regulated by IL2 neurons, Nature Neuroscience Volume 15, 107-112 (2) Rock Pulak, (2006) Techniques for Analysis, Sorting, and Dispensing of *C. elegans* on the COPAS™ Flow-Sorting System, Methods in Molecular Biology Volume 351, pp 275-286.

676C. Regulation of the Egg-Laying Behavioral Response to Hypoxia. **Corinne Pender**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139.

Response to changes in levels of oxygen is a fundamental process in human physiology and plays a major role in pathologies as diverse as cardiovascular disease, stroke and cancer. More generally, the capacity to respond to fluctuations in oxygen is an important adaptation for many organisms. Diminished access to oxygen can elicit metabolic, developmental and behavioral responses. Much remains unknown about the molecular and neural mechanisms underlying behavioral modifications triggered by chronic exposure to low oxygen. We are using egg-laying behavior of *C. elegans* as a model for studying behavioral responses to decreased oxygen concentrations. Upon exposure to hypoxia (0.5% oxygen), worms decrease their egg-laying rate. The conserved prolyl hydroxylase EGL-9 is a key component of the response to hypoxia. *egl-9* was originally identified in a *C. elegans* screen for mutants defective in egg laying. EGL-9 defines an evolutionarily conserved family of enzymes that hydroxylate the transcription factor HIF-1 using available oxygen, thus targeting HIF-1 for degradation. Increase in HIF-1 activity as a result of reduced inhibition by EGL-9 under hypoxic conditions is the basis for many adaptations to hypoxia by *C. elegans*. Additionally, *hif-1(lf)* mutations suppress the egg-laying defect of *egl-9(lf)* mutants, indicating that *egl-9* function requires *hif-1* activity. Thus, it is likely that the inhibition of egg laying under hypoxia is controlled by the *egl-9/hif-1* pathway. Previous work has demonstrated that the sites of action of EGL-9 for controlling egg laying are in the nervous system and the uv1 cells. To find downstream effectors of HIF-1 that control egg laying in response to hypoxia, we are conducting screens using an *egl-9(lf)* background to identify mutations that suppress the egg-laying defect of *egl-9* mutants. As *hif-1(lf)* suppresses the egg-laying defect of the *egl-9(lf)* mutant, we expect downstream effectors of *hif-1* in the control of egg laying to similarly suppress this defect. We have identified 17 suppressors of the *egl-9* egg-laying defect, at least 13 of which are not alleles of *hif-1*. These suppressors might represent mutations in new genes required for behavioral adaptation to hypoxia.

677A. A male-specific neuropeptide, FLP-23, is necessary for sperm transfer in *C. elegans*. Renee Miller[1,2], Inna Hughes[1], Teigan Ruster[1,2], Andrew Spitzberg[1,2], Steven Husson[3], Tom Janssen[4], Liliane Schoofs[4], **Douglas Portman**[1]. 1) Ctr Neural Dev & Disease, Univ Rochester Sch Med Dent, Rochester, NY; 2) Dept. of Brain and Cognitive Sciences, Univ. of Rochester, Rochester, NY; 3) Dept. of Biology, Univ. of Antwerp, Antwerp, Belgium; 4) Dept. of Biology, K.U. Leuven, Leuven, Belgium.

Neuropeptides play important, conserved roles in modulating and optimizing animal behaviors, including reproductive behaviors. In *C. elegans*, males execute a stereotyped, multistep mating program upon contact with receptive hermaphrodites. Classical neurotransmitters (e.g., acetylcholine), as well as neuromodulators (monoamines and neuropeptides), have been shown to mediate various steps of male mating behavior. Using a peptidomic approach, we identified FLP-23 as a candidate male-specific member of the FMRF family of neuropeptides. Using reporter genes, we found that *flp-23* is expressed in two neurons in the male tail (provisionally identified as DVE and DVF), but expression is undetectable in the hermaphrodite. Interestingly, *flp-23* mutant males appear morphologically normal but fail to sire cross-progeny. *flp-23* males execute the early steps of mating normally, but never transfer sperm to the hermaphrodite uterus. We are currently working to identify the exact behavioral step(s) that are defective in these animals, and are taking candidate-based approaches to identifying a FLP-23 receptor. Along with recent evidence from *Drosophila*, our work suggests that neuropeptides may be conserved mediators of sperm transfer.

678B. A Potential Role for Palmitoylation in the Acute Response to Ethanol. **R Raabe**, A.G. Davies, J.C. Bettinger. Virginia Commonwealth University, Richmond, VA.

An individual's naïve level of response (LR) to ethanol is predictive of their lifetime likelihood to abuse alcohol, and this initial LR is genetically influenced. This suggests that the genes that are responsible for LR may also be central to the development of abuse disorders, making them particularly attractive

targets for research. Our laboratory uses C. elegans to investigate the genetic influences on responses to acute ethanol exposure. We have focused on two components of ethanol response: initial sensitivity, measured as the change in mean velocity over the first ten minutes of treatment, and the development of acute functional tolerance (AFT), measured as an increase in the mean velocity at thirty versus ten minutes of exposure. We have previously found that SLO-1, the large-conductance potassium (BK) channel, is likely to be a direct target of ethanol. *slo-1* mutants are profoundly resistant to ethanol, and are defective in AFT. Recently, we used a forward genetic screen for mutations that disrupt the ability of worms to develop AFT to identify *ctbp-1*, a transcriptional repressor that affects AFT through its negative regulation of a triacylglyceride lipase, *lips-7*. *lips-7* mutants have the opposite phenotype to *ctbp-1*; enhanced development of AFT. Preliminary analysis of the lipid profile of the *lips-7* mutant versus wild type has suggested that *lips-7* mutants may have an increase in palmitic acid. This led us to ask if and how palmitic acid may be involved in AFT. Palmitoylation, the reversible addition of a lipid to a protein, can alter ion channel activity directly and indirectly. Mouse mSLO-1 has multiple palmitoylation sites that affect localization, internalization, activity, and trafficking. This suggests one model for AFT in which the effects of ethanol on targets such as SLO-1 are modulated by palmitoylation. We have tested the ethanol responses of a combination of RNAi and loss-of-function mutations in various genes related to the synthesis, degradation, or use of palmitic acid. We have identified several suspected palmitoylation-related genes that alter both initial sensitivity and AFT. Our goal is to better understand how the palmitoylation machinery modifies acute responses to ethanol.

679C. PKC-1 mediates responses to 5-HT-dependent behavioral adaptation in *C. elegans*. **Seth Ronk**, Eric Foss, Lucinda Carnell. Dept. of Biological Sciences, Central Washington University, Ellensburg, WA.

We are interested in understanding the cellular and molecular mechanisms involved in 5HT-dependent behavioral adaptation. We have found that overnight exposure to 3 mg/ml serotonin (5-HT) results in a recovery of speed from the inhibitory acute effect on locomotion. Acute exposure results in average speeds of 41 mm/s that increases to 158 mm/s after adaptation, which is a 73% recovery compared to untreated control speeds (215 mm/s). Through a forward genetic screen we identified a mutant that is defective in adaptation to 5-HT, which has an additional phenotype observed in the absence of 5-HT. Upon reaching the edge of the bacteria lawn they cease movement and only move when the food source is entirely exhausted. We refer to this behavior as depressed foraging. Animals deficient in *pkc-1*, an ortholog to the mammalian PKC epsilon, also display depressed foraging. Therefore, we tested *pkc-1* mutants for defects in behavioral response to acute and chronic responses to 5-HT. We found the *pkc-1* (*nj3*) mutant is deficient in behavioral adaptation, displaying increases in speeds to only 62 mm/s, compared to acute speeds of 23 mm/s, representing a recovery of 31%. *pkc-1* (*nj3*) also appears to display hypersensitivity to 5-HT compared to wild-type animals with speeds of 22 mm/s and 41 mm/s at 3 mg/ml concentrations, respectively. This hypersensitivity to 5-HT could contribute to the depressed foraging behavior observed when untreated worms are grown on a bacterial lawn. The worms could be displaying a type of enhanced slowing with food due to increased release of 5-HT or increase function of 5-HT pathways. These results offer insight to a pathway that is involved in 5-HT-dependent behavioral adaptation. Both animals pre-treated with 5-HT and *pkc-1* mutants have been shown to be resistant to aldicarb, suggesting both influence acetylcholine release in the motor neurons of NMJ (Sieburth et al. 2005). Further studies will be performed to examine the role of the chronic effects of 5-HT on acetylcholine release.

Sieburth, D., Madison, J.M. & Kaplan J.M. (2007) PKC-1 regulates secretion of neuropeptides. Nat Neurosci 10, 49-57.

680A. Internal metabolic status modulates pheromone-mediated neural plasticity in *C. elegans*. **L. Ryu**, K. Kim. DGIST, Daegu, South Korea.

The nervous system senses and processes environmental signals in the context of internal and/or external signals to generate proper behaviors. Behavioral plasticity such as habituation, sensitization, and short/long-term memory is essential for survival and evolution of animals and can be mediated by changes at the sensory receptor and/or neuronal circuit levels. However, the molecular and cellular mechanisms underlying these mechanisms of behavioral plasticity are not well understood. *C. elegans* provides an ideal system in which to explore the molecular and neural basis of behavioral flexibility. Previously, we and others found that adult hermaphrodites specifically avoid the ascaroside pheromone C9 (ascr#3) (Srinivasan et al., 2008; Macosko et al., 2009; Jang et al., 2012). The ADL chemosensory neurons detect and drive repulsion to C9 (Jang et al., 2012). *In vivo* Ca^{2+} imaging experiments showed that addition of C9 induces a rapid increase in ADL intracellular Ca^{2+} levels which returns quickly to baseline even in the presence of C9. When animals were exposed to 10-second pulses of C9 at 10-second intervals, ADL exhibited Ca^{2+} transients to the addition of only the first pulse of C9 but not to following pulses indicating that ADL quickly habituates to C9 (Jang et al., 2012). We now find that when animals are starved for just 5 mins, ADL exhibits Ca^{2+} transients to not only the first pulse but also to a second pulse of C9. This observation suggests that C9 habituation of ADL may be modulated by internal feeding status. To investigate how internal metabolic conditions influence ADL pheromone responses, we are currently performing a series of experiments including behavioral assays, candidate gene searches and genetic screens.

681B. Identification of molecules interacting with the insulin/PI3K pathway involved in salt chemotaxis learning. **N. Sakai**[1], M. Tomioka[1], T. Adachi[2], T. Jiang[1], Y. Iino[1]. 1) Dept. of Biophys. and Biochem., Grad Sch. of Sci., Tokyo, Japan; 2) Dept. of Biol. Sci., Fac. of Sci., Kanagawa Univ., Kanagawa, Japan.

The insulin/PI 3-Kinase (PI3K) signaling pathway is widely conserved among many species including mammals, and regulates a variety of phenomena such as tumor progression and metabolism. Recently, this pathway is reported to be involved also in learning and memory. However, so far, a mechanism by which this pathway regulates neuronal plasticity is poorly understood.

Previously, we have found that *C. elegans* changes responses to external salt concentrations depending on food conditions. When worms are cultivated on a medium that contains sodium chloride (NaCl) and bacterial food, they are attracted to the NaCl concentration at which they are grown. In contrast, after exposure to NaCl under starvation conditions, they learn to avoid the NaCl concentration. This behavioral change is called “salt chemotaxis learning”.

Mutants of the insulin/PI3K pathway components show strong defects in salt chemotaxis learning. Although the DAF-16/FOXO-dependent transcription is a major output of the insulin/PI3K pathway in the control of developmental decision and aging, other machinery downstream of the insulin/PI3K pathway has been predicted in salt chemotaxis learning .

In this study, we aimed to dissect the molecular mechanisms downstream of the insulin/PI3K pathway in chemotaxis learning by using both forward and reverse genetics approaches. Through a screen for suppressors of the abnormal behavior in *daf-18*, which is a negative regulator of the insulin/PI3K pathway, we obtained 4 suppressors and found that the three of them have point mutations in *goa-1*, which encodes an alpha subunit of Go. Therefore

communication between the Go protein signaling and the PI3K signaling may be important for salt chemotaxis learning. Furthermore, we also found other putative signaling molecules downstream of insulin/PI3 kinase pathway using a reverse genetic approach.

682C. Regulation of Behavioral Suspended Animation and Mitochondrial Dynamics in Response to Oxygen Deprivation. **N. Salazar-Vasquez**, P. Ghose, E.C. Park, A. Tabakin, C. Rongo. Waksman Institute of Microbiology , Rutgers, The State University of New Jersey , Piscataway, NJ.

Oxygen is essential for the function and viability of neurons. Neurons have high energy demands, and unlike other cells, neurons do not store glycolytic reserves, relying instead on oxidative phosphorylation by their mitochondria. Mitochondrial size exists in a dynamic equilibrium through balanced processes of organelle fission and fusion. Using the *C. elegans* command interneurons as a model, we report that the mitochondrial dynamic fusion/fission machinery is a target of regulation during oxygen deprivation. Animals subjected to anoxia (no oxygen) conditions undergo mitochondrial fission and behavioral and developmental arrest. Upon reoxygenation, their mitochondria reconstitute and normal behavior and development resumes. We believe this response is modulated by the hypoxia response pathway. Mutants for the proline hydroxylase gene *egl-9* show faster behavioral recovery and faster and increased mitochondrial fusion, termed “hyperfusion”. This hyperfusion phenotype requires the hypoxia inducible factor HIF-1 and STL-1, the orthologue of the mammalian stomatin-like protein SLP-2. Our findings suggest that these mitochondrial and behavioral responses to oxygen deprivation allow the animal to survive anoxic conditions. Understanding more about the exact mechanism used by the nematode during anoxia might help us understand how neurons react to oxygen stress and how mitochondria contribute to neuronal damage and neurodegeneration in humans.

683A. The *C. elegans* interneuron ALA is a nociceptor. **Jarred Sanders**[1], Stanislav Nagy[2], Graham Fetterman[2], Charles Wright[2], David Biron[2,3]. 1) Committee on Genetics, Genomics, and Systems Biology, The University of Chicago, Chicago, IL 60637; 2) The Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL 60637; 3) Department of Physics and the James Franck Institute, The University of Chicago, Chicago, IL 60637.

To avoid tissue damage and survive harsh environments, it is essential for all animals to appropriately respond to noxious stimuli. Such stimuli are detected by specialized neurons, called nociceptors, which are evolutionarily conserved. We have found that in the nematode *C. elegans*, the unilaterally placed single interneuron ALA acted as a nociceptor: it was required for the inhibition of egg-laying in response to noxious touch, but not for immediate avoidance responses. Moreover, ALA exhibited distinct physiological responses to anterior and posterior noxious touch, suggesting that it could distinguish between spatially separated stimuli. These responses required neither neurotransmitter nor neuropeptide release from potential upstream neurons. In contrast, the long, bilaterally symmetric processes of ALA itself were required for producing its physiological responses: when they were severed, responses to stimuli administered between the cut and the cell body were unaffected, while responses to stimuli administered posterior to the cut were abolished. Our findings suggest that ALA can autonomously sense noxious touch and is thus a novel nociceptor.

684B. G protein coupled receptor SRTX-1 is a key component for thermosensation in AFD ensuring temperature sensation range. **Hiroyuki Sasakura**[1,3], Hiroko Ito[1], Kyogo Kobayashi[1], Keita Suzuki[1], Ikue Mori[1,2,3]. 1) Nagoya University; 2) Institute for Advanced Research; 3) CREST, JST, Japan.

Temperature is a ubiquitous stimulus that affects numerous biological phenomena such as homeostasis, metabolism, behavior and aging. Although TRP ion channels play pivotal roles to detect a wide range of temperatures, molecular machinery of TRP independent thermosensation is largely unknown. *C. elegans* senses the environmental temperature by two pairs of sensory neurons, AFD and AWC to exhibit thermotaxis. Signal transduction pathway for temperature in AFD and AWC is similar to that in visual and olfactory system in mammals (Mori et al., 2007; Kuhara et al., 2008). A recent study in *Drosophila* larva showed that Rhodopsin GPCR known as a light sensor plays critical role for thermosensation (Shen et al., 2011). Thus, it is plausible to hypothesize that GPCRs evolutionarily play an important role for sensing the environmental temperature. Previous reports showed that *srtx-1* encodes the GPCR specifically expressed in AFD and AWC (Colosimo et al., 2004; Biron et al., 2008). To reveal the role of SRTX-1, we isolated *srtx-1* null mutants and examined thermotaxis (Hedgecock and Russell, 1975). We found that *srtx-1* null mutants exhibited abnormal thermotaxis. The ability to migrate higher or lower regions on a thermal gradient is severely lost in *srtx-1* mutants, whereas wild type animals migrated up or down the gradient to reach the past cultivation temperature. The abnormal thermotaxis was rescued by expressing *srtx-1cDNA* only in AFD and Ca^{2+} imaging showed that AFD response to temperature is decreased in *srtx-1* mutants. In contrast, the overexpression of *srtx-1cDNA* in AFD caused wild type animals to disperse on thermal gradient, which is the opposite phenotype to the narrowed migration phenotype of *srtx-1* null mutants. We suggest that SRTX-1 ensures AFD to sense a wide range of environmental temperature.

685C. Control of sleep-like behavior by the G-alpha(q) gene *egl-30*. **Juliane Schwarz**, Henrik Bringmann. Sleep & Waking, Max Planck Institute for Biophysical Chemistry, Göttingen, Niedersachsen , Germany.

Sleep-like states are characterized by massively reduced behavioral and neural activity. Nearly nothing is known about the genetic control of sleep-like behavior. It is also not clear how general activity levels during wake-like behavior influence activity levels during sleep-like behavior. We studied sleep-like behavior during Lethargus in larvae of *C. elegans* and looked through a set of known mutants with altered activity levels. Using hydrogel microchambers we could easily monitor behavior and measure neuronal activity levels across the sleep- and wake-cycle. We found that activity levels are generally correlated for almost all mutants: increased activity levels during wake-like behavior typically resulted in increased activity during sleep-like behavior. Among these hyperactive mutants was a gain-of-function mutant of the conserved heterotrimeric g protein subunit g-alpha(q) gene *egl-30*. Unexpectedly we found a loss-of-function mutant of *egl-30* with less activity during their wake state but virtually no sleep-like activity: mutants lacked prolonged periods of complete immobility, had less reduced basal as well as evoked neural activity and showed less reduction of response upon stimulation. Egl-30 is a highly conserved key regulator of behavior. The data demonstrate that Egl-30 not only controls general activity levels, but also differences between wake and sleep-like behavior.

686A. Identifying Novel BK Channel Modulators. **Luisa Scott**[1], Sangeetha Iyer[1], Scott Davis[2], Ashley Philpo[1], Angela Shen[1], Sarah Nordquist[2], Jon Pierce-Shimomura[1]. 1) WCAAR, University of Texas at Austin, Austin, TX; 2) Institute of Neuroscience, University of Texas at Austin, Austin, TX.

The large-conductance calcium-activated potassium (BK) channel is an evolutionarily conserved target of ethanol. Physiologically relevant concentrations

of ethanol increase BK channel opening. Additionally, genetic manipulation of BK channel function affects behavioral sensitivity to ethanol in multiple animal models. These findings suggest that the BK channel is a viable therapeutic target for alcohol intoxication and addiction. To begin to test this, we have developed a screen to identify peptides that alter BK channel function. First, we screened 30 million 9 amino acid peptide sequences using a monovalent phagemid display technique. Twenty-seven unique peptides remained after panning for sequences that bind to the human BKa channel but not the rat SK2 channel or human glycine a1 receptor. Sequences with clusters of positively charged amino acids were enriched 100-300 fold. Three motifs were enriched 3000-6000 fold. Next, select peptides were rapidly screened for functional effects using the nematode, C. elegans. Several peptides with highly enriched motifs affected an ethanol- and BK-channel dependent behavior in this animal. The behavioral assay suggests that peptide pskan4 enhances the ethanol mediated increase in BK channel opening, but does not alter channel activity in the absence of ethanol. Peptide pskan1 had BK channel- but not ethanol-dependent effects. In contrast, a peptide without a highly enriched motif had non-specific effects, and a 9 amino acid peptide not selected in the panning procedure did not alter locomotion. Finally, we have begun to confirm the function of the peptides electrophysiologically. Preliminary recordings support the putative action of peptides pskan1 and pskan4 as BK channel openers. Overall, these findings show that we have developed and successfully employed a screen for identifying and characterizing novel peptides that alter BK channel-dependent behavior in the presence of physiologically relevant concentrations of ethanol. This screening technique can be applied to identify modulators of other ion channels.

687B. The *C. elegans* male regulates movement direction during mating through cholinergic control of the sex-shared command cells. **Amrita Laxman Sherlekar**[1], Abbey Janssen[1], Meagan Siehr[1], Laura Caflisch[1], May Boggess[2], Robyn Lints[1]. 1) Biology Dept., Texas A&M University, College Station, TX; 2) School of Mathematical and Statistical Sciences, Arizona State University, Tempe, AZ.

C. elegans male mating behavior represents a tractable paradigm for understanding the neural bases of sex-specific behaviors, decision-making and sensorimotor integration. The first phase of mating behavior, the vulva search, is triggered when the male physically contacts a potential mate with his rays, male-specific sensilla associated with the male genitalia. In contrast to male movement in the absence of a mate, the contact-based search is conducted with backward, rather than forward, locomotion. However, when the male senses the vulva with his hook sensillum, movement ceases and the male attempts copulation. How does mate contact induce this dramatic change in movement direction and how does the hook terminate this behavior? Using optogenetics, cell-specific ablation- and mutant behavioral analyses, we find that the male exploits the sex-shared locomotory system to drive search locomotion. The male renders this system responsive to mating cues through male-specific inputs. This pathway consists of the male sensory rays, interneuron PVY and it's auxiliary neuron PVX. Although PVY and PVX synapse with both forward and backward command cells, PVY preferentially stimulates the backward cells because of a bias in synaptic inputs and in the distribution of relevant neurotransmitter receptors, in favor of the backward command neurons. Unlike many sex-shared sensory pathways, PVY/PVX regulation of the command cells involves cholinergic, rather than glutamatergic, transmission mediated by receptors containing ACR-18, ACR-16 and UNC-29 subunits. The use of cholinergic transmission likely contributes to response specificity and may serve to coordinate directional control with other cholinergic-dependent motor behaviors of the mating sequence. Termination of movement with detection of the vulva involves down-regulation of PVY activity by the hook. These findings, together with our dissection of the hook-to-PVY/PVX signaling mechanism, will be presented.

688C. Is sleep conserved? Making the case in *D. melanogaster* and *C. elegans*. **Komudi Singh**, Jennifer Y. Ju, Melissa B. Walsh, Michael A. DiIorio, Anne C. Hart. Neuroscience, Brown University, Providence, RI, 02912.

Sleep is a ubiquitous behavior. However, it is unclear if the genes and pathways regulating this process are conserved across animal species. Limited evidence of conservation exists; Notch, EGF pathways and cGMP kinase regulate sleep across species. To test conservation more broadly, we surveyed the *Drosophila* literature and shortlisted eighteen genes required for *Drosophila* sleep-like behavior. Sixteen orthologous *C. elegans* genes were identified based on similarity. The impact of these sixteen *C. elegans* genes on molting lethargus quiescence and arousal was tested using previous defined assays (Singh *et al*., 2011, WBPaper00038400). All of the genes that affected *Drosophila* sleep also altered *C. elegans* L4/adult quiescence. In fact, the change in quiescence was precisely what would be predicted from the *Drosophila* literature, with one exception- suggesting deep conservation of regulatory mechanisms. Next, we aimed to connect these conserved genes in a pathway and establish site of action in the nervous system of *C. elegans*. The wake-promoting function of D1 dopamine receptor is conserved as animals lacking D1 dopamine receptors had increased sleep-like behavior in *Drosophila* and *C. elegans*. We find that the *C. elegans* G_sa protein is also required for lethargus quiescence. Further, G_sa, cAMP, PKA, and CREB likely all function in D1 dopamine receptor expressing neurons to regulate *C. elegans* quiescence. Additionally, we find that serotonin regulates quiescence and likely functions through the G_oa and the DAG kinase pathway in quiescence, extending previous studies (Yuan *et al*., 2006, Guo *et al*., 2011). Analysis of *C. elegans* orthologs of *Drosophila* genes expands the list of conserved genes that regulate sleep across species. The striking conservation observed in these two disparate invertebrate animals suggests that these genes and pathways regulate sleep in all species.

689A. Expanding the Spectrum of Dopamine Regulators: Swip-10, the (m)BLAC Sheep of the Family. **C. Snarrenberg**, S. Whitaker, Q. Han, E. Pohl, J.A. Hardaway, R.D. Blakely. Dept. of Pharmacology, Vanderbilt University.

Several neurological disorders are associated with disrupted dopamine (DA) signaling including Parkinson's disease, schizophrenia and addiction. In C. elegans, an inability to clear synaptic DA results in swimming induced paralysis (Swip). In a screen for animals that display DA-dependent Swip, we identified two mutants that support DA-dependent Swip, vt29 and vt33, and that lie within a previously undescribed gene F53B1.6, now named swip-10. Sequence analysis revealed highly conserved mouse and human orthologs, Mblac1 and MBLAC1 respectively, named for the presence of metallo-b lactamase domains. Swip-10 promoter:GFP fusion constructs yielded expression in C. elegans glial cells in vivo. Consistent with this localization, rescue of Swip was achieved by expressing swip-10 under either its own promoter or the ptr-10 glial promoter. In the mammalian brain, glial cells regulate DA neurons and DA-modulated circuits by clearing extracellular glutamate via expression of high affinity glutamate transporters. Immunocytochemical studies reveal high levels of Mblac1 expression in the mouse brain in areas associated with glutamatergic control of emotional and reward behaviors including the prefrontal cortex, hippocampus, nucleus accumbens, habenula and amygdala. In keeping with a role of swip-10 in regulating glutamate-dependent DA neuron excitation, we found that mutation of multiple GLTs generates DA-dependent Swip and that swip-10 and glt-4 interact genetically to trigger Swip.

Additionally, loss of the glutamate receptors, glr-4, glr-6 and mgl-1, individually and in combination diminished the Swip of swip-10. Our results suggest that swip-10 is a novel regulator of extracellular glutamate via altered glutamate transport. We hypothesize that knockout of swip-10 elevates tonic extracellular glutamate, increases excitation of DA neurons, and increases DA release. Further analysis of swip-10 and its mammalian orthologs may lead to novel insights into brain disorders linked to altered glutamate signaling, including those for which perturbed glutamate-control of DA signaling has been documented. F31MH09312 to JAH and R01MH095044 to RDB.

690B. Quantitative trait loci mapping of temperature-dependent behaviour in *Caenorhabditis briggsae*. **Gregory W. Stegeman**[1], Asher D. Cutter[1], William S. Ryu[2,3]. 1) Department of Ecology and Evolutionary Biology, University of Toronto; 2) Donnelly Centre, University of Toronto; 3) Department of Physics, University of Toronto, Toronto, Ontario, Canada.

In order to understand how evolution acts on complex traits like behaviour, we need to define the causative genetic variants underlying these traits of interest. To further this aim we are working to determine the genetic basis of natural variation in temperature-dependent behaviour among *Caenorhabditis briggsae* isolates from temperate and tropical groups. Compared to *C. elegans*, *C. briggsae* has a strong phylogeographic pattern which indicates latitudinal differentiation. Most *C. briggsae* wild isolate strains fit into one of two genetically similar groups which have been named the 'tropical' clade or temperate 'clade' according to the latitudes at which strains from these groups have been isolated. We found that worms of strain AF16 from the 'tropical' clade tolerate a higher temperature and are able to continue locomotion at higher temperatures than worms of strain HK104 from the 'temperate' clade. We developed a droplet-based behavioral assay where the swimming behaviour of individual worms is monitored while the temperature of the system is programmatically controlled. Because we suspect that natural variation in behaviour is multigenic we took a quantitative genetic approach and assayed the *C. briggsae* AF16-HK104 recombinant inbred lines (RIL) (Ross, J.A., et al., 2011. PLoS Genetics, 7(7), e1002174) using our droplet assay. We found a gradient of behavioural variation along with some transgressive segregation among the RIL phenotypes. According to our quantitative trait loci (QTL) mapping analysis, two loci may explain the natural thermal behaviour variation observed between AF16 and HK104. The most striking aspects of behaviour which differ most between the "temperate" and "tropical" parents, map to the center of Chromosome 2 and a larger region covering the left and centre region of Chromosome 5. This analysis suggests this temperature-dependent behaviour is at least digenic and possibly involves more loci and further work will attempt to resolve these loci and ultimately locate the causative nucleotide differences.

691C. Odorant choice behavior and systematic reverse genetics approach to reveal molecular mechanisms underlying the behavior. **Y. Suehiro**, S. Mitani. Department of Physiology, Tokyo Women's Medical University, Shinjuku, Tokyo, Japan.

Animals integrate sensory signals, make decisions and choose proper behaviors. Although the mechanisms of the behavioral choice have been studied in various vertebrates, molecular bases underlying it have not been identified because of the complex nervous systems. *Caenorhabditis elegans* has simple nervous system with only 302 neurons and can perform behavioral choice. To reveal the molecules required for the behavioral choice systematically, we performed screening of genes using *C.elegans*. In our laboratory, 5214 mutant strains have been already isolated. Among the mutants, we selected and analyzed 1479 strains carrying mutations in genes, which are expressed in head neurons or are suggested to be involved in neural function. For the screening, we developed a novel choice assay using odorants. *C. elegans* shows chemotaxis toward odorants such as diacetyl (DAc) and benzaldehyde (Bz). Because an increase of DAc is received by AWA neurons and a decrease of Bz is received by AWC neurons, we performed following methods to stimulate AWA and AWC neurons simultaneously. Bz and DAc were dropped on the left and right side of an agarose plate, respectively. Then, we put worms on the spot where the diluted Bz was dropped, and counted the worms on both sides of the plate 1 hour after. During this assay, it is estimated that worms receive a decrease of Bz and increase of DAc simultaneously by moving from the initial point. When the 1:200 diluted Bz and 1:1000 diluted DAc were used as stimuli, approximate half of wild type worms were attracted to the DAc and the other worms stayed around the initial point where Bz was dropped, suggesting that our assay can be used to quantitate the behavioral choice. Under the condition, we searched for mutants, which showed abnormal choice, and found 143 mutants as candidates. To exclude mutants, which have defects in chemotaxis, we used ethanol instead of each odorant and assayed. Finally, we obtained 72 candidate genes, which have not been reported to be involved in behavioral choice. Now we are examining expression patterns of candidate genes and analyzing cell functions of some interneurons related to the odorant chemotaxis by genetic ablation.

692A. Sensory inputs are centrally integrated to modulate nociception in *Caenorhabditis elegans*. **Philip Summers**, Amanda Ortega, Richard Komuniecki. University of Toledo, Biological Sciences, Toledo, OH.

In *C. elegans*, sensory neurons synapse onto a layer of interneurons that, in turn, synapse onto the command interneurons to modulate sensory-mediated locomotory decision-making. This study is focused on understanding how multiple sensory inputs are integrated by the AIB interneurons to modulate aversive responses to dilute octanol mediated by the ASH sensory neurons. Ablation of the AIBs decreased the rate of spontaneous reversal, but, in contrast, decreased the time taken to initiate reversal in responses to dilute octanol. In fact, inhibition of AIB signaling using a variety of approaches also decreased the time taken to initiate an aversive responses. The ASH, ADL, ASE, and AWC sensory neurons provide the major glutamatergic inputs into the AIBs, so to examine the role of glutamatergic signaling from these neurons on ASH-mediated behaviors, *eat-4* that encodes a vesicular glutamate transporter was selectively overexpressed on the assumption that *eat-4* overexpression would increase tonic glutamatergic signaling. Selective *eat-4* overexpression in the ASHs, ADLs or ASEs decreased the time taken to initiate reversal off food, while *eat-4* overexpression in the AWCs abolished the 5-HT stimulation of aversive responses. To identify the glutamate receptors involved, *eat-4* was selectively overexpressed in animals with null alleles for glutamate-gated anion and cation channels and once the appropriate receptors were identified, their specific roles in the AIBs were confirmed by AIB-specific knockdown. As predicted, ASE signaling inhibited the AIBs by activating the glutamate-gated Cl- channel encoded by *avr-14* and AWC signaling inhibited the 5-HT stimulation of aversive responses by activating the AMPA-like glutamate gated channel, GLR-1. Indeed, the AIB-specific overexpression of *avr-14* decreased the time taken to initiate reversal off food and the AIB-selective overexpression of *glr-1* abolished food or 5-HT stimulation. These studies are continuing to examine how multiple sensory inputs are integrated in the modulation of both spontaneous and sensory-evoked reversals and may provide useful insights into disorders associated with the altered sensory integration. Supported by NIH grant AI-145147.

693B. Exploring the role of *rapsyn-1* in regulating *C. elegans* behavior. **Ada Tong**, Sreekanth Chalasani. MNL-SC, Salk Institute, La Jolla, CA.

Acetylcholine (ACh) signaling is a well-studied pathway in the neuromuscular junction (NMJ), but its role in the nervous system is poorly characterized. Rapsyn is a 43-kD protein that clusters and anchors acetylcholine receptors (AChR) in the postsynaptic membrane of the NMJ. Mutations in rapsyn have been associated with neuromuscular diseases in humans. For example, the N88K mutation in the *RAPSN* gene causes congenital myasthenic syndrome (1). In mice, targeted disruption of the RAPSN gene causes death within a few hours of birth (2). In *C. elegans*, however, *rapsyn-1 (rpy-1)* mutants are viable and appear wildtype in their locomotory ability. We plan to combine genetics and behavioral analysis to understand the role of *rpy-1* in the worm.

We find that *rpy-1* mutants display a gain-of-function behavioral phenotype in the local search assay. They make twice as many turns in the first 15 minutes off food when compared to N2 wildtype animals. In order to test where *rpy-1* functions, we performed knockdown experiments by expressing sense and antisense *rpy-1* transcripts in muscles or in neurons. Surprisingly, neuronal knockdowns of *rpy-1* are similar to *rpy-1* mutants, while muscle knockdowns appear wildtype. This suggests that *rpy-1* is required in the neurons rather than in the muscle. We will use transgenic rescue experiments to identify the cellular sites of *rpy-1* action. We also plan on using candidate gene mutants and biochemical methods to identify components of the *rpy-1* signaling pathway. These studies will provide insights into ACh signaling in the nervous system.

(1) Dunne, V., Maselli, R. A., Hum. Genet. 49: 366-369 (2004)

(2) Gautam, M., et al., Nature 377: 232-236 (1995).

694C. Dopaminergic control of gait switching in C. elegans. **Stephen M. Topper**, Sara Aguilar, Layla Young, Jonathan Pierce-Shimomura. ICMB, The University of Texas at Austin, austin, TX.

The ability to switch between different forms of locomotion is critical to many aspects of survival, whether it is switching from walking to running to evade predators, or switching to a slower gait to obtain food. However, the neuronal mechanisms of gait switching are not well understood. We recently showed C. elegans, displays distinct crawl and swim gaits, mediated by dopamine and serotonin, respectively (Vidal-Gadea et al., 2011). Further investigation into the role of dopamine signaling in the transition to crawl has indicated the D1-like dopamine receptor dop-4 as a key component. Laser microablation of dop-4-expressing neurons has revealed single neurons that are required for the transition to crawl. Optogenetic activation of dop-4-expressing neurons via the light-activated GPCR optoXRB2 induces crawl-like behavior in swimming worms. Finally, expression of dop-4 in specific subsets of dop-4 neurons is sufficient to rescue the swim to crawl transition. We are now combining noninvasive imaging of these neurons in freely moving worms with optogenetics to dissect how dopamine controls gait switching at the circuit level. A genetic analysis dopaminergic locomotory in C. elegans may reveal fundamental principles on gait switching with implications for many fields, including the study of human ailments such as Parkinson's disease.

695A. Ethanol induces state-dependent behavioral transition. **Stephen M. Topper**, Sara Aguilar, Layla Young, Andres Vidal-Gadea, Jonathan Pierce-Shimomura. ICMB, The University of Texas at Austin, austin, TX.

Alcohol, the most widely abused drug in the world, has a wide range of effect on individuals and there are many factors that predispose one to addiction. Chief among them are stress, depression, and anxiety, all of which include altered monoamine signaling. It remains unclear how behavioral states differentially influence responses to ethanol. C. elegans displays distinct behavioral states associated with locomotion (crawling and swimming) that are mediated by dopamine and serotonin respectively. We found that ethanol-induced responses in C. elegans depended on crawl or swim behavioral state. Whereas alcohol non-specifically impaired locomotion and feeding in crawling worms on land, alcohol induced a specific transition from swim to crawl state in worms submerged in ethanol. This response was dependent on dopamine. Loss of the D1-like dopamine receptor DOP-4 impaired the EtOH-induced transition to crawl, while optogenetic inhibition of DOP-4 receptor-expressing neurons via the Arch chloride pump recovered the swim state. These results suggest that alterations of dopamine-dependent behavioral states can drastically influence EtOH-induced behaviors with implications for prevention of addiction. Acknowledgements: CGC and the Knockout Consortium for strains, Bruce/Jones Award for Alcohol and Addiction Research, Karl Deisseroff for reagents.

696B. Identification of genes involved in the pheromone signaling that regulates olfactory plasticity. **H. Toriyabe**[1], K. Yamada[1,2], Y. Iino[1]. 1) Dept.of Biophy. and Biochem., The University of Tokyo, Tokyo, Japan; 2) RIKEN, BRC, CED, Japan.

Pheromones of *C. elegans* were recently identified as mixtures of sugar derivatives called ascarosides. Ascaroside signaling regulates various behaviors, such as sex-specific attraction, repulsion, dauer formation and aggregation. Olfactory plasticity is also regulated by the pheromones. *C. elegans* is attracted to a series of odorants; however, after prolonged exposure to the odor in the absence of food, worms stop approaching the odorant and disperse from it. We recently reported that abundant pheromone is required for olfactory plasticity. We further identified a key gene involved in this regulation, *snet-1*, which encodes a neuropeptide. Low concentration of pheromone results in an over-production of the SNET-1 neuropeptide, which in turn appears to inhibit the olfactory plasticity. *snet-1* is expressed in a subset of head neurons, including the pheromone-sensing neurons ASI, where expression of *snet-1* is observed only in the absence of the pheromone. By observing the expression of *snet-1p::venus* in ASI neurons of mutant animals, we found that some of the known pheromone signaling molecules also regulate the *snet-1* expression: the *tax-4* cGMP pathway and the *daf-2* insulin pathway promote *snet-1* expression, while the *daf-7* TGF-b pathway represses the expression. To identify novel genes participating in the relay of pheromonal signals in adult worms, we mutagenized *snet-1p::venus* animals by ethyl methanesulfonate (EMS) and screened for mutants that showed constitutive expression of the Venus reporter in ASI neurons. In the screen, we obtained six candidate mutants. Pheromone-sensing neurons may modulate the response of olfactory sensory neurons or downstream neurons by changing the expression of *snet-1* in pheromone-sensing neurons through the signaling pathways described above.

697C. Why do sleeping worms look like hockey sticks? **Nora Tramm**, Naomi Oppenheimer, Efraim Efrati, Stanislav Nagy, David Biron. Department of Physics, JFI / IBD, The University of Chicago, Chicago, IL 60637.

At the transition between larval stages, *C. elegans* exhibits a sleep-like behavior during a stage termed lethargus. During lethargus, *C. elegans* adopts a characteristic hockey stick-like posture: a single anterior bend in an otherwise straight body. A characteristic posture is considered one of the behavioral

hallmarks of sleep, and typically includes functional features such as support of a substrate for the limbs and the head and shielding of sensory organs. What might be the function of a hockey stick-like posture? It was previously noted that during lethargus, *C. elegans* larvae abruptly rotate about their longitudinal axis. Plausibly, these "flips" facilitate ecdysis by assisting the disassociation of the old cuticle from the new one. However, the question of how longitudinally directed body wall muscles could generate a rotation about the anterior-posterior body axis was not previously addressed. Using a fluorescent calcium indicator expressed in body wall muscles, we found that body bends during lethargus quiescence were actively maintained, rather than tension being maintained passively, e.g. in analogy to the "catch state" in mollusks. Detailed analysis revealed that flips occurred preferentially when the animals exhibited a posture with a single body-bend during a bout of quiescence; when flips occurred the bend was preferentially in the anterior or middle section of the body. The inability of posterior body bends to drive flips raised the possibility that muscle control in the anterior and mid-body sections may be more refined than in the posterior sections. We developed a model in which body wall muscles at the bend, when actuated in appropriate relative phases, could drive longitudinal rotation. Our analysis and observations suggest that, rather than being a consequence of restricted motion, posture during lethargus quiescence may serve a distinct developmental role in facilitating molting.

698A. The roles of biogenic amines on feeding state-dependent thermotactic behavior in *C. elegans*. **Satomi Tsukamoto**, Shunji Nakano, Ikue Mori. Graduate School of Science, Nagoya University, Nagoya, Japan.

Biogenic amines are important neurotransmitters or neuromodulators that regulate behavior in both vertebrates and invertebrates. To study the mechanisms by which biogenic amines regulate neural circuits, we are investigating thermotaxis behavior in *C. elegans*. After cultivation at a certain temperature with food, the animals migrate to that cultivation temperature, whereas they move randomly on the thermal gradient after cultivation without food. A simple neural circuit has been identified for this feeding state-dependent thermotactic behavior (Mori and Ohshima, 1995; Kuhara, Okumura et al., 2008). Also, we previously reported that exogenous serotonin and octopamine can mimic well-fed or food-deprived states, respectively (Mohri et al., 2005), which suggested that endogenous serotonin and octopamine can modulate thermotaxis.

In the current study, we found that octopamine-deficient *tbh-1* mutants stayed at the cultivation temperature even when they were starved, while serotonin-deficient *bas-1* and *tph-1* mutants dispersed from the cultivation temperature in fed condition. Thus, octopamine and serotonin are required for starvation and food signaling, respectively. To identify octopamine receptor required for thermotaxis, we evaluated thermotactic behavior of mutants for previously discovered octopamine receptor genes (*ser-3, ser-6, octr-1, tyra-3*) and found that only *octr-1* mutants showed a phenotype similar to that of octopamine-deficient *tbh-1* mutants. We so far showed that octopamine and serotonin are physiological modulators of thermotaxis and drive animals to the opposite behavioral states. We are currently investigating how these two amines regulate the neural circuit of thermotaxis. Our study will shed light onto a circuit-level mechanism for biogenic amine signaling in the brain.

699B. Caenorhabditis elegans can detect and avoid from rare earth ions, which have toxic effects on the worm locomotion, growth, and development. **Tokumitsu Wakabayashi**, Yuta Nakano, Yui Nojiri, Miwa Watanabe, Hiroshi Tomita. Iwate Univ, Morioka, Iwate, Japan.

Rare Earth (REs) are a set of elements including scandium (Sc), yttrium (Y), and fifteen lanthanides. Because of their unique chemical properties, they are used for many High-Tech devises which support our daily life. Despite their name, REs are relatively plentiful in earth crust. Since REs are widely dispersed in soil, organism may encounter these elements during the evolutional history of life. However, effects of REs on biological systems, especially in multicellular organisms, are poorly understood. In this study, we have explored the impact of REs on the behavior, locomotion and growth of organisms by using *Caenorhabditis elegans* as a model system.

To see the effect of REs on the worm growth and development, wild-type eggs were soaked in RE solutions for 24 hr. In the presence of 100 mM RE, a significant part of eggs were unhatched and the large part of larval worms hatched was dead. The effects were observed for all REs except radioactive promethium which we have not tested. MAPK mutants which were hypersensitive to heavy metal (HM) ions such as Cu^{2+} and Cd^{2+} showed hypersensitivity to REs in both eggs and adult worm, suggesting that the worm used common signaling pathway for RE- and HM-stress responses. We also tested the behavior of the worm against REs using radial concentration gradient formed on an agar plate. Wild-type worms showed an avoidance behavior against all REs examined. The avoidance behavior was abolished in the chemosensory-defective *che-2* mutant worms, indicating that the worm can detect REs via the function of chemosensory neurons. By using cell-specific rescue strain of *dyf-11* mutant and cell-specific genetic cell ablation lines, we found that REs were detected by overlapped but distinct subset of sensory neurons that used for HM avoidance.

700C. Small Molecule Communication: C. elegans and bacterial chemical signals. **Kristen Werner**[1], Lark Perez[2], Martin Semmelhack[3], Bonnie Bassler[1,4]. 1) Molecular Biology, Princeton University, Princeton, NJ; 2) Chemistry, Rowan University, Glassboro, NJ; 3) Chemistry, Princeton University, Princeton, NJ; 4) Howard Hughes Medical Institute.

Bacterial group behaviors are governed by a process called quorum sensing, in which bacteria produce, secrete, and detect extracellular signal molecules called autoinducers (AIs). Vibrios produce multiple AIs, some enable intra-species communication and others that promote inter-species communication. Vibrio cholerae produces an intra-species AI called CAI-1 that is a 13 carbon long fatty acyl molecule and the interspecies signal called AI-2 that is a boron-containing furanone. The information contained in the AIs is funneled into a shared phosphorelay signaling cascade that controls virulence, biofilm formation, and other traits. The bacteriovorous nematode, Caenorhabditis elegans, also uses small molecules to interpret its environment. A class of C. elegans-derived molecules called ascarosides influence nematode behaviors including attraction, repulsion, and mating. The presence of bacteria stimulates chemotaxis, egg-laying, and feeding in C. elegans, however, the bacteria-produced molecules that the nematode detects to control these phenotypes are largely unknown. We demonstrate that in addition to playing a vital role in quorum-sensing-regulated behaviors in V. cholerae, CAI-1 also influences behavior in C. elegans. C. elegans is more strongly attracted to V. cholerae than to its food source E. coli HB101 and C. elegans prefers V. cholerae that produces CAI-1 over a V. cholerae mutant for CAI-1 production. Consistent with this finding, robust chemoattraction occurs to synthetic CAI-1. CAI-1 is detected by the sensory neuron AWCON. Laser ablation of this cell, but not other amphid sensory neurons, abolished chemoattraction to CAI-1. To define which moieties of CAI-1 are crucial for recognition by C. elegans, we synthesized CAI-1 analogs and tested whether they promote chemoattraction. The fatty-acid chain length as and the precise position of the CAI-1 ketone group are the key features required for mediating CAI-1-

directed nematode behavior. Together, these analyses define a bacteria-produced signal and the nematode detection apparatus that permit interkingdom communication.

701A. The monoamine neurotransmitter serotonin shows evolutionarily divergent effects on feeding behavior in *Pristionchus pacificus*. **Martin Wilecki**, James W Lightfoot, Ralf J Sommer. Max Planck Institue for Developmental Biology, Tübingen.

The ecology and behavior of the diplogastrid nematode *Pristionchus pacificus* differs substantially from *C. elegans*. While both nematodes can be cultured on bacteria, *P. pacificus* is able to exploit alternative food sources. Tooth-like denticles in the mouth enable it to feed on other nematodes and fungi. We are interested in the neurobiological basis behind the predatory vs. bacterial feeding in *P. pacificus*. Monoamine neurotransmitters regulate several aspects of feeding behavior in *C. elegans*. Although antibody stainings reveal similar expression patterns in both nematodes, the response to these neurotransmitters in pharmacological experiments differ substantially. Most tested neurotransmitters elicit opposite responses in pharyngeal pumping assays. For example, serotonin is known to stimulate pharyngeal pumping in *C. elegans*, but it dramatically decreases pharyngeal pumping in *P. pacificus*. Serotonin also triggers movement of the claw-like dorsal tooth in *P. pacificus*, which makes it an interesting candidate for more detailed studies of the neurobiology of the predatory feeding. The rate-limiting serotonin synthesis enzyme tryptophan hydroxylase (TPH-1) and the serotonin transporter MOD-5 are highly conserved between *P. pacificus* and *C. elegans*. We are using reporter constructs to visualize expression of the underlying genes and use cell ablation experiments to elucidate key neurons involved in regulating the complex feeding behaviors in *P. pacificus*. We also plan to generate mutants of *tph-1* and *mod-5* using TALENs for phenotypic analysis of their effects on feeding behaviors.

702B. Root-knot Nematode Behavior, Pheromones, and Genetics. **Valerie Williamson**[1], George Bruening[1], Jacinta Gimeno[1], Sylwia Fudali[1], Frank Schroeder[2]. 1) Plant Pathology, University of California, Davis, CA; 2) Boyce Thompson Institute, Ithaca, N.Y.

Root-knot nematodes (*Meloidogyne* spp.; RKN) are sedentary endoparasites that infect many plants and cause substantial crop loses worldwide. L2 hatch from eggs in the soil and must locate and invade host roots to complete their life cycle. They are attracted to the root zone of elongation, but what attracts them to this region is unknown. After invasion, nematodes migrate to the vascular cylinder where they cause differentiation of host cells into multinucleated giant cells that serve as the source of nutrients and cause the formation of a characteristic “root-knot.” We have developed an assay system using a gel of Pluronic F-127 and demonstrated L2 attraction to the elongation zone as well as to volatile and soluble components of root tip exudates. Our goal is to analyze these extracts to identify chemicals responsible for modifying nematode behavior. We are also pursuing a genetic/genomic approach using the northern RKN *Meloidogyne hapla*. The genome of this species has been sequenced and annotated, and a set of F2 lines, which, due to the unusual reproductive mode, resemble recombinant inbred lines, has been produced and used to develop a sequence-anchored molecular map. These lines are maintained as cultures on tomato plants and have been used to assess behaviors including clumping and host attraction. The two parental strains differ in relative attraction to Arabidopsis and to the model legume *Medicago truncatula*. We assessed attraction of each of 90 F2 lines to each host and preliminary analysis has identified QTL contributing significantly to each phenotype, but different QTL were identified for the two host species suggesting that different nematode genes modulate attraction to each host. The clumping and aggregation behavior of RKN as well as their accumulation near root tips, suggests that chemical communication occurs among L2. We have identified ascarosides previously identified from *C. elegans* in exudates from RKN L2. Interestingly, several peaks were seen that had elution properties suggesting that they could be ascarosides not present in *C. elegans*. Potential roles of these ascarosides in RKN communication are under investigation.

703C. Genetic analysis of dopamine signaling for repulsive odor learning. **Shuhei Yamazaki**, Kotaro Kimura. Department of Biological Sciences, Osaka Univ., Toyonaka, Osaka, Japan.

Through learning, an animal can optimize its chances for survival and reproduction by modifying its behavior based on prior experiences. Multiple types of learning, and the molecular mechanisms that mediate learning, have been studied in both vertebrates and invertebrates. Nevertheless, How sensory behavior is modulated by learning and how specific molecules are involved in this process are not well understood. Previously, we have shown that dopamine signaling is required for non-associative learning of odor avoidance behavior of worms. Worms exhibit an enhanced avoidance behavior to 2-nonanone after preexposure to the odor, and this enhancement is regulated in RIC neurons by dopamine signaling via the D2-like dopamine receptor DOP-3 (Kimura et al., 2010, J. Neurosci.). Currently, we are working towards identifying new genes that can genetically interact with the dopamine-signaling pathway to regulate and/or enhance 2-nonanone avoidance. We have found some mutant strains that exhibit behavioral defects that are similar to those exhibited by dopamine mutants. We plan to identify these mutations with whole-genome sequencing, and reveal the physiological role of their gene products by using our integrated microscope system (Tanimoto et al., this meeting).

704A. A forward screen to identify genes involved in the blockage of olfactory adaptation by food in *C. elegans*. Amanda Cha[1], Ghazal Ghafari[1], Laine Janzen[1], Stephanie Summers[1], Pilar Stinson[1], Marie Engelhardt[1], Kelsi Kettellapper[1], Jamie Knight[1], Noelle L'Etoile[2], **Jared Young**[1]. 1) Dept Biol, Mills Col, Oakland, CA; 2) Department of Cell and Tissue Biology, University of California, San Francisco, CA.

C. elegans is attracted to specific odors sensed by the paired AWC olfactory sensory neurons; however, the attraction decreases if exposure occurs in the absence of food, referred to as olfactory adaptation. We seek to further explore odor processing and its interaction with food sensory signals in the model organism *C. elegans*. Adaptation in *C. elegans* to particular odorants does not occur when exposure is accompanied by *Escherichia coli*, a food source for the worm. Here we report results from a forward screen to identify genes involved in the block of olfactory adaptation by food. In the forward screen, F2 mutants were isolated based on their lack of attraction to an odor after the odor was presented along with food. The screen was carried out and 33 worms isolated as possibly interesting mutants. Further analysis, aimed at identifying lines with robust phenotypes, is focused on 7 of these lines.

705B. Investigating the neural mechanisms underlying a hypertonic response in Caenorhabditis elegans. **Jingyi Yu**[1,2], Yun Zhang[1,2]. 1) Department of Organismic and Evolutionary Biology; 2) Center for Brain Science, Harvard University, Cambridge, MA.

Environmental osmotic changes can interfere with molecular and cellular functions by influencing cytoplasmic osmolarity. Preventing this damage is an

important survival skill for animals that live in environments with unstable osmolarity. It is known that in addition to hormonal responses, mammals also use behavioral strategies to maintain a stable cellular osmolarity , such as water-seeking behaviors driven by thirst. However, little is known about the molecular mechanisms or neural circuits underlying these behavioral responses. Previously, it has been shown that Caenorhabditis elegans generates an acute aversive response to high osmotic shock (Bargmann et al. 1990). Here, we show that C. elegans also produces an aversive response when exposed to prolonged hypertonic conditions. Compared with the response to high osmotic shock, the hypertonic response is slower, evoked by lower osmolarity, and involves increased turning rate over time. It does not require the same molecular mechanism as the response to high osmotic shock, since osm-9 mutants show a wild-type response in the hypertonic condition (Liedtke et al. 2003). The behavioral response is specific to osmotic stimuli, and common mechanosensory or chemosensory mutants do not show any defect. Currently, we are looking for the genetic and circuit mechanisms that regulate this behavior.

706C. Neurotoxic unc-8 mutants encode constitutively active DEG/ENaC channels that are blocked by divalent cations. Ying Wang[1], Lu Han[1], Cristina Matthewman[1], Tyne Miller[2,3], David Miller[2,3], **Laura Bianchi[1]**. 1) Dept Physiology & Biophysics, Univ Miami, Miami, FL; 2) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 3) Neuroscience Program, Vanderbilt University, Nashville, TN.

Hyperactivation of ion channels of the DEG/ENaC family can induce swelling and degeneration of the cells in which they are expressed. A genetic screen for uncoordinated mutants in C. elegans identified dominant mutations in the DEG/ENaC channel subunit UNC-8 that cause neuronal swelling. Here we show that the movement defect of these unc-8d mutants is correlated with the selective death of DA and DB cholinergic motor neurons in the ventral nerve cord (VNC); GABAergic motor neurons are not affected. Our finding that < 25% of DA and DB motor neurons degenerate in unc-8(d) mutants may explain the original suggestion that neuronal swelling is transient and does not result in cell death (Shreffler et al, 1995). We performed electrophysiology experiments in Xenopus oocytes expressing the mutant proteins UNC-8 (G387E) and UNC-8(A586T) to confirm that these UNC-8d channels induce hyperactive cation transport. Our data show that currents are small due to blockage by extracellular divalent cations (Ca++, Mg++). Indeed, removal of extracellular divalent cations produces larger currents and results in the swelling and eventual death of oocytes expressing UNC-8(G387E). We suggest that the UNC-8 channel is intrinsically sensitive to extracellular divalent cations and that this property limits UNC-8(d) toxicity in vivo. Since the local concentration of extracellular calcium is likely to be decreased by the neurotransmitter release mechanism, regulation of external Ca2+ levels by neuronal activity may contribute to the selective death of neurons that express UNC-8d channels.

707A. A circuit for working memory in *C. elegans*. **Adam Calhoun[1,2]**, Tatyana Sharpee[1], Sreekanth Chalasani[1]. 1) UC San Diego, San Diego, CA; 2) Salk Institute, San Diego, CA.

How do organisms use neural circuits to learn about their environment and then use that knowledge to guide behavior? The nervous system of *C. elegans* contains 302 neurons whose connectivity is fully mapped, simplifying the process of investigating neural circuits. These animals are faced with the common problem of how to best find new sources of food when they are transferred to a food-free plate. Their strategy is to produce many turns to stay in a small area when it believes food is nearby and to suppress those turns when it cannot find food. We have found that *C. elegans*' off-food search strategy is dependent on the size of the food patch it has most recently been exploring suggesting that it uses its prior experience to guide search behavior.

Using a dimensional reduction technique, we have identified the relevant information in the on-food behavior and in the structure of the bacterial patch that predicts off-food search. The information is decoded by two sensory neurons, ASI and ASK, whose responses to bacteria match the relevant on-food statistics. Downstream of these sensory neurons, we identify several interneurons required for learning: removal of any individual neuron prevents the animal from learning the patch size. We additionally identify a downstream dopaminergic neuron, CEP, which is crucial for learning. Dopamine interacts with the broader circuit via two D1-like receptors, *dop-1* and *dop-4* on sensory neurons and postsynaptic interneurons respectively. We also find that the CREB homologue, *crh-1*, is required in the same interneurons as *dop-4*. The neurons required for learning overlap with the neurons required to perform a search suggesting that the same network that generates behavior also contains within itself the ability to modify it.

708B. Protein with tau-like repeats regulates neuronal integrity and lifespan in *C. elegans*.. **Yee Lian Chew[1]**, Xiaochen Fan[1], Jürgen Götz[2], Hannah Nicholas[1]. 1) School of Molecular Bioscience, University of Sydney, Australia; 2) Centre for Ageing Dementia Research (CADR), Queensland Brain Institute (QBI), University of Queensland, Australia.

Protein with tau-like repeats (PTL-1) is the sole *Caenorhabditis elegans* homolog of tau and MAP2, which are members of the mammalian family of microtubule-associated proteins (MAPs). In particular, tau plays a crucial role in pathology, as elevated levels lead to the formation of tau aggregates in several neurodegenerative conditions, including Alzheimer's disease. We used PTL-1 in *C. elegans* to model the biological functions of a tau-like protein without the complication of functional redundancy which is observed among the mammalian MAPs. Using fluorescent reporter lines, we visualised the touch receptor and GABAergic neurons in two distinct *ptl-1* mutant strains as they aged, and scored for the presence of abnormal structures such as blebbing along the axon, or branching from the cell body or axon. We observed that these changes in neuronal morphology are present at a higher frequency in young *ptl-1* mutant animals compared with wild-type controls. These structures were recently noted by others to characterise neuronal aging in *C. elegans* (Tank *et al.*, 2011, J. Neurosci.; Pan *et al.*, 2011, PNAS; Toth *et al.*, 2012, J. Neurosci.). Therefore, our data indicate that PTL-1 is important for the maintenance of neuronal integrity as animals age. In addition, we observed that *ptl-1* mutant animals have a shorter median lifespan compared with wild-type, indicating that PTL-1 may play a related or additional role in regulating lifespan. We found that gene dosage of PTL-1 is critical, as variations from wild-type levels are detrimental both in terms of neuronal integrity and whole organismal aging. Finally, human tau is unable to robustly compensate for loss of PTL-1, although phenotypes observed in tau transgenic animals are dependent on the presence of endogenous PTL-1. Our findings suggest that some of the effects of tau pathology may result from the loss of physiological tau function, and not solely from a toxic gain-of-function due to accumulation of tau.

709C. Regulation of Coincident Activity is an Efficient Strategy for Reversible Modulation of Arousal in *C. elegans* Sleep. **Julie Cho**, Paul Sternberg. Biol, Caltech, Pasadena, CA.

Sleep is an essential physiological state characterized by its circadian timing, behavioral quiescence, homeostasis, increased arousal threshold, and rapid reversibility. Behavioral evidence suggests that our perception is dramatically dampened during sleep, and that there are physiological changes within individual neurons and their connections to each other. However, there are many ways to dampen arousal, and despite studies spanning mammalian and non-mammalian species, the circuit modifications that promote sleep behavior and dynamic waking during the sleep state are largely unknown. Previously, we have systematically dissected components of a sensory motor circuit to identify changes in the flow of information from sensory to inter- to motor-neuron that implement sleep like behavior in the nematode *Caenorhabditis elegans*. Here we use optogenetic techniques to further expand upon this work and specifically manipulate and perturb the neural circuit in specific subsets to show that sleep in the worm is a general state independent of individual circuits and independent of stage. Furthermore, this state-dependent change is reflected by coincident activity at the second layer of sensory processing. Coordinated coincident activity is essential in normal awake activity and Hebbian learning. Although C. elegans neurons do not exhibit spiking behavior, we show that coordination of command interneuron activity, as measured by calcium, to be necessary for normal awake behavior and reflects the dynamics of wake behavior.

710A. *In vivo* optical recording of action potentials in *C. elegans* body wall muscles using the voltage sensitive fluorescent protein ArcLight. Liping He[1], Julian Wooltorton[2], Brian Salzberg[2,3], **Chris Fang-Yen[1,2]**. 1) Dept. of Bioengineering, University of Pennsylvania, Philadelphia, PA; 2) Dept. of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3) Dept. of Physiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

How the worm's sinusoidal locomotion is coordinated by the motor circuitry and body wall muscles (BWMs) remains poorly understood. Electrophysiological studies have found that BWMs fire action potentials, both spontaneously and in response to nerve stimulation. However, these recordings require dissection of the animal and can only be obtained from one or two cells simultaneously. In order to probe the encoding of locomotory states in the BWMs and motor circuitry, we have expressed the voltage sensitive fluorescent protein, ArcLight-A242 in body wall muscle cells using the *myo-3* promoter. ArcLight is trafficked efficiently to the muscle cell membrane and the animals' locomotory behavior appears normal. Using epifluorescence microscopy we record activity of muscles in nanoparticle-immobilized worms at 100 frames per second. We find oscillations in fluorescence similar to bursts of action potentials reported previously, with frequency in a band centered at approximately 3 Hz. These oscillations are particularly prominent in the head. We find that activity in opposing dorsal and ventral muscle cells are anticorrelated, consistent with their functional roles. We are developing a dual-view imaging system to simultaneously record muscle activity and behavior in freely moving worms.

711B. Transport mechanisms involved in pH regulation of *C. elegans* amphid sheath glia. **Jeff Grant**, Rachele Sangaletti, Laura Bianchi. Physiology and Biophysics, University of Miami, Miami, FL.

Glia are essential for maintenance of the pH and ionic composition of the synaptic microenvironment. Perturbations of intracellular and extracellular pH of both glia and neurons affect the function of several types of ion channels, thus modulating the neuronal excitability. The amphid sheath glia of *C. elegans* are closely associated with 12 amphid sensory neurons. Our lab has previously shown that the pH sensitive DEG/ENaC channel ACD-1 is expressed in these glia, and that the activity of this channel is involved in modulation of chemosensory behavior in *C. elegans*. This highlights the likely importance of glial pH regulation in chemosensory signaling. However, to date nothing is known regarding the mechanisms by which the amphid sheath glia regulate pH. To examine the mechanisms of intracellular pH regulation in amphid sheath glia, the GFP-based pH sensor Phlourin was expressed in amphid sheath glia under the control of the glial-specific promoter PT02B11.3, and *in vivo* fluorescent pH imaging was performed. Incisions were made in the cuticle of the animals to allow for perfusion of solutions of different compositions over the amphid sheath glia. Analysis of the rate of acid extrusion after an acid-load in these cells revealed that they possess both HCO_3^--dependent and independent mechanisms of acid removal. Application of the anion exchange blocker DIDS (250mM) or removal of extracellular Na^+ significantly dampened HCO_3^--dependent acid extrusion in the sheath glia, indicating the likely presence of Na^+-coupled HCO_3^- transporters in these cells. Furthermore, Cl^- removal inhibited HCO_3^--dependent acid extrusion after an acid load in these cells and also caused a transient alkalization under baseline conditions. These data indicate that Cl^-/HCO_3^- exchange activity is functional in the sheath glia. To determine the molecular identity of the transporters involved in amphid sheath glia HCO_3^- transport, we are now using single cell RT-PCR. Once we have identified the transporters expressed in amphid glia, we will determine the role they play in glial pH regulation using RNAi techniques. Furthermore, we will test whether disrupting glial pH regulation by knock-down of these transporters affects sensory perception.

712C. Imaging the brain. Wafa Amir, Nicholas Swierczek, **Rex A. Kerr**. Janelia Farm Research Campus, Asburn, VA.

By coupling light-sheet microscopy with nuclear-localized GCaMP6 and nls-RFP for a reference channel, we can image essentially the entire brain at rates of up to 10 Hz. We find that the brain appears relatively quiet in animals that are not moving, and that only a small number of neurons show strong activity correlated with movement. However, without a robust method to identify neurons, the technique does not definitively determine which neurons are active. Nor do we know for certain which neurons are truly uninvolved and which merely have low activity levels or small calcium transients, though nls-GCaMP6 does robustly report calcium transients in gentle touch neurons. We are attempting both to use restricted expression patterns to simplify the identification problem, and to improve automated (or manual!) methods of identification, and will report on our progress in this regard.

713A. Temperature experience-inducing cold tolerance is regulated by insulin signaling in intestine and neuron. Akane Ohta, Satoru Sonoda, Tomoyo Ujisawa, Yuko Kobayashi, Hayato Nakamoto, **Atsushi Kuhara**. Dept Biology, Grad. school of Sci., Konan University, Kobe, Japan.

Temperature is critical environmental stimuli and cause biochemical changes. Animals therefore can respond and habituate to the changes in ambient temperature. We are studying about molecular mechanism underlying temperature experience-dependent cold tolerance in C. elegans.
20°C-cultivated animals were died by cold stimuli 2°C, whereas 15°C-cultivated animals can survive at 2°C. Mutants defective in DAF-2/insulin receptor and its downstream molecules showed abnormal cold tolerance. DAF-2 is the sole insulin receptor in C. elegans, while there are about 40 ligands for insulin

receptor. We have found that at least three insulin, DAF-28, INS-6 and INS-1, are essential for cold tolerance. Two insulin, DAF-28 and INS-6, are both positive agonists and work redundantly for DAF-2/insulin receptor in cold tolerance. Genetic epistasis analysis indicated that INS-1/insulin genetically inhibits DAF-2/insulin receptor through negative regulation of DAF-6/insulin.
Abnormal cold tolerance in daf-28 mutant was strongly rescued by specific expression of daf-28 cDNA in ASJ sensoryneuron that is known as a light and pheromone-sensing neuron. By contrast, abnormality of daf-28 mutant was partially rescued by expressing daf-28 cDNA in other sensoryneurons. These suggest that DAF-28/insulin functions cell non-autonomously, but releasing DAF-28 from ASJ is essential for temperature experience-dependent cold tolerance. Unexpectedly, cell specific rescue experiments revealed that DAF-2/insulin receptor in both intestine and neuron is required for cold tolerance. These results suggest that a single sensoryneuron ASJ regulates temperature experience-dependent cold tolerance through insulin signaling in the intestine and neuron. Since abnormal cold tolerance of daf-2 mutant was suppressed by mutation in transcriptional factor FOXO/DAF-16, we are investigating downstream molecules of insulin signaling for cold tolerance by DNA microarray analysis.

714B. Mechanosensitive innexin channels in *C. elegans* touch neurons. **R. Sangaletti**, L. Bianchi. Department of Physiology and Biophysics, Miller School of Medicine, University of Miami, 1600 NW 10th Ave, Miami, FL, 33136.

In vertebrates, cell-cell communication is achieved through the formation of gap junction channels. Vertebrate gap junctions are formed by 2 homo- or hetero-multimeric hexamers of connexin proteins protruding from the plasma membrane of two adjacent cells. Vertebrate pannexins have no sequence similarity with connexins, but display similar channel properties (large conductance and permeability to small molecules in addition to ions). In contrast to connexins, pannexins function as plasma membrane hemi-channels, rather than cell-to-cell channels. In invertebrates, pannexin homologs are called innexins and are thought to function both as gap junction and plasma membrane hemi-channels. There are 25 innexin genes in the *C. elegans* genome, some of which have been shown to play a role in gap junction formation. However, no *C. elegans* innexin has been so far shown to form plasma membrane hemi-channels. Using electrophysiological and dye uptake techniques, we found that gentle touch neurons cultured in vitro express a mechanosensitive innexin channel. More specifically, using the inside-out configuration of the patch-clamp technique, we observed a non-selective channel activated by membrane stretch, with large conductance (1 nS) and subconductance states. The channel was more active in the presence of high extracellular K+ and was blocked by carbenoxolone, a pannexin/innexin channel blocker. We also performed dye uptake experiments on cultured touch neurons. We exposed the cells to a hypotonic solution to activate the channels and measured the uptake of Ethidium Bromide into the cells, in the presence and absence of carbenoxolone. We found that Ethidium Bromide uptake into touch neurons was through a carbenoxolone-sensitive pathway. Our results support that *C. elegans* touch neurons express plasma membrane hemi-channels with properties resembling innexins. Future experiments include identifying the gene encoding the innexin channel expressed in touch neurons and determining its role in touch neuron function.

715C. A Genome Wide Analysis of Na^+/Ca^{2+} Exchanger Genes in *C. elegans*. **Vishal Sharma[1]**, Chao He[1], Julian Sacca-Schaeffer[1], Eric Brzozowski[1], Damien O'Halloran[1,2]. 1) Department of Biological Sciences, George Washington University, Washington, DC; 2) Institute for Neuroscience, George Washington University, Washington, DC.

Ca^{2+} signaling controls a variety of cellular processes, ranging from neurotransmission to apoptosis. Ca^{2+} ions may be transported into cells from the extracellular environment or released from intracellular storage compartments. Despite its important regulatory functions, excessive overload of intracellular Ca^{2+} has an adverse effect on cell health. Accordingly, organisms have evolved mechanisms to rapidly extrude intracellular Ca^{2+} through proteins embedded in the plasma membrane. Na^+/Ca^{2+} exchangers (NCX) are low affinity, high capacity transporters, which couple extrusion of one Ca^{2+} ion with the influx of three Na^+ ions. $Na^+/Ca^{2+}/K^+$ exchangers (NCKX) are structurally similar to the NCX proteins but extrude one K^+ ion alongside one Ca^{2+} ion, in exchange for Na^+ ion influx. Another, less well-characterized branch in the Na^+/Ca^{2+} exchanger family tree, are the calcium/cation exchanger (CCX) channels, which can catalyze both Na^+/Ca^{2+} and Li^+/Ca^{2+} exchange. NCX, NCKX and CCX comprise the three main branches of the Na^+/Ca^{2+} exchanger superfamily in animals. They are distributed across various tissue and cell types in mammals and implicated in several brain and cardiac pathologies such as Alzheimer's and heart failure. Despite their wide distribution and central roles, a full description of their agency is still lacking. Here we provide the first genome wide analysis of the NCX, NCKX, and CCX family members in the model system *Caenorhabditis elegans*. We infer their phylogeny, use transcriptional GFP reporter fusions to provide a comprehensive description of their expression patterns, and also examine mutants in several transporters where we find defects in muscle contraction, lipid accumulation and neural development. Our results show that Na^+/Ca^{2+} exchanger proteins are widely expressed in a number of tissues and cell types in *C. elegans* including sensory neurons, interneurons, motor neurons and muscle cells. We propose that *C. elegans* may be an ideal model system to learn more about the biology of Na^+/Ca^{2+} exchange.

716A. Isolating genes for temperature experience-dependent cold tolerance. **Satoru Sonoda**, Yukari Kinoshita, Shoko Furukawa, Mikiko Endo, Yushuke Uehara, Akane Ohta, Atsushi Kuhara. Dept Biology, Grad. school of Sci., Konan University , Kobe, Japan.

We are utilizing temperature experience-dependent cold tolerance as a model for studying temperature sensation and habituation. After cultivation at 20°C, wild-type were destroyed by cold stimuli. In contrast, after cultivation at 15°C, most of animals can survive. To isolate genes involved in the cold tolerance, we are using four approaches, (1) DNA microarray, (2) Natural variation, (3) Artificial evolution and (4) EMS-mutagenesis.

(1) We tested cold tolerance of mutants defective in genes isolated from DNA microarray analysis (Ajilent array). Several genes such as protein protease PP1 and laminin are involved in cold tolerance. Since PP1/GSP-3 is involved in sperm development and motility, and mutants impairing sperm genes showed abnormal cold tolerance. We are now investigating the relationship between sperm genes for cold tolerance and known-cold tolerance signaling pathway, such as insulin signaling and G protein-coupled temperature signaling in ASJ neuron.

(2) Natural C. elegans isolated from various area showed variety of cold tolerance. Responsible gene for natural variation between Bristol N2 and California CB4854 are genetically mapped on X-chromosome. Using deep DNA sequencer and SNP analysis, candidates of responsible genes are narrowed down to ~20 genes.

(3) C. elegans has strong advantage for artificial evolution analysis, since life cycle is short and strains can be preserved at -80°C. We are maintaining wild-type at 15 or 23°C for gradual accumulation of mutations. So far, 87 generations are frozen, and we found that cold tolerance was notably changed at

61 generation. We are planning to decode whole genome by using deep DNA sequencer. (4) Through 2000 genomes screen by using EMS, we isolated 10 cold tolerance mutations. One of these has been mapped on X-chromosome.

717B. A single neuron class with contrasting sensory tuning curves enables sex-specific attraction in *C. elegans*. **Jagan Srinivasan**[1,2], Anusha Narayan[2,3], Omer Durak[4], Neelanjan Bose[5], Frank C Schroeder[5], Paul W Sternberg[2,3]. 1) Biology and Biotechnology, Worcester Polytechnic Institute, Worcester, MA; 2) Biology Division/HHMI, California Institute of Technology, Pasadena CA 91125; 3) McGovern Institute for Brain Research/Dept. of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge MA 02139; 4) Neuroscience Graduate Program, Massachusetts Institute of Technology, Cambridge MA 02139; 5) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853.

In the model organism *Caenorhabditis elegans*, a class of endogenously produced small molecule signals termed ascarosides mediates a wide variety of social behaviors such as male attraction, aggregation and olfactory learning. We are interested in understanding the neural mechanisms underlying gender-specific behaviors. Two of the previously isolated ascarosides ascr#3 and ascr#8, secreted by hermaphrodites are attractive exclusively to *C. elegans* males in a two-spot behavioral assay. Males are attracted at specific concentrations of the chemicals leading to the generation of a behavioral tuning curve. Our cell ablation experiments indicate that male response to ascr#3 requires two classes of neurons, ASK and CEM. ASK neurons are part of the core sensory architecture of the worms whereas the CEM neurons are specific to males. ascr#8 is mediated primarily by CEM neurons. To better understand the sensory properties of the CEM neuron in response to different concentrations of ascarosides, we are adopting an electrophysiological approach in combination with cell-ablation and genetic analyses. We find that ascaroside responses in CEMs can be depolarizing or hyperpolarizing with a defined probability independent of anatomical identity. These opposing responses are tuned to different concentrations with varying kinetics. Worms with one intact CEM show no concentration preference, and reducing synaptic transmission strongly disinhibits all CEM responses. Our results suggest that the CEM class collectively encodes ascaroside concentration preferences and synaptic modulation is necessary to this process.

718C. Screening a Million Mutations to Identify Novel Ciliary Proteins. **Tiffany A. Timbers**[1], Victor L. Jensen[1], Katherine Lee[1], Stephanie Garland[2], Mark Edgley[2], Donald G. Moerman[2], Michel R. Leroux[1]. 1) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada; 2) Department of Zoology, University of British Columbia, Vancouver, British Columbia, Canada.

Cilia are cellular organelles that enable sensory physiology (*e.g.* vision) and modulate various developmental signaling pathways (*e.g.* Wnt). Disrupting the function of these organelles results in a wide range of ailments, and is associated with many human genetic disorders, including polycystic kidney disease and Bardet-Biedl syndrome. To identify novel proteins required for the formation and function of primary cilia we have undertaken an innovative high-throughput screen of the "Million Mutation Project" (MMP; http://genome.sfu.ca/mmp) *C. elegans* library for phenotypes related to the dysfunction of cilia. This resource consists of 2007 chemically mutagenized worms, all of which have been sequenced to a depth of 15x genome equivalents, and collectively harbor 826,810 single nucleotide variants in 20,115 genes (Thompson et al. submitted).

As the nature of the disrupted gene is known the library facilitates gene cloning, the greatest bottleneck in traditional mapping-cloning approaches after chemical mutagenesis. From screening the library we have identified strains with previously unidentified mutations that result in gross defects in cilia structure. These were uncovered using a dye-filling assay. Abrogated ciliary functions were revealed by probing cilia-dependent behaviors such as CO_2 avoidance and dauer formation. To date, we have screened 20% of the library (400 strains) using the dye-filling assay and have identified 10 strains in which no neurons fill with DiI, and 30 strains in which the staining of the dye-filled neurons is weaker. 10 strains appear to have novel mutations. SNP mapping and rescue experiments will be used to identify the causative mutation. Detailed molecular analyses of these novel ciliary proteins will provide new insights into the factors required for cilia formation and function, fundamental cellular processes with important biomedical implications.

719A. System identification for thermosensory neuron encoding thermal environment. **Y. Tsukada**[1], N. Honda[2], A. Murase[2], T. Shimowada[1], O. Noriyuki[3], A. Kuhara[4], S. Ishii[2], I. Mori[1]. 1) Graduate Sch Sci, Nagoya Univ, Nagoya, Japan; 2) Graduate Sch Info, Kyoto Univ, Kyoto, Japan; 3) Hiroshima Univ, Hiroshima, Japan; 4) Dept Biology, Konan Univ, Hyogo, Japan.

A central goal in neuroscience is the full characterization of the information processing of neural circuits. Despite the knowledge of the complete connectivity and the molecules important for the specific mechanisms of neural circuits, it remains understood about the mechanism of information processing in the neural circuit of *Caenorhabditis elegans*. Here, we focus on the response of thermosensory neuron AFD during thermotaxis and its change in different conditions. We used automated tracking system to capture freely moving single animals on thermal gradient (about 0.5 °C/cm). Combining the tracking system with a calcium imaging system, we monitored behavior and activity of AFD thermosensory neuron during thermotaxis with genetically encoded calcium indicator, cameleon YC 3.60. The time course of temperature for the tracked worm was estimated by recorded xy-coordinates and thermography. Thus we quantified exact temperature input, AFD activity, and behavioral state of freely moving animal during thermotaxis. As we previously reported, wild-type N2 animals on an agar plate with thermal gradient showed thermotaxis behavior: they migrate to the cultivated temperature region when we keep the worms in a constant temperature during cultivation. Then we estimated response functions of neural activity of AFD for input of temperature. The estimated response functions indicate differential detection of temperature by AFD. We also found that the response functions of the worms cultivated in different temperatures are different forms, but not depending on food conditions. After the quantitative measurements of AFD response functions, we reconstructed AFD responses with temperature inputs and the estimated response functions. Then we compared the reconstructed responses with real data. The results showed reproductive high correlation, and thus we conclude that estimated response functions express dynamic property of AFD thermosensory neuron. We suggest that such quantitative approach has potential to understand non-linear characteristic of neural circuits.

720B. Photo and pheromone sensoryneuron regulates temperature experience-dependent cold tolerance. **Tomoyo Ujisawa**, Satoru Sonoda, Tomohiro Ishiwari, Akane Ohta, Atsushi Kuhara. Dept Biology , Grad. school of Sci., Konan University, Kobe, Japan.

Animals have habituation mechanism against temperature changes. We are using temperature experience-dependent cold tolerance of C. elegans, as a model to study its molecular mechanism. After cultivation at 20°C, wild-type animals can not survive at 2°C. By contrast, after cultivation at 15°C, most of

animals can survive at 2°C. To investigate whether cold tolerance is established in the specific developmental stage, we used temperature shift experiments using larvae. We found that a temperature-experience of larval stages did not affect the cold tolerance in adult animal. We next used temperature shift experiments at adult stage. Only three hours after the cultivation temperature was changed from 20 to 15°C, the cold tolerances were established. These indicate that temperature experience in formation of cold tolerance can be replaced for three hours.

To determine the cells and genes for temperature experience-dependent cold tolerance, we utilized various mutants. tax-4 mutant defective in cGMP-gated channel showed abnormal cold tolerance. This abnormality is rescued by specific expression of tax-4 cDNA in a single sensoryneuron ASJ, known as a photo and pheromone sensoryneuron. We found that ASJ responds to temperature stimuli by calcium imaging analysis using genetically encodable calcium indicator, cameleone. Defects in known-thermotaxis neural circuit did not affect the cold-tolerance. These results suggest that ASJ is involved in the cold-tolerance.

We measured the cold tolerance of the mutants defective in photo signal transduction of ASJ. The mutants impairing trimetric G protein alpha subunit, guanylyl cyclase or phosphodiesterase involved in photo signal transduction in ASJ showed abnormalities of cold tolerance. lite-1 mutant lacking photoreceptor protein did not show abnormal cold tolerance, suggesting that temperature is received by other receptor. Altogether, molecular and physiological analysis demonstrated that photo- and pheromone-sensing neuron regulates temperature experience-dependent cold tolerance through G protein-coupled pathway.

721C. BBS-4 and BBS-5 function redundantly to regulate IFT recycling in cilia. **Yuxia Zhang**, Qingwen Xu, Yan Hang, Qing Wei, Qing Zhang, Yujie Li, Zeng Hu, Kun Ling, Jinghua Hu. MAYO CLINIC.

The BBSome proteins are a group of eight conserved proteins whose etiologies are associated with Bardet-Biedl syndrome, a ciliopathy disorder whose symptoms include obesity, retinal degeneration, and nephropathy. Our previous finding identified that the BBSome as an entity acts as the key player regulating IFT assembly and turnaround in cilia. Interestingly, neither bbs-4 nor bbs-5 single mutant shows any ciliary defect phenotype. Here, we found that bbs-4 and bbs-5 play redundant role in regulating cilia formation and signaling. bbs-4; bbs-5 double mutant show identical cilia-related defects as other bbs mutants, such as strong IFT-B accumulation at the ciliary tip, mislocalization of cargo proteins and so on. Using Bimolecular Fluorescence Complementation (BiFC) analyses, we were able to observe fluorescence complementation between BBS-4 and BBS-5, indicating that BBS-4 and BBS-5 localize close to each other in the endogenous BBSome. Further in vitro GST pull-down assay reveals the domain in BBS-4 responsible for interacting with BBS-5. Considering that BBS-4 and BBS-5 are two proteins without shared domains or conserved structure, the redundant role between them is unexpected. Our further work in mammalian BBS proteins demonstrates that the interaction between BBS4 and BBS5 and their redundant role are probably highly conserved. What's more, one mutation identified in human BBS4 protein can significantly reduce the association between BBS-4 and BBS-5. Taken together, these observations potentially provide mechanistic insights into the complicated genetic heterogeneity and pathogenesis underlying some BBS patients.

722A. Identification and characterisation of novel genes involved in the development of the *C. elegans* stem-like seam cells. **Peter J. Appleford**, Alison Woollard. Laboratory of Genes and Development, Department of Biochemistry, University of Oxford, South Parks Road, Oxford, UK.

The *C. elegans* seam cells provide a useful paradigm for the stem cell mode of division as a selection of differentiated cell types are derived from them during development, whilst the seam cell number is expanded and maintained by means of a tightly-programmed pattern of symmetric and asymmetric divisions. At hatching, wild type worms possess 10 seam cells per side; throughout larval development most of these then divide asymmetrically 4 times with the anterior daughter typically adopting a differentiated fate and the posterior daughter retaining the stem-like seam fate. An additional symmetric (proliferative) division at L2 provides the worm with its full complement of 16 seam cells which fuse at L4 to give rise to a seam syncytium. Seam cell divisions must be tightly regulated; both in a spatial sense (as not all seam cells share the same lineage fates) as well as in developmental time, to achieve the final adult arrangement of cells.

In conjunction with Union Biometrica, we have developed a methodology whereby automated sorting of mutagenised worms can be achieved using the Biosorter; from these screens we have identified mutants having a variety of seam cell defects, including seam hyperplasia and spacing abnormalities. Seam cell hyperplasia may result from reiteration of the L2 symmetrical division (as in heterochronic pathway retarded phenotypes), or symmetrisation of otherwise asymmetric divisions. Spacing mutants often result from a failure of the daughter cells to extend and contact their neighbours following division. We have also identified a third class of seam cell mutants whereby sister nuclei fail to separate following division, and remain in contact in rosettes, rather than adopting a linear alignment along the A-P axis. Currently we are using Whole Genome Resequencing and the Cloudmap pipeline to map mutant alleles.

723B. Systematic quantification of developmental phenotypes at single-cell resolution during embryogenesis. Julia Moore, Zhuo Du, **Zhirong Bao**. Developmental Biology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Current imaging technology provides an experimental platform in which complex developmental processes can be observed at cellular resolution over an extended time-frame. New computational tools are essential to achieve a systems level understanding of this high content information. We have constructed a structured approach to systematically analyze complex in vivo phenotypes at cellular resolution, which decomposes the task into a panel of statistical measurements of each cell in terms of cell differentiation, proliferation and morphogenesis, followed by their spatial and temporal organization in groups and the cohesion within the whole specimen. We demonstrate the approach to C. elegans embryogenesis with in toto imaging and automated cell lineage tracing. We define statistical distributions of the wild-type developmental behaviors at single-cell resolution based on over 50 embryos, cumulating in over 4,000 distinct, developmentally based measurements per embryo. These methods enable statistical quantification of abnormalities in mutant or RNAi-treated embryos and a rigorous comparison between embryos by testing each measurement for the probability that it would occur in a wild type embryo. We demonstrate the power of this structured approach by uncovering quantitative properties including subtle phenotypes in both wild type and perturbed embryos, transient behaviors that lead to new insights into gene function and a previously undetected source of developmental noise and its subsequent correction.

724C. Robustness of the vulval cell fate pattern to pathway dosage modulation and cryptic evolution of *lin-3* regulatory sequences. **Michalis Barkoulas**, Alexandre Peluffo, Marie-Anne Félix. IBENS, Paris, France.

Biological systems can perform reproducibly to generate invariant outcomes, despite external or internal noise. Over the last few years, the *C. elegans* vulva has become a model system to explore phenotypic robustness, as the cell fate pattern is highly invariable and the underlying developmental mechanisms well described. Although most of the key molecular factors underpinning vulval formation have now been identified, still very little is understood in quantitative terms. Through experimental perturbations of the two main pathways (EGF and Notch) involved in *C. elegans* vulval patterning we investigated some quantitative aspects of developmental robustness and system behaviour. We established a dose-response curve for the EGF pathway in the vulva and show that the system can tolerate a four-fold variation in genetic dose of the upstream signalling molecule, *lin-3/egf*, without phenotypic change in cell fate pattern. Using comparative single molecule FISH hybridisation, we show that levels of *lin-3* expression are conserved within *C. elegans* isolates and *C. briggsae*, but differ in more distant nematodes, such as *C. angaria*. We demonstrate that LIN-3 plays a key role in vulval induction in other *Caenorhabditis* species despite substantial evolution of some cis-regulatory elements required for correct spatiotemporal expression in the *C. elegans* context.

725A. Exploring the genetic regulation of a stochastic cell decision using Mutation Accumulation lines in C. elegans and C. briggsae. **Fabrice BESNARD**, Marie-Anne Félix. IBENS, ENS, PARIS, France.

Two isogenic individuals in the same environment may develop differently: such stochastic behavior remains intriguing, in particular compared to the generally reproducible development of animals. In C. elegans, most cell lineages are quasi-invariant but exceptions include the stochastic cell decision that specifies the epidermal P3.p cell. P3.p belongs to a competence group of 6 Vulval Precursor Cells (VPC, P3p-P8p cells) that form a row of cells in the ventral midbody region and acquire different fates during vulva formation. While (P4p-P8p) VPCs perform a robust sequence of cellular decisions, the anterior P3.p cell displays an outstanding stochastic behavior: at the end of the larval L2 stage, P3.p can either divide once or fuse directly with the hypodermis without dividing. In an isogenic population of worms grown in the same environment, the P3.p division frequency measures the outcome of this stochastic decision in a population. Further analysis showed that P3.p division frequency varies readily with the environment and the genetic background. Thus, P3.p division decision is sensitive to all three types of variations: stochastic, environmental and genetic. So far, the molecular basis of P3.p sensitivity is still unclear. To address the sensitivity of P3.p to mutation, we use a set of Mutation Accumulation Lines in the two species C. elegans and C. briggsae, including two progenitor genotypes for each species. Analysis of vulval phenotypes of these MAL have shown that random mutations can easily alter P3.p division frequency. We propose to unravel the nature of these mutations to understand how they skew P3.p division frequency and sensitivity to noise, without affecting the other VPCs. Using whole-genome re-sequencing, we are characterizing accumulated mutations and isolating candidate mutations by back-crossing into the progenitor line of the MAL of interest. Identified genes will then be tested by functional studies. This study will give insight into the mechanisms driving the remarkably fast evolution of this very variable, likely neutral, P3.p behavior.

726B. Environmental flexibility of C. elegans vulval signalling pathways. Stephanie Grimbert, **Christian Braendle**. Insitut de Biologie Valrose, CNRS, INSERM & University of Nice, Nice, France.

We are interested in how environmental variation affects the functioning of developmental systems and their underlying genetic networks. As a model system, we study the process of C. elegans vulval patterning - a mechanistically well-defined system involving Ras, Notch and Wnt pathways. We previously showed that vulval patterning generates an invariant phenotypic output across various environments although the underlying signalling pathways turned out to be highly environmentally sensitive. In particular, development in starvation conditions or dauer passage strongly suppresses the Vulvaless phenotype of the reduction-of-function mutations in the Egf/Ras/Mapk pathway. We are characterizing the mechanisms by which environmental signals alter activities and interaction of vulval signalling pathways, and how such changes impact the precision of developmental outcomes. We focus on the starvation suppression of lin-3(rf), which cause a strong hypoinduced vulval phenotype under normal conditions. We present our current results indicating that (a) environmentally induced changes in signalling pathway activities are mediated by internal physiological cues rather than external sensory cues and (b) starvation effects suppressing the lin-3(rf) mutations are unlikely due to the developmental delay caused by the starvation treatment. We are currently testing to what extent the relative contribution of Ras and Wnt pathways to the vulval inductive signal changes with the environment.

727C. Muscles from Ectoderm: Possible Cases of *in vivo* Reprogramming. **Kirk B. Burkhart**, Nick Burton, Shuo Luo, Bob Horvitz. HHMI, Dept. Biology, Massachusetts Institute of Technology, Cambridge, MA.

Differentiated cells can be transformed into different cell types by a process termed cell-fate reprogramming. For example, the forced expression of transcription factors can directly change cultured fibroblasts into neurons or into induced pluripotent stem cells. However, very little is known about the natural mechanisms of reprogramming during animal development. During *C. elegans* development, the founder cell AB gives rise to predominantly ectodermal cells. However, a few cells break this lineage pattern. For example, the left intestinal muscle cell, the anal depressor muscle cell, the sphincter muscle cell, and the body wall muscle ABprpppppaa are derived from AB. We hypothesize that these changes in lineage are caused by *in vivo* reprogramming events. To test this hypothesis, we are using genetic screens to identify the machinery that controls the development of these ectoderm-derived muscle cells, determining the mechanisms by which these cell fates are established, and profiling the epigenetic changes that occur during these cell-fate decisions. The *C. elegans* homolog of Twist, CeTwist (encoded by *hlh-8*), is required for the development of the intestinal muscles and the anal depressor cell (Corsi *et al.*, Development, 2000). Twist is a basic helix-loop-helix transcription factor that is required for the epithelial-mesenchymal transition (EMT), which is a natural transformation in cell lineage important for animal development. Thus, the development of the intestinal muscle cells and the anal depressor cell bear similarity to EMT in both their striking change in lineage and in their genetic requirements. Identifying the machinery that controls the development of ectoderm-derived muscle cells might reveal new factors that can reprogram cell fate as well as factors involved in EMT.

728A. Y39G10AR.7 is a putative MPK-1 Erk target during excretory duct cell fate specification. **Preston Chin**, Phil Cheng, Christian Rocheleau. Departments of Medicine and Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada.

The Ras/MAPK signaling pathway determines several developmental processes in Caenorhabditis elegans, including determination of the excretory duct cell fate during embryogenesis and vulval cell fates during larval stages. Scaffolding proteins KSR-1 and KSR-2 mediate the formation of signaling complexes and are redundantly required for proper excretory duct and vulval cell fates. Without an excretory duct cell, early stage larvae are incapable of excreting waste fluid and die with a phenotypically distinct rod-like lethality. *ksr-1* null mutants are fundamentally wild-type, but are highly sensitive to any decreases in Ras/MAPK signaling. In a mutagenesis screen for enhancers of *ksr-1* lethality, we identified the *vh20* mutation as having a ~50% rod-like lethal progeny in the *ksr-1(n2526)* background. A combination of SNP mapping and whole genome sequencing identified a missense mutation in *Y39G10AR.7* as a candidate for *vh20*. Interestingly, we had previously identified *Y39G10AR.7* as a weak enhancer of *ksr-1* (6% rod-like lethality) in a genome-wide RNAi screen for *ekl* genes (Rocheleau and Sundaram, unpublished). BLASTp analysis only identifies Y39G10AR.7 homologs in other Caenorhabditis species. Protein alignments revealed a cluster of eight consensus Erk phosphorylation sites and four Erk docking sites (D-box and FXFP) strongly suggesting that Y39G10AR.7 might be a downstream target of MPK-1 Erk activity. The *vh20* E422K mutation disrupts stretch of negatively charged residues in the C-terminus suggesting this domain may be functionally important. We are currently performing transgenic rescue of *vh20* with a genomic clone of *Y39G10AR.7*. We will investigate the genetic relationship between vh20 and other mutations in the Ras/MAPK pathway and downstream MPK-1 Erk substrates LIN-1 and EOR-1.

729B. An RNAi screen for maternal factors influencing endoderm specification. **Hailey H. Choi**, Morris F. Maduro. University of California, Riverside 900 University Ave. Riverside, CA 92521.

Department of Cell, Molecular, and Developmental Biology, University of California, Riverside[1] Department of Biology, University of California, Riverside[2]

Gene expression between otherwise identical cells can vary due to stochastic factors, such as differences in the number of transcription factor molecules available to bind a promoter. We have developed a system to examine how gene networks buffer noise using the well-studied endoderm specification network. In this network, input from maternal and zygotic genes ultimately influences activation of the embryonic E specification genes *end-1* and *end-3*. Activation of these genes leads to activation of *elt-2* and *elt-7*, which direct endoderm differentiation. Activation of *elt-2* has been hypothesized to occur as a result of accumulation of threshold amounts of *end-1* mRNA (Raj et al., 2010). To examine the phenotypic outcomes from near-threshold activation of gut specification, we have generated “partial gut specification” strains in which gut is specified by single-copy transgenes of *end-1* and/or *end-3* in which the binding sites for the MED-1,2 factors have been mutated. One of these strains, carrying a MED site (-) *end-1* in a double mutant *end-1,3(-)* background, makes gut in ~25% of embryos. Using an integrated *elt-2*::GFP reporter to identify fully committed gut cells (even in arrested embryos), we are using RNAi to screen for maternal factors that may influence the ability of this strain to specify gut. We hypothesize that such genes might act generally in gene expression, or may be more restricted to endoderm specification. Using the Vidal feeding library, we have selected a subset of genes that are known to be expressed in the germline. In a pilot screen of ~10% of these clones, we have found several genes that, when knocked down, cause an increase in the proportion of embryos making gut over controls. One of these, *hda-1*, has been previously shown to cause an increase in gut specification when the endoderm network is compromised (Calvo et al., 2001). We will report on further progress with the screen.

730C. Characterization of a BMP negative regulator in the extracellular matrix. Y.J. ANG, **King-Lau CHOW**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

The conserved Bone Morphogenetic Protein (BMP) pathway impacts on a myriad of developmental processes in animals through the receptor relay molecules that target nuclear genes expression. In worm, this pathway uses the ligand, DBL-1, to control the body length. We are interested in investigating the negative regulatory control of this pathway with a focus on LON-2. We first studied the temporal requirement of *lon-2* by profiling the body length of *lon-2* mutant and its gene expression throughout the life cycle. The body length of nematode is regulated through endoreduplication in the hypodermis where *lon-2* is expressed. We tested the cell specific requirement of *lon-2* function by ectopically expressing *lon-2* in different tissues in mutant worms. The rescue results confirm that *lon-2* functions effectively non-restrictive to hypodermis to control hypodermal differentiation. In addition, LON-2 is hypothesized to regulate DBL-1 through physical interaction on the hypodermis through specific functional domain(s). We investigated the *in vivo* interaction of both proteins by co-immunoprecipitation of epitope-tagged LON-2 and DBL-1 so as to map the minimal functional structure of this molecule. We further evaluate the importance of LON-2 secretion by testing functions of LON-2 protein without signal peptide. Removal of GPI linkage and immobilization of LON-2 on the hypodermal membrane will also be examined for its impact on LON-2 biological activity. Observation from of these experiments will elucidate the regulatory mechanism of *dbl-1* pathway executed through physical interaction of LON-2 domain in a cell specific context. (This study is supported by Research Grants Council, Hong Kong).

731A. The role of the claudin-like gene *nsy-4* in *C. elegans* sensory ray development. **King L. Chow**, Kei C. Fan. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

During the ray development, homeobox protein CEH-43 and T-box transcription factor TBX-2 are key transcription factors regulating the cellular assembly of the organ structure. Yet, downstream structural genes that execute the process are largely unknown. Here we present our recent findings on a potential structural gene, *nsy-4*. Based on our genome-wide RNAi screen, *nsy-4* is required for the ray differentiation. We showed with structural cell (Rnst)-specific marker that Rnsts of most missing ray in *nsy-4RNAi* males are born, indicating that *nsy-4* is essential for the assembly but not the lineage of ray cells. Reporter gene analyses revealed that *nsy-4* is expressed in both the hypodermis and Rnst. In Rnst, *nsy-4* expression is positively regulated by *ceh-43* and *tbx-2*. The *nsy-4* reporter signal in both *ceh-43(RNAi)* and *tbx-2* mutants was reduced significantly, suggesting that the expression of *nsy-4* is dependent of CEH-43 and TBX-2. These results collectively imply *nsy-4* to be a ray structural gene downstream of this transcription factor network. As for its function, though *nsy-4* is a worm-specific protein with no obvious homolog outside nematodes, it structurally resembles claudin in the vertebrates. Studies done on claudin-like molecules in *C. elegans* had shown that they have similar functions as their mammalian counterparts in establishing distinct apical junctions. This type of cell junctions are found in rays and is important for the adhesion between ray cells. Using two translational reporters, NSY-4

was confirmed to be subcellularly localized at the apical junctions in all rays throughout the assembly process. Phenotypic analysis reveals morphological defects in the developing ray, including malformation of papillae and lack of Rnst in the extending rays, both of which are associated with improper apical junction establishment. We will continue to confirm the function of *nsy-4* as a claudin in establishing cellular adhesion between ray cells during the ray development. Ultimately the study would shed light on detailed mechanism guiding the assembly of this simple sensory organ. (The study is supported by Research Grants Council, Hong Kong).

732B. Transcriptional Regulation of the Hox gene *lin-39* by LIN-31, a Winged-Helix Transcription Factor Involved in C. elegans Cell Fate Specification. **A. Dewey**[1], F. Meza Gutierrez[2], C. Morris-Singer[3], L. Miller[1]. 1) Biology, Santa Clara University, Santa Clara, CA; 2) TETRAD, UCSF, San Francisco, CA; 3) Cell and Developmental Biology, Harvard University, Cambridge, MA.

LIN-31, a winged-helix transcription factor, acts as an effector of the conserved RTK/Ras/MAP kinase signaling pathway and is required for vulval development in the nematode C. elegans. *lin-39*, a LIN-31 and Ras dependent Hox gene involved in extracellular pathways during vulval development, was identified as a downstream target because LIN-31 is able to gel-shift fragments of the *lin-39* promoter (Wagmaister *et al.* 2006). These previous gel-shift/EMSA experiments demonstrated that LIN-31 can bind to multiple subfragments within a 1.3kb fragment of the *lin-39* promoter. Because LIN-31 is a forkhead/winged-helix transcription factor, it is likely to bind specific sites containing conserved forkhead core binding sequences within this 1.3kb *lin-39* promoter fragment. The goal of this project is to express and purify GST::LIN-31 from E. coli and then develop a non-radioactive gel-shift assay to determine the specific sites that LIN-31 can bind. Once these sites have been identified, they can be correlated with function within C. elegans to determine which are necessary to express a downstream gene by using a GFP reporter construct. Based on these results, investigations of the interactions between LIN-31 and other proteins and its effect on cell-fate specification, can move forward. Identifying these binding sites will provide insight into the mechanisms by which a transcription factor at the end of a signaling pathway can elicit a specific cell fate.

733C. Elucidating the role of *nmy-2* in seam cell division patterns. **Siyu S Ding**, Peter J Appleford, Alison Woollard. Department of Biochemistry , University of Oxford, Oxford, United Kingdom.

C. elegans seam cells are multipotent neuroectodermal cells that undergo both symmetric and asymmetric divisions during larval development, thus providing a paradigm for the stem cell mode of division. Reiterative seam cell asymmetric division typically produces an anterior daughter that rounds up and a posterior daughter that elongates after division. The anterior daughter then moves out of the seam line and differentiates whereas the posterior daughter further elongates to re-form the seam line and maintains the stem fate. Non-muscle myosin is fundamental to processes such as cellular reshaping and migration, hence it emerges as a potential regulator of seam cell asymmetric divisions. A precedent for the role of non-muscle myosin 2 (NMY-2) in *C. elegans* post-embryonic development has been established in the Q neuroblast lineage (Ou et al., 2010). This further sparked our interest in the role for NMY-2 in seam cells. While post-embryonically abrogating *nmy-2* function either using temperature sensitive mutants or by RNA interference failed to produce significant changes in seam cell numbers, combining the two treatments robustly reduced terminal seam cell number from 16 in wild-type animals to 11.6 in treated animals. Progressive cell loss was observed in each round of asymmetric division. Our current focus is to understand the cell molecular processes by which *nmy-2* influences seam cell asymmetric divisions using live imaging and video lineage analysis.

734A. Imaging developmental landscape of *C. elegans* embryos. **Zhuo Du**, Anthony Santella, Fei He, Michael Tiongson, Zhirong Bao. Sloan-Kettering Institute, New York, NY.

Fluorescence microscopy at single-cell resolution offers exciting opportunities for investigating complex in vivo processes. In *C. elegans*, this allows systematic tracking of every cell at every minute through embryogenesis and the gathering of thousands of quantitative measurements of individual cell behavior per embryo. A major challenge lies in translating the information into a mechanistic understanding of development. We report an automated pipeline to infer the developmental landscape based on live imaging and single-cell phenotype analysis. The resulting depiction of the landscape includes: differentiated states, paths traversing the states and genetic pathways and cell-to-cell signaling events that regulate path choices. Our pipeline includes a series of algorithms to (1) digitize embryogenesis by constructing a cell lineage and determining the single-cell expression patterns of cell fate markers; (2) define differentiated states based on combinatorial gene expression patterns and detect state changes in mutants through pattern matching; (3) infer the decision points regulating binary state choices in development using detected homeotic transformations; and (4) predict genetic modules and cell-to-cell signaling events based on systems-level analysis of mutual information between genes and cells across multiple perturbations. These algorithms are general rules for automated reasoning not relying on prior knowledge of gene function or mechanisms. We have validated our approach by dissecting the developmental landscape underlying the specification of early progenitor cells. The resulting systems-wide mechanistic model recapitulates current knowledge of regulation and provides new insights into gene functions, hidden developmental paths and the decision points regulating state transitions.

735B. The *let-7* microRNA is dispensable for early vulva cell fate specification, but is required for later stages of vulva development. **Matyas Ecsedi**, Helge Grosshans. Friedrich Miescher Institute, Maulbeerstrasse 66, 4058 Basel, Switzerland.

Members of the *let-7* microRNA (miRNA) family are well-known for their role in the heterochronic pathway, which times cell proliferation and differentiation in the hypodermal seam cells. In addition to retarded hypodermal phenotypes, *let-7* mutants show lethal vulva bursting. The underlying cause of vulva bursting and the developmental role(s) of the *let-7* family in the vulva are largely unknown. As *let-60/RAS*, a major regulator of vulva cell fates, is a well-established *let-7* target, we sought to determine *let-7*'s role in vulva precursor cell (VPC) fate specification. Whereas *let-60* deregulation would be predicted to cause mis-specification, we unexpectedly find that early vulva cell fate specification at the L3 stage is executed normally in *let-7* or *mir-84* animals as assessed by the 1° and 2° cell fate markers, *egl-17::cfp* and *lin-11::gfp*. To resolve this issue, we examined post-transcriptional regulation of the *let-60* 3'UTR using a single-copy integrated reporter. We observed repression of this reporter in the hypodermis and in the anchor cell at the L4 stage, but not in the VPCs in earlier stages. Furthermore, expression of a functional *let-60* rescue construct without a 3'UTR results in normal number of apparently wild-type vulvae, indicating that *let-60* is not a relevant *let-7* target in the vulva. To determine whether *let-7* activity in the vulva is necessary at all, we expressed a *let-7* rescue fragment in selected tissues from single-copy integrated transgenes in the *let-7(null)* background. Exclusively hypodermal

expression of *let-7* alone was not sufficient, but required presence of *let-7* in VPCs to rescue lethality of the *let-7* mutation. Indeed, we find high expression of *mir-48/84* and *let-7* in the vulva at the L4 stage as assessed by single-copy integrated promoter::GFP reporters. Taken together, these results suggest that the vulva morphogenesis defects and bursting observed in *let-7* and *let-7 family* mutants are caused by altered gene expression during vulva morphogenesis at the L4 stage. We are currently characterizing in detail vulva morphogenesis in *let*-7 family mutants using time lapse imaging and testing which genes must be down-regulated by *let-7* to ensure formation of a wild-type vulva.

736C. Suppressors of *pos-1* identify a novel function for GLP-1 and new players, *gld-3*, cyclin E and *spos-1*, involved in endoderm specification. **Ahmed Elewa**, Masaki Shirayama, Sandra Vergara, Takao Ishidate, Craig Mello. University of Massachusetts Medical School, Worcester, MA.

Maternal mRNAs program much of early embryogenesis, however the mechanisms that control their remain mysterious. POS-1 is a Cys3His zinc finger protein that regulates the translation of maternal RNAs. In *pos-1* mutants the fates of the P2 and EMS blastomeres are misspecified leading to incomplete pharyngeal development and an absence of both endodermal cells and germ cells and . In addition, *pos-1* mutants exhibit a defect in the Notch-signaling-dependent specification of the ABp blastomere. While the ABp defects in *pos-1* mutants are correlated with missexpression of maternal mRNAs encoding the Notch signaling components GLP-1 and APX-1, nothing is known at present about what mechanisms underlie the *pos-1* defects in EMS and P2 development. To begin to address this question we undertook RNAi and genetic screens for factors that restore endoderm specification in *pos-1* mutants. The RNAi screen identified the KH domain gene (*gld-3*), Cyclin E (*cye-1*) and the novel gene F32D1.6 (which we have named "*s*uppressor of *pos-1*" *spos-1*). Forward mutagenesis screens identified alleles of two genes *gld-3* and *glp-1*. In each case suppression results in restored endoderm and pharyngeal-mesoderm differentiation, but does not restore germ-line specification or Notch signaling. The *glp-1(ne4298)* lesion alters a conserved amino acid in the 4th swi6 motif resulting in a strong temperature sensitive *glp-1* loss of function phenotype. Surprisingly, the temperature-sensitive period (TSP) for *glp-1* suppression of *pos-1* occurs prior to fertilization, indicating that *glp-1(ne4298)* alters a maternal function that influences endoderm specification several hours later during early embryogenesis. The *spos-1* gene has no obvious conserved motifs. However, *spos-1* single mutants exhibit a maternal effect lethal phenotype with properly specified and well-differentiated tissues. We are investigating whether POS-1, GLP-1 and other *pos-1* suppressors regulate *spos-1* expression. We will present our characterization of SPOS-1 and ongoing efforts to understand the surprisingly complex nature of endoderm specification.

737A. Defining genetic pathways of disease through genetic suppression screening in *C. elegans*. **Amy Fabritius**, Andy Golden. NIDDK/LBG, NIH, Bethesda, MD.

Orthologs of genes responsible for many human monogenic diseases exist in *C. elegans*. We are using mutants in these orthologs in *C. elegans* to perform EMS mutagenesis suppressor screens to find genes that are involved in various disease pathways. I am using mutants in the *C. elegans* type IV collagens and the ubiquitin-activating enzyme in suppressor screens to identify potential novel therapeutic targets.

Humans have six type IV collagens (alpha1-alpha6) and *C. elegans* has two type IV collagens (*emb-9* and *let-2*). The type IV collagens in both species are a major constituent of basement membranes and play an important role in signaling and cell adhesions. Type IV collagens form a heterotrimer along their long helical central domain and higher order net-like structures through interactions of the amino and carboxyl termini. Mutations in the human type IV collagens can cause Alport syndrome or brain small vessel disease. Mutations in either gene in *C. elegans* lead to embryonic lethality or larval arrest (Kramer, 2005).

The ubiquitin-activating enzyme in human (UBA1or UBE1) and in *C. elegans* (*uba-1*) is required for activation of ubiquitin for downstream ligation of ubiquitin by the E2 and E3 ubiquitin ligases and subsequent protein degradation by the proteasome. Mutations in UBA1 cause early-onset spinal muscular atrophy and some cancers. UBA-1 is the only known ubiquitin-activating enzyme in *C. elegans* and *uba-1(it129ts)* allele causes embryonic lethality, larval lethality, sperm-specific lethality and change in size at restrictive temperature (Kulkarni and Smith, 2008).

We have obtained and are characterizing potential suppressors of an allele of *emb-9*. We are also continuing mutagenesis screens to find additional interactions and suppressors of *uba-1* and additional disease genes. These results will help further define genetic pathways and potentially identify genes that could serve as therapeutic targets to suppress disease symptoms.

738B. Asymmetric regulation of the human VAX ortholog homeobox gene ceh-5 during early neurogenesis in Caenorhabditis elegans. **Umesh Gangishetti**, Lois Tang, Johan Henriksson, Thomas R. Burglin. Dept. of Biosciences and Nutrition Karolinska Institutet Huddinge Sweden.

Bilateral symmetry of an animal's body plan is one of the unique features of higher animals. The nervous system is largely bilaterally symmetric on a morphological level, but often displays striking degrees of functional left-right asymmetry. In a 4D microscopy expression screen, we have identified a homeobox gene ceh-5 that displays a unique asymmetrical expression. It is the ortholog of the human VAX genes, and is expressed in three different groups of cells during gastrulation. Two of these are bilaterally symmetric groups and interestingly ceh-5 is expressed much stronger in the right than in the left. The third group is expressed in the very anterior starting about two cell cycles later. Little is understood about cell fate specification and how left-right asymmetries are generated during gastrulation. ceh-5, therefore, provides an excellent entry point to study these phenomena. We have dissected the promoter region of ceh-5 to understand how the distinct spatio-temporal ceh-5 expression is generated in the two bilaterally symmetric cell groups. A series of promoter deletions in ceh-5 fused to GFP were used to make transgenic animals. We examined their expression using the 4D imaging microscopy framework Endrov (see abstract by Henriksson et al., www.endrov.net). We identified a minimal 900bp region up-stream of the ATG that can initiate and maintain expression of ceh-5 during embryogenesis. About 70 bp upstream of ATG is a 21bp element containing an E-box motif essential for the regulation of ceh-5. When mutated the lateral expression is abolished. This motif is highly conserved among different Caenorhabditis species and is known to be a binding site for basic helix-loop-helix (bHLH) transcription factors. We examined the effect of knocking-down C. elegans bHLH factors in ceh-5::GFP lines. In this fashion, we have so far identified the bHLH factors NGN-1, CND-1, and HLH-2 as required for the lateral expression of ceh-5. Not all bHLH factors have been examined yet, so we are now continuing the RNAi experiments to determine if additional factors play a role.

739C. Investigating the role of SEM-4/SALL in development of the postembryonic mesoderm. **Vikas Ghai**, Chenxi Tsian, Jun Liu. Cornell Univ, Ithaca, NY.

The *C. elegans* postembryonic mesoderm (M) lineage is derived from a single pluripotent precursor, the M mesoblast, which will produce 32 cells, including 14 bodywall muscles (BWMs), two coelomocytes (CCs), and two sex myoblasts (SMs) that produce 16 sex muscles. SEM-4, the sole C. elegans member of the SALL family of C2H2 zinc finger transcription factors is required for both CC and SM fates, as both are transformed to BWM cells in *sem-4* null mutants (Basson and Horvitz, 1996). Here we investigate the role of SEM-4 during the development of the M-lineage. Based of analysis of additional *sem-4* mutants, only a subset of highly conserved zinc fingers (5-7) seems to be required for the M-lineage phenotypes. Indeed, *sem-4* is expressed in the M-lineage precursors that will give rise to both the CCs and SMs as well as the SM descendants. This restricted expression requires input from M lineage intrinsic factors, such as FOZI-1, and SYS-1/beta-catenin, which is downstream of the Wnt/beta-catenin asymmetry pathway. It also requires the presence of large intronic sequences in the *sem-4* locus. Forced expression of SEM-4 throughout the M-lineage is able to produce extra SMs, suggesting that while *sem-4* is necessary for both the CC and SM fate, it is sufficient to specify the SM fate. The mis-expression phenotype of *sem-4* is similar to the loss-of function phenotype of *mls-2*/Hmx mutants, suggesting that MLS-2 might negatively regulate *sem-4*. However, analysis of *sem-4*; *mls-2* double mutant phenotypes did not suggest a simple epistatic relationship between the two genes. The M lineage phenotype and expression pattern of *sem-4* regarding SM development resemble those of *sem-2/*SoxC mutants, suggesting a a potential interaction. Results from molecular epitasis and yeast-two hybrid experiments suggest that *sem-2* and *sem-4* do not regulate each other's expression in the M-lineage or physically interact with each other. However, *sem-2* is required for SEM-4 to convert additional cells to the SM fate when mis-expressed. We are currently testing how SEM-4 and SEM-2 may interact to correctly specify the SM fate, what SEM-4 interacts with to specify the CC fate, and what are downstream of SEM-4 in both fate specification events.

740A. Defining the role of the *Caenorhabditis elegans* homeobox protein, PAL-1, in the development of the stem-like seam cells. **Sophie P. R. Gilbert**, Charles Brabin, Peter J. Appleford, Alison Woollard. Department of Biochemistry, University of Oxford, Oxford, Oxfordshire, United Kingdom.

pal-1, the *C. elegans* homologue of *Drosophila caudal*, has previously been shown to have an important role in establishing posterior patterning during embryogenesis [1]. Here we investigate a novel role for *pal-1* during seam cell development. *pal-1* was identified in a small scale candidate RNAi screen to detect genes acting redundantly with *rnt-1*, a gene already known to be essential for maintaining correct seam cell divisions. Synthetic lethality of *pal-1* with *rnt-1* was observed, indicating that the two gene products may physically interact during embryogenesis. Although *pal-1* null animals are embryonic lethal, worms homozygous for a *pal-1* mutant allele *e2091* survive embryogenesis but on hatching display an uneven distribution of seam cells along their length, together with other seam defects. Analysis of this strain has previously identified two point mutations in the last intron of *pal-1* [2]; we have found that a wild-type copy of this intron is capable of driving specific seam GFP expression, whereas an intron bearing the two mutations fails to express correctly, suggesting a separate seam-specific role for *pal-1*. We have demonstrated that the last intron of *pal-1* contains an enhancer element (perturbed in *e2091*) required to correctly specify *pal-1* expression in the seam and enable correct seam cell development and orientation. To identify transcription factors that bind to this tissue-specific intronic enhancer we are carrying out a yeast one-hybrid screen using both the wild-type and *e2091* mutant version of the *pal-1* intron. We will also report our phenotypic analysis of *pal-1(e2091)* with respect to the cell biology of the seam cells.

1. Edgar, L.G., et al., Dev Biol, 2001. 229(1): p. 71-88.
2. Zhang, H. and S.W. Emmons, Genes Dev, 2000. 14(17): p. 2161-72.

741B. When two become one: Sperm-egg fusion during *C. elegans* fertilization. **Boaz Gildor[1]**, Meital Oren[1,2], Benjamin Podbilewicz[1]. 1) Faculty of Biology, Technion- Israel Institute of Technology, Haifa 32000, Israel; 2) Department of Biochemistry and Molecular Biophysics, Columbia University NY, NY.

Cell-cell fusion is a morphogenetic process that shapes a variety of tissues in multicellular organisms including muscles, bones, the placenta and the fertilized zygote. About one third of the somatic cells of the worm body undergo cell-cell fusion during development, a fact that has facilitated the discovery of the first eukaryotic fusogens in *C. elegans*. Our long term goal is to identify the fusogens that drive sperm-egg fusion in *C. elegans* using bioinformatics, biochemistry, genetics and molecular biology. The fact that *C. elegans* worms can be hermaphrodites has been used to study fertilization and has proven useful for isolating many mutants specifically affecting different stages in the process of fertilization. We have started the screening process by building a candidate list through a bioinformatic genome-wide analysis of the worm genome. Our two assumptions are that the fusogen will be a transmembrane protein essential for fertility. The TMHMM prediction software was utilized to filter non-transmembranal proteins and genes required for fertility were selected according to Ste phenotype described in wormbase in mutant strains or in RNAi experiments. The resulting ~500 genes were manually prioritized according to known gene function, proteomic data and mRNA expression in the germline. We are in the process of cloning the candidate genes into heat shock promoter plasmids, and injecting them into *eff-1* mutant worms. *eff-1* is an established fusogen, sufficient for fusion in worms or heterologous systems. We hypothesize that over-expression of the sperm-egg fusogen will result in hypodermal fusion events that can be easily monitored. Because sperm-egg fusion may require more than one gene, we will also inject pools of genes together. This methodology is also being used to test the fusogenic activity of other candidate fusogens. One of these candidates is the *eff-1* paralog, C26D10.7. Despite their 88% identity, no function for C26D10.7 has been discovered so far. We hope that by searching for novel fusogens we will eventually identify a real sperm-egg fusogen for the first time.

742C. The Mediator subunit CDK-8 is a dual negative/positive regulator of EGFR-Ras-MAPK signaling. **Jennifer M Grants**, Lisa TL Ying, Stefan Taubert. CMMT, University of British Columbia, Vancouver, BC, Canada.

The epidermal growth factor receptor-Ras-mitogen activated protein kinase (EGFR-Ras-MAPK) signaling pathway controls many fundamental cellular processes such as proliferation and differentiation, and thus is a tightly regulated pathway. The transcription factors LIN-1/Ets and LIN-31/Forkhead are important effectors downstream of EGFR-Ras-MAPK; however, their coregulators remain poorly understood. The conserved transcriptional coregulator complex, Mediator, may be an important regulator of these transcription factors. Here, we describe the identification of Mediator subunit cyclin dependent kinase 8 (CDK-8) as a novel regulator of the EGFR-Ras-MAPK pathway in *Caenorhabditis elegans*. CDK-8 is an intriguing Mediator subunit as its mammalian ortholog is known to be both a coactivator and a corepressor of transcription. Using *C. elegans* vulval induction as measure of EGFR-Ras-MAPK signaling activity, we demonstrate that CDK-8 negatively impacts this pathway. Specifically, *cdk-8* null mutation causes vulval hyperinduction that is significantly enhanced by mutations in synthetic multivulva (synMuv) genes, well-characterized negative regulators of EGFR-Ras-MAPK. Genetic epistasis

analysis places *cdk-8* downstream of the receptor *let-23/EGFR*. In line with this position, we find evidence that CDK-8 may act as a corepressor for the transcription factor LIN-1, as loss of *cdk-8* enhances the low-penetrance vulval hyperinduction phenotype caused by *lin-1* RNAi knockdown. Unexpectedly, CDK-8 also takes on a positive regulatory role in the EGFR-Ras-MAPK pathway in the absence of transcription factor LIN-31, as *cdk-8* is required for the expression of the vulval hyperinduction phenotype of *lin-31* null mutants. Thus, *cdk-8* acts downstream of *lin-31* in this positive regulatory role, suggesting that CDK-8 may be an important coactivator for another transcription factor downstream of EGFR-Ras-MAPK. Overall, this work identifies both positive and negative regulatory roles for CDK-8 within an important developmental and oncogenic signaling cascade. Furthermore, this provides the first indication that CDK-8 can act as a dual negative/positive transcriptional coregulator within a single gene network.

743A. Genetic analysis of vulval development in *C. briggsae*. **Bhagwati P. Gupta**[1], Devika Sharanya[1], Bavithra Thillainathan[1], Cambree J. Fillis[2], Kelly A. Ward[2], Edward M. Zitnik[2], Molly E. Gallagher[2], Helen M. Chamberlin[2]. 1) Biology, McMaster University, Hamilton, ON, Canada; 2) Molecular Genetics, Ohio State University, Columbus, OH, USA.

In both *C. elegans* and *C. briggsae*, vulval development results from the division of 3 of 6 multipotential vulval precursor cells (VPCs) that are induced to adopt primary (1^0) and secondary (2^0) fates in a 2^0-1^0-2^0 pattern. The induced VPCs divide to form 22 progeny that make up the adult vulval structure. The remaining VPCs do not contribute to the vulval tissue and fuse with the surrounding hypodermal syncytium. Genetic screens in *C. elegans* have recovered mutations that cause Vulvaless (Vul, fewer than 3 VPC induction) and Multivulva (Muv, more than 3 VPC induction) phenotypes. Analyses of these mutants have resulted in identification of components of several signal transduction pathways including EGF, Notch and Wnt.

The apparent similarity of cellular events in vulval development in *C. elegans* and *C. briggsae* has suggested that the underlying genes and genetic networks would be conserved. We have tested this assumption by conducting genetic screens in *C. briggsae* for mutants with abnormal vulval development. A set of Vul mutants has been recently reported (Sharanya et al., G3, 2012). These screens have also identified at least 7 genes that mutate to the Muv phenotype. Three of the Muv genes include orthologs of *pry-1*, *lin-1* and *lin-31*. Mutations in the 4 remaining genes all exhibit a dependency on EGF signalling, as the Muv phenotype is suppressed by treatment with the MEK inhibitor U0126. However, work from *C. elegans* does not suggest which genes might be affected. Using polymorphism mapping, all 4 unknown genes have been mapped to genomic regions that lack obvious candidate genes, including any orthologs of known *C. elegans* synMuv genes. We are currently using whole genome sequencing methods to identify the mutant genes, and GFP reporter transgenes to characterize the cell lineage defects in these mutants. Overall, these results argue that despite the developmental and morphological similarity in wild-type vulval development, *C. elegans* and *C. briggsae* exhibit notable differences in the set of genes that contribute to this developmental process.

744B. A role for UNC-55 in AS motor neuron subtype specification. **Michael Hart**[1,2], Oliver Hobert[1,2]. 1) Dept Biochemistry, Columbia Univ, New York, NY; 2) Howard Hughes Medical Institute.

The C. elegans ventral nerve cord (VNC) contains cholinergic and GABAergic motor neurons that are divided into distinct subtypes based on muscle innervation, morphology, developmental lineage, and molecular markers. The orphan nuclear hormone receptor unc-55 is expressed in the GABAergic VD and cholinergic AS motor neurons in the VNC of C. elegans. Unc-55 is known to play a role in the VD motor neuron subtype through repression of GABAergic DD motor neuron expressed genes involved in synaptic remodeling. AS cholinergic motor neuron fate is controlled by the COE transcription factor unc-3, however, factors involved in further specification into the AS motor neuron subtype are unknown. To interrogate a potential role for UNC-55 in AS motor neuron subtype specification, we examined GFP-reporters for both generic motor neuron genes and motor neuron subtype specific genes in unc-55 mutants. We found that unc-3-dependent DA specific GFP-markers were ectopically expressed in AS motor neurons in unc-55 mutants. Derepression of DA motor neuron genes in unc-55 mutants was specific, as general cholinergic motor neuron markers and other subtype specific markers were unaffected in unc-55 mutants. Unc-3-dependent GFP-markers that are shared by both DA and AS motor neurons were also unaffected in unc-55 mutants. We have also characterized the repression of a DA specific gene by UNC-55 through mutagenesis of predicted UNC-55 binding sites. These results support a repressive role for UNC-55 in differentiating AS motor neurons from DA motor neurons and a repressive model for generation of cholinergic motor neuron subtypes in the C. elegans VNC.

745C. Identification of *hcf-1* as a *dsh-2* suppressor. Kyla Hingwing, Tammy Wong, Jack Chen, **Nancy Hawkins**. Dept Mol. Biol. & Biochem, Simon Fraser Univ, Burnaby, BC, Canada.

In *C. elegans*, many asymmetric cell divisions are regulated by Wnt signalling. We have identified a Wnt/CWN-1, a Frizzled/MOM-5 and a Dishevelled/DSH-2 that function to control asymmetric neuroblast division. Loss of both maternal and zygotic *dsh-2* function results in asymmetric neuroblast division defects and embryonic/early larval lethality, while loss of zygotic *dsh-2* function disrupts asymmetric cell division of the somatic gonadal precursor cells (SGPs), Z1 and Z4. To identify genes that function with *dsh-2* in asymmetric division, we undertook a genetic screen to isolate suppressors of *dsh-2* lethality and isolated over 60 dominant suppressors. We focused our characterization on *Sup305*, which we demonstrated was also a strong suppressor of both asymmetric neuroblast and SGP division defects. Asymmetric division of Z1/Z4 involves the reciprocal asymmetric localization of the transcription factor, POP-1/TCF, and its co-activator, SYS-1/b-catenin, in the proximal and distal daughters respectively, resulting in a high SYS-1 to POP-1 ratio in the distal daughter and the specification of distal cell fate. *dsh-2* mutants disrupt both POP-1 and SYS-1 asymmetry. We analyzed the expression of the POP-1 and SYS-1 GFP reporters in *dsh-2; Sup305*. The suppressor was able to partially re-establish both POP-1 and SYS-1 asymmetry. Thus, a sufficient SYS-1 to POP-1 ratio is likely restored to specify distal cell fate. Genetic mapping experiments placed *Sup305* on the middle of chromosome IV. From a combination of whole genome sequencing and genetic mapping experiments, we determined that *Sup305* was a G to A mutation in *hcf-1* resulting in a Proline to Serine amino acid substitution. Loss of *hcf-1* rescued *dsh-2* phenotypes suggesting that *Sup305* is a dominant negative mutation in *hcf-1*. *hcf-1* is a transcriptional cofactor that bridges transcription factors to the chromatin modifying machinery. In *C. elegans*, *hcf-1* has been previously shown to modulate cell cycle, stress and lifespan. Our results indicate that *hcf-1* is also a novel Wnt pathway interactor. Further work will determine the mechanism of *hcf-1* suppression of *dsh-2*.

746A. HAM-1: An asymmetrically localized transcriptional regulator? **Khang Hua**[1], Amy Leung[2], Maria Wu[1], Nancy Hawkins[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada; 2) Boreal Gemonics, Vancouver, British Columbia, Canada.

Asymmetric cell division is a process in which a mother cell divides to produce two daughter cells with distinct cell fates. This process is biologically important for generating the cellular diversity required during embryonic development. In *C. elegans* all 302 neurons are generated through asymmetric cell division. To investigate the molecular mechanisms controlling asymmetric neuroblast division we have focused on HAM-1, an asymmetrically localized protein required for many asymmetric neuroblast divisions during embryogenesis. By antibody staining the protein is found exclusively at the cell cortex. However, in transgenic embryos expressing a functional GFP::HAM-1 fusion protein localization in the nucleus is observed. Consistent with this localization, HAM-1 contains a putative winged-helix DNA binding domain and two nuclear localization signals. We also have evidence that HAM-1 nuclear localization is required for proper function. A deletion analysis of HAM-1 revealed that the last 50 amino acids are essential for function but not localization. Within this sequence is a Tyrosine residue that is predicted to be part of a SH2 domain binding motif. Using site-directed mutagenesis, we have shown that this residue is essential for function, indicating that phosphorylation by a Tyrosine kinase may regulate HAM-1 function. We also have evidence that HAM-1 may interact with the Wnt signalling pathway. By yeast two hybrid, HAM-1 physically interacts with two *C. elegans* Dishevelled homologs, DSH-1 and MIG-5. We are currently generating a *dsh-1 mig-5* double mutant to investigate the biological relevance of this interaction. In addition, we have recently shown that GFP::HAM-1 is delocalized from the cell cortex in *mom-5*, a Frizzled receptor mutant. Thus, we propose a model in which HAM-1 is asymmetrically distributed between daughter cells, where it can then mediate a differential transcription program.

747B. The small GTPase Ral signals via an Exocyst-GCK-2/MAP4K-p38-MAPKAPK cascade. **Rebecca E.W. Kaplan**, Channing J. Der, David J. Reiner. University of North Carolina, Chapel Hill, NC.

We previously published that during EGF- and Notch-dependent developmental patterning of the C. elegans vulval epithelium, Ras switches between effectors Raf and RalGEF-Ral to promote mutually antagonistic 1° and 2° cell fates, respectively. Mammalian Ral signaling is poorly understood mechanistically but well validated in oncogenesis and metastasis. We therefore candidate-screened proteins loosely associated with Ral signaling. Three subunits of the Exocyst complex have been previously shown to bind Ral proteins; we found that loss of Exo84 but not Exo70 or Sec5 caused a phenotype similar to loss of Ral and blocked the activity of transgenic mutationally activated Ral. A CNH domain-containing MAP4 kinase has been shown to interact with the Exocyst by yeast two-hybrid and in human cells. We found that loss of a structurally related MAP4K, GCK-2, conferred effects similar to loss of Exo84. The MAP4K kinase domain mediates the putative Ral signal, but evidence of CNH domain MAP4K genetic complexity implies signaling duality, analogous to the aforementioned Ras signaling duality; antagonistic signals mediated by the same protein may contribute to developmental fidelity. Piecemeal studies on these vertebrate and invertebrate MAP4K proteins argue that they generally signal through the p38 MAP kinase cascade. We found that PMK-1/p38 functions in the same cascade as GCK-2/MAP4K, and demonstrated that the MLK-1 but not MTK-1 MAP3K functions similarly. We could not identify a MAP2K, perhaps due to redundancy amongst seven candidate paralogs. Thus we have identified a MAP kinase cascade probably initiated by Ral binding the Exocyst. We have additionally shown that a MAK-2/MAPKAP kinase, a well-known output of p38 kinases, functions in this GCK-2/MAP4K cascade and is expressed only in EGF-induced vulval precursors. We are currently investigating nuclear/cytoplasmic trafficking of p38 and MAPKAPK as an output of GCK-2/MAP4K cascade activity and plan to examine p38 activation in Ral-dependent cancer cell lines.

748C. MEX-5 positively regulates *mex-3* mRNA at anterior blastomere in early *C. elegans* embryo. **Hiroyuki Konno**, Koki Noguchi, Yuji Kohara. National Institute of Genetics, Mishima, Japan.

Post-transcriptional regulation of maternal genes is essential for proper development of early embryo. mRNA localization is one of such regulation, which is observed in diverse organisms. Although the study of translational and post-translational regulation of maternal mRNA in *C. elegans* has advanced significantly during the last decade, knowledge of regulation of mRNA localization is still limited. We analyzed *mex-3* mRNA localization as a model of mRNA localization to anterior half of embryo. After fertilization, the *mex-3* mRNA is gradually localized to the anterior half during the one-cell stage, and is predominantly localized to the anterior AB cell at the two-cell stage (1). We searched RNA binding protein whose loss of function perturbs *mex-3* mRNA localization. We found that the *mex-3* mRNA asymmetry was reduced in *mex-5* mutant and that, in *mex-5; mex-6* double mutant, the mRNA localization to anterior blastomere was not observed; *mex-3* mRNA was distributed uniformly and detected at low levels. Furthermore, a forced expression of MEX-5 protein at plasma membrane by the expression of a PH::mCherry::MEX-5 construct in *mex-5; mex-6* double mutant resulted in the localization of the endogenous *mex-3* mRNA to plasma membrane. We identified U rich sequence in *mex-3* 3' untranslated region as a *cis*-regulatory element that is required and sufficient for its localization to anterior half of embryo. The sequence fulfills the features of binding site of MEX-5 (2). These results suggest that MEX-5 positively regulates *mex-3* mRNA at anterior blastomere. (1) Bruce W. Draper et al. *Cell* **87**, 205-216 (1996). (2) John M. Pagano et al. *J. Biol. Chem.* **282**, 8883-8894 (2007).

749A. Structure-function of SYS-1/b-catenin, an effector of Wnt-directed asymmetric cell divisions. **Koonyee Lam**, Michael Molumby, Jennifer Hutchinson, Lori Adams, Bryan Phillips. Department of Biology, University of Iowa, Iowa City, IA.

Wnt/b-catenin signaling is an important component in embryonic development as well as homeostasis of adult tissues. A related Wnt/b-catenin Asymmetry (WbA) pathway controls binary cell fate decisions during the many *C. elegans* asymmetric cell divisions (ACD). WbA requires the proper regulation and function of the transcriptional activator in this pathway, the b-catenin SYS-1. ACD produces two daughter cells with different SYS-1 expression profiles, with high SYS-1 expression in the Wnt responsive cell and low SYS-1 expression in the Wnt nonresponsive cell. Despite poor sequence conservation, the SYS-1 crystal structure shows close structural similarities with mammalian b-catenin, namely twelve armadillo repeats with a specific TCF binding domain. SYS-1 lacks an unfolded C-terminal domain (CTD) that is utilized by mammalian b-catenin for the recruitment of several transcriptional co-activators such as DPY-22/MED12 and CBP-1/CBP. Genetic analyses of these co-activators indicate they are required for proper ACD, suggesting they may be recruited to Wnt target genes by SYS-1 via a novel interaction. Likewise, SYS-1 lacks GSK3b and CK1a regulatory sites, which facilitate degradation of b-catenin. We therefore have undertaken a structure-function approach to identify SYS-1 transactivation and regulatory domains. Yeast one-hybrid assays with SYS-1 fragments show that the unfolded N-terminal domain (NTD) and the first four armadillo repeats, but not the CTD repeats, are able to separately

activate transcription. In worms, these two activation domains, when linked to the POP-1/TCF DNA binding domain, are sufficient to induce the Wnt signaled fate in both daughters of an ACD. Consistent with a role in transcription, NTD, but not the CTD, fragments preferentially localize to *C. elegans* nuclei after ACD. Finally, no SYS-1 fragment has yet been identified that is sufficient to drive degradation in Wnt inactive cells, suggesting any modification sites are distinct from regulatory binding sites and that a targeting scaffold may be involved in SYS-1 degradation. These studies will help identify SYS-1 regulatory and transactivation domains, which will aid in identifying novel mechanisms of Wnt target gene regulation.

750B. Towards sequencing key cells of the developing Caenorhabditis elegans vulva. **James Lee**, Pei Shih, Paul Sternberg. Biology, Calif Institute of Technology, Pasadena, CA.

Vulval precursor cell (VPC) development has already demonstrated a clear and direct impact on vertebrate tumorigenesis, intercellular signaling, and progenitor differentiation. While the signaling pathways involved in VPC development are well characterized, the transcriptional responses that establish 1o, 2o, and 3o cell fates are poorly understood. Traditional approaches, such as genetic screens, become difficult where complex interactions and functional redundancies arise in the VPC program. In their place, multiple pairwise interaction studies have contributed to a draft regulatory network for late-stage vulval development, but such studies by themselves are tightfisted and rely on RNAi screens that suffer high false-negative rates. Next-generation methods, including microarray, ChIP, enhanced yeast one-hybrid, and PAB-1 mRNA pull-down are also problematic in vulval development because of their low cell-to-cell resolution. Instead, we are using a modified post-embryonic cell dissociation technique to collect single VPC cell types for transcriptome sequencing. By analyzing the expression profiles, genetic functions, and multiple pairwise interactions of developmentally meaningful transcription factors, we plan to answer two previously unapproachable questions: what is the gene regulatory network underlying 1o and 2o VPC identity, and how are EGF, Notch, and Wnt signals routed into this network? Of great interest are the differences and overlaps of these three signaling pathways in their effects on VPC fate patterning.

751C. UNC-40 positively modulates BMP signaling independent of netrin signaling. Chenxi Tian[1], Herong Shi[1], Shan Xiong[3], Fenghua Fu[1,2], Wen-Cheng Xiong[3], **Jun Liu[1]**. 1) Dept of Mol Biol and Gen; 2) Weill Inst of Mol and Cell Biol, Cornell University, Ithaca, NY 14853; 3) Inst of Mol Med and Gen and Dept of Neurology, Medical College of Georgia, Georgia Regents Univ, Augusta, GA 30912.

UNC-40 is the sole *C. elegans* homolog of DCC and neogenin, and is well known for its role as a netrin receptor important in axon and cell migration. We have found that UNC-40 is a positive modulator of the BMP-like Sma/Mab pathway. We have previously shown that loss-of-function mutations in the zinc finger-containing protein SMA-9 cause a dorsal to ventral fate transformation in the postembryonic mesodermal M lineage. This phenotype can be specifically suppressed by mutations in the Sma/Mab pathway. In a *sma-9* suppressor screen, we found that mutations in *unc-40* can suppress the *sma-9* M lineage defect. Phenotypic and genetic epistasis analyses showed that *unc-4*0 mutants are small and UNC-40 functions in the signal-receiving cells at the ligand/receptor level in the Sma/Mab pathway to regulate body size. This function of UNC-40 is independent of the axon guidance molecule UNC-6/netrin and the receptor UNC-5, as *unc-6* and *unc-5* mutants do not display Sma/Mab pathway mutant phenotypes. Instead, UNC-40 functions together with the RGM (repulsive guidance molecule) protein DRAG-1, a putative co-receptor of the Sma/Mab pathway. UNC-40 physically interacts with DRAG-1, and we have identified the key residues in the extracellular domain of UNC-40 that are critical for UNC-40-DRAG-1 interaction in vitro and UNC-40 function in vivo. Surprisingly, the extracellular domain of UNC-40 is sufficient to mediate Sma/Mab signaling, in clear contrast to the requirement of the intracellular domain of UNC-40 in mediating axon and cell migration. We showed that the mammalian homologs of DRAG-1 and UNC-40 can respectively rescue the Sma/Mab pathway phenotypes of *drag-1* and *unc-40* mutants. Moreover, an intracellular domain-deleted version of the UNC-40 homolog neogenin is capable of mediating BMP signaling in mammalian cell culture. Thus, in addition to mediating axon and cell migration, UNC-40 has an independent but evolutionarily conserved function in modulating BMP signaling.

752A. Regulation of serotonergic neuron patterning in *C. elegans* by Wnt signaling genes. **Curtis M. Loer**, Erin Williams. Dept of Biology, University of San Diego, San Diego, CA.

In *C. elegans,* the homeotic complex (HOM-C) gene *lin-39* is necessary for proper specification of many cells in the central body, including neurons in the ventral nerve cord (VNC). In the male, six central body VNC neurons (CP1 - CP6) normally express the neurotransmitter serotonin. In *lin-39* mutant males, these cells fail to make serotonin or die. We have found that in *lin-39; ced-3* double mutants - in which programmed cell death is blocked - all surviving CPs fail to make serotonin. Furthermore, the loss of serotonergic fate in the CPs in *lin-39* mutants is complete: four different genes required for making and using serotonin (*tph-1, bas-1, cat-1* and *cat-4*) all fail to be expressed normally in *lin-39* mutants, as assessed by GFP reporters. Other serotonergic neurons express serotonin and these genes normally in *lin-39* mutants. To find other genes that function similarly to *lin-39* in specifying serotonergic fate in the CP neurons, we are knocking down candidate genes by RNAi. Since *lin-39* is known to be regulated in vulval development by Wnt signaling, RTK signaling, and HOM-C protein regulators, we have screened these and other genes for effects of knockdown on serotonergic marker expression in CP neurons. We have found that knockdown of *hmp-2, sys-1,* and *sem-4* all reduce serotonergic marker gene expression. Both *hmp-2* and *sys-1* function in Wnt/MAPK signaling, encoding b-catenin-like proteins. The *sem-4* gene encodes a zinc-finger protein previously shown to interact with *lin-39* in vulval development in the central body. At the same time, we are testing which 'neuronal RNAi enhancing' mutants or transgenics - such as *uIs69* (with *unc-119::sid-1* - neuronal expression of dsRNA transporter SID-1) with or without a *sid-1* mutation - work best to increase RNA interference in these neurons (and/or their precursors).

753B. The role of the *C. elegans* Jarid1 histone lysine demethylase RBR-2 in vulva cell fate determination. **Yvonne C. Lussi**, Toshia R. Myers, Anna Elisabetta Salcini. Biotech Research and Innovation Centre (BRIC), University of Copenhagen, Denmark.

Regulation of histone modifications is critical for correct gene expression during development and differentiation, and aberrant activity of chromatin-modifying enzymes has been associated with diseases such as cancer. Among the chromatin-modifying enzymes, histone lysine demethylases have recently been identified as key players in eukaryotic gene transcription. The *C. elegans* JARID1 family member RBR-2 was shown to demethylate H3K4me3/me2, a mark associated with active chromatin, and to play a role in postembryonic development, as *rbr-2* mutant animals display defects in

vulva formation. We examined the role of RBR-2 in specification of the vulva precursor cells (VPCs) using different cell fate markers and found that RBR-2 is necessary for the proper patterning of 2° lineage cells. 1° and 3° cell fates appeared essentially unaffected in the *rbr-2* mutant worms. In absence of RBR-2, 2° lineage cells fail to acquire the correct identity. Expression of a genomic *rbr-2* construct under its endogenous promoter in a *rbr-2* mutant background rescued the 2° lineage defects, demonstrating a specific role for RBR-2 in vulva development. The failure in the execution of 2° cell fate points to a role of RBR-2 in the LIN-12/Notch signaling, a determinant of 2° cell fate. This notion is further supported by the finding that inactivation of *rbr-2* partially suppressed the pseudovulva phenotype of *lin-12* gain-of-function mutant animals and exacerbated misspecification of 2° vulval cells in *lin-12* hypomorphic animals. Taken together, our data suggest that RBR-2 function is critical for the correct execution of 2° cell fate identity during vulval development and implicate RBR-2 in Notch signaling during vulva organogenesis. In future, we will address the role of RBR-2 during cell fate determination and its putative role in Notch signaling by genetic studies as well as ChIP-sequencing and microarray analysis.

754C. Animal to Animal Variability During Vulval Cell Fate Specification. **Sabrina Maxeiner**, Daniel Roiz, Alex Hajnal. Institute of Molecular Life Sciences, Winterthurerstrasse 190, Zurich, Switzerland.

Cell fate determination and differentiation depend on the precise temporal and spatial regulation of intercellular signaling pathways (Euling et al. 1996). During vulval development, the anchor cell in the gonad induces the primary (1°) vulval cell fate by secreting an epidermal growth factor LIN-3 that activates the EGFR/RAS/MAPK signaling pathway in the nearest vulval precursor cell (VPC) P6.p (Sternberg 2005). A lateral signal from P6.p then induces the secondary (2°) cell fate in P5.p and P7.p by activating the LIN-12 NOTCH pathway. The distal VPCs (P3.p, P4.p and P8.p) receiving low levels of inductive and lateral signals adopt the tertiary (3°) fate. In particular, the connection between cell cycle progression and the NOTCH pathway ensures that 1°, 2° and 3° cell fates are specified in a temporally defined manner (Nusser-Stein et al. 2012). Although the vulval cell fate patterning exhibits an invariable outcome, the progression of fate acquisition by individual VPCs occurs with a certain degree of variability (Barkoulas et al. 2013). Defective coupling of signaling and cell cycle progression as it occurs in heterochronic mutants leads to mistakes in the timing of VPC division and cell fate determination (Ambros et al. 1984). To investigate the variability of EGFR/RAS/MAPK and LIN-12 NOTCH signaling during vulval fate specification quantitatively, we are establishing branched DNA single molecule fluorescence in situ hybridization (bDNA FISH) in *C. elegans*. bDNA FISH exhibits higher sensitivity and robustness compared to oligo DNA single molecule FISH (smFISH) allowing quantification at single RNA molecule resolution with improved signal-to-noise-ratio (Player at al. 2001, Raj et al. 2008). We are applying bDNA FISH to measure the dynamics and variability of 1° and 2° cell fate markers during VPC fate specification in L2 larvae. We assume that early during the cell fate acquisition process before the establishment of a stable fate pattern, the VPCs exhibit a certain degree of randomness in EGFR/RAS/MAPK and LIN-12 NOTCH signaling strength. To test this hypothesis, we are quantifying VPC fate specification in heterochronic mutants, where VPC induction is precocious (*lin-28(lf)*) or retarded (*lin-14(gf)*).

755A. Dynein Heavy Chain-1: a novel negative regulator of LET-23 EGFR induced vulva induction. **Jassy Meng**, Olga Skorobogata, Christian Rocheleau. Departments of Medicine and Anatomy and Cell Biology, McGill University, Montreal, Quebec, Canada.

The LET-23 EGFR/ LET-60 Ras/ MPK-1 Erk signaling pathway is required for vulval cell fate specification. The LIN-2 Cask/ LIN-7 Veli /LIN-10 Mint complex is required for the basolateral localization of LET-23 EGFR in the vulval precursor cells. Mutations in *lin-2*, *lin-7* or *lin-10* result in a very strong Vulvaless (Vul) phenotype, which can be strongly suppressed by loss of a negative regulator. We previously found that the late endosomal regulator, RAB-7, is a strong negative regulator of LET-23 EGFR signaling. A *rab-7* deletion allele, *ok511*, is a strong suppressor of the *lin-2* Vul phenotype as well as maternal effect embryonic lethal. To identify additional regulators that function with RAB-7 we conducted a clonal forward genetic screen for essential suppressors of the *lin-2* Vul phenotype. We identified *vh22*, which in addition to being a strong suppressor of *lin-2*, is Dpy and temperature sensitive embryonic lethal with early cell division defects. Using SNP mapping and whole genome sequencing we identified two missense mutations in *dhc-1*. Confirming that *vh22* is an allele of *dhc-1*, we find the *vh22* fails to complement the *dhc-1 or195* and *or352* alleles for the dead egg phenotype, and a DHC-1::GFP transgene strongly rescues the *vh22* dead egg and suppression of *lin-2* Vul phenotypes. Furthermore, *or195*, *or352* and *dhc-1(RNAi)* are able to suppress the *lin-2* Vul phenotype suggesting that *dhc-1* is a negative regulator of LET-23 EGFR signaling. Genetic epistasis experiments indicate that DHC-1 antagonizes LET-23 EGFR signaling downstream or in parallel to *lin-3 EGF*. DHC-1 is the Heavy Chain of the Dynein minus-end directed microtubule motor that among many functions is recruited by the Rab7 GTPase to mediate endosome to lysosome trafficking. We are currently testing if DHC-1 might exert its function by regulating LET-23 EGFR trafficking and/or degradation.

756B. Control of LET-23 localization by PRMT-1 during vulval development. **Sabrina Kathrin Merkle**[1], Juan Miguel Escobar-Restrepo[1], Tobias Schmid[1], Fabienne Largey[2], Alex Hajnal[1]. 1) Institute of Molecular Life Sciences, University of Zurich, Switzerland; 2) Clinic for Neurology, University Hospital Zurich, Switzerland.

Studies in multiple types of human cancers revealed that elevated RAS levels are often caused by overexpression or an enhanced activity of the Epidermal Growth Factor Receptor (EGFR) (Harper *et al.,* 2001). The *ras* signalling pathway is highly conserved in metazoans and, amongst others, controls cell fate specification during *C. elegans* vulval development. LET-23, which is the ortholog of EGFR, is retained on the basolateral membrane of the vulval precursor cells (VPCs) where it receives the inductive LIN-3 EGF signal secreted by the anchor cell (reviewed in Kim, 1997). Activation of LET-23 in the VPC P6.p leads to acquisition of the 1° cell fate via the LET-60/MPK-1 pathway. A lateral signal from P6.p then activates in its neighbours, P5.p and P7.p, the LIN-12 NOTCH pathway to specify a 2° fate (Sternberg and Horvitz, 1986). To identify novel components that regulate LET-23 EGFR signalling, we performed a pull-down assay using the intracellular domain of LET-23 (LET-23intra) followed by mass-spectrometry analysis. This approach identified PRMT-1 as a new LET-23intra binding protein. *prmt-1* encodes a type 1 arginine methyltransferase which modulates its substrates by asymmetric di-methylation at arginine residues (Fukamizu *et al.*, 2011). We examined the expression and subcellular localization of LET-23::GFP in *prmt-1(ok2710)* mutants and found an apical enrichment of the receptor in the VPCs. Human PRMT5 has been shown to modulate EGFR/RAS/ERK signalling by addition of a methyl group to the kinase domain of EGFR (Hung *et al.*, 2010). We are currently performing epistasis experiments with components of the LET-23/LET-60/MPK-1 pathway to test if the LET-23::GFP miss-localization observed in *prmt-1(ok2710)* mutants affects signalling and if this effect may be modulated by methylation of LET-23.

757C. The role of Wnt and FGF signaling in *C. elegans* vulval cell lineage polarity. **Paul Minor**, Paul Sternberg. HHMI, Division of Biology, Caltech, Pasadena, CA.

During *C. elegans* vulval development the anchor cell induces 3 of 6 multipotent vulval precursor cells (VPCs). The closest VPC generates a 1° lineage; the flanking VPCs, P5.p and P7.p, each generate a 2°, mirror symmetric, lineage. Two Wnt signals from the anchor cell promote the wild-type, anterior-facing, P7.p orientation, whereas the Wnt/EGL-20 signal from the tail promotes the daughter cells of P7.p to face the posterior. We show Wnt/EGL-20 acts through a member of the LDL receptor superfamily, LRP-2, along with Ror/CAM-1 and Van Gogh/VANG-1. All three transmembrane proteins control orientation through the localization of the b-catenin-like transcriptional coactivator SYS-1. We show that the Fibroblast Growth Factor (FGF) pathway acts in concert with LIN-17/Frizzled to regulate the localization of SYS-1/b-catenin, a component of the Wnt/ b-catenin asymmetry pathway. The source of the FGF ligand is the 1° vulval precursor cell (VPC), P6.p, which controls the polarity of the neighboring 2° VPC, P7.p, by signaling through the sex myoblasts (SMs), activating the FGF pathway. The Wnt, *cwn-1*, is expressed in the posterior body wall muscle of the worm as well as the SMs, making it the only Wnt expressed on the posterior and anterior sides of P7.p at the time of the polarity decision. Both sources of *cwn-1* act instructively to influence P7.p polarity in the direction of a morphogen gradient. Using single molecule FISH (smFISH), we show the FGF pathway leads to the regulation of *cwn-1* transcripts in the SMs. These results illustrate the first evidence of the interaction between FGF and Wnt in *C. elegans* development and vulval cell lineage polarity as well as highlight the promiscuous nature of Wnt signaling within *C. elegans*.

758A. Role of the $CRL2^{LRR-1}$ ubiquitin ligase in regulating LIN-12/Notch signaling in *C. elegans* vulva development. **Madhumati Mukherjee**, Edward T. Kipreos. Dept of Genetics, University of Georgia, Athens, GA.

The Ras and Notch signaling pathways are important in many aspects of animal development. Oftentimes they act antagonistically to specify particular developmental fates. The *Caenorhabditis elegans* vulva is one of the classic systems used to study these interactions. It is patterned by the concerted action of a Ras-mediated 'inductive' signal and a LIN-12/Notch-mediated 'lateral' signal. Of the set of six vulval precursor cells (P3.p-P8.p), only P5.p, P6.p, and P7.p form the mature vulva. The anchor cell (AC) in the gonad activates the Ras pathway in P6.p to induce the 1°cell fate. The adjacent P5.p and P7.p cells adopt 2° fates due to the activation of the LIN-12/Notch pathway. For proper cell fate patterning, Ras activity in P6.p must downregulate the LIN-12/Notch pathway, and LIN-12/Notch signaling must antagonize the Ras pathway in P5.p and P7.p. A defect in this antagonism can give rise to abnormal vulval fates. We find that loss of the substrate recognition subunit (SRS) LRR-1, of the cullin-RING ubiquitin ligase 2 (CRL-2) complex, results in a multivulva (Muv) phenotype at a penetrance of ~1%. The expression of the 1°cell fate marker EGL-17::CFP in the VPCs and their descendants is similar in *lrr-1* mutants and wild type, however expression of the 2°cell fate marker LIP-1::GFP is upregulated two-fold in P6.p and their descendants in *lrr-1* mutants. Inactivation of LRR-1 significantly increases the percentage of Muv animals of the *lin-12(n302)* mild hypomorph. RNAi inactivation of the Ras pathway inhibitors *lst-2*, *lst-3*, *lst-4* and *dpy-23* can suppress the Muv phenotype in *lrr-1* mutants. In *lrr-1* mutants, the level and localization of the full-length LIN-12/Notch receptor is not affected, however, the level of the cleaved Notch intracellular domain (NICD) protein is significantly elevated in the P6.p cell. NICD is known to be targeted for degradation by the SCF-SEL-10 ubiquitin ligase. *sel-10(null)* mutants do not have a Muv phenotype. When we combine *lrr-1 RNAi* with a *sel-10(null)* mutant the Muv phenotype increases to 6%, suggesting that the two genes function in separate pathways. Currently we are attempting to understand whether LRR-1 directly or indirectly regulates NICD accumulation.

759B. Tousled-like Kinase Is Required to Generate a Bilateral Asymmetry in the *C. elegans* Nervous System. **Shunji Nakano**[1], Bob Horvitz[2], Ikue Mori[1]. 1) Div. Biological Science, Nagoya University; 2) HHMI, Dept. Biology, MIT.

Bilateral asymmetry in *C. elegans* can arise from left-right asymmetric cell lineages. The single left-right unpaired MI neuron descends from the right side of an otherwise left-right symmetric cell lineage: the homologous cell on the left side becomes the e3D epithelial cell.

To identify the mechanisms that generate the MI-e3D asymmetry, we screened for mutants in which the MI-e3D asymmetry is lost. We isolated 16 mutations in 7 genes that transform MI into an e3D-like cell, thereby generating symmetry in a normally left-right asymmetric cell lineage. We previously showed that the establishment of the MI-e3D asymmetry requires left-right asymmetric expression of a CEH-36/NGN-1/HLH-2 transcriptional cascade (Nakano et al., Development, 2010). CEH-36 homeodomain protein is expressed in the MI grandmother cell but not in the e3D grandmother cell. CEH-36 induces asymmetric expression of two bHLH proteins, NGN-1 and HLH-2, in the MI mother cell but not in the e3D mother cell. We also showed that replication-coupled chromatin assembly is necessary for the MI-e3D asymmetry (Nakano et al., Cell, 2011). Reduction of the activity of the CAF-1 complex, a histone chaperone that deposits histone H3-H4 onto replicating DNA, causes the loss of the MI-e3D asymmetry.

Here we report that two mutations isolated from our screens, *n5342* and *n5351*, are alleles of the gene *tlk-1*. These mutant animals carry mutations in the *tlk-1* locus. Animals carrying a *tlk-1* deletion allele, *tm2395*, also show the loss of the MI-e3D asymmetry. *tlk-1* encodes an evolutionarily conserved serine-threonine kinase implicated in several chromatin-based processes, including transcriptional regulation, chromosome segregation and DNA damage responses. We are planning to test whether *tlk-1* is involved in CAF-1-mediated chromatin assembly or regulates expression of CEH-36, NGN-1 or HLH2. We hope that continued characterization of these genes will further reveal the mechanisms that establish bilateral asymmetry in the *C. elegans* nervous system.

760C. TORC2 Signaling Antagonizes SKN-1 to Induce C. elegans Mesendodermal Embryonic Development. Vanessa Ruf[1], Christina Holzem[1], Tobias Peyman[1], Gerd Walz[1,2], T. Keith Blackwell[3], **Elke Neumann-Haefelin**[1]. 1) Department of Medicine, Renal Division, University Hospital Freiburg, Freiburg, Germany; 2) Center for Biological Signaling Studies (bioss), University of Freiburg, Germany; 3) Joslin Diabetes Center, Harvard Stem Cell Institute, and Harvard Medical School Department of Genetics, Boston, MA.

The evolutionarily conserved target-of-rapamycin (TOR) kinase controls fundamental metabolic processes to support cell growth. TOR functions within the context of two distinct complexes, TORC1 and TORC2. The TORC2 with its specific component Rictor has been recently implicated in aging and regulation of growth and metabolism. Here, we identify rict-1/Rictor as a new regulator of embryonic development in C. elegans. The transcription factor skn-1 is essential to establish the development of the mesendoderm in embryos and is required for cellular homeostasis and longevity in adult worms. Loss

of skn-1 function in the embryo leads to mis-specification of the mesendodermal precursor consequently lacking intestine and pharynx. We found that genetic inactivation of rict-1 re-stored mesendodermal specification in skn-1 deficient embryos and thereby suppressed skn-1-associated lethality. Moreover, inactivation of the TORC2 components but not TORC1 partially rescued skn-1 embryonic lethality. SGK-1 mediated these functions downstream of rict-1/TORC2 as a sgk-1 gain of function mutant suppressed the rict-1 mutant phenotype. These data indicate that TORC2 and SGK-1 antagonize SKN-1 during embryonic development.

761A. Identification of mechanisms by which the expression of *lim-4* homeodomain gene is regulated to specify the SMB motor neuron fate. **Jisoo Park**, Jihye Yeon, Kyuhyung Kim. Daegu Gyeongbuk Institute of Science & Technology, Daegu, South Korea.

A cascade of transcriptional control confers neuron-specific morphologies and functions of individual neuronal cell types. The precise spatiotemporal regulation of transcription factors is essential for this neuronal differentiation and specification. However, the developmental mechanisms by which transcription factors are regulated to specify neuronal cell fate are not well known. We identified a *lim-4* LIM homeodomain gene as a 'terminal selector' transcription factor (Hobert, 2008) that specifies the SMB motor neuron fate (See Jinmanh Kim's abstract). From the promoter analyses of the *lim-4* gene, we identified several cis-regulatory sequences that are necessary for the expression of *lim-4* in the SMB neurons. These sequences include a motif responsible for autoregulation of *lim-4* in the SMB neurons (SMBDL/R and SMBVL/R). Interestingly, we also identified two other regulatory motifs that regulate the expression of *lim-4* in the SMB dorsal (SMBDL/R) and SMB ventral (SMBVL/R) neurons, respectively, suggesting that the expression of *lim-4* in SMB dorsal and ventral neurons is independently controlled. To explore the nature of differential expression of *lim-4* in SMB, we are generating transgenic animals expressing GFP in the SMB dorsal neurons as well as mCherry in the SMB ventral neurons and performing mutant screens to identify upstream molecules, such as transcription factors. *Key words: SMB motor neurons, *lim-4*, terminal selector.

762B. SOS-1 is required for remodeling epithelial junctions during the G1 excretory pore cell's transition from epithelial tube to neuroblast. **Jean Parry**, Meera Sundaram. Genetics, University of Pennsylvania, Philadelphia, PA.

Epithelial cells from the excretory system, rectum, and epidermis are remodeled and migrate away to form neurons during the course of normal *C. elegans* larval development (Jarriault et al., 2008, Sulston and Horvitz, 1977). The *C. elegans* excretory system is made-up of three epithelial, unicellular tubes that comprise a functioning organ system when the initial excretory pore cell (G1), undergoes this dedifferentiation and migration. In a precisely coordinated process, the G1 cell must be replaced by a neighboring epithelial cell (G2) while a cuticle-lined lumen is maintained for excretion of waste. During this process, the G1 cell remodels its intracellular and intercellular adhesions and alters its cytoskeletal dynamics, a process that can be visualized *in vivo* at single cell resolution. Here we show that the temporally precise wild-type process of G1 dedifferentiation and migration is marked by dorsal directed movement, loss of junctional markers including HMR-1 and AJM-1 starting from the ventral base, and redistribution of the actin cytoskeleton from the junctions to the leading edge of the migrating cell. Previous work in our lab showed that the extracellular leucine-rich repeat protein LET-4 was required for maintaining apical extracellular matrix (ECM) and junction integrity between the G1 pore and excretory duct (Mancuso et al., 2012). Intriguingly, LET-4 also has dynamically altered localization during the detachment of the G1 cell from the excretory duct. This remodeling and migration process in the excretory system is dependent on the Ras-GEF, SOS-1. In temperature sensitive *sos-1(cs41)* mutants, a shift to non-permissive temperature shortly after hatching prevents the G1 cell from completing remodeling and detachment. Corresponding to the blockage in detachment, LET-4 fails to re-localize in *sos-1(cs41)* mutants. These results lead us to suggest a model in which Ras dependent remodeling of apical ECM might be required for the detachment of the G1 cell from the excretory duct. In support of this model, a mutagenesis screen carried out in our lab found that another extracellular leucine-rich repeat protein, PAN-1, is required for detachment of the G1 cell from the excretory duct.

763C. Cell Fate Restriction and Reprogramming in *C. elegans*. **Tulsi Patel**[1], Oliver Hobert[2]. 1) Genetics and Development, Columbia University Medial Center, New York, NY; 2) Biochemistry and Biophysics, Columbia University Medical Center, New York, NY.

While the plasticity of cells in a multicellular organism is progressively lost during development, the differentiated state of a cell is not irreversible. Studies have shown that many transcription factors (TFs) that have been identified as master regulators or terminal selector genes (TSGs) for specific cell types, are sufficient to reprogram other differentiated cells. However, ectopic expression of a TSG leads to a context-dependent transformation of other cells, i.e., while some host cells can be converted, most cell types cannot. Likewise, in *C. elegans* we find that the ectopic misexpression of the Zn-finger TF, CHE-1, which is the TSG for the ASE neuron fate, is only able to induce expression of ASE genes in a few other neurons in the adult worm, suggesting that all other cells are refractory to CHE-1 activity. We have been using this context dependency in the activity of induced CHE-1 to study cell fate restriction in *C. elegans*. Firstly, we are identifying the cells in the adult worm that do respond to CHE-1 in order to understand why only these cells are permissive. So far we have found that the RIS, CEPDR/L, and CEPVR/L neurons ectopically express ASE genes in response to ubiquitous CHE-1. We are testing whether these neurons express a cofactor that allows CHE-1 to activate its targets, or if they have a chromatin structure that is inherently more plastic. Secondly, we hypothesize that other cell types remain refractory to CHE-1 because their respective TSGs create a non-permissive chromatin landscape. We have tested this hypothesis in the cholinergic motor neurons (MNs) and the GABAergic MNs, for which *unc-3* and *unc-30* are the TSGs respectively. We find that in *unc-3* mutants, CHE-1 is able to induce expression of ASE genes in a significant number of cholinergic MNs. However, GABAergic MNs are not more responsive in *unc-30* mutants. This data suggests that UNC-3 has a role in making the cholinergic MNs refractory to CHE-1. As it is possible that removal of *unc-3* leads to the derepression of a CHE-1 cofactor or that *unc-3*(-)MNs have some other property that makes them accessible to CHE-1, other TSGs are being tested to determine if this hypothesis applies to numerous cell types.

764A. Regulation of Post-embryonic Seam Cell Proliferation and Identity by the Non-Receptor Tyrosine Kinase, FRK-1. **Aaron Putzke**, Danielle Mila, Katherine Genzink, McLane Watson, Caroline Askonas, Kelsey Moore. Biology, Hope College, Holland, MI.

A hallmark of stem cell proliferation is asymmetric division resulting in one daughter maintaining the stem cell state and the other daughter differentiating into a non-stem cell identity. These divisions are crucial for building tissues and have become an important area of study for human disease. We have characterized a non-receptor tyrosine kinase, FRK-1, and hypothesize that it is required for maintenance of the asymmetric divisions in the stem

cell-like seam cells of the hypodermis. Our data show a genomic knockout of *frk-1* (allele *ok760*) results in severely uncoordinated larvae that arrest at the L1 stage. Homozygous *frk-1(ok760)* larvae have an excess number of lateral hypodermal cells which appear to have lost the asymmetry in the stem cell-like divisions of the seam cell lineage. *frk-1(ok760)* mutants show that the lateral hypodermal cells are consistent in size to the anterior daughter of a normal asymmetric seam cell division. Using *scm::gfp* and *elt-5::gfp* we observe a decrease in seam cell numbers, as well as an intermittent loss of alae. Furthermore, crossing *frk-1(ok760)* with transgenic lines containing non-seam hypodermal GFP markers, such as *elt-3*, *dpy-7* and later markers such as *col-19*, show a proportion of lateral hypodermal cells appear to precociously differentiate when compared to wildtype. Interestingly, our data also show a role for FRK-1 in seam cell proliferation, as eliminating FRK-1 during the L3-L4 transition results in supernumerary seam cells that are dependent on asymmetric Wnt signaling. Finally, we have observed a significant change in the expression of heterochronic regulators in *frk-1(ok760)* homozygous larvae, including up-regulation of *let-7* and *lin-4* and down-regulation of *lin-14*, *lin-28* and *hbl-1*, supporting a requirement for FRK-1 in preventing precocious differentiation. We are currently investigating whether there is a dependence on FRK-1 kinase activity and translocation to the nucleus during the stem cell-like divisions of seam cells. In conclusion, our data suggest a requirement for FRK-1 during larval development in maintaining the asymmetric divisions of seam cells, thereby preventing precocious differentiation prior to adulthood.

765B. Multiple Aspects of C. elegans Germ Cell Development are Regulated by XND-1. **Mainpal Rana**, Judith Yanowitz. Magee Women Research Institute, University of Pittsburgh, Pittsburgh, PA.

Despite the central importance of germ cells for the transmission of genetic material between generations, very little is known about the molecular programs that regulate their development. Defects during germ cell formation and differentiation can lead to infertility, birth defects, and formation of germ cell cancers. Work in our lab has identified *xnd-1* (X nondisjunction factor-1) as a key regulator of germ cell development in the nematode, *Caenorhabditis elegans*. Our analysis has revealed that *xnd-1* is one of the first genes turned on in the primordial germ cells (PGC) suggesting it may be a key regulator of PGC development. Consistent with this hypothesis, *xnd-1* mutant embryos exhibit multiple defects in PGC development including aberrant PGCs number and size, mislocalization, premature proliferation and missegregation of P-granules. Furthermore, co-depletion of XND-1 and NOS-2, a C. elegans Nanos homolog important for PGC development, reveals a synthetic sterile phenotype. The sterility is specific to *nos-2*, as it is not seen in *nos-1* or *nos-3* double mutants. These results suggest that *xnd-1* and *nos-2* function in parallel pathways for germline specification. Using time lapse imaging, we are exploring the causes and consequences of the PGC defects. We have also identified key embryonic regulations affecting XND-1 expression in the PGC.

766C. Netrin expression in *P. pacificus*. Brent Wyatt, Kelly Mahoney, **David Rudel**. Department of Biology, East Carolina University, Greenville, NC.

Netrin (UNC-6) is a secreted cell guidance cue recognized by two molecularly unrelated cell surface receptors: UNC-40, a member of the DCC receptor family, and UNC-5. In *Caenorhabditis elegans* it provides a signal guiding axon extension and cell migrations, including the migrations of the gonadal distal tip cells (DTCs) and subsequently the extension of the gonadal arms during larval development. It has been shown that Netrin receptors are necessary for the dorsal migration of the DTCs. In the nematode *Pristionchus pacificus*, the gonadal arms make a second dorsal to ventral migration during gonad arm extension. We hypothesize this may also involve Netrin receptors. We have identified a *Ppa-unc-40* homolog, but to date no *Pristionchus unc-5* gene has been identified. To begin characterization of the Netrin pathway in *P. pacificus* we have isolated the *Ppa-unc-40* genomic locus and characterized the mRNA using RT-PCR. Not surprisingly, preliminary RNA in situ hybridization results suggests that *Ppa-unc-40* may localize to the DTCs. We are constructing transcriptional fusion plasmids for future expression studies. We hypothesize based upon previous work that *Ppa*-UNC-40 expression in the DTCs may be correlated with the phases of gonad arm extension.

767A. A *C. elegans* FerT Kinase Regulates Developmental Cell-Cell Fusion to Direct Cell Identity. **R. Mako Saito**, David Tobin, Sarah Roy. Genetics, Dartmouth Med Sch, Hanover, NH.

Cell-cell fusion is integral to the development of several tissues and lends itself well for genetic study (reviewed in Podbilewicz, Wormbook). Cell fusions play a prominent role in the development of the hypodermis. The majority of the hypodermis is contributed by hyp7, a large syncytial cell that arises from multiple fusion events in both embryonic and larval development. hyp7 is formed during embryogenesis by the fusion of 23 cells, and additional cells, including descendents of the V and P cell lineages, contribute during larval development. Here, we identified and characterized a gene promoting fusion, examined a role for cell-cell fusion in determining cell fate and are investigating additional developmental regulators of cell-cell fusion identified in a genome-wide RNAi screen. A genetic screen to identify cell cycle regulators isolated several mutant strains with extra VPCs. We determined that one strain was actually fusion defective and the extra VPCs were in fact posterior Pn.p cells that failed to fuse with hyp7 (Ref phenotype). This finding led us to ask if cell-cell fusion was the cause or effect of the vulva vs. hypodermal decision. Using 2 functional readouts for VPC identity, the production of pseudovulvae in response to activated *lin-12* or *let-60*, we determined that posterior Pn.p cells that fail to fuse with hyp7 because of *eff-1(lf)* assumed the vulva fate. These studies revealed cell-cell fusion as a novel mechanism for developmental signals to determine cell identity. The mutation disrupting cell fusions was recently found to alter an Fps/Fes kinase family member and experiments are ongoing to analyze its function. Lastly, we completed a genome-wide RNAi screen to identify genes that are necessary for the normal developmental fusion between the Pn.p and hyp7 cells. The screen revealed 78 genes and the majority have not been previously associated with cell-cell fusion. However since several genes, such as *nhr-25* and *egl-27*, were previously described to influence fusion, we are confident that these genes are enriched for components of a genetic network regulating Pn.p-hyp7 fusion. Our progress on the analyses of this newly identified regulatory organization will be presented.

768B. Differential regulation of HLH-2/E2A stability during gonadogenesis in *C. elegans* . **Maria Sallee**, Iva Greenwald. Dept. of Genetics and Development, Columbia University, New York, NY.

The bHLH transcription factor HLH-2 has distinct roles during hermaphrodite gonadogenesis. hlh-2 endows two “a” cells with the potential to adopt the Anchor Cell (AC) fate; is required for cell-cell interactions that specify one a as an AC and the other as a Ventral Uterine precursor cell (VU); and is required for the differentiated functions of the AC. The interaction between the a cells is mediated by LIN-12/Notch, and engages feedback mechanisms to ensure that LIN-12 accumulates in one cell and promotes VU fate while HLH-2 accumulates in the other and promotes AC fate.

Previous work revealed that hlh-2 is transcribed in both cells, but a post-transcriptional mechanism leads to downregulation in the presumptive VU (Karp and Greenwald, *G&D* 2003). We have now determined that this mechanism involves differential protein stability, such that HLH-2 is preferentially destabilized in the VU, and have performed a structure/function analysis to identify cis-acting determinants of this difference. Surprisingly, we have found that mutations that disrupt dimerization as well as dimers forced through tethering both lead to stability in the VU. In addition, we have identified a region in the carboxy terminus that is required for HLH-2 downregulation in the VU. Some of the mutant or dimeric forms also affect the AC/VU decision and/or AC function. The structure/function information leads to testable models for how LIN-12 activation leads to instability of HLH-2 in the VU. We are also testing candidate trans-acting factors that may influence HLH-2 stability.

HLH-2 is the ortholog of mammalian E2A, an important oncogene. There is striking conservation between HLH-2 and E2A in the regions we have identified as important for differential stability. We are currently testing whether E2A is differentially regulated during the AC/VU decision, potentially providing insight about conservation.

769C. Quantitative proteome analysis of maternal gene *spn-4* mutant in *Caenorhabditis elegans*. **Aimi Tomita**[1], Yukako Toshato[1], Toshiya Hayano[2], Masahiro Ito[1]. 1) Depart. of Bioinfo, College of Life Sci, Ritsumeikan Univ, Shiga 525-8577, Japan; 2) Biomed. Sci, College of Life Sci, Ritsumeikan Univ, Shiga 525-8577, Japan.

Maternal genes in *Caenorhabditis elegans* determine the polarity of the embryo before the 12-cell stage. The maternal gene *spn-4* encodes a protein containing an RNA-binding domain and is required for normal cytokinesis and spindle orientation in early embryos. SPN-4 binds the region in the 3'-UTR and forms a complex with POS-1. This complex is required for maternal *glp-1* mRNA translation in anterior blastomeres of early embryos. Furthermore, SPN-4 regulates the translation of other maternal genes. However, many molecular mechanisms of the maternal genes are still unknown. We therefore performed shotgun proteomic analyses with isobaric tags for relative and absolute quantitation (iTRAQ), to identify the group of genes whose translation is regulated by SPN-4. iTRAQ analysis was performed once for each of the 2 samples, corresponding to embryonic and adult stages, and1121 proteins were identified. The proteins were classified intoup-regulated and down-regulated expression levels of the proteins in *spn-4* mutant compared to that in wild-type. We focused on 61 proteins whose expressions were down-regulated at the embryo stage in the mutant. STRING and NEXTDB databases were used to investigate the functional clusters with protein-protein interactions and *in situ* hybridization patterns, respectively, among the proteins. We obtained 6 functional clusters by using STRING. Among them, RNA-binding protein CAR-1, which is required for early embryonic cytokinesis, and *spn-4* interacting protein Y18D10A.11 form a cluster. The other cluster includes kinetochore-binding proteins, KBP-3 and KBP-4, which are expressed in mitotic spindles. These 4 proteins are related to mitotic division. Furthermore, we confirmed mRNA expression of 61 genes in the gonad by using NEXTDB. The expression patterns of 37 genes indicated that these genes were maternal. There are 19 genes, including *car-1*, *kbp-3*, and *kbp-4*, with the embryonic lethal phenotypes on WORMBASE. Collectively, our results suggest that these 3 genes are important for cell-fate determination and that their translation is closely regulated by SPN-4.

770A. Role of *sox-2* in postembryonic lineage progression. **Berta Vidal Iglesias**[1,2], Oliver Hobert[1,2]. 1) Howard Hughes Medical Institute; 2) Biochemistry & Molecular Biophysics, Columbia University Medical Center, New York, NY.

sox-2 is a highly conserved transcription factor that is well known to be a stem cell master regulator and is one of the Yamanaka factors required for the generation of induced pluripotent stem cells. *sox-2* has also been extensively associated to neurogenesis and plays an important role in the maintenance of neural stem cells. Using a fosmid-based reporter gene we have seen that *sox-2* is expressed broadly during *C. elegans* embryogenesis, being expressed in several neuronal progenitors, although not exclusively neither in all of them. The *sox-2* null mutant is L1 lethal and has the pharynx unattached; however, quantification of a panneuronal reporter does not show any neuronal specification defects, indicating that *sox-2* is dispesable for *C. elegans* embryonic neurogenesis. Interestingly, at the L1 stage, *sox-2* is also expressed in several postembryonic blast cells, which have to keep dividing during larval development to give rise to different postembryonic lineages that generate neurons among other cell types. Being *sox-2* an important pluripotency factor, we hypothesized that it might be required in the L1 blast cells to retain their developmental potential. Using mosaic analysis strategies to overcome the L1 lethality of the *sox-2* null allele, we have so far identified defects in three different postembryonic lineages in the absence of *sox-2*: the V5 lineage, which originates the postdeirid, the K lineage that gives rise to the DVB neuron and the B lineage, which generates the spicules in males. These results support the idea that postembryonic blast cell competence is compromised in the absence of *sox-2*. The extent of *sox-2* functions in different postembryonic lineages and its mechanism of action to control lineage progression are currently being investigated.

771B. Centrosomal localization of SYS-1/beta-catenin is required for proper expression patterns during asymmetric cell division. **Setu M. Vora**, Bryan T. Phillips. Biology, University of Iowa, Iowa City, IA.

The Wnt/b-catenin asymmetry pathway is responsible for carrying out a number of different asymmetric cell divisions throughout C. elegans development. These divisions give rise to daughter cells with differential activity of Wnt signaling components. SYS-1/b-catenin is a principal transcriptional effector in this pathway and allows daughter cells from an asymmetric division to adopt distinct transcriptional profiles and according cellular fates. Before division, SYS-1 symmetrically localizes to the centrosomes and is asymmetrically expressed between daughter cells after division. Interestingly, vertebrate b-catenin also displays centrosomal localization during division, but it is unknown what role centrosomal localization plays in any b-catenin regulation. Through yeast two-hybrid screening, we have identified the centrosomal scaffold protein RSA-2 (regulator of spindle assembly) as a strong positive interacting partner of SYS-1. RNAi knockdown of RSA-2 results in decreased centrosomal targeting of SYS-1. We are using rsa-2 (RNAi) to examine the fate of centrosomally uncoupled SYS-1 during asymmetric divisions. Our research shows that in rsa-2 (RNAi), SYS-1 relocalizes near kinetochore microtubules during division, suggesting a trafficking mechanism for targeting of nuclear SYS-1 to the centrosomes. Furthermore, uncoupling SYS-1 from the centrosome promotes Wnt-signaled cell fate and disrupts patterns of SYS-1 asymmetry after division. Taken together, these results suggest that SYS-1 localization to the centrosome results in SYS-1 clearance from the mother cell before cytokinesis to allow faithful regulation of de novo SYS-1 in the daughter cells. These analyses will allow us to understand dynamic expression patterns of SYS-1 during asymmetric division and explore how subcellular localization relates to regulation of cell fate determinants during development.

772C. ceh-36 regulates cell fate patterning during embryogenesis. **Travis Walton**, John Murray. Genetics, University of Pennsylvania, Philadelphia, PA.

We previously used lineage tracing analysis to define the expression patterns of transcriptional and translational reporters of over 100 transcription factors (TFs) in embryos through the 350-cell stage. One finding of this work was the unexpected prevalence of "lineally repetitive" TF expression patterns in multiple lineages not related by terminal fate. This suggests that these TFs may mediate the conversion of lineage identity into patterns of cell fate. We have focused on one lineally repetitive TF, the Otx homeodomain factor ceh-36, which is expressed in several lineages that generate 251 cells with diverse cell fates. Although there are three Otx TFs that are functionaly redundant in some cell types, previous genetic studies demonstrated roles for ceh-36 distinct from its paralogs ttx-1 and ceh-37. Therefore, we investigated the requirement of ceh-36 as a regulator of lineage identity during embryogenesis. We tested for defects in cell cycle events and cell position at single-cell resolution by 4D microscopy and lineage tracing of ceh-36(-) mutant embryos through comma stage and comparison with our quantitative model of wildtype embryogenesis. We analyzed two putative null alleles, ceh-36(ok795) or ceh-36(ky646), which are both characterized by partially penetrant larval lethality. Quantitative analysis identified at least 29 cells with defective lineage patterns or positions, which included failed cell death and cell migrations to inappropriate left/right bilateral position. Most defects were partially penetrant, possibly due to redundancy with other regulators. Cells identified by our quantitative analysis have been validated using cell-type specific reporters and indicate defective cell-fate specification. Our work demonstrates that ceh-36 plays a broad role specifying diverse cell-types whose common feature is shared lineage ancestry. Our analysis likely underestimates the number of cells that require ceh-36 function because it did not identify other cells whose identity, but not position or lineage pattern, are known to be defective in ceh-36(-).

773A. Wnts and VANG-1/Van Gogh control cell fates in the Q lineage. **Falina J Williams**, Jerome Teuliere, Gian Garriga. MCB, UC Berkeley, Berkeley, CA.

Asymmetric cell division (ACD) is a process that generates cell diversity. Both intrinsic and extrinsic mechanisms can distribute developmental potential asymmetrically to generate daughter cells of different fates and position the cleavage furrow asymmetrically to generate cells of different sizes. Wnts are evolutionarily conserved secreted glycoproteins that are utilized throughout development and play a role in ACD.1 During C. elegans development, Wnts regulate asymmetric divisions by controlling the distributions of the b-catenin SYS-1 and the LEF/TCF POP-1.2 They can also regulate the orientation of the spindle.3 In other organisms, Wnts can also regulate cytoskeletal dynamics via the Planar Cell Polarity (PCP) pathway, which allows polarization of neighboring cells along an axis orthogonal to the apical-basal axis within an epithelial sheet.4 The ACDs of the Q.a and Q.p neuroblasts to give rise to a larger daughter that lives and a smaller cell destined to die. We find that two Frizzled homologs LIN-17 and MOM-5 together are necessary for both apoptotic fates, for the asymmetric distribution of POP-1 in the Q.a and Q.p daughter cells, and for the asymmetric position of the Q.a and Q.p furrows that produce daughter cells of different sizes. Reduction of Wnt signaling, however, fails to generate the same robust disturbance of POP-1 distribution and does not affect the furrow localization. Instead, reduction of Wnt signaling can result in a reversal of POP-1 asymmetry, a phenotype that is enhanced by loss of the Van Gogh homolog VANG-1, a component of the PCP pathway. 1.Munro and Bowerman. CSH Perspectives. 2009. 2.Mizumoto and Sawa. Trends in Cell Biology. 2007. 3.Walston and Hardin. Seminars in Cell and Developmental Biology. 2006. 4.Segalen and Bellaiche. Seminars in Cell and Developmental Biology. 2009.

774B. Glucose 6-phosphate dehydrogenase (G6PD) deficiency impairs early embryogenesis in C. elegans. **Hung-Chi Yang**[1], Meng-Hsin Ou[1], Szecheng J. Lo[2], Daniel Tsun-Yee Chiu[1]. 1) Department of Medical Biotechnology and Laboratory Sciences, Chang Gung University, Taoyuan, Taiwan; 2) Department of Biomedical Sciences, Chang Gung University, Taoyuan, Taiwan.

Human glucose 6-phosphate dehydrogenase (G6PD) deficiency, also called favism, is manifested mainly by hemolytic anemia. In a mouse model, severe G6PD deficiency causes embryonic lethality. Using the established G6PD-knockdown Caenorhabditis elegans (C. elegans) animal model (Yang et al. Cell Death & Disease, 2013 in press), we seek to go beyond cellular physiology and delineate the role of G6PD in embryonic development. Under DIC microscopy, embryos derived from G6PD-knockdown C. elegans (refers to G6PD-knockdown embryos) exhibited drastic morphological alterations upon challenge with osmotic stress. Such embryos also demonstrated differential permeability to small molecule dyes. Since G6PD knockdown causes severe hatching defect, we tested the hypothesis that impaired early embryogenesis caused by G6PD deficiency contributes to defective hatching. Compared with mock embryos, a portion (10%) of G6PD-knockdown embryos showed symmetric division at two-cell stage. More importantly, compared with the hallmark events of early embryogenesis in mock embryos, G6PD-knockdown embryos (one-cell to four-cell stage) exhibited differential embryonic defects. The mild defects (type 1) included absence of perivitelline space, failure of forming cortical ruffling and pseudocleavage, the maternal and paternal pronuclei fused centrally, polarity defect in first division and synchronous cytokinesis in second division. In addition to these morphological defects, type 1 embryos required a longer time course during early embryonic development (average 42 minutes from one-cell to four-cell stage) compared to mock embryos (average 29 minutes). The severe defects (type 2) included the maternal and paternal pronuclei which fused slower than that of mock embryos and arrested at one-cell stage for more than 40 minutes as compared to mock embryos (average 17 minutes from one-cell to two-cell stage). Taken together, these findings suggest that G6PD deficiency impairs early embryogenesis in C. elegans.

775C. Single-blastomere transcriptome profiling after the first embryonic division. Erin Osborne Nishimura[1,2], **Jay C. Zhang**[1,3], Adam Werts[3], Bob Goldstein[3], Jason D. Lieb[1,3]. 1) Carolina Center for Genome Sciences; 2) Lineberger Comprehensive Cancer Center; 3) Department of Biology, UNC Chapel Hill, NC.

We are interested in understanding how reproducible patterns of RNA inheritance and abundance arise in dividing cells, and for what purpose. The *C. elegans* early embryo represents a unique biological system to study this problem because the lack of zygotic transcription removes the potential complication of transcriptional fluctuations. Following fertilization and prior to the onset of zygotic transcription, the *C. elegans* zygote cleaves asymmetrically to create the anterior AB and posterior P1 blastomeres. To identify asymmetrically distributed transcripts, we dissected the AB and P1 blastomeres and performed low-input RNA-seq. Our results are consistent with a recently published study with a similar experimental design (Hashimshony et al, 2012 Cell Reports). We developed a scoring method for ranking transcripts, verified asymmetric patterns using qRT-PCR and *in situ* hybridization, and narrowed our analysis to a subset of transcripts that represent the most asymmetric RNAs in each cell at the 2-cell stage.

AB and P1 are the founders of two lineages with distinct fates. We found that transcripts enriched in AB tend to encode proteins that function in mitosis and epithelium development, whereas transcripts segregated to the P1 are associated with translational regulation. To test whether any of the genes in

our AB-enriched or P1-enriched subsets play a functional role in asymmetric cell division or downstream embryonic patterning, we performed controlled RNAi phenotyping and video microscopy to categorize embryological defects. Using these methods, we identified *F32D1.6*, an AB-enriched (anterior) transcript with a role in anterior-specific morphological events. In addition, two posterior enriched transcripts, *T04A8.7* and *puf-3*, were found to exhibit noteworthy early embryological defects. We are currently identifying sequence elements and RNA binding proteins that are required for this process with the goal of elucidating the mechanisms behind asymmetric RNA partitioning.

776A. Genome-wide landscape of hybrid incompatibility (HI) between *Caenorhabditis briggsae* and *C.* sp.9 . **Zhongying Zhao**, Cheung Yan, Yu Bi, Xiaoliang Ren, Dongying Xie. Department of Biology, Hong Kong Baptist University, Hong Kong.

HI serves as reproductive barrier limiting gene flow between different species. Characterization of HI remains the key to understanding of speciation. Inter-species HI loci have been isolated primarily between model organisms and its related species. However, *C. elegans* has been prevented from isolation of inter-species HI loci due to lack of a sister species with which it can mate and produce viable progeny. To circumvent the limitation, we set to isolate HI loci between *C. briggsae* and its sister species, *C.* sp. 9 which has recently been isolated. We first generated 80 stable transgenic strains expressing GFP markers in *C. briggsae*. We next mapped the GFP integration sites through repeated back-crossings/introgression into *C.* sp.9 followed by PCR genotyping. The markers demonstrate roughly even distribution over *C. briggsae* genome. We then rendered the introgression fragment homozygous if possible in an otherwise *C.* sp.9 background. We were able to produce a total of nine viable lines carrying independent homozygous introgression fragments with four on autosome and five on X chromosome. The remaining introgression fragments seem inviable as homozygote. The sizes of introgression fragment range from 0.5 Mb to as big as 10 Mb. We characterized the HI for all the homozygous introgressions as well as for 17 other heterozygous introgressions, representing nearly 80% of *C. briggsae* genome. Surprisingly, most of the homozygous introgressions on autosome dramatically reduce viabilities while those on X chromosome demonstrate modest incompatibilities, inconsistent with large X theory. Strikingly, none of the homozygous introgression lines significantly affects male sterility or viability though the Haldane's rule is widely obeyed during the initial introgression. Nevertheless, we did observe that introgressions leading to male sterilities are mainly located in the middle of X chromosome and mapped male viability loci onto an approximately 1Mb region of X chromosome. In addition, we found that the HI loci are largely recessive. Our work build a framework that enables nematode species to be used as a model for investigation of the mechanisms of inter-species HI that has so far not been successful.

777B. Perturbation of NAD+ salvage biosynthesis causes a distinct death program in a neuroendocrine cell. **Awani Awani**, Matt Crook, Wendy Hanna-Rose. Genetics, Pennsylvania State University, State College, PA.

While all cells require NAD+ for general cellular metabolism, perturbations in biosynthesis of NAD+ and/ or the levels of its metabolites can have surprisingly tissue-specific consequences in physiology and development.A nicotinamidase encoded by the pnc-1 gene converts nicotinamide (NAM) to nicotinic acid (NA) in the first step of the invertebrate salvage pathway for biosynthesis of NAD+. In pnc-1 mutants, the uterine-vulva 1 (uv1) cells die with typical necrotic morphology in response to excess NAM, a by-product of NAD+ consumption by NAD+ consumer enzymes that is typically cleared by PNC-1 activity. The uv1 cells are neuroendocrine cells that are thought to promote egg laying in response to swelling of the uterus upon accumulation of eggs. We have used genetic and pharmacological approaches to test the role of known pathways in the uv1 death program. In contrast to the well-studied degenerin/ mec-4d-induced touch cell necrosis program, uv1 cell death is not blocked by genetic or pharmacological disruption of ER Ca2+ storage or release. However, knockdown of a calpain (CLP-1) can ameliorate the penetrance of the death phenotype. These results indicate that ER Ca2+ signaling is not involved in initiating the death program, but Ca2+ is likely involved downstream during execution of the death program. Because bafilomycin A1 and knockdown of aspartyl protease ASP-4 partially rescue uv1 death, acidification of the cytoplasm and lysosomal proteases are likely key components of the death execution machinery.We have also used genetic and metabolic approaches to identify novel regulators of cell death in this system. While we found no role for ER Ca2+ signaling in uv1 necrosis, glutamate signaling is critical for uv1 cell death. Knockdown of glutamate receptors GLR-3 or GLR-6 or a protein involved in trafficking of glutamate (EAT-4) can partially rescue uv1 cell death. uv1 cell death also strictly requires the TRP channel OCR-4. We have shown that NAD+ salvage biosynthesis perturbation is an insult capable of initiating necrosis and that multiple pathways, both characterized and unknown, converge and diverge downstream of necrosis initiating insults in the execution of this cell death program.

778C. A *C. elegans* model for TDP-43-induced motor neuron pathology. **J. C. Chaplin**, M. Mangelsdorf, R. Narayanan, R. Wallace, M. A. Hilliard. Queensland Brain Institute, Brisbane, Queensland, Australia.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder that causes progressive muscle weakness and wasting, and is characterized by the presence of ubiquitinated protein inclusions in the motor neurons of patients. A major component of these inclusions is the RNA binding protein TDP-43, and over forty different mutations in the gene encoding TDP-43 have been reported in ALS patients. Despite this, the function of TDP-43 in the nervous system remains unknown, and its role in the pathogenesis of ALS is unclear. We have generated a *C. elegans* model for TDP-43 pathology by individually expressing human wild-type TDP-43 or TDP-43 carrying ALS-causing mutations (A315T and M337V), specifically in the GABAergic motor neurons. The effect of the expression of these molecules was investigated by analysing the morphology of the neurons, as well as their neuromuscular synapses. We found that while the wild type form of TDP-43 did not induce significant changes in neuronal morphology or structural maintenance, the expression of either mutated allele of TDP-43 produced clear axonal degeneration defects. These were evident as gaps in both the dorsal and ventral nerve chords, as well as on circumferential commissures of the animal. The presynaptic loci of these neurons, visualized using a tagged vesicle associated protein, RAB-3, were disrupted upon expression of mutant alleles of TDP-43, while remained largely intact in animals expressing wild type protein. Both these phenomena were adult onset and progressive in nature. To investigate TDP-43 localization in *C. elegans* we generated transgenic strains in which TDP-43 is tagged with GFP and expressed in the same GABAergic motor neurons. We observed for both wild type and mutant alleles that TDP-43 is localized predominantly in the nucleus with some expression in the cytoplasm and in puncta consistent with synaptic loci. This resembles the subcellular localization observed in mice, with TDP-43 shuttled between the nucleus and the synapses. We are now using a candidate gene approach and forward genetic screening to identify new molecules able to rescue or enhance the observed degeneration defect, and/or alter the TDP-43 subcellular localization.

779A. Elucidating the mechanism by which C.elegans KRI-1 regulates damaged-induced germline apoptosis. **Eric M. Chapman**[1,2], W. Brent Derry[1,2]. 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada.

kri-1 is necessary for promoting DNA damaged-induced apoptosis in the germline by a cell non-autonomous mechanism that acts in parallel to the p53-like gene cep-1 (Ito et al., 2010). kri-1 is the homologue of human KRIT1, which when mutated leads to the neurovascular disease in humans Cerebral Cavernous Malformations. The C.elegans ERK homologue, MPK-1, is phosphorylated and activated in the germline upon irradiation, and this activation is necessary for apoptosis. To determine if kri-1 is involved in MAPK regulation I ablated gla-3, an inhibitor of MPK-1, by RNAi, and found that apoptosis was restored. To determine if kri-1 is required to modulate the MAPK pathway, I immunostained germlines for activated MPK-1 and found reduced levels in kri-1(0) worms compared to wild type. Since KRI-1 is expressed in the soma, this suggests that kri-1 is somehow able to activate MAPK signalling in the germline. Currently, I am exploring various transport mechanisms, such as the vitellogenesis pathway, to determine how KRI-1 signals from the soma to germline. In order to identify downstream targets of kri-1 in the apoptosis signaling pathway, I plan to use an unbiased mutagenesis suppressor screen.

780B. Using *Caenorhabditis elegans* to Fight Human Neurodegenerative Diseases. **Xi Chen**[1], Brian C Kraemer[2], Jeff Barclay[1], Robert D Burgoyne[1], Alan Morgan[1]. 1) Department of Cellular and Molecular Physiology, Physiological Laboratory, Institute of Translational Medicine, University of Liverpool, Crown St, Liverpool, L69 3BX, United Kingdom; 2) Geriatric Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108, USA.

Age-associated neurodegenerative disorders (NDs) remain a significant and unresolved financial and social burden facing ageing populations. Therapies for these devastating and eventually fatal disorders are currently lacking as there are only palliative treatments available. The use of the nematode *Caenorhabditis elegans (C. elegans)* as a primary platform to probe the poorly defined disease mechanisms of several major NDs and a more rapid means towards novel gene and drug discovery has recently escalated. A diverse set of informative *C. elegans* ND models have been developed manifesting abnormal phenotypes that partially recapitulate the salient cellular, molecular and pathological aspects of several distinct human NDs processes. Recent studies have identified *dnajc5* encoding cysteine-string protein a (CSPa) as the disease-causing gene of a rare autosomal dominant human ND known as adult-onset neuronal ceroid lipofuscinosis (ANCL). The null animal models of CSP are characterised by impaired neurotransmission, pre-synaptic neurodegeneration and premature mortality. Simple model organisms may therefore shed light on potential therapeutic approaches for ANCL and other NDs. In this study, we are integrating multiple well-defined *C. elegans* ND models to uncover potential therapeutic interventions that target shared pathogenic pathways. Using locomotion behaviour and lifespan as read-outs, a pilot screen for chemical suppressors of neurodegeneration-induced phenotypic dysfunctions uncovered one promising candidate drug that not only rescued the short lifespan of a *C. elegans* null mutant model of ANCL, but also ameliorated the mobility defect and short lifespan of a distinct worm tauopathy model based on transgenic expression of mutant human tau. These findings should encourage further screening and characterisation of other neuroprotective compounds, and ultimately may assist in accelerating the clinical evaluation and development of drugs for the treatment of NDs.

781C. C. elegans peroxidasin, pxn-1 is essential for epidermal attachment of muscles and neurons. **Jeong H. Cho**, Juyeon Lee. Dept of Biology Education, Chosun University, Gwangju, South Korea.

Peroxidasins are heme-containing extracellular peroxidases, with highly conserved structure, and serving diverse functions in plants and animals. Peroxidases catalyze oxidation of various substrates (proteins and lipids) and tyrosine cross-linking of extracellular matrix (ECM). Caenorhabditis elegans have two peroxidasin homologues (pxn-1 and pxn-2). According to a recent study, PXN-2 is necessary during muscle-epidermal attachment and embryonic morphogenesis. Here we show the C. elegans pxn-1 expression, localization and function. pxn-1 is expressed in motor neurons, hypodermis, and reproductive muscles. PXN-1 translational fusions (PXN-1::GFP) localize to the ECM of neuron and vulval muscles. pxn-1 deletion mutants however do not display defects in normal conditions (at 20oC). However, under temperature stress at 25oC, the pxn-1 mutants display epidermal detachment. Interestingly, pxn-1 mutant neuron is misguided and PXN-1 overexpression may cause axonal outgrowths, branching while missing their destination. Taken together, these results suggest that a putative extracellular peroxidase, C. elegans Pxn-1, has an essential role in epidermal attachment of muscles and neurons.

782A. Analysis of the Function and Dysfunction of the Human Amyotrophic Lateral Sclerosis Gene *C9ORF72* Using *C. elegans*. **Anna Corrionero**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

An expansion of a GGGGCC hexanucleotide repeat in an intronic region of the human gene *C9ORF72* is the most common known genetic cause of familial amyotrophic lateral sclerosis (ALS). Little is known about the normal function of *C9ORF72*, or, importantly, about how the repeat expansion causes ALS as well as frontotemporal dementia (FTD). Given the current understanding of other neuromuscular disorders caused by expansions of nucleotide repeats, three mechanisms have been proposed for the pathogenic effects of the *C9ORF72* hexanucleotide expansion: (1) loss of wild-type *C9ORF72* gene function, (2) altered function of the repeat-containing RNA, and (3) altered function caused by a polypeptide containing dipeptide repeats encoded by the hexanucleotide repeat.The *C. elegans* genome contains an uncharacterized gene homologous to *C9ORF72*, *F18A1.6*. We are analyzing the normal expression pattern and determining the biological function and molecular genetic pathway of action of F18A1.6. Using a translational reporter, we have determined that F18A1.6 is expressed from early in embryogenesis to adulthood in epithelial, muscle, hypodermal and intestinal cells and mainly localizes in the cytoplasm. Preliminary observations of mutants carrying different *F18A1.6* alleles suggest that *F18A1.6* loss-of-function mutants are altered in locomotion and lifespan. We are also trying to identify phenotypic abnormalities caused by the expression in neurons of the expanded *C9ORF72* GGGGCC repeats. Animals expressing a pathogenic but not a wild-type number of repeats show locomotion defects. We will design genetic screens to identify genes that mediate or mitigate the behavioral and cellular defects of *F18A1.6* mutations or of GGGGCC repeat expansions. These screens could identify molecular genetic pathways of action of normal and abnormal *C9ORF72* and thereby define new potential therapeutic targets for ALS and FTD.Our goal is to help elucidate how the most common ALS-causing mutation, a non-coding hexanucleotide repeat expansion in a conserved gene of unknown function, exerts its pathogenic effects. More generally, we hope to contribute to the understanding of how repeat-expansion mutations can lead to neuromuscular

disorders.

783B. Blocking NAD+ salvage biosynthesis sensitizes specific mechanosensory neurons to nutritional conditions and predisposes them to death. **Matt Crook**, Wendy Hanna-Rose. Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA.

While all cells require NAD+ for general metabolism, perturbations in biosynthesis of NAD+ can have surprisingly tissue-specific consequences in physiology and development. We use C. elegans to study the role of NAD+ biosynthetic pathways in cell survival. PNC-1 converts nicotinamide (NAM) to nicotinic acid (NA) in the first step of the invertebrate NAD+ salvage pathway. In pnc-1 mutants, the OLQ neurons, mechanosensory cells involved in head withdrawal and foraging, are sensitized to nutritional conditions and prone to death. OLQ cells die at a low penetrance when grown under normal culture conditions, but OLQ death increases significantly on UV-killed E. coli. Neither accumulation of the PNC-1 substrate NAM nor the lack of PNC-1 product NA is sufficient to cause OLQ death, which suggests that both low NAM and normal NA levels are required for survival. OLQ cells die with a stereotypical progression; the dendrites first bleb, then the cytoplasm swells dramatically in a manner characteristic of a necrotic cell, followed by dendrite and cell body clearance. We sought to compare OLQ death to the well-studied degenerin/ mec-4d-induced touch cell necrosis model. We found that loss of calreticulin function, which suppresses degenerin-induced death, significantly increased OLQ death. Two unc-51/Atg1 alleles, which also suppress degnerin-induced death by blocking autophagy, also increased OLQ death. UNC-51 regulates both autophagy and axon guidance. Knockdown of two other autophagy genes, bec-1 and lgg-1, had no effect. However, mutation of unc-14, an unc-51 axon guidance partner, also showed increased OLQ death, suggesting that correct axon guidance may be essential for OLQ survival. Finally, we found that asp-4(lf) allele, a key effector of necrosis, did not affect OLQ death. These results suggest that OLQ death in pnc-1 animals does not follow a canonical necrosis pathway, yet it is not an apoptotic event, as OLQ death increased in ced-4; pnc-1 double mutants. OLQ death offers a model to probe a novel death pathway in a system with powerful genetic and metabolomic tools at hand.

784C. EGF and phosphocholine; a novel mechanism to prevent necrosis. **Matt Crook**, Wendy Hanna-Rose. Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA.

Necrosis is not a chaotic uncontrolled reaction to cellular trauma, but a finely controlled cell death program. We have developed a model for analysis of genetic control of necrosis. In our model, nicotinamide induced necrosis of the uterine-vulval uv1 cells in pnc-1 animals is robustly rescued by overactivation of EGF signaling. LET-23, an EGF receptor, has many functions in C. elegans development, and two signaling pathway are known to mediate LET-23 signaling effects; one that signals through LET-60/ Ras and a second through ITR-1. LET-60 signaling downstream of LET-23 is necessary but not sufficient for rescue of necrosis and ITR-1 is neither necessary nor sufficient. These results suggest that an as yet unidentified pathway downstream of LET-23 mediates rescue in concert with the LET-60 pathway. We carried out a targeted RNAi screen and have discovered two genes, pmt-1 and cdk-2, that are required for let-23gf rescue of uv1 necrosis. PMT-1 is a phosphoethanolamine methyltransferase that catalyses the first step of the conversion of ethanolamine to phosphocholine, followed by conversion to phosphatidylcholine. To confirm the relevance of this pathway we knocked down pmt-2, downstream of pmt-1, and supplemented pmt-1 and pmt-2 RNAi animals with choline, an alternative substrate for phosphocholine synthesis. pmt-2 RNAi phenocopied pmt-1 RNAi and choline supplementation restored rescue . However, choline supplementation in a pnc-1 background did not rescue uv1 necrosis, which confirms that phosphocholine synthesis via the PMT pathway is required but not sufficient for rescue. We hypothesised that the PMT pathway is required for let-23gf uv1 rescue to maintain LET-23 cell membrane localization and activity. To test this we investigated the effect of pmt-1 and pmt-2 RNAi on two let-23gf phenotypes; excess vulval induction and excess uv1 cell specification. We found that neither pmt-1 nor pmt-2 RNAi affected either of these phenotypes, which suggests an alternative hypothesis; that phosphocholine itself is required for a LET-23 survival signal. This was supported by the failure of cept-1 or Y49A3A.1 RNAi, both genes required for phosphatidylcholine synthesis, to affect rescue.

785A. BRAP-2 is necessary for the regulation of DNA damage induced germ line apoptosis in *C. elegans*. **Dayana R. D'Amora**, Terrance J. Kubiseski. Department of Biology, York University, Toronto, Ontario, Canada.

Genotoxic stress caused by prolonged exposure to ionizing radiation (IR) and endogenous metabolic by-products such as reactive oxygen species, can cause diverse types of damage, from point mutations to double-strand breaks (DSBs). The DNA damage response (DDR), comprised of cell cycle arrest, DNA repair, and programmed cell death (apoptosis), protects genomic integrity from mutations that can lead to cell cycle dysfunction and cancer. BRCA1 (Breast cancer susceptibility gene 1) is a tumour suppressor with several functions in the DDR including DNA repair, transcriptional regulation, and ubiquitination. The role of the BRCA1 ortholog BRC-1, in DNA repair is conserved in *C. elegans*, forming an E3 ubiquitin ligase with its binding partner BRD-1, that localizes to DSBs. Previous studies have shown that following exposure to IR, the loss of either *brc-1* or *brd-1* results in persistent DNA damage causing high levels of germ cell death. However, the mechanisms of BRCA1 action in apoptosis, potential interactions and substrates, have yet to be determined. A novel protein known as BRAP2 (BRCA1 associated binding protein 2) has been characterized as a Ras effector protein, an E3 ubiquitin ligase, and as a cytoplasmic retention protein that can bind to the nuclear localization signal of BRCA1. We previously found that a deletion mutant of *brap-2* (EEED8.16) was highly sensitive to oxidizing conditions and demonstrated L1 larval arrest at low concentrations of paraquat that was dependent on *brc-1*. To investigate the role of *brap-2* in DNA damage induced apoptosis, we quantified the levels of germ cell death in *brc-1;brap-2* mutants following IR. A significant reduction in germ line apoptosis was apparent, with no defects in the proliferation of mitotic nuclei. Following IR, elevated *skn-1* (Nrf2 homolog) and reduced pro-apoptotic *ced-13* (BH3-only) mRNA expression levels were observed in this double mutant strain, indicating that SKN-1 may mediate *brap-2* function. Furthermore, *brap-2* mutants experience increased radiation sensitivity in both meiotic pachytene and mitotic nuclei. Our findings reveal that functional BRAP-2 is necessary for the BRC-1 mediated activation of DNA damage induced repair and germ line apoptosis.

786B. The Possible Role of Autophagic Cell Death in the Regulation of Excitotoxicity in *C. elegans*. **John S Del Rosario**, Towfiq Ahmed, JunHyung An, Tauhid Mahmud, Itzhak Mano. Physiol, Pharm & Neurosci, Sophie Davis Biomed Sch, City College, City University of New York, New York, NY.

Stroke is the third leading cause of death in the United States. This neurodegenerative condition is caused by a lack of blood supply to areas of the brain. The lack of oxygen and glucose generates a neurodegenerative cascade in a process called brain ischemia. Normally, the excitatory neurotransmitter

glutamate (Glu) is cleared from the synaptic cleft by Glu Transporters (GluTs). However, the malfunction of GluTs leads to a buildup of Glu in the synapses, over-stimulating the Glu receptors (GluRs) on the post-synaptic cells. This overstimulation causes the degeneration of the post-synaptic neurons in a process called excitotoxicity. The molecular mechanisms that lead to neurodegeneration by excitotoxicity remain unclear. We developed a model of excitotoxicity in the nematode *Caenorhabditis elegans* by knocking-out the GluT gene *glt-3* in a sensitive background. Recent reports link autophagy with a numbers of related forms of neurodegeneration, but the extent of the involvement of autophagy in excitotoxic neurodegeneration is still poorly understood. We now examine the possible role of autophagy in excitotoxic neurodegeneration and elaborate on its mechanisms by taking two approaches: 1) we are observing if autophagy takes place, by monitoring it with an autophagy fluorescent flag (using the LGG-1 autophagy protein); 2) we are testing whether autophagy is *required* for excitotoxic neurodegeneration by blocking two master-regulators of the autophagy pathway, *unc-51* and *atg-16.2*. Preliminary results suggest that the classic trigger of autophagy *unc-51* partially modulates excitotoxicity. In addition, LGG-1 expression confirms a moderate activation of autophagy in nematode excitotoxicity. This suggests that autophagy is not completely required, but might accompany excitotoxic neurodegeneration. Understanding the molecular mechanisms that regulate excitotoxicity in *C. elegans* might help us to suggest new strategies to mitigate stroke damage in humans.

787C. Investigation of SMN1 structure-function relationship in neuronal degeneration. **A. Donato**[1], I. Gallotta[1], G. Battaglia[2], M. A. Hilliard[3], P. Bazzicalupo[1], E. Di Schiavi[1]. 1) Institute of Genetics and Biophysics, CNR, Naples, Italy; 2) Neurological Institute C. Besta, Milan, Italy; 3) The University of Queensland, QBI, Brisbane, Australia.

Spinal muscular atrophy (SMA) is an autosomal recessive disease characterized by specific degeneneration of lower spinal cord motor neurons and caused by mutations in the *Smn1* gene (Survival of MotoNeurons 1). The SMN protein is an RNA binding protein mainly involved in the assembly of snRNPs, which are crucial for pre-mRNA splicing. This function is exerted through the interaction of the SMN Tudor domain with Sm proteins. Despite these well-known interactions, it is still unclear which domains of SMN are important to prevent neuronal degeneration. Indeed, other SMN protein domains can play key roles in neuron specific processes, such as in neurite outgrowth (Bergeijk *et al.*, 2007). Moreover an isoform of SMN, comprising only the N-terminal region (a-hSMN), is preferentially found in motor neuron axons but its role in SMA is not clear (Setola *et al.*, 2007). In this study we aim at investigating the role of the human protein (hSMN) *in vivo* and to define which protein region is important to prevent neurodegeneration. We took advantage of a *C. elegans* SMA model, obtained by knocking-down *smn-1* in GABA motor neurons by cell-specific RNAi. In this experimental model we observed an age-dependent GABAergic motor neurons specific degeneration that is rescued by pan-neuronal expression of the full length hSMN and not by a-hSMN, suggesting that the N-terminal region alone is not sufficient to rescue the *smn-1* knock-down effects. We next confirmed this result by expressing fl-hSMN in the *smn-1* null mutant *ok355* (Briese *et al.*, 2009) where it successfully extended the lifespan and rescued the impaired locomotion. This analysis confirms the functional conservation between the human and nematode proteins and establishes *C. elegans* as a powerful animal in which to investigate SMN function *in vivo*. We are now further characterizing the structure-function relationship of hSMN protein focusing on the role of other domains, such as the C-terminus, which is known to exert a role in inhibiting the onset of apoptosis (Vyas *et al.*, 2002), and of point mutations found in SMA patients.

788A. *ced-11* is Required for the Morphological Appearance of Apoptotic-Cell Corpses. **Kaitlin B. Driscoll**[1], Gillian Stanfield[2], Bob Horvitz[1]. 1) Dept. Biology, MIT, Cambridge, MA; 2) Dept. Human Genetics, University of Utah, Salt Lake City, UT.

Programmed cell death is a fundamental process that is required for proper development and tissue homeostasis in many organisms. Genetic analyses of programmed cell death in *C. elegans* led to the discovery of an evolutionarily conserved genetic pathway that regulates the activation of apoptosis. A cell dying by apoptosis undergoes a series of morphological changes that results in the appearance of round refractile cell corpses, as visualized by Nomarski optics. *ced-11* was identified in a screen in our laboratory for mutations that alter the morphological appearance of cell corpses in *C. elegans*. The corpses of *ced-11* mutant embryos are non-refractile as visualized by Nomarski optics.

We have found that while mutations in *ced-11* do not cause a cell-death defect, they can enhance the cell-death defect of weak alleles of other cell-death mutants in the ventral cord. This observation indicates that *ced-11* plays a role in the cell death process. *ced-11* acts downstream of the CED-3 caspase and appears not to have an effect on engulfment. *ced-11* encodes a protein with similarity to members of the TRP family of non-selective cation channels. As TRP channels are often permeable to calcium, we tested if *ced-11* regulates calcium during apoptosis. We used GCaMP3, a genetically-encoded calcium indicator, to monitor calcium in dying cells. In wild-type embryos refractile corpses that express GCaMP3 have bright fluorescence throughout the corpses. In *ced-11* corpses that express GCaMP3 there is a reduction of fluorescence in the nucleus, suggesting that *ced-11* might act as a calcium-permeable channel to regulate the entry of calcium into the nucleus of cells undergoing apoptosis. Alternatively, *ced-11* might regulate the breakdown/integrity of the nuclear envelope and thus allow calcium into the nucleus of apoptotic corpses. We plan to determine how *ced-11* affects the entry of calcium into the nucleus of apoptotic corpses. Better understanding of the role *ced-11* in apoptosis might help elucidate the role of calcium downstream of caspase activation and the mechanism of nuclear degradation in apoptotic cell death.

789B. Diapause protects neurons from degeneration and promotes axonal regrowth. **Andres Fuentes**, Andrea Calixto. Universidad Mayor, Santiago, Chile.

The downregulation of the insulin pathway during the dauer state protects mechanosensory neurons from degeneration triggered by hyperactivated degenerins (*mec-4d* and *deg-1*). We proposed that this protection was due to metabolic changes such as caloric restriction and enhanced expression of antioxidants (Calixto et al., 2012). We found that at the time of dauer entry 36 to 48 hours after food deprivation, many *mec-4d* expressing touch receptor neurons (TRNs) had already degenerated. However, one week after dauer entry the TRNs appear functionally and morphologically intact. This raised the question whether the neuronal protection observed in dauers and mutants of the insulin pathway were due to the regeneration of neurons undergoing degeneration. We found that degenerating TRNs in a *daf-2* mutant background showed growth cones and reinforcement of the neuronal processes. To test whether diapause formation and DAF-2 downregulation promoted neuronal regeneration of somas and axons, we scored the integrity of *mec-4d* expressing AVM TRN at dauer entry and thereafter every 24 hours to assess the regrowth of processes. We found that the dauer state not only halts the degeneration process but also causes regeneration of the TRNs expressing MEC-4d. To test whether *daf-2* downregulation induced regeneration of the

touch receptor neurons we performed combinatorial *daf-2(RNAi)* with genes that are required for regeneration in mechanosensory neurons (Yan et al., 2009). *dlk-1(RNAi)* reduced the *daf-2(RNAi)* induced protection from degeneration while *dlk-1(RNAi)* alone was no different from controls. Furthermore, reduction of *efa-6*, known to prevent regeneration (Chen et al., 2011), protects from the *mec-4d* induced degeneration and enhances the protection provided by *daf-2(RNAi)*. These results suggest that repression of genes that inhibit regrowth may override the deleterious effects of persistant prodegenerative stimuli and that dauer formation promotes regeneration of neurons.

790C. UNC-105 activation causes mitochondrial dysfunction and CED-4 dependent caspase-mediated protein degradation in terminally differentiated *C. elegans* muscle. **C. J. Gaffney**[1], F. Shephard[1], J. Chu[2], D. L. Baillie[2], A. Rose[3], D. Constantin-Teodosiu[1], P. L. Greenhaff[1], N. J. Szewczyk[1]. 1) MRC/ARUK Centre for Musculoskeletal Ageing Research, University of Nottingham, Nottingham, United Kingdom; 2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 3) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

UNC-105 is an ENaC/Degenerin family ion channel. A dominant gain-of-function mutation in *unc-105* was recently reported to cause aberrant muscle protein degradation. We found this degradation and the movement defect reduced in *unc-105* mutants also containing either an intragenic premature stop codon or a mutation in *let-2*, which encodes a collagen that appears to gate the hyperactive UNC-105 channel. The degradation was not suppressed by mutations or drugs known to suppress proteasome-, autophagy-, or calpain-mediated degradation or by mutations known to suppress neurodegeneration in response to hyperactivation of other degenerin channels in *C. elegans*. However, protein degradation, but not the movement defect, was decreased by treatment with caspase inhibitors or RNAi against *ced-3* or *ced-4*. To test if ion influx affected mitochondria, thereby triggering CED-4 release and CED-3 (caspase) activation, we examined mitochondria in wt, *unc-105*, and *unc-105; let-2* animals. Adult *unc-105* animals displayed a time dependent fragmentation of the mitochondrial network which was associated with impaired mitochondrial membrane potential both *in vivo*, as assessed by time dependent loss of JC-10 from muscle mitochondria, and *in vitro*, as assessed by decreased JC-1 uptake by isolated mitochondria. This loss of membrane potential correlated with decreased rates of maximal ATP production in mitochondria isolated from *unc-105* mutants. Lastly, reduced levels of CED-4 were observed in mitochondrial isolations from *unc-105* mutants and, interestingly, mitochondrial cytochrome C levels were also reduced. Thus, just as in mammalian cells, constitutive cationic influx into *C. elegans* muscle leads to pathological changes in mitochondrial architecture and function, release of mitochondrial proteins to the cytoplasm, and subsequent caspase activation.

791A. Neurodegeneration and death induced by neuron-specific knock-down of *smn-1*, the homolog of the gene responsible for Spinal Muscular Atrophy. **I. Gallotta**[1], A. Donato[1], N. Mazzarella[1], P. Bazzicalupo[1], M. Hilliard[2], E. Di Schiavi[1]. 1) Institute of Genetics and Biophysics, CNR, Naples, Italy; 2) The University of Queensland, QBI, Brisbane, Australia.

Spinal Muscular Atrophy (SMA) is a neurodegenerative disease characterized by degeneration and death of spinal cord motor neurons. All classical forms of SMA are associated with mutations in the survival of motor neuron gene 1 (*Smn1*). Using traditional genetic mutants two models of SMA have been developed in *C. elegans*. However degeneration and death of neurons were not observed in these models (Briese *et al.*, HMG, 2009; Sleigh *et al.*, HMG, 2010). Using a transgene-based RNAi strategy (Esposito *et al.*, Gene, 2007), we generated two different models of SMA by reducing the function of *smn-1* in two distinct classes of *C. elegans* neurons, the GABA motor neurons and the touch receptor neurons. When *smn-1* is silenced in the GABA neurons, we observed the degeneration and death of these cells in otherwise viable and fertile animals. Neuronal degeneration correlated with defects in backward movements, as expected from loss of these neurons; it was detected as disappearance of presynaptic markers, and reactivity to different genetic and chemical cell-death markers. We found that neurodegeneration was age-dependent and that cell death induced by *smn-1* silencing required molecules of the apoptotic pathway. These phenotypes were rescued by the expression of human *Smn1*, indicating a strong functional conservation between the two genes. Furthermore, using a candidate gene approach we identified several genes that when mutated are able to modulate the death of GABA motor neurons. We are also testing candidate drugs to slow down the degenerative process. Finally we developed a second model in which *smn-1* is silenced specifically in touch receptor neurons. Also in this case we observed functional alteration and degeneration of touch receptor neurons. Unlike GABA motor neurons, however, touch receptor neurons degeneration started with a retraction of the axons that mimics a dying-back phenotype and showed a slower progression toward neuronal death. These results suggest that, as observed in humans, intrinsic features of the two classes of neurons determine different responses to the loss of *smn-1*.

792B. Chronic alcohol exposure induces toxicity and neurodegeneration in C. *elegans*. **Lina Gomez**[1], Sangeetha Iyer[2], Ashley Crisp[3], Jesse Cohn[3], Jon Pierce-Shimomura[4]. 1) Institute for Neuroscience The University of Texas at Austin, Austin, TX; 2) Section of Neurobiology The Univesity of Texas at Austin, Austin, TX; 3) Institute of cellular and molecular biology The University of Texas at Austin, TX; 4) Waggoner Center for Alcohol and Addiction Research The Univesity of Texas at Austin, Austin, TX.

Alcohol abuse is an enormous problem causing death and disability to over 43 million people worldwide each year (WHO). Chronic alcohol consumption also contributes to abnormal brain morphology and significant brain volume loss indicative of neurodegeneration in adults and fetuses. Until there are effective treatments to alter maladaptive behavioral patterns in alcohol abuse, more research is needed to prevent alcohol-induced toxicity and degeneration. We used C. elegans as a model system to identify genetic modulators of alcohol toxicity and explored whether prolonged alcohol exposure damages the nervous system. In our study, we exposed L4-stage worms to varying concentrations of ethanol for three days and found a dose-dependent deficit in crawling and developmental delay. Furthermore, we evaluated degeneration by assessing the health of neurons using fluorescent reporters. Compared to the untreated group, we found that ethanol-exposed worms had significant neurodegeneration. Previous findings using C. elegans have suggested that the innate immune pathway may protect against neurodegeneration caused by drug toxicity (Schreiber & McIntire, 2011). We find that deletion of either the innate immune gene nsy-1 (ortholog to the mammalian ASK-1 MAPKKK) or pmk-1 (ortholog to the mammalian p38 MAPK) caused hypersensitivity to ethanol toxicity. Conversely, boosting innate immune signaling via gain-of-function mutation in nsy-1 produced resistance to ethanol toxicity. Our findings indicate that prolonged exposure to ethanol leads to behavioral impairments, developmental delay and neuronal degeneration in C. elegans. We are currently studying how the p38 MAP kinase pathway alleviates toxicity and neurodegeneration in response to ethanol.

793C. Functional analysis of VPS41-mediated protection from b-Amyloid cytotoxicity. **Edward F Griffin**, Christopher Gilmartin, Kim A Caldwell, Guy A Caldwell. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

According to the Alzheimer's Association 2012 report, 13% of Americans over 65 years of age suffer from Alzheimer's Disease (AD), resulting in an estimated cost of 200 billion dollars for related health care. Additionally, AD is the most prevalent dementia, and the sixth leading cause of death in the United States. Thus, investigating mechanisms of pathophysiology and identifying potential therapeutic targets for AD is significant. AD is characterized by the formation of plaques, composed primarily of b-amyloid 1-42 (Ab) in the brain, resulting in neurodegeneration. Our lab has observed that over-expression of VPS41 in *C. elegans* provides neuroprotection from Ab toxicity, and that deficiency of VPS41 in worms expressing Ab increases the toxicity of Ab (unpublished data). In yeast, VPS41 has been demonstrated to function in the tethering of vesicles, late endosomes, and AP-3-coated vesicles from the late Golgi, to the lysosome for degradation. Previously, our lab has shown that over-expression of human VPS41 is neuroprotective in a transgenic worm model of Parkinson's Disease, wherein dopaminergic neurodegeneration is induced by a-synuclein overexpression (Harrington et al., 2012, *J. Neurosci.*). VPS41-mediated neuroprotection from a-synuclein was dependent on the phosphorylation of VPS41 as well as its interactions with AP-3d, RAB7, VPS core proteins, and VPS39. Through this study, and others, we can conclude that VPS41 has a role in lysosomal trafficking. Yet, how this specifically relates to the processing and ameliorates the cellular impact of neurotoxic proteinaceous aggregates is undetermined. Here we report the results of a systematic RNAi screen whereby we knocked down the core components involved in lysosomal trafficking and categorized their requirement for Ab protein toxicity. Preliminary results indicate that Ab is trafficked to through the endosomes rather than through AP-3-coated vesicles from the late Golgi. In this regard, further analysis of functional effectors of Ab protein processing via the lysosomal pathway, along with subsequent evaluation in our worm neuronal model of AD (Treusch et al, 2011, *Science*), will assist in the elucidation of the underlying mechanism involving VPS-41.

794A. SGK-1 promotes germline apoptosis by a cell non-autonomous mechanism. **Madhavi Gunda**, W.Brent Derry. Developmental and Stem Cell Biology Department, The Hospital for Sick Children, Toronto, Ontario, Canada.

The *Caenorhabditis elegans sgk-1* ortholog of the serum and glucocorticoid-inducible kinase SGK belongs to the AGC family of serine/threonine kinases that are activated in response to a number of extracellular stimuli. SGK-1 has a variety of physiological functions in *C. elegans* which include development, life span, lipid storage and stress response. SGK-1 regulates these biological processes by mechanisms that are both dependent and independent of the DAF-16/FoxO transcription factor. Here we describe a novel function for *sgk-1* in regulating DNA damage-induced germline apoptosis. We found that *sgk-1* is required to promote damage induced apoptosis by a cell non-autonomous mechanism that is also independent of *daf-16* and the canonical insulin pathway. Ablation of *sgk-1* by RNAi or mutation results in complete resistance to radiation-induced germline apoptosis. Since *sgk-1* likely functions in parallel to the canonical insulin signaling pathway, we carried out genetic epistasis analysis with components of the TORC2 pathway and found that *rict-1* functions in the same pathway as *sgk-1* to regulate DNA damage induced apoptosis in the germline. This relationship has also been observed in the regulation of lipid storage and development (Jones *et al.,* 2009). By quantitative real time PCR we found that *egl-1* transcript is induced to normal levels in response to irradiation in both *sgk-1* and *rict-1* mutants, indicating that these genes function in parallel or downstream of *cep-1* to promote apoptosis. Since we previously showed that the insulin pathway regulates DNA damage-induced germline apoptosis through the Ras/MAPK signaling pathway (Perrin *et al.,* 2013) we also asked if *sgk-1* also modulates Ras signaling. Ablation of *sgk-1* completely suppressed the hypersensitivity of let-60/Ras gain-of-function mutants to damage induced apoptosis, suggesting that SGK-1 and the TORC2 pathway are important determinants of Ras signaling in the germline. We are currently investigating the levels of phosphorylated MPK-1/Erk in *sgk-1* mutants which will help us better understand the cross-talk between these pathways. Currently, we are also focusing on how *sgk-1* interacts with the core cell death components to regulate cell death.

795B. The Sodium-Potassium ATPase alpha subunit EAT-6 promotes programmed cell death. **Tsung-Yuan Hsu**, Meng- I Lee, Yi-Chun Wu. Molecular & Cell Biology, National Taiwan University.

Genetic analyses have identified and characterized several genes that function in the execution of programmed cell death in *C. elegans*. However, how the cell death process is regulated is not clear. We found that EAT-6, a sodium-potassium ATPase alpha subunit, promotes programmed cell death. A sodium-potassium ATPase pumps sodium out of cells, while pumping potassium into cells, in an ATPase-dependent manner and functions to maintain the membrane potential across plasma membrane. A typical mammalian sodium-potassium ATPase is composed of two alpha subunits containing an ATPase activity, two beta subunits and one gamma subunit. *C. elegans* has two alpha subunit genes, *eat-6* and *catp-4*, and three beta subunit genes, *nkb-1*, *nkb-2* and *nkb-3*, but no gamma subunit gene. We found that loss of any beta subunit gene does not result in a detectable embryonic cell death defect, while loss of the alpha subunit gene *eat-6*, significantly reduces embryonic cell corpses. This result suggests differential involvement of alpha and beta subunits in programmed cell death. In addition, overexpression of *eat-6* results in ectopic cell death, even in the absence of beta subunit. Furthermore, we identified two kinases that function downstream of eat-6 to promote programmed cell death. Using a bimolecular fluorescence complementation (BIFC) analysis and a yeast two-hybrid assay, we detected an interaction between the EAT-6 intracellular domain and one of these kinases. This result suggests that EAT-6 may relay the cell-death promoting signal to the kinase by protein-protein interaction. We are currently doing experiments to examine whether the sodium-potassium pumping activity of EAT-6 is essential for the cell-death promoting function and to determine the relationship of *eat-6* with respect to the core cell death genes in promoting programmed cell death.

796C. Innate immune signaling protects against patterned neurodegeneration in Alzheimer's disease. **Sangeetha V. Iyer**, Ashley Crisp, Anushri Kushwaha, Jon Pierce-Shimomura. Section of Neurobiology, University of Texas, Austin, TX.

Alzheimer's disease (AD) affects more than 5 million people in the U.S. At the cellular level, AD is typified by the development of extracellular plaques formed due to aggregation of amyloid precursor protein (APP) fragments. AD can be caused in humans by a single extra copy of the APP gene as observed in Down syndrome. Because APP is widely expressed throughout the brain; it is unclear why this ubiquitously expressed gene leads to a selective pattern of degeneration starting with cholinergic neurons important for memory. To address this question, we generated a novel C. elegans model of AD that expresses a single copy of the human APP gene. Like human AD, our model displays age-dependent degeneration of specific cholinergic neurons. The progressive cholinergic neurodegeneration correlated significantly with the levels of accumulated APP. Identical results were obtained upon over-

expression of the orthologous worm gene, apl-1, demonstrating that the basis for this is conserved. Since the initiation of APP accumulation coincided with the onset of immunosenescence, we hypothesized that innate immune signaling may play a role in APP accumulation and degeneration. We found that deletion of any member of a conserved core of ASK1/p38MAPK innate immune pathway led to the selective degeneration of the same cholinergic neurons. Conversely, boosting innate immunity protected against APP accumulation and degeneration cell autonomously. To boost innate immune signaling pharmacologically, we tested a candidate AD drug, the retinoid X receptor agonist bexarotene, on our AD worms. Treatment with bexarotene as well as other nuclear hormone receptor agonists significantly reduced APP accumulation and cholinergic degeneration. Although bexarotene was recently proposed to provide protection via upregulation of the AD-risk gene ApoE in mice, worms do not possess a true homolog of ApoE. Thus, our results imply that bexarotene also protects via pathways independent of ApoE. Together, our data suggest that immunosenescence may unexpectedly sculpt the timing and cellular pattern of neurodegeneration caused by APP, and that the conserved ASK1/p38MAPK innate immune pathway may be harnessed for promising therapeutic outcomes in AD.

797A. Sirtuin Mediated Neuroprotection and its Association with Autophagy and Apoptosis: Studies Employing Transgenic C. elegans Model. **Pooja Jadiya**, Aamir Nazir. Division of Toxicology, CSIR - Central Drug Research Institute, Lucknow, U.P., India.

Sirtuins, NAD+-dependent protein deacetylases, are well known for their role in longevity. Aging is the major known risk factor for the onset of Parkinson's disease (PD). In our previous studies, SIRT1 induction by calorie restriction (CR) diet regimen has been shown to protect against dopaminergic neurodegeneration via sir-2.1 mediated pathway. Taking the studies forward, we endeavoured to understand the role of nicotinamide adenine dinucleotide activated protein deacetylase Sir2p/Sirt1 in calorie restriction mediated prevention of Parkinsonism employing transgenic Caenorhabditis elegans expressing human alpha synuclein. Our findings provide evidences towards the role of calorie restriction in reducing a-synuclein aggregation, mitochondrial, lipid content and ROS in human a-synuclein expressing strain of C. elegans. RNAi of sir-2.1 enhanced aggregation of alpha synuclein but sir-2.1 silenced worms raised on reduced calorie diet didn't show protective effect in reducing protein aggregation which concluded that protective effects of calorie restriction was mediated by NAD-dependent histone deacetylase activity of sir-2.1. We next focused on the mechanism of the sir-2.1 mediated neuroprotective effect via autophagy. Assessment of autophagy in C. elegans was performed using a transgenic strain DA2123 strain, expressing a GFP-tagged LGG-1 protein. Worms with RNAi induced gene silencing of sir-2.1 showed decreased expression of GFP-tagged LGG-1 protein and decreased mRNA level of different autophagy genes including lgg-1,bec-1,atg-5,atg-7 and atg-13 in qPCR studies. To further examine the signaling pathways which could link SIRT1 to the regulation of autophagic degradation, we analyzed the expression of different apoptosis genes including ced-4, cep-1, lin-35, jkk-1 and jnk-1. In our studies, silencing of sir-2.1 showed significantly up-regulation of ced-4 (Apaf-1) & cep-1 (p53 ortholog- DNA damage pathway) apoptosis genes. Our study emphasizes the protective role of sir-2.1 on autophagosome formation, which is associated with the p53 and apaf-1 dependent signaling pathways which are well-known stress resistance mediators.

798B. LIN-3/EGF promotes programmed cell death by transcriptional activation of the pro-apoptotic gene *egl-1*. **Hang-Shiang Jiang**, Yi-Chun Wu. Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.

Programmed cell death (PCD) is the physiological death of a cell mediated by an intracellular suicide program. Although key components of the PCD execution pathway have been identified, how PCD is regulated during development is poorly understood. Here, we report that the EGF-like ligand LIN-3 acts as an extrinsic signal to promote PCD. Loss of *lin-3* reduces PCD during embryogenesis, whereas overexpression of *lin-3* by a *lin-3* transgene causes ectopic cell deaths. This observation suggests that the level of LIN-3 signaling is important for the precise fine-tuning of the life-versus-death fate of cells. Mis-expression of *lin-3* in the intestinal cells, where *lin-3* is not normally expressed, rescued the cell death defect of the *lin-3* mutant. Since more than 90% PCD occurs in the head region, this suggests that LIN-3 secreted from the intestine can act at a distance to induce cell death in other parts of the embryo. We found that *lin-3* acts upstream of *let-23*, which encodes the EGF receptor, to promote PCD. In addition, mutants defective in the LET-60-MPK-1 pathway, which signals downstream of *lin-3* and *let-23* during vulva differentiation, also have reduced numbers of apoptotic cells during embryogenesis. LIN-1 and LIN-31 are two transcription factors that are regulated by the LET-60-MPK-1 pathway during vulval differentiation. We found that *lin-1*, but not *lin-31*, is required for embryonic PCD. Using Quantitative real-time reverse transcriptase PCR, we found that the abundance of *egl-1* transcripts correlated well with the level of *lin-3* activity. *egl-1* transcripts were less abundant in *lin-3* and *let-23* mutants than in wild-type animals, while *lin-3* overexpression caused an increase in *egl-1* transcript levels. Using EMSA and transgenic bypass experiments, we demonstrated that LIN-1 can directly bind to the *egl-1* promoter *in vitro* and that this binding is important for *lin-3* to elicit its cell death-promoting function *in vivo*. We propose that the extrinsic cell death-promoting signal LIN-3 is transduced through LET-23 to activate the LET-60-MPK-1 pathway and LIN-1, and the activated LIN-1 binds to, and activates transcription of the key pro-apoptotic gene *egl-1*, which in turn leads to PCD.

799C. Assisted Suicide: a Caspase- and Engulfment-Dependent Cell Death. **Holly Johnsen**, Bob Horvitz. HHMI, Biology, MIT, Cambridge, MA.

Programmed cell death occurs during the development of many organisms. The *C. elegans* cell-death pathway has been extensively studied and is evolutionarily conserved. During programmed cell death, caspases are activated in the dying cell. The cell corpse is engulfed by a neighboring cell and degraded. Almost all cell deaths are "suicides"—they are cell-autonomous, caspase-dependent and can occur even in engulfment-defective animals.

In the *C. elegans* male, the cells B.alapaav and B.arapaav are generated during the late L3 stage. During the early L4 stage one of these cells dies, and the other survives and adopts an epithelial fate. These two cells form an equivalence group; the decision of which cell dies and which survives is stochastic and takes place during the L3/L4 lethargus. The cell that dies is engulfed by the neighboring cell P12.pa. In contrast to most *C. elegans* cell deaths, the B.al/rapaav death is engulfment-dependent; if engulfment is blocked by a mutation in one of the genes in the engulfment pathway, both B.alapaav and B.arapaav survive. Furthermore, we have found that if the engulfing cell P12.pa is ablated, the B.al/rapaav death fails to occur in approximately 60% of animals. These observations suggest that cell interactions between B.alapaav and B.arapaav as well as between B.al/rapaav and P12.pa are involved in this cell death, leading some to suggest that P12.pa "murders" B.al/rapaav.

We are investigating the control and execution of the B.al/rapaav cell death. When the B.al/rapaav cell death is blocked by engulfment defects or P12.pa ablation, the undead cell still initiates the cell-death pathway. Similar to other dying cells, the undead cell looks round by Nomarski and EM and exposes

phosphatidylserine on its surface. *egl-1* and *ced-3* are required for the B.al/rapaav cell death and are expressed in the undead cell, suggesting that the core cell-death pathway is required but not sufficient for this cell death, i.e. that this death is an assisted suicide. We hope our studies will provide insight into new mechanisms of programmed cell death, cell-cell signaling, and fate determination within equivalence groups.

800A. Modulation of *mec-10(d)*-induced necrosis by ER chaperone NRA-2. **Shaunak Kamat**[1], Shrutika Yeola[1], Monica Driscoll[1], Laura Bianchi[2]. 1) Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ; 2) Department of Physiology and Biophysics Miller School of Medicine University of Miami Rm 5133, Rosenstiel 1600 NW 10th Ave Miami, FL 33136.

ER chaperones play a major role in cellular homeostasis by regulating the assembly of polypeptides, intracellular Ca^{2+} signaling, and degradation of misfolded proteins. Here we report on an ER - resident chaperone NRA-2 that modulates hyper-activated ion channel-induced necrosis by regulating surface expression of a death-inducing DEG/ENaC channel family member subunit MEC-10(d). *nra-2* was previously identified in a screen for nicotinic acetylcholine receptor (nAChR) - interacting proteins and was found to regulate the subunit composition of the AChR receptor in *C. elegans* muscle[1]. We found that loss of function of *nra-2* led to a significant increase in *mec-10(d)* - induced necrosis in *C. elegans* touch receptor neurons (TRNs), and this enhancement was rescued by TRN-specific transgenic expression of *nra-2*. We observed that loss of *nra-2* led to a significant increase in surface localization of MEC-10(d)::GFP and a significant decrease in ER localization. Electrophysiological experiments in *Xenopus* oocytes revealed that NRA-2 suppresses amiloride-sensitive currents induced by hyperactivated MEC channels. Our study suggests a role for NRA-2 as an ER quality control protein that inhibits surface expression of mutant MEC-10(d) channels and is thus neuroprotective against hyperactivated ion channel-induced necrosis. 1. Ruta B. Almedom, Jana F. Liewald, Guillermina Hernando, Christian Schultheis, Diego Rayes, Jie Pan, Thorsten Schedletzky, Harald Hutter, Cecilia Bouzat, and Alexander Gottschalk. **An ER-resident membrane protein complex regulates nicotinic acetylcholine receptor subunit composition at the synapse.** *The EMBO Journal*, 28(17):2636-2649, July 2009.

801B. Mutations in progranulin and cell death genes confer organismal stress resistance. M. Judy, A. Nakamura, H. McCurdy, A. Huang, H. Grant, C. Kenyon, **A. Kao**. University of California San Francisco, San Francisco, CA.

As animals move about their world, they encounter changing and sometimes harsh environmental conditions. To cope with environmental stress, animals have evolved protective mechanisms. At the local or cellular level, these responses include apoptosis to remove damaged cells. On a systemic or organismal level, animals can alter metabolism or gene expression to promote stress resistance. Because neurodegeneration involves cell death and dysfunction modulated by both genetic and environmental factors, we are interested in the link between stress response and programmed cell death (PCD).

Mutations in the progranulin gene link to familial forms of the neurodegenerative disease frontotemporal lobar degeneration. Earlier, we showed that *C. elegans* lacking progranulin (*pgrn-1*) exhibit accelerated clearance of apoptotic cells (Kao, PNAS 2011). More recently, we found that *pgrn-1* mutants were specifically resistant to heat, osmotic and ER stress, but not to oxidative, UV or pathogen stress. Because *pgrn-1* normally regulates PCD, we wondered whether other cell death mutations affected the stress response. Indeed, a similar pattern of stress resistance was induced by mutations that either block cell death (*ced-3, ced-4*) or slow apoptotic cell engulfment (*ced-1, -2, -5, -6, -7*). Double mutants of *pgrn-1* and *ced-3* or engulfment mutants were not additive in ER stress resistance, suggesting a common mechanism of enhanced stress response.

These results demonstrate that mutations perturbing PCD in different ways have similar effects on environmental stress resistance. We speculate that these cell death regulators converge on a common stress regulatory mechanism. We are currently using transcriptional analysis to examine the genetic pathways altered in cell death mutants and investigating whether cleavage of progranulin into granulins is changed in cell death mutants. These findings may lead to new therapeutic targets in the treatment and prevention of neurodegenerative disease.

802C. A C. elegans model of Adult-onset Neuronal Ceroid Lipofuscinosis reveals a sir-2.1 independent protective effect of resveratrol. **Sudhanva Kashyap**[1], James Johnson[1], Mimi Ayala[2], Jeff Barclay[1], Bob Burgoyne[1], Alan Morgan[1]. 1) Cellular and Molecular Physiology, University of Liverpool, Liverpool, Merseyside, United Kingdom; 2) Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, Merseyside, United Kingdom.

As human populations age, neurodegenerative diseases are becoming an increasing burden. The underlying mechanisms that cause age-dependent loss of neurons in these diseases remain unclear. As a result, therapies for these debilitating and eventually fatal disorders are lacking. One way to address this problem is by the use of model organisms like C. elegans to understand the mechanisms of neurodegeneration and for screening novel drug targets. Here we describe a new C. elegans model for age-dependent neurodegeneration caused by mutation of the dnj-14 gene. DNJ-14 is the worm homologue of cysteine string protein (CSP), a neuronal chaperone protein which prevents the misfolding of presynaptic proteins. Mutations in CSP cause Adult-onset Neuronal Ceroid Lipofuscinosis (ANCL), a human neurodegenerative disease. CSP knockout mice have early mortality and exhibit age-related neurotransmission defects, sensorimotor dysfunction and presynaptic neurodegeneration. We show here that mutations in C. elegans' dnj-14 also result in a significant reduction in lifespan, small reduction in locomotion and resistance to aldicarb (a behavioural read-out of cholinergic neurotransmission). The worms also show age-dependent defects in chemosensation which correlated to the loss of sensory neurons at a later age. Treatment with resveratrol, a polyphenol that has been previously shown to be neuroprotective in several neurodegenerative disease models, rescues the lifespan and the chemosensation defects in the dnj-14 mutant worms. Resveratrol also rescues the lifespan of dnj-14 mutants lacking sir-2.1. Similarly, a PDE-4 inhibitor, rolipram also rescues the lifespan of dnj-14 worms. This suggests that resveratrol acts in a PDE-4 inhibition dependent and sir-2.1 independent manner. Hence we show that this new worm model could be useful in screening novel neuroprotective compounds.

803A. Post-transcriptional control of C. elegans germ cell apoptosis by RNA-binding proteins. **Martin Keller**[1,2], Michael O. Hengartner[1]. 1) Institute of Molecular Life Sciences, University of Zurich, Switzerland; 2) Molecular Life Sciences PhD program, Life Science Zurich Graduate School, ETH/University of Zurich, Zurich, Switzerland.

Post-transcriptional control of mRNAs by RNA-binding proteins (RBPs) has a prominent role in the regulation of gene expression. RBPs interact with mRNAs to control their biogenesis, splicing, transport, localization, translation and stability. Defects in such regulation can lead to a wide range of human

diseases from neurological disorders to cancer. Many fundamental biological pathways related to such disorders are conserved between Caenorhabditis elegans and humans. Therefore, studying RBPs associated with apoptosis in C. elegans could potentially give insight into processes underlying human diseases. Several RBPs are known to regulate apoptosis in the adult C. elegans germline. How these RBPs control apoptosis is however largely unknown. To gain a more comprehensive understanding of the general involvement of RBPs in the regulation of germ cell apoptosis, we performed an RNAi screen to identify novel RBP candidates that affect germ cell death. We verified our RNAi results via loss-of-function mutants and focus on the following confirmed candidates: glh-1, wago-4 and wago-5. In order to understand how these RBPs control apoptosis, the target mRNAs and the RNA-binding motifs will be identified with the recently developed in-vivo PAR-CLIP technique. Furthermore, RBP-protein interaction will be dissected using co-immunoprecipitation experiments. Our approach will allow us to build up a model of the germ cell apoptosis RNA regulon and broaden our understanding on how RBPs orchestrate cellular events.

804B. Investigating neuroprotecive genes on *S. venezuelae* toxicity in *C. elegans*. **H. Kim**, G.A. Caldwell, K.A. Caldwell. Biological Sciences, The University of Alabama, Tuscaloosa , AL.

One of the main features of Parkinson's disease (PD) is the loss of dopaminergic (DA) neurons. Only 5-10% of PD has been associated with a genetic cause, and it is thought that the environment, or a combination of environment and genetic factors, might lead to the loss of DA neurons. We have identified a species of soil bacteria, *Streptomyces venezuelae*, which produce a metabolite that causes neurodegeneration of *C.elegans* DA neurons and cultured human DA neurons (Caldwell et al., 2009, *PLoS ONE*). We hypothesize that exposure to this metabolite might be associated with PD, in association with a genetic predisposition to neurodegeneration. In this regard, we have previously identified gene products associated with DA neurodegeneration that are involved in ER to Golgi, or autophagy-lysosomal trafficking (Gitler et al., 2008, *PNAS*, Hamamichi et al., 2008, *PNAS*, and Gitler et al., 2009, *Nature Genet*). These gene products include SEC-22, PARK9, VPS41, ATG7, Rab 1, Rab3A, Rab8A, and ULK2/UNC-51. In this study, the DA neurons of *C.elegans* with mutations in theses genes were examined for their response to exposures with the bacterial metabolite. Animal containing a mutation in a gene involved in ER to Golgi trafficking, *sec-22(ok3053)*, displayed exacerbated DA neurodegeneration in combination with the metabolite. Mutations in genes associated with autophagy-lysosomal trafficking also displayed enhanced DA neurodegeneration in the presence of the metabolite; however, the response observed in these mutants [*unc-51(e369)* and *catp-6(ok3473)*] was less pronounced and delayed. Current studies on the remaining gene products, where mutants are unavailable, are being investigated using DA neuron-specific RNAi for differential neuroprotective activity against the metabolite. Candidates that, when depleted, display a toxicity phenotype, will also be overexpressed in DA neurons and assayed for neuroprotective activity against the metabolite. Taken together, pathway-specific candidates that have a toxin-modulating effect will assist in assigning a functional role for the metabolite within neurons.

805C. Using artificial insemination to identify genes involved in linker cell death and corpse removal. **Lena M Kutscher**, Nima Tishbi, Shai Shaham. Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

The male-specific *C. elegans* linker cell dies post-embryonically after leading male gonad elongation. Linker cell death occurs in the absence of all known apoptotic cell death genes, and dying linker cells are ultrastructurally distinct from apoptotic cells. Previously, a genome-wide RNAi screen revealed six genes involved in linker cell death, including one encoding a polyglutamine-containing protein. Subsequently, we also identified genes whose inactivation by mutation, but not by RNAi, prevents linker cell death. These results suggest that a forward genetic screen may reveal novel genes required for linker cell death and clearance. Our initial attempts at such a screen proved unsuccessful because of the possibility of male sterility, and the low recovery of males from clonal isolates of mutagenized Him strains. To circumvent these limitations, we artificially inseminated wild-type hermaphrodites with sperm derived from mutagenized F2 males with a surviving linker cell. We performed 66 successful artificial inseminations (from 181 attempts), resulting in 44 independent strains with linker cell death defects. The mutants are varied; they seem to block linker cell death, delay it, affect aspects of the morphology of the dying linker cell, or prevent engulfment. Linker cell engulfment mutants are of particular interest as this process is independent of all known engulfment genes. Whole genome sequencing of several of our mutants suggests that we have indeed identified novel cell death genes. Progress towards cloning some of these will be discussed.

806A. Identification of interacting partners of a poly-glutamine protein involved in non-apoptotic cell death. **Lena M. Kutscher**, Shai Shaham. Laboratory for Developmental Genetics, The Rockefeller University, New York, NY.

Cell death is an important cellular process in development and disease. While caspase-dependent apoptosis has been extensively studied, cell death still occurs in animals lacking these proteases, suggesting that cells must be able to engage non-apoptotic cell death pathways. One such example is the non-apoptotic cell death of the *C. elegans* linker cell, which leads the elongation of the developing male gonad during larval development. At the L4-to-adult transition, when the linker cell reaches the cloaca, it dies. Linker cell death is not apoptotic, as assessed by mechanism and morphology, and relies in part on a poly-glutamine protein, PQN-41C. We are interested in identifying binding partners of PQN-41C to determine its functions during linker cell death and outside the linker cell. We used a yeast two-hybrid screen to isolate 33 putative PQN-41C interactors. Gene ontology and protein domains identify five subclasses of interactors affecting proteolysis (*asp-3*, *F32A5.3*, *K10C2.1*, *F57F5.1*, *nep-17*), or possessing coiled-coil domains (*pqn-41, pqn-59, R11A8.7, pqn-85, npp-4*), ubiquitin-related domains (*tag-214, pqn-59, F52G3.1, sao-1*), and RNA-related (*etr-1, asd-1, asd-2, pes-4*), or DNA-associated domains (*chd-7, zfp-1, tag-153, eef-2, T19D12.2, pqn-85*). We tested the functions of the interacting proteins in linker cell death by examining whether RNAi against each candidate promotes linker cell survival. Of the genes we tested thus far, seven appear to have a function in linker cell death, with linker cell survival rates ranging from 12% to 33% upon RNAi knockdown. Two candidates may cause enhanced linker cell death or cell clearance upon knockdown. Our results therefore suggest that PQN-41C may act in complex with other proteins to promote linker cell death.

807B. Necrotic Cells Share a Similar Mechanism with Apoptotic Cells in being Recognized by Engulfing Cells in *C. elegans*. **Zao Li**[1], Victor Venegas[1], Prashant Raghavan[1], Yoshinobu Nakanishi[2], Zheng Zhou[1]. 1) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas; 2) Graduate School of Medical Science, Kanazawa University, Kanazawa, Ishikawa, Japan.

Necrosis is the premature death of cells caused by external factors, such as acute cell injury or trauma. In contrast to apoptosis, the programmed cell

death, necrosis is caspase-independent and necrotic cells are morphologically distinct from apoptotic cells. Although these two categories of cell deaths are genetically different, it has been suggested that necrotic cell corpses are actively removed by the same set of genes required in apoptotic cell removal indicating they might share a similar clearance mechanism. In the nematode *Caenorhabditis elegans*, gain-of-function mutations in certain ion channel subunits result in necrotic-like cell death of six touch neurons. Necrotic touch neurons are subsequently engulfed and degraded inside engulfing cells. However, it is unclear how necrotic cells are recognized by phagocytes. Phosphatidylserine (PS) is an important apoptotic cell surface signal that attracts engulfing cells. Using ectopically expressed MFG-E8, a high-affinity PS-binding protein, we observed that PS was actively present on the surface of necrotic touch neurons. In addition, phagocytic receptor CED-1, whose function is needed for the efficient clearance of apoptotic cells, also acts as a phagocytic receptor for necrotic cells. We demonstrate that necrotic cells, like apoptotic cells, rely on cell-surface PS as an "eat me" signal to attract CED-1. We further found CED-7, the worm homolog of mouse ABC1 transporter, was necessary for PS-exposure on necrotic cell surfaces. Moreover, we discovered another protein contributing to the presentation of PS on necrotic cell surfaces and acting in a parallel pathway to CED-7. Our findings suggest between two distinct cell deaths, a conserved mechanism may exist for the recognition of cell corpse.

808C. A small metabolite isolated from *Streptomyces venezuelae* enhances age-dependent proteotoxic stress in *C. elegans* models of neurodegenerative diseases. **B. A. Martinez**, A. Ray, G. A. Caldwell, K. A. Caldwell. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Aging is a ubiquitous process that deteriorates critical homeostatic pathways. This is reflected in a higher incidence of neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's Disease (AD) among aging individuals. Though much work has been done studying genetic modifiers of age-related disorders, the majority of cases are idiopathic; and even among those with similar genetic dispositions, the age of onset can differ. These observations suggest that the environment contributes synergistically to disease progression. Recent evidence has correlated rural living with increased incidence of contracting idiopathic neurodegenerative diseases, possibly through greater exposure to the agricultural environment. We previously identified that a small compound excreted from a soil bacterium *Streptomyces venezuelae* causes neuronal degeneration in both invertebrate models and cultured human neurons. Therefore, this metabolite could be a possible contributor to age-dependent neurodegeneration seen in PD or AD. In this study, we report that this metabolite enhances age-dependent proteotoxic stress initiated by pathogenic expression of neurodegenerative disease-related proteins a-synuclein, amyloid-b, and polyglutamine repeats in *C. elegans* models. To explore the mechanism underlying these age-associated observations, we analyzed the effect of the metabolite on a reporter for the insulin/IIS transcription factor, DAF-16. In response to treatment, the DAF-16::GFP fusion protein relocalized to the nucleus, suggesting a possible explanation for the age-associated phenotypes observed when animals are treated with the metabolite. Further exploration included the Nrf-2 homologue, SKN-1, a redox and stress sensitive transcription factor. Surprisingly in our assays, SKN-1 does not appear to be recruited to the nucleus, implicating a possible route of toxicity that bypasses SKN-1, but not DAF-16, during neurodegeneration. Continued analyses for a synergistic role of the metabolite with protein misfolding should yield further insights into neurodegenerative processes.

809A. Unraveling the role of MOAG-4 in protein aggregation. A.T. van der Goot, **A. Mata-Cabana**, E. Stroo, E.A. Nollen. ERIBA, UMCG, Groningen, The Netherlands.

Several age-related neurodegenerative disorders, such as Alzheimer's, Parkinson's and Huntington's diseases, are characterized by the formation of proteins aggregates in the brain. How aggregates form during aging is poorly understood. Recently, we identified a modifier of aggregation, MOAG-4, as a positive regulator of aggregation in *C. elegans* models for neurodegenerative diseases. The role of MOAG-4 is conserved in the human orthologs SERF1A and SERF2. MOAG-4/SERF appears to regulate age-related proteotoxicity through a previously unexplored pathway. How this regulator works needs to be established. With this aim we are searching for partners and endogenous substrates for MOAG-4. Two different approaches are being carried out. On one hand, we have performed a mutagenesis screen to find mutants that increase the PolyQ aggregation in the *moag-4* mutant strain. We found 22 mutations encoding, at least, four different genes able to increase aggregation in the absence of *moag-4*. So far, we identified *unc-30*, a neuron-specific transcription factor involved in the synthesis of GABA, and *unc-89*, required for myosin organization, as Suppressor of MOAG-4 (SMAG). However, both proteins seem to act in parallel of MOAG-4 since their deletion results in increased aggregation in the presence of MOAG-4 as well. We are still analyzing mutants for which we didn't identify the causative mutations yet or their relationship with MOAG-4 during the aggregation process. On the other hand we also are performing different proteomic approaches to identify putative substrates and proteins that may form functional complexes with MOAG-4. With this regard, we have carried out a yeast two hybrid screening using a human library to find SERF interacting proteins. Hence, we found 40 interactors, 23 of which have homologous proteins in *C. elegans*. In order to analyze their role on protein aggregation we are performing RNAi experiments in worms expressing a PolyQ stretch. The integration of the results obtained from these complementary approaches will unravel the endogenous role of MOAG-4, the mechanism by which it acts, and this will contribute to our understanding of how cells cope with toxic, aggregation-prone proteins during aging.

810B. Neuronal expression of wild-type and A152T mutant tau cause distinct patterns of toxicity in *C. elegans*. **Helen L. McCurdy**, D. Cox, B. Bliska, Aimee W. Kao. Univ of California, San Francisco, San Francisco, CA.

Mutations in tau underlie multiple neurodegenerative disorders, yet pathophysiological mechanisms are unclear. Mutations in tau link to frontotemporal lobar degeneration (FTLD). While most FTLD- related tau mutations are found in the protein's C-terminal microtubule-binding domains, a new mutation has recently been described outside these domains. This Ala to Thr variant at position 152 (A152T tau) is in an unstructured region of tau (Kovacs 2011, Coppola 2012).

Tau regulates microtubule functions, which are critical to neuronal health. To investigate what mediates A152T tau toxicity, we created *C. elegans* strains expressing human tau variants in neurons. We found that animals expressing neuronal A152T tau displayed a distinct phenotype from those expressing wild-type (WT) tau. A152T tau animals have delayed embryonic and larval development compared to both N2 and WT tau controls. Strikingly, while WT tau conferred toxicity in thrashing assays, A152T tau did not. Directly adjacent to Ala 152 is Thr 153, a residue that can be phosphorylated during mitosis. To investigate whether mutant Thr 152 is phosphorylated and therefore responsible for the developmental phenotype of A152T tau animals, we generated a strain with a phosphomimetic glutamate (E) at position 152. We found that these A152E tau animals phenocopied A152T tau in delayed development. Interestingly, the A152E tau animals resembled A152T with normal thrashing as day 1 adults, however at day 4, they exhibited impaired

thrashing, suggesting age-related toxicity of phosphorylation in this region.

These studies show that 1) A152T tau mutation confers toxicity distinct from WT tau and 2) aberrant phosphorylation may be responsible for the pathogenicity of this mutation. We are currently generating additional phosphoinhibitory and mimetic mutants and testing a series of candidate kinase inhibitors. With these studies, we will further determine the consequences of A152T mutation on tau phosphorylation and function. Understanding the basis of A152T tau toxicity may lead to novel therapeutic approaches.

811C. Neuronal Exophers: a Novel Mechanism for Removal of Neurotoxic Cytoplasm Components. **Ilija Melentijevic**[1], Marton Toth[1], Christian Neri[2], Monica Driscoll[1]. 1) Molecular Biology, Rutgers, New Brunswick, NJ; 2) INSERM, Paris, France.

We discovered a new, previously unknown feature of young adult C. elegans neurons—neurons can extrude substantial packets of cellular contents, which can include aggregated human neurodegenerative disease proteins or mitochondria, but no nuclear DNA. We currently call these extrusions "exophers". The ability to jettison cell contents appears to change with age, and extrusion is increased when protein turnover or autophagy is inhibited. Thus, this pathway may constitute a novel neuronal protection mechanism that serves to maintain protein/organelle homeostasis when other systems are compromised. We propose that the neuronal extrusion phenomenon constitutes a significant but currently unknown conserved pathway by which healthy neurons maintain their functions, and speculate that, in disease, this pathway may malfunction to promote spread of pathology. We will present the basic characterization of neuronal exopher production and our latest data on genetic influences on exopher generation.

812A. Progressive degeneration of dopaminergic neurons through TRP channel-induced necrosis. **Archana Nagarajan**[1], Ye Ning[1], Oliver Hobert[2,3], Maria Doitsidou[1,4]. 1) Centre for Organelle Research, University of Stavanger, Stavanger, Norway; 2) Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY, USA; 3) Howard Hughes Medical Institute, New York, NY, USA; 4) Norwegian Center for Movement Disorders, Stavanger University Hospital.

Dopaminergic neurons are involved in various behaviours and their loss leads to pathological conditions like Parkinson's disease. We use C. elegans to study mechanisms of dopaminergic neuronal degeneration, and to identify pathways that slow down or prevent neuronal loss. In a semi-automated forward genetic screen for C. elegans with abnormal dopaminergic system we isolated two mutants in which the dopaminergic neurons robustly and progressively degenerate in a dominant fashion. Using high-throughput mapping and whole genome sequencing, we identified in both mutants a missense mutation in the trp-4 locus, which we show to be the causal mutation for the degeneration phenotype. trp-4 encodes a cation channel of the Transient Receptor Potential (TRP) channel family and was previously shown to be involved in proprioception in C. elegans. We have characterized the progression of degeneration in trp-4(d)=(dominant) mutants for the various classes of dopaminergic neurons. We show that in trp-4(d) mutants, the classic apoptotic pathway is dispensable for degeneration and instead, neuronal cell death occurs through necrosis. Through a hypothesis driven approach, we show that loss of function of an ER calcium chaperon, calreticulin (crt-1) significantly suppresses degeneration in trp-4(d) mutants. Furthermore, manipulation of intracellular Ca2+ levels through genetic or pharmacological means results in altered severity of degeneration. In conclusion, we introduce a novel model for neurodegenerative channelopathy, showing that gain of function mutations in a TRP ion channel cause progressive loss of dopaminergic neurons. Since human midbrain dopaminergic neurons also express several members of the TRP ion channel family, we are currently testing whether mutations in these channels confer susceptibility to PD. Furthermore, we are conducting high-throughput suppressor screens on trp-4(d) mutants to identify novel pathways that halt dopaminergic neuronal degeneration.

813B. MEC-17 protects from axonal degeneration, maintaining mitochondrial organization and axonal transport. **Brent Neumann**, Massimo Hilliard. Queensland Brain Institute, The University of Queensland, St Lucia, Queensland, Australia.

Maintenance of axonal structure is critical for neuronal function. Under normal conditions, the axon is maintained in a healthy state through a constant supply of materials via its attachment to the cell body. This connection is lost after nerve injury and becomes compromised in a number of neurodegenerative diseases, leading to degeneration of the axon. Despite the depth of knowledge that has emerged about how axons are maintained and how they degenerate, only a handful of genes that trigger axonal degeneration have been identified. From forward genetic screening in *C. elegans*, we have identified the a-tubulin acetyltransferase gene *mec-17* as causing spontaneous, adult-onset, and progressive axonal degeneration. MEC-17/aTAT1 was recently identified as an a-tubulin acetyltransferase enzyme conserved across all ciliated organisms and found to be critical for microtubule organization, stabilizing their number and length, and defining protofilament number. We demonstrate that MEC-17 is essential for maintenance of axonal structure in the mechanosensory neurons, with mutants displaying degeneration of the axon in up to 45% of adult animals. Loss of MEC-17 led to microtubule instability, a reduction in mitochondrial number, and disrupted axonal transport, with altered distribution of both mitochondria and synaptic components. Furthermore, our results demonstrate that *mec-17*-mediated axonal degeneration occurs independently from its acetyltransferase domain, is enhanced by mutation of the tubulin-associated molecule COEL-1, and is strongly influenced by the animal's body length. Finally, in addition to its role in axonal maintenance we reveal that MEC-17 is crucial for axonal regeneration after injury. This study therefore identifies a critical role for the conserved microtubule-associated protein MEC-17 in preserving axon integrity and preventing axonal degeneration, as well as mediating axonal regeneration.

814C. Axonal degeneration in *C. elegans* proceeds independently from the WldS pathway. **Annika L. A. Nichols**, Brent Neumann, Ellen Meelkop, Massimo A. Hilliard. Queensland Brain Institute, The University of Queensland, Brisbane, QLD, Australia.

Distal axons of severed neurons undergo a stereotypic degeneration process consisting of thinning, beading, and fragmentation, which is commonly referred to as Wallerian Degeneration (WD). In mice, a dominant mutation causing the chimeric protein WLDS (Slow Wallerian Degeneration), that includes part of the ubiquitin fusion degradation protein 2a and the complete sequence of nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1), confers a strong delay of axonal degeneration. Through the identification and molecular cloning of *WldS*, axonal degeneration has been shown to be an active process that is controlled by a genetic pathway. Furthermore, studies on axonal degeneration in mice, rats, fruit flies, and zebrafish have shown that this process is highly conserved, and have identified several of the genes involved, including *Nmnat2/nmat-2, Sarm1/tir-1* and *Wallenda/dlk-1*. However, although severed *C. elegans* axons present a morphologically similar degeneration pattern to other organisms, the existence of a similar active axonal

degeneration pathway in this tractable model have yet to be investigated. To address this gap, we have characterized axonal degeneration in the PLM mechanosensory neurons and developed strains overexpressing WLDS as well as the endogenous *C. elegans* Nmnat homologs (NMAT-1 and NMAT-2) in these cells. We have investigated the effects of these molecules, as well as of TIR-1 and DLK-1, on axonal degeneration following laser-induced injury. Across a wide range of experimental paradigms, including differing expression levels, larval stages, and neuronal classes, we do not find a delay in axonal degeneration. However, we find that WldS overexpression can protect the neuron against necrosis induced by the dominant mec-4d mutation (which causes the MEC-4 calcium channel to be constitutively open), similarly to previous work with NMAT-2 (Calixto *et al.*, 2012). Therefore we propose that axonal degeneration in *C. elegans*, does not proceed through the active molecular mechanisms of the WD pathway as seen in mammals, zebrafish and fruit flies, suggesting that in *C. elegans* either this is a passive process or that there is another axon destruction program yet to be determined.

815A. Cell-Nonautonomous Inhibition of Radiation-Induced Apoptosis by Dynein Light Chain 1 in *Caenorhabditis elegans*. Tine H Møller, Anna Dippel Lande, **Anders Olsen**. Molecular Biology and Genetics, Aarhus Universtiy, Aarhus, Denmark.

Programmed cell death, apoptosis, is essential for development of multi-cellular organisms and is also a protective mechanism against cellular damage. We have identified dynein light chain 1 (DLC-1) as a new regulator of germ cell apoptosis in *C. elegans*. The DLC-1 protein is highly conserved across species and part of the dynein motor complex. In mammalian cells DLC-1 is important for cellular transport, cell division, regulation of protein activity and it has been implicated in cancer. There is, however, increasing evidence for dynein-independent functions of DLC-1 and our data describes a novel dynein-independent role. In C. elegans we find that knockdown of *dlc-1* by RNAi induces excessive apoptosis in the germline but not in somatic cells during development. We show that DLC-1 mediates apoptosis through the genes *lin-35*, *egl-1* and *ced-13*, which are all involved in the response to IR-induced apoptosis. In accordance with this, we show that IR cannot further induce apoptosis in *dlc-1(RNAi)* animals. Furthermore, we find that DLC-1 is functioning cell-nonautonomously through the same pathway as *kri-1* in response to IR-induced apoptosis. Interestingly, we find that the DLC-1 protein localizes to the cell membrane of apoptotic cells. Our results strengthen the notion of a highly dynamic communication between somatic cells and germ cells in regulating the apoptotic process. The possible functions of DLC-1 in this process will be discussed.

816B. TPPP/p25a causes degeneration of dopaminergic neurons in *C.elegans*. Katrine Christensen, Lotte Vestergaard, Rikke Kofoed, **Anders Olsen**. Molecular Biology and Genetics, Aarhus Universtiy, Aarhus, Denmark.

The presence of Lewy bodies and the degeneration of dopaminergic neurons are pathological hallmarks of Parkinson's disease (PD). Lewy bodies are mainly composed of a-synuclein (a-syn). Recently, the Tubulin Polymerization Promoting Protein (TPPP/p25a) was found to be associated with a-syn in Lewy bodies. The role of TPPP/p25a is largely unknown, but it has been shown to have a pro-aggregatory effect on a-syn. Furthermore, overexpression of TPPP/p25a in the presence of a-syn causes apoptosis in a rat oligodendroglial cell line. To study how TPPP/p25a may be causally involved in the neurodegeneration observed in PD and other a-synucleinopathies we have built a transgenic worm that overexpresses human TPPP/p25a fused to GFP specifically in dopaminergic neurons. This is the first multicellular organism overexpressing TPPP/p25a. We find that overexpression of TPPP/p25a results in neuronal atrophy in an age dependent manner. This is manifested by a change of the cell body shape going from a relatively round to an elongated, diffuse structure which eventually disappears. This phenotype can be observed already in the late larval stages. Overexpression of TPPP/p25a in an apoptosis deficient mutant, *ced-4*, results in the same morphological changes, showing that the phenotype is not due to apoptotic cell death. The use of a genetic approach where a number of double mutants have been constructed excludes involvement of the following genes: dopamine transporter *dat-1*, receptor tyrosine kinase *daf-2* and serine/threonine kinase *jnk-1*. Interestingly simultaneous overexpressing a-syn and TPPP/p25a rescues the dopaminergic ADE neurons from TPPP/p25a toxicity. We are currently investigating if this could be the result of a regulatory effect at the post-translational level.

817C. Bacterial metabolite causes mitochondrial dysfunction and oxidative stress in a *C. elegans* Parkinson's disease model. **A. Ray**, B.A. Martinez, G.A. Caldwell, K.A. Caldwell. Biological Sciences, Univ Alabama, Tuscaloosa, AL.

Parkinson disease (PD), the second most prevalent neurodegenerative disorder, is characterized by loss of dopaminergic (DA) neurons and the formation of protein inclusions that contain the a-synuclein (a-syn) protein. In *C. elegans*, overexpression of human a-syn, specifically in the eight DA neurons, causes neurodegeneration in an age- and dose-dependent manner, similar to that in PD patients. We are using this *C. elegans* model of PD to identify the causative factors of this disease. Growing experimental evidence suggest that exposure to environmental toxins may increase the risk of susceptibility to PD. Previously, our lab reported that a bacterial metabolite produced by *Streptomyces venezuelae* caused age- and dose-dependent DA neurodegeneration in *C. elegans* and human neurons (Caldwell et al., 2009, PLoS ONE). We hypothesized that the metabolite could exacerbate neurodegeneration in combination with PD susceptibility gene mutations. Indeed, we report hypersensitivity to the metabolite in *C. elegans* DA neurons expressing human a-syn in two PD-associated gene mutant backgrounds, *pdr-1* (*parkin*) and *djr-1.1* (*DJ-1*). Using another PD toxin model, 6-hydroxydopamine (6-OHDA), we demonstrate that exposure to more than one environmental risk factor has an additive effect in causing DA neurodegeneration. Evidence from the literature suggests that PD-related toxicants cause mitochondrial dysfunction. Thus, we are examining the impact of the metabolite on mitochondrial function and oxidative stress. Our findings show that DA neurodegeneration induced by the metabolite can be rescued by the mitochondrial complex I activator, Riboflavin and the complex II activator, D-beta-hydroxybutyrate (D-b-HB). Currently, we are further investigating the involvement of mitochondrial complex chains using genetic mutant studies. Using an *in vitro* assay, we determined that the bacterial metabolite causes excessive production of reactive oxygen species in *C. elegans*. Also, the anti-oxidant, Probucol, fully rescued metabolite-induced DA neurodegeneration *in vivo*. Taken together, this study suggests that the *S. venezuelae* metabolite causes mitochondrial dysfunction and oxidative stress, and ultimately, this leads to neuronal cell death.

818A. A Small-Molecule Screen for Linker Cell Death Inhibitors. **Andrew Schwendeman**, Shai Shaham. Rockefeller University, New York, NY.

Programmed cell death has a central role in development and disease. Apoptosis is the best characterized molecular cell death mechanism, but may not account for all deaths. Our lab has described a novel cell death program utilized to kill the male-specific linker cell of *C. elegans* at the L4-adult transition.

These studies show that linker cell death is independent of all known apoptotic genes, and exhibits morphological characteristics distinct from apoptotic cells, including open chromatin, nuclear envelope crenellation, and swollen mitochondria. Cell deaths exhibiting these features have been described in the developing vertebrate nervous system and in pathologies such as Huntington's disease. We have developed a high-throughput small-molecule screen to identify specific inhibitors of linker cell death. These reagents could be used as tools for studying the death mechanism and to test mechanistic overlap between linker cell and vertebrate cell death and degeneration. Animals expressing a linker cell::GFP reporter are grown on *E. coli* OP50 until the L4 stage, washed, and then incubated for 10 hours in multi-well plates with compounds derived from small molecule libraries. A fluorescence plate reader automatically counts linker cells, allowing preliminary hits to be identified. Visual examination of wells allows identification of spurious results caused by precipitation of compounds or autofluorescence. Identified small molecules are analyzed for their IC_{50} and for the specificity of their effects on linker cell death. A pilot screen of 1280 compounds identified several that result in persistent fluorescent linker cells. One of these, Tyrphostin A9, has an IC_{50} in the nanomolar range and is ideally suited for use as a positive control for screening larger libraries. Screening of additional libraries using this assay is currently ongoing.

819B. *C. elegans* clathrin and its adaptor epsin promote apoptotic-cell engulfment through regulating cytoskeleton remodeling. **Qian Shen[1]**, Bin He[1], Nan Lu[1], Barbara Conradt[2], Barth D. Grant[3], Zheng Zhou[1]. 1) Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX, USA; 2) Center for Integrated Protein Science, Department of Biology II, Ludwig-Maximilians-University, Munich, 82152 Planegg-Martinsried, Germany; 3) Department of Molecular Biology and Biochemistry, Rutgers, the State University of New Jersey, New Jersey, USA.

The engulfment and subsequent degradation of apoptotic cells by phagocytes is an evolutionarily conserved process that efficiently removes dying cells from animal bodies during development. Here we reported that clathrin heavy chain (CHC-1), key component of a vesicle coating protein clathrin, and its adaptor protein epsin (EPN-1), play crucial roles in removing apoptotic cells in *C. elegans*. Clathrin is a coat protein well known for its function in receptor-mediated endocytosis, but unknown for acting in phagocytosis, neither are epsins or other clathrin adaptors. Our study has identified the novel roles of clathrin and epsin in phagocytosis. Inactivating *epn-1* or *chc-1* specifically reduces the speed of engulfment through impairing actin polymerization, the driving force for engulfment. Clathrin-actin crosstalk not only induces membrane curvature, but also directs actin polymerization and drives pseudopod extension around apoptotic cells. Epistasis analysis places *epn-1* and *chc-1* in the same genetic pathway as *ced-1, ced-6, ced-7,* and *dyn-1* for cell-corpse engulfment. The CED-1 signaling pathway is necessary for the pseudopod enrichment of EPN-1 and CHC-1. As a result, CED-1, CED-6, and DYN-1, like EPN-1 and CHC-1, are essential for the assembly and stable maintenance of actin fibers along pseudopods, indicating that in addition to driving 'focal exocytosis' for membrane expansion, the CED-1 signaling pathway also regulates the remodeling of the cytoskeleton for cell-corpse engulfment. Our work identified a novel mechanism employed by clathrin and its adaptor to promote pseudopod extension and the engulfment of apoptotic cells, and ties the CED-1 signaling pathway to the actin cytoskeleton.

820C. Glucose influences aging, proteotoxicity and stress response in *C. elegans*. **Arnaud Tauffenberger[1,2,3]**, Alexandra Vaccaro[1,2,3], J. Alex Parker[1,2,3]. 1) CRCHUM; 2) Département de Pathologie et Biologie cellulaire, Université de Montréal; 3) Center of excellence in neurosciences.

In developed countries, it is believed that over consumption of carbohydrates and fat is responsible for many metabolic disorders, including obesity, type 2 diabetes and coronary diseases. These disorders exact enormous costs on health systems and research into mechanisms and therapeutic approaches are of obvious importance. Conditions that reprogram metabolism, like dietary restriction, have become active areas of investigation. Work from yeast to primates has demonstrated that dietary restriction may not only increase lifespan, but also more importantly, maintain healthspan. Aging societies are also burdened by the increasing incidences of age-related diseases including late onset neurological disorders including Alzheimer's disease, Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS). Metabolic dysfunction in neurodegeneration, and in particular the role of glucose metabolism is not completely understood. To investigate the role of glucose metabolism in aging and proteotoxicity, we used *C. elegans* transgenic models expressing human protein TDP-43 in the motor neurons. Mutations in TDP-43 are causative for ALS leading to the loss of motor neurons in patients. Our transgenic TDP-43 worms display motility defects leading to age-dependent paralysis and the degeneration of GABAergic motor neurons. Surprisingly, we have observed that glucose has the capacity to rescue age-dependent proteotoxicity, perhaps by restoring global protein homeostasis. However, excess glucose has deleterious effects on organismal lifespan. Many of the genes required for the glucose-induced phenotypes are in common with dietary restriction pathways suggesting that the two conditions use the same pathways but with opposite endpoints. A summary of our recent data will be presented.

821A. Cortical HAM-1 positions the cleavage furrow in myosin-dependent asymmetric neuroblast divisions that generates apoptotic cells. **Jerome Teuliere[1]**, Nancy Hawkins[2], Gian Garriga[1]. 1) MCB, Univ California, Berkeley, Berkeley, CA, USA; 2) MBB, Simon Fraser University, Burnaby, BC, Canada.

While the mechanisms that execute apoptosis in *C. elegans* are understood, less is known about how somatic cells choose the apoptotic fate. Cell divisions that produce apoptotic cells are asymmetric, generating a larger cell that lives and a smaller cell that dies. Two examples are the anterior (Q.a) and posterior (Q.p) daughters of the Q cell. Distinct mechanisms, however, shift the position of the cleavage furrow in these two divisions to produce an anterior apoptotic cell (Q.aa) division and a posterior apoptotic cell (Q.pp) [3]. Protrusion of the posterior membrane and anterior localization of myosin II produce an anteriorly-shifted Q.a cleavage furrow. Protrusion of the anterior membrane and posterior displacement of the mitotic spindle produce a posteriorly-shifted Q.p furrow. We find that the winged-helix protein HAM-1 specifically controls the Q.a furrow position. Q.a divided with a reversed polarity in *ham-1* mutants to produce a larger anterior and a smaller posterior daughter, a Q.p division pattern. As in the wild-type Q.p division, the anterior Q.a membrane protrudes and the spindle is displaced posteriorly. The asymmetric distribution of myosin II remained asymmetric in QR.a, indicating that while myosin asymmetry is required for an anteriorly positioned Q.a cleavage furrow [3], it is not sufficient in QR.a. A functional GFP::HAM-1 transgene had a Q.a-specific expression, consistent with the *ham-1* phenotype. Although HAM-1 antibodies only detected cortical HAM-1 in dividing cells [4], we detected HAM-1::GFP at the cortex and in the nucleus of both embryonic cells and in the Q lineage. Misexpression of HAM-1 in Q.p led to daughter size asymmetry defects. We observed this defect with either wild-type HAM-1 or a mutant that does not accumulate in the nucleus, suggesting that HAM-1 acts at the cortex to position the cleavage furrow. The spectrum of *ham-1* mutant defects suggests that HAM-1 primarily regulates a subset of divisions

that produce anterior cells fated to die. [3] Ou, G et al. Science 330, 677-680 (2010) [4] Guenther, C & Garriga, G. Development 122, 3509-3518 (1996).

822B. The DNA binding protein *dpff-1* is required to trigger starvation-induced germ cell apoptosis. **Angel E. Villanueva Chimal**, Rosa E. Navarro. Departamento de Biología Celular y Desarrollo, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México. México.

In *C. elegans*, physiological apoptosis eliminates fifty percent of germ cells during oogenesis. Higher levels of germ cell apoptosis can be triggered by stressful conditions such as DNA damage, starvation, heat shock, oxidative and osmotic stress. DNA damage-induced apoptosis requires, EGL-1, the p53 protein and the DNA damage repair machinery. Heat shock, oxidative and osmotic stresses trigger germ cell apoptosis by the MAPKK pathway, and through an EGL-1 and CEP-1 independent mechanism. The mechanisms that regulate physiological apoptosis and starvation-induced germ cell apoptosis are still unknown.

Our aim is to understand how physiological and starvation-induced germ cell apoptosis is regulated. In an ongoing screening to identify proteins that participate in these types of apoptosis we found DPFF-1, a member of the D4 zinc and double PHD fingers transcription factor family.

In semi-quantitative RT-PCR experiments we found that *dpff-1* mRNA is expressed in somatic and germ cells under normal growing conditions, but its accumulation is lower when animals are starved for 6 h. To study DPFF-1 *in vivo* expression in *C. elegans*, we generated a transgenic strain carrying a GFP::DPFF-1 construct by MosSCI. The GFP signal was detected in nuclei of embryos, larval and adult animals in germ, neuronal, hypodermal, intestinal and somatic gonad cells.

dpff-1(RNAi) animals do not have defects in fertility, but they do have a decrease in the number of oocytes per gonad. Also, *dpff-1*(RNAi) animals showed defects on the onset of mitosis and meiosis. *dpff-1*(RNAi) well-fed animals have higher levels of physiological apoptosis (1.95) when compared to control. On the contrary, *dpff-1*(RNAi) animals did not respond to starvation-induced germ cell apoptosis. We are currently studying *dpff-1*'s role in germ cell apoptosis.

823C. Characterization of a Chloride-Mediated Cell Death Pathway in *C.elegans* PLM Neurons. **Claudia M. Wever**, Aamna Kaul, Miles Byworth, Joseph A. Dent. Biology, McGill University, Montreal, Quebec, Canada.

Sodium-mediated cellular excitotoxicity is process in which cells with hyperactive cation channels die by necrotic cell death. This cell death pathway has been studied in *C.elegans* and involves a characteristic swelling of affected cells followed by removal of the cell corpse through autophagy. Chloride-mediated cell death, however, has not been well characterized. We have generated a transgenic strain that expresses the ivermectin (IVM)-sensitive chloride channel subunit AVR-15 in mechnosensitive cells under the mec-7 promoter. We have also engineered this strain to have GFP-labeled mechanosensitive neurons. Upon exposure to IVM, the AVR-15 channels are activated, causing excessive chloride influx into the touch receptor cells, including the GFP-tagged PLM neurons. In this strain, the PLM neurons die upon IVM treatment but the PLM neurons cells do not resemble the swollen, vacuole-filled cells characteristic of sodium-mediated cell death. Rather, the PLM neurons shrink, forming a button-like morphology characteristic of apoptosis, indicating that the chloride-mediated cell death pathway differs from the excitotoxic pathway. To determine if chloride-mediated cell death shares components of known cell death pathways, we are examining chloride-mediated cell death in genetic backgrounds defective in apoptosis (ced-3, ced-4 ced-5, ced-7) and resistant to necrosis (unc-51, unc-68, crt-1). To determine the specificity of this effect to chloride permeability, we are looking at the effect of mutations in the kcc-2 chloride transporter. We have also constructed a cation-selective AVR-15 and will test whether it results in cell swelling or cell shrinkage when exposed to IVM. Preliminary results indicate that increased chloride permeability represents a unique cell death pathway distinct from necrosis and possibly downstream of apoptosis.

824A. Genetic modifiers of amyloid-beta toxicity in *C. elegans* Alzheimer's disease models. **Xiaohui Yan**, Adam L. Knight, Kim A. Caldwell, Guy A. Caldwell. Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL, 35487.

The insulin/insulin-like growth factor 1 signaling (IIS) pathway regulates both aging and proteotoxicity and is conserved from invertebrates to mammals. In humans, several age-related neurodegenerative disorders such as Alzheimer's disease (AD) and Parkinson's disease (PD) are associated with protein misfolding or aggregation. Others and we have shown that the reduction in IIS ameliorates the proteotoxicity associated with amyloid b (Ab) aggregation in AD, and a-synuclein (a-syn) aggregation in PD *C. elegans* models. Therefore, we hypothesized that there could be common cellular mechanisms to maintain the proteostasis for counteracting a-syn and Ab misfolding during aging. In this regard, we screened sixty age-related genetic factors that modify a-syn aggregation, in a *C. elegans* AD model in which temperature-inducible muscle expression of human Ab_{42} leads to a reproducible paralysis phenotype. We identified seven genes that accelerate Ab-induced paralysis upon RNAi. These modifiers include components of the protein clearance and select metabolic factors, including two genes encoding independent but functionally related enzymes, the serine hydroxymethyltransferase (SHMT) and the aminomethyltransferase (AMT) of the glycine cleavage system (GCS). The reactions catalyzed by SHMT and GCS generate one-carbon units for folate-dependent one-carbon metabolism whose normal function supports a variety of cellular activities. When AMT was overexpressed in our *C. elegans* neuronal AD model, where Ab_{42} accumulation induces an age-dependent degeneration of glutamatergic neurons (Treusch et al., 2011, Science), the neuronal loss was significantly rescued. This study provides the first evidence of a role for SHMT and GCS in modulating a-syn and Ab related proteotoxicity. Interestingly, alterations in one-carbon metabolism have been linked to epidemiological and genetic association studies for AD risk, yet, the underlying cellular mechanisms are largely unknown. Taken together, these findings will potentially expand our mechanistic understanding of the well established, but poorly understood, interaction between metabolism, especially the folate-dependent one-carbon metabolism, and age-associated neurodegenerative diseases.

825B. Comparisons of three *C. elegans* DNase II activities *in vitro* and *in vivo*. **Hsiang Yu**[1], Szecheng J. Lo[1,2]. 1) Graduate Institute of Biomedical Sciences, Chang Gung university, Taoyuan city, Taiwan; 2) Department of Biomedical Sciences, Chang Gung university, Taoyuan city, Taiwan.

DNase II is an acidic DNase which plays an important role in the degradation of apoptotic cell DNA from a wide spectrum of animals. In *C. elegans*, three DNase II genes, *nuc-1*, *crn-6* and *crn-7*, are identified, in contrast two DNase II (a and b) are found in mammals. There are three waves of cell apoptosis, embryonic, larval stage and oogenesis during *C. elegans* development. The role of *nuc-1* and *crn-6* in embryonic and larval development have been

demonstrated; however enzymatic activities of three DNase II remain obscure. Here, we report three methods for detecting NUC-1, CRN-6 and CNR-7 activities *in vitro* and *in vivo*. First, using metachromatic agar-diffusion assay (MADA), DNase II activity in embryonic extracts from wild-type and mutant animals were examined. Results showed that NUC-1 had the highest DNase II activity while CRN-6 and CRN-7 exhibited 10 fold lower activity than NUC-1. Second, recombinant proteins of GST fusion with NUC-1, CRN-6 and CRN-7 were generated in *E. coli*. The enzymatic activity of purified DNase II fusion proteins were analyzed using non-denaturing PAGE. DNA digestion by NUC-1 and CRN-7 was demonstrated in gels, but little or no DNA digestion by CRN-6. Thirdly, a technique which directly labels the DNase II-type breaks with a fluorescent probe was applied to detect the DNA fragmentation in embryos. Results showed that the fluorescent signals in *nuc-1* mutant are twice less than those in wild-type, *crn-6* and *crn-7* embryos. This result is consistent to the TUNEL assay in embryo as demonstrated before. In the future, the technique of direct libeling DNase II-type breaks can be employed to study roles of three DNase II in germ-line apoptosis.

826C. Functional characterization of *lin-41* and its targets. **Hrishikesh Bartake**. Friedrich Miescher Institute, Basel, Switzerland.

lin-41 belongs to the TRIM-NHL class of proteins, which are defined by the presence of a TRIM domain comprising RING finger, B-Box, Coiled-coil motifs and NHL repeats. In *C. elegans*, *lin-41* serves as an important developmental regulator involved in switching between larval and adult cell fates. *lin-41* loss-of-function mutation causes hypodermal seam cells to cease proliferation and prematurely differentiate, whereas its continued expression leads to over-proliferating cells. The human LIN-41 ortholog TRIM71 is highly expressed in stem and progenitor cells and its knockdown results in increased cell differentiation. Recent studies revealed functions of TRIM71 involving protein ubiquitylation and post-transcriptional gene silencing. However, little is known about how *C. elegans lin-41* mechanistically executes cell cycle exit and terminal differentiation during larval-to-adult transition and so far its molecular targets have remained unknown. To understand the molecular and developmental roles of *C. elegans lin-41*, we have generated a worm line expressing tagged LIN-41 protein, which rescues the *lin-41* null mutant phenotype. We confirm previous results showing expression of LIN-41 in hypodermis, neurons, muscle cells and somatic gonad. We also find that LIN-41 is localized to cytoplasm, where it shows a speckled distribution as well as localization to the nuclear envelope. Currently we are performing co-immunoprecipitation to identify protein binding partners of LIN-41. Constructs with TRIM or NHL domain deletions will allow us to determine their function *in vivo* by observing phenotypes in mutant animals, which should help shed light on the pathways regulated by *lin-41*. Moreover, to complement our immunoprecipitation data we will perform HITS-CLIP and assess the mRNAs that are associated with LIN-41 protein The results that we obtain in this study will further contribute to our understanding of the roles played by *lin-41* in maintaining critical balance between cell proliferation and differentiation.

827A. Activity and functional interactions of the leucine-rich protein PAN-1 during larval development. **Derrick L. Cardin**, Chris R. Gissendanner. College of Pharmacy, University of Louisiana at Monroe, Monroe, LA. 700 University Avenue Monroe, La 71201.

PAN-1 is a novel nematode-specific leucine-rich (LRR) protein that exhibits complexity in both structure and function. The pan-1 gene encodes three protein isoforms: a predicted type I transmembrane protein with extracellular LRRs and two cytoplasmic proteins, one with a smaller number of LRRs and one lacking LRRs. PAN-1 participates in multiple developmental processes. It associates with P-granules in the germline (Gao et al, 2012) and is also required for larval development. For the latter, pan-1 is necessary for early larval development and the L4 to adult transition. pan-1 promotes growth of the germline, somatic gonad, and vulva during later larval stages and is required for progression of the L4 to adult molting cycle. To better understand the function of PAN-1 during larval development we have initiated a series of studies to address the cellular activities of this protein. Expression of the transmembrane isoform of PAN-1 lacking a cytoplasmic domain is dominant-negative, indicating that PAN-1 may dimerize and transduce an intracellular signal. Using these dominant-negative effects as an assay, we are performing conditional expression of different cytoplasmic domain-deleted constructs to identify critical regions of the intracellular domain. We are also characterizing interactions between pan-1 and other genes. We have found that lin-29 loss of function suppresses the molting progression and vulva development phenotypes in pan-1 RNAi animals. We are further characterizing this interaction to determine how pan-1 intersects with lin-29 in molting regulation. In addition, loss of function of lron-12, which encodes a secreted LRR protein, enhances the pan-1 RNAi phenotype. lron-12 mutants exhibit a larval growth phenotype indicating that pan-1 and lron-12 function together to regulate larval development. We propose that PAN-1 participates in a novel nematode-specific signaling pathway regulating life cycle progression.

828B. Roles of *C. elegans* LIN-28 in hermaphrodite fertility and embryonic viability. **Sungwook Choi**, Anna Zinovyeva, Victor Ambros. Program in molecular medicine, U Mass medical school, Worcester, MA.

lin-28 was first characterized as a developmental timing regulator in *C. elegans*. *lin-28* encodes an RNA-binding protein whose functions include regulating the level of *let-7* microRNA. We found that *C. elegans lin-28* loss of function (*lin-28(lf)*) mutants exhibit temperature sensitive fertility defects. *lin-28(lf)* hermaphrodites have an average brood size of 20 progeny at 20°C, and are essentially sterile at 25°C. Our data show that *lin-28(lf)* mutants not only produce fewer embryos than wild type, but *lin-28(lf)* embryos also exhibit about 72% lethality. We found that *lin-28(lf)* hermaphrodites exhibit ovulation and spermathecal exit defects. Some *lin-28(lf)* oocytes contain endomitotic DNA, which is a characteristic of ovulation mutants. Many *lin-28(lf)* embryos become trapped in the spermatheca, suggesting defects in spermathecal exit. Moreover, we found that *lin-28(lf)* embryos are more permeable to liphophilic dye than wild type embryos. This finding implies that *lin-28(lf)* embryos have abnormal egg shell integrity, which contributes to the embryonic lethality of *lin-28(lf)* mutants. Investigating the genes downstream of *lin-28* in fertility function, we found that *lin-28(lf);let-7(lf)* double mutants show increased brood size compared to *lin-28(lf)* mutants, indicating that the fertility defects are partially caused by *let-7* hyperactivity. Also, loss of *lin-29,* a gene downstream of *let-7* in the heterochronic pathway can partially suppresses the fertility defects of *lin-28(lf)* mutants. We are now investigating whether the fertility defects of *lin-28(lf)* hermaphrodites originate from their development abnormalities, and we are determining the anatomical focus of action of LIN-28 for hermaphrodite fertility.

829C. Significant Transcription in Zygote Pronuclei and 1-4 Cell Embryos Drives Early Development in the Nematode, *Ascaris suum*. **Richard E. Davis**, Julianne Roy, Jianbin Wang. Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Aurora, CO.

During early *C. elegans* development, zygote maturation and early embryonic development are typically characterized by an absence of mRNA

transcription, and regulation of gene expression during this period is primarily post-transcriptional. We took advantage of the availability of distinct stages of zygote maturation (prior to pronuclear fusion) and early embryo development to provide a unique and comprehensive time course of mRNA expression, turnover, and translation in early development of the parasitic nematode *Ascaris suum*. RNA-seq data on zygotes undergoing maturation prior to pronuclear fusion and 1, 2, 4-cell, and later stages of early development strikingly demonstrate that a large number of genes are transcribed during zygote maturation and in the 1-4 cell embryos of *A. suum*. This differs from *C. elegans* and the general view that transcription is quiescent until at least the 2-cell stage in metazoa. Much less maternal mRNA is contributed from the oocytes in *Ascaris* compared to that in *C. elegans*. We find that the orthologs of many maternal *C. elegans* mRNAs are not maternally contributed in *A. suum*, but are transcribed during *A. suum* zygote maturation prior to pronuclear fusion and in the early embryo. Ribosome profiling of 1-cell, 4-cell, 32-64 cell, and 250 cell embryos mRNAs demonstrated that, in general, mRNAs do not appear to be made and stored for subsequent translation, but are directly translated following their synthesis. Our data indicate that the roles of maternally contributed and zygote transcribed genes differs between *A. suum* and *C. elegans* despite the fact that the two nematodes appear to exhibit identical morphological patterns in early development. In *Ascaris*, maternal mRNA contribution is minimal, and newly transcribed genes appear to drive early development. This suggests that mechanisms used for controlling the timing of the expression of key conserved genes has been altered between the two nematodes, illustrating significant plasticity in the regulatory networks that play important roles in developmental outcomes in nematodes.

830A. Regulation of Developmental Timing and Cell-Fate Determination by MAB-10 and LIN-29. **Akiko Doi**, Bob Horvitz. Dept. Biology, MIT, Cambridge, MA.

For the proper development of a multicellular organism, appropriate cell-fate decisions must occur with spatial and temporal specificity. To reveal fundamental mechanisms that control cell fate decisions, we are studying the heterochronic pathway of C. elegans. This evolutionarily conserved pathway controls the temporal progression of development by regulating the activities or gene-product levels of a succession of genes. Many components of this pathway have mammalian homologs that play critical roles in stem cell maintenance and differentiation and are emerging as central to a variety of basic problems in biology such as carcinogenesis and aging. Although some of the key regulators have been identified, the mechanisms through which they act are not well understood.

For example, the mechanisms of action and regulation of the conserved heterochronic genes mab-10 and lin-29 have yet to be identified. mab-10 was discovered in our laboratory to encode the C. elegans NGFI-A-binding protein (NAB) transcriptional co-factor; MAB-10 is involved in the terminal differentiation of the hypodermal stem-like seam cells and more generally in the larval-to-adult transition (Harris & Horvitz, *Development* 138, 4051, 2011). LIN-29, the master regulator of the larval-to-adult transition, was shown to be an early growth response (EGR) protein that acts together with MAB-10 to control the expression of genes that regulate the onset of adulthood and terminal differentiation in the hypoderm. MAB-10/NAB and LIN-29/EGR are critical to the control of developmental timing in C. elegans, and understanding the function and regulation of MAB-10 and LIN-29 should provide important insights concerning development in mammals. To this end, we are analyzing the mechanisms by which MAB-10 and LIN-29 exert their functions and are screening for factors that enhance or suppress the mab-10 and lin-29 mutant phenotypes.

831B. A *lin-42* null allele: highly penetrant defects in developmental timing and molting. **Theresa L B Edelman**[1], Katherine A McCulloch[1], Angela Barr[1], Christian Frøkjaer-Jensen[2], Erik M Jorgensen[2], Ann E Rougvie[1]. 1) Genetics, Cell Biology and Development Department, University of Minnesota, Minneapolis, MN; 2) Biology Department, University of Utah, Salt Lake City, UT.

The *C. elegans* heterochronic gene *lin-42* and the *period* family of circadian rhythm genes from flies and vertebrates provide an intriguing example of gene conservation paralleled by functional conservation; all are involved in biological timing mechanisms. LIN-42 times stage-specific developmental programs and molting cycles during larval development, while Per proteins synchronize various processes with the 24-hr clock. Remarkably, LIN-42, like its Period homologs, exhibits a dynamic, oscillatory expression pattern with levels peaking once in each larval stage. LIN-42 and Period proteins share multiple regions of homology, notably a PAS domain that mediates protein-protein interactions and smaller SYQ and LT domains of unknown function. *lin-42* encodes four isoforms, two of which do not overlap. One of these non-overlapping isoforms contains the PAS domain, while the other contains the SYQ and LT domains; the remaining two isoforms contain all of these homology domains. Previously described *lin-42* mutations leave at least one isoform intact, complicating genetic and phenotypic analyses. We generated a null allele using mosDEL technology to delete the entire *lin-42* coding region. *lin-42(0)* mutants display a strong precocious heterochronic phenotype similar to *lin-42(lf)* alleles. In addition, *lin-42(0)* mutants have severe molting defects, similar to those seen in *lin-42(ok2385)*, a deletion that spans the SYQ and LT domains. The majority of *lin-42(0)* animals arrest during early larval development, a phenotype present in *lin-42(ok2385)* mutants and likely to be a result of failed ecdysis. However, the larval arrest phenotype is statistically more penetrant in the null, suggesting that the PAS-containing isoform still present in *lin-42(ok2385)* mutants contributes to regulation of molting. The SYQ-LT containing isoform can rescue the null when over-expressed, indicating it is key to LIN-42 function, while the other isoforms may play more regulatory role(s). A null allele now allows epistasis analysis and will facilitate placement of *lin-42* in the heterochronic and molting pathways.

832C. Hypodermis integrates nutrient signaling to regulate blast cell quiescence. **M. Fukuyama**[1], K. Kontani[1], A. Rougvie[2], T. Katada[1]. 1) University of Tokyo, Bunkyo, Tokyo, Japan; 2) University of Minnesota, MN, USA.

Nutritional regulation of stem and blast cell quiescence underlies growth and tissue homeostasis. Caenorhabditis elegans larvae hatched under nutritionally poor conditions can maintain developmental quiescence in stem and blast cells until ample food (E. coli) is supplied (L1 diapause or L1 arrest). Although previous studies suggested that the insulin/IGF signaling (IIS) pathway plays an important role in this nutritional response, little is understood about how the nutritional availability is sensed and relayed to trigger release from the developmental quiescence via the pathway. We found that feeding ethanol and ectopic activation of the IIS pathway in the hypodermis together can release neuroblasts and mesoblast from L1 diapause. Feeding ethanol and amino acids can induce not only exit from L1 diapause, but also expression of several insulin-like peptides, like E. coli. In addition to insulin-like peptides, the hypodermis probably senses amino acids, since constitutive activation of raga-1 in the tissue, whose mammalian orthologues mediate amino acid signaling to TORC1, can also cause neuroblasts and mesoblast to initiate development in the presence of ethanol. These findings suggest that the hypodermis mediates sensing the nutrient availability to coordinately release quiescence in neuroblasts and mesoblast via the IIS-TORC1 pathway.

833A. Dynamically-expressed prion-like proteins form a cuticle in the pharynx of *Caenorhabditis elegans*. **J.B. George-Raizen**[1], K.R. Shockley[2], A.L. Lamb[1], D.M. Raizen[1]. 1) Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 2) Biostatistics Branch, National Institute of Environmental Health Sciences, NIH, Department of Health and Human Services, Research Triangle Park, NC.

In all molting animals, a cuticular extracellular matrix forms the first barrier to infection and other environmental insults. In *C. elegans* there are two types of cuticle, a well-studied collagenous cuticle that lines the body, and a poorly-understood chitinous cuticle that lines the pharyngeal lumen and buccal cavity. By performing a transcriptional profiling experiment in precisely staged developing larvae, we find strong induction during the molt of *abu/pqn* genes encoding secreted prion-like (P) glutamine (Q) and asparagine (N) rich PQN proteins. 32 cysteine-rich PQN proteins form an ABU/PQN paralog group and of the 30 *abu/pqn* genes represented on the affymetrix array, 27 are transcriptionally induced during periods of cuticular synthesis. Transcriptional reporters for eight randomly selected *abu/pqn* genes are expressed in pharyngeal muscle. Translational fluorescent reporters for ABU/PQN proteins are expressed on the cuticular apical side of pharyngeal muscle and are shed during the molt. Disrupting *abu/pqn* gene function results in abnormal pharyngeal cuticular structures and abnormal feeding. Prior reports of modest transcriptional activation of *abu/pqn* genes in response to genetic, infectious, and chemical perturbations, can be explained by the unappreciated presence of molting animals in those experiments. Our findings suggest that the expression of the *abu/pqn* genes is regulated by a developmental timing program in the *C. elegans* pharynx where they promote the assembly and function of unique cuticular structures.

834B. LEP-2/Makorin represses LIN-28 to keep nematode tail tip differentiation on schedule. **R. Antonio Herrera**, Karin Kiontke, David Fitch. Center for Developmental Genetics, Biology, New York Univ, New York, NY, 10003.

Heterochronic genes regulate the timing of the larval-to-adult (L/A) switch. During the L/A switch to adulthood, the gonad reaches reproductive maturity and somatic tissues (seam cells, body epidermal cells, the hermaphrodite vulva and male tail tip) undergo terminal differentiation. During terminal differentiation (morphogenesis) of the tail tip, the four posterior cells fuse and change shape to produce a round tail tip in adult males. We found a new gene in the heterochronic pathway, *lep-2*. Loss-of-function *lep-2(*lf*)* mutants have a delay in the onset of tail tip morphogenesis from L4 into adulthood, leading to adults with pointed larval-like ("leptoderan") tail tips. *lep-2(*lf*)* also leads to a failure in the cessation of larval molts and the production of adult cuticle. Contrary to other known heterochronic genes, *lep-2* has no effect on seam cell differentiation. Given the delayed developmental phenotypes of *lep-2(*lf*)* mutants we propose that *lep-2* acts in the heterochronic pathway to schedule tail tip morphogenesis. Our previous work showed that *let-7* and *lin-41* regulate this process; here, we show that the L/A switch is scheduled in the male tail tip by core heterochronic genes *lin-4*, *lin-14* and *lin-28*. Using genetic epistasis analysis of double mutants and scoring for male tail tip phenotypes, we find that *lin-14* represses *lep-2*, which represses *lin-28*. Consistent with these results, *lep-2* mutant animals fail to downregulate LIN-28 post L2. We mapped *lep-2*, and found that it is a Makorin (Mkrn) zinc finger RING domain protein. Mkrns are conserved eukaryotic genes from humans to nematodes to sea squirts. Indeed, the nematode function of Mkrn in differentiation may be conserved; Mkrn1 promotes differentiation in human stem cells (hSCs). Heterochronic genes (*lin-4*/mir-125a and *let-7* and LIN-28 and LIN-41/TRIM71) have been shown to regulate the cellular switches between pluripotent and differentiated cellular states in hSCs. We speculate that LEP-2/Mkrn and other conserved heterochronic genes regulates the cellular differentiation switch in eukaryotes.

835C. The *C. elegans* plasminogen/HGF-like protein SVH-1 is required for larval developmental growth. **Naoki Hisamoto**, Motoki Yoshida, Chun Li, Kunihiro Matsumoto. Dept Biological Sci, Nagoya Univ, Nagoya, Aichi, Japan.

The plasminogen family consists of both the active proteases including plasminogen and the protease-inactive growth factors including hepatocyte growth factor (HGF). *C. elegans* plasminogen/HGF-like protein SVH-1 acts as a growth factor-like molecule that regulates axon regeneration via an HGF receptor-like protein SVH-2 and KGB-1 MAP kinase cascade (Li et al., *Nature Neurosci*. 15, p551, 2012). Interestingly, we found that the *svh-1* null mutation caused the larval lethal phenotype, which was not observed in *svh-2* or *kgb-1* null mutants. The *svh-1* null mutant worms arrested at L2 stage with the starved appearance, suggesting that the mutant worms have a defect in taking foods. The feeding assay using the fluorescent beads and the observation of the pharyngeal pumping revealed that *svh-1* mutant worms exhibited the progressive feeding defect associated with abnormal pharyngeal contraction. Rescue experiments showed that protease activity was essential for larval growth but not for axon regeneration. These results suggest that SVH-1 is involved in the regulation of pharyngeal contraction via its protease activity.

836A. BLMP-1/BLIMP1 - a novel substrate of the DRE-1/FBXO11 SCF complex that regulates *C. elegans* developmental timing. **Moritz Horn**, Christoph Geisen, Adam Antebi. Max-Planck-Institute for Biology of Ageing, Cologne, Germany.

The precise timing of distinct cellular events is fundamental to organismal development. In *C. elegans* temporal selector genes, called heterochronic loci, regulate stage specific cellular programs during larval development. Many heterochronic genes are evolutionarily conserved and control temporal patterning of cell division, migration and differentiation events across taxa. *dre-1* specifies *C. elegans* late larval development by preventing precocious expression of adult specific seam cell fates, and encodes a highly conserved F-Box protein orthologous to human FBXO11 (Fielenbach et al., Dev Cell 2007). F-box proteins function as substrate recognition components of SCF E3-ubiquitin ligase complexes. Recently, Pagano and colleagues identified two substrates of human FBXO11: BCL-6 (Duan et al., Nature 2012) and CDT-2 (Rossi et al., Mol Cell 2013). However, the DRE-1/FBXO11 substrate(s) involved in developmental timing remains largely unknown. From RNAi-based suppressor screens, we identified the Zn finger transcription factor BLMP-1 as a novel substrate of the $SCF^{DRE\text{-}1/FBXO11}$ E3-ubiquitin ligase complex. *blmp-1* depletion strongly suppressed *dre-1* mutant developmental timing defects as well as those of other precocious heterochronic loci such as *hbl-1*, *lin-41* and *lin-42*. *blmp-1* loss of function mutants showed alterations in seam cell fates and gonadal outgrowth establishing *blmp-1* as a heterochronic gene. Consistent with BLMP-1 being a DRE-1 substrate, BLMP-1 protein levels were strikingly elevated upon *dre-1* depletion. *blmp-1::GFP* expression was regulated in a stage and tissue specific manner consistent with the corresponding phenotypes. Importantly DRE-1 and BLMP-1 protein interacted in vivo, and the mammalian counterparts co-precipitated in cell culture, revealing an evolutionary conserved interaction between these two proteins. Mammalian BLIMP1 is the key determinant of B cell maturation and has been implicated in stem cell biology and cancer. Our studies show that post-translational regulation of *C. elegans* BLMP-1 by DRE-1/FBXO11 is critical for coordination of

developmental timing and maturation, suggesting that these two proteins may work together to regulate related processes throughout taxa.

837B. Ascaroside signals suppress heterochronic phenotypes of the *daf-12(rh61)* mutant. **Orkan Ilbay**[1], Zhiji Ren[1], Jagan Srinivasan[2], Frank C. Schroeder[3], Victor Ambros[1]. 1) Molecular Medicine, Umass Medical School, Worcester, MA; 2) Dept of Biol and Biotech, WPI, Worcester, MA; 3) Boyce Thompson Institute and Dept of Chem and Chem Biol, Cornell University, Ithaca, NY.

During animal development, cells divide and adopt specific fates and progressively differentiate into distinct cell types. The relative timing of a series of developmental events determines morphological endpoints and may affect the physiology of an organism. In *Caenorhabditis elegans* development, there are four larval stages (L1-L4), and all cell division and differentiation events at all stages have been mapped. Heterochronic genes, whose products include developmentally-expressed microRNAs and transcription factors, control the temporal patterning of developmental events, and contribute to developmental robustness. One of the components of the heterochronic pathway, DAF-12, promotes L2-to-L3 cell fate transitions by positively regulating the transcription of certain *let-7* family microRNAs. Under unfavorable conditions, such as crowding, scant food supplies, or elevated temperature, *C. elegans* L2 larvae can commit to developmental arrest as the stress-resistant dauer larva. In such unfavorable conditions, DAF-12 negatively regulates progression to L3 cell fates, in part by repressing expression of *let-7* family microRNAs, and promotes dauer formation. Several *daf-12* mutations have been characterized as causing defective dauer regulation or heterochronic phenotypes, or both. In particular *daf-12(rh61)* results in expression of a truncated form of DAF-12. *daf-12(rh61)* mutant animals are dauer-defective and have penetrant heterochronic phenotypes. Dauer formation is induced by ascarosides, signaling molecules produced by *C. elegans* that serve as measures of population status for individual worms. Here, we report that a combination of three dauer-inducing ascarosides, namely ascr#2, ascr#3, and ascr#5, suppresses heterochronic phenotype of *daf-12(rh61)*. Interestingly, ascr#5 alone can suppress the *rh61* heterochronic phenotype, while only moderately inducing dauer formation in N2 animals. This suggests that while signaling from all three ascarosides contributes to the dauer program, suppression of *rh61* heterochronic phenotype is mainly regulated by ascr#5 signaling.

838C. Post-dauer regulation of developmental timing. Stephen Domingue, Benjamin Prout, **Xantha Karp**. Dept of Biology, Central Michigan University, Mt Pleasant, MI.

During each larval stage cells divide and differentiate under precise temporal control. For example, lateral hypodermal seam cells (V1-V4, V6) divide once or twice per stage in self-renewing divisions that generate additional seam cells and hyp7 nuclei. The particular cell lineage pattern at each stage is called stage-specific cell fate. At adulthood, seam cells exit the cell division cycle and express adult specific characteristics including the COL-19 collagen and adult alae. The timing of these developmental events is controlled by heterochronic genes that function as a molecular timer. This timer includes transcription factors that promote early cell fates and microRNAs that promote cell fate progression.

In favorable environments, development proceeds continuously through four larval stages to adulthood. In contrast, adverse environments promote entry into the stress-resistant dauer stage that interrupts development after the second larval molt. Dauer larvae exhibit both developmental and cellular quiescence, remaining paused in their development with no cell divisions. If dauer larvae encounter favorable environmental conditions, they recover and re-initiate development, going through post-dauer L3 and L4 stages. Strikingly, stage-specific cell fates are identical in continuous and post-dauer larval stages. However, the genetic mechanisms that regulate stage-specific cell fate appear divergent in continuous and post-dauer development, as many heterochronic genes that are required during continuous development are dispensable after dauer. In addition, the activity of certain heterochronic microRNAs is enhanced during or after dauer, relative to continuous development. The genes responsible for these differences remain largely unknown. In order to discover novel genes that regulate stage-specific cell fate during dauer-interrupted development, we have performed a genetic screen for mutants that display heterochronic phenotypes after dauer. We have isolated seven independent alleles and are currently characterizing them genetically and molecularly.

839A. Heterochronic gene *lin-46*: protein expression and interaction with HBL-1. **Kevin Kemper**, Bhaskar Vadla, Eric G. Moss. Molecular Biology, UMDNJ, Stratford, NJ.

Alteration of the heterochronic pathway results in the abnormal timing of developmental events. *lin-46* is a heterochronic gene discovered by its suppression of *lin-28* and *lin-14* precocious phenotypes. *lin-46* mutations result in retarded hypodermal phenotypes, specifically reiteration of L2 seam cell divisions in the L3 and gaps in adult alae. LIN-46 protein resembles Gephyrin from mammals and MoeA from bacteria. Our studies of LIN-46 have identified both its expression pattern and interaction with another member of the heterochronic pathway. LIN-46 expression is restricted spatially and temporally, the basis being both transcriptional and post-translational. Using a GFP reporter we found LIN-46 expression in the cytoplasm and nucleus of lateral hypodermal seam cells, peaking around the time of the molts. Substituting other hypodermal promoters, such as *col-10*, showed similar restriction. Use of the pan-neuronal promoter *rgef-1* indicates the temporal restriction is specific to the hypodermis. Preliminary qPCR data indicates periodic expression of the *lin-46* mRNA also. *lin-46* null mutants display a cold sensitivity. Temperature shift analysis specifies this cold sensitive period for all phenotypes to be two hours prior to the L2 molt. Genetic data places *lin-46* in parallel with *lin-28*, potentially acting on common targets. LIN-28 has two mechanisms of action: supporting expression of HBL-1 and blocking let-7 accumulation. By qPCR, early let-7 levels in a *lin-28(0); lin-46(0)* mutant were unchanged from a *lin-28(0)* mutant alone, indicating no direct role for LIN-46 in let-7 regulation. A yeast two-hybrid screen using LIN-46 isolated a portion of the HBL-1 protein. The region isolated includes two C-terminal C2H2 zinc-fingers, a motif shown in related proteins to be involved in dimerization. Further analysis of the binding proved it to be specific. Injection of this fragment into wildtype animals caused a weakly retarded phenotype similar to *lin-46(0)*. Injection into *lin-46(0)* animals caused a weakly precocious phenotype, indicating an interaction with other targets. These data indicate a role for LIN-46 in the post-translational regulation of HBL-1, possibly by inhibiting its interaction with other targets.

840B. A comparative analysis of the genetic basis of molting in the necromenic nematode Pristionchus pacificus. **Victor Lewis**, Maryn Cook, Justin Alonso, Ray Hong. California State University Northridge, Northridge, CA.

Development in nematodes appears to be highly conserved, consisting of four larval stages. Although previous evidence has implicated the disruption of

multiple molecular cues with defective ecdysis in C. elegans, the conserved mechanisms required for proper molting across the phylum Nematoda remains in question. The necromenic nematode Pristionchus pacificus bridges the gap between free living and parasitic nematodes. While P. pacificus retains the conserved four juvenile stages observed in all nematodes, its first molt from J1 to J2 occurs prior to hatching. This defining characteristic makes it an ideal model system for comparative analysis of genetic control for molting in nematodes. Additionally, it is not well understood whether the same genetic controls in P. pacificus are responsible for proper molting both before and post-hatching, and what possible developmental advantages may be conferred by the J1-J2 embryonic molt. We performed a forward mutagenesis screen for molting deficient mutants in which ecdysis could not be completed properly. We identified 6 ecdysis deficient mutants which exhibit a variety of phenotypes including embryonic molting deficiencies, the inability to complete ecdysis, abnormal pharynx formation, incomplete expulsion of the pharyngeal cuticle, and improper cuticle formation. We also identified a possible ortholog of Cel-mlt-10 that peaks in expression prior to each post-embryonic molt. The presence of both embryonic and post-embryonic molting deficiencies in the same mutant line indicates that at least some of the genetic controls necessary for proper molting in P. pacificus are required at every developmental stage.

841C. Low population density increases lifespan and delays egg laying of *C. elegans* hermaphrodites. **Andreas H. Ludewig**[1], Frank C. Schroeder[2], Frank Doering[1]. 1) Molecular Prevention, CAU Kiel, Germany; 2) Boyce Thompson Institute/Cornell University, Ithaca, NY.

In *C. elegans*, high population density (overcrowding) has been shown to trigger developmental arrest and dauer formation within early larval stages (Golden and Riddle 1982). The actual population density signal sensed by the worms includes a synergistic mixture of ascaroside pheromones as well as small signalling peptides (Ludewig and Schroeder, 2013; Yamada et al., 2010). However, many aspects of population density-dependent signaling events remain poorly understood. Here, we compared low and intermediate dense *C. elegans* populations grown under *ad libidum* feeding conditions. We found that the number of worms per plate strictly determined their average lifespan whereat the number of worms was inverse correlated to mean lifespan. Isolated worms lived around 4 (!) days longer than animals grown up in larger groups. In 1999, Gems and Riddle demonstrated strong dependence of lifespan on population density for males but not for hermaphrodites. However, in those assays worms were placed on experimental plates as L4 larvae, whereas our assays started with eggs, hinting on some defining events during developmental stages L1-L3 in hermaphrodites. We also found that the time point of first egg lay was correlated with the number of worms per plate. Worms that grew up in isolation laid their first egg 9-11 hours later than worms grown up in groups of 25 or more animals per plate. The overall time course of egg laying from isolated worms was shifted towards later time points and the total number of eggs was reduced by 45%. This population dependent early egg laying (= *eel*) phenotype is completely abolished in *daf-16(mu86)* mutants and partially abolished in *daf-12(rh61; rh411)* mutants. Unexpectedly, we also found precocious first egg laying under mild dietary restriction (DR). Evidence from previous studies suggests that small-molecule signalling may trigger DR early egg laying and population dependent longevity (Ludewig and Schroeder, 2013; Yamada et al., 2010; Yzraelit, 2012). To test this hypothesis, we currently examine candidate small molecules for their influence on those phenomena.

842A. The *C. elegans period* homolog *lin-42* regulates the timing of heterochronic miRNA expression. **K.A. McCulloch**, A.E. Rougvie. GCD, Univ Minnesota, Minneapolis, MN.

The heterochronic gene pathway of *C. elegan*s ensures that developmental programs are executed in the correct sequence during larval development. Many of the genes identified as members of this pathway are conserved, including *lin-42*, the *C. elegans* homolog of the circadian clock gene *period*. Strikingly, like *period*, *lin-42* mRNA and protein levels cycle. However, *lin-42* levels cycle with larval stages rather than the 24-hr clock. *lin-42* mutants have a precocious phenotype where adult specific fates occur too early. Also, *lin-42* regulates the molting cycle; null and strong *lin-42(lf)* mutants undergo a prolonged lethargus, molt asynchronously, and are defective in ecdysis. These defects cause developmental delays and often result in larval arrest. Genetic analyses reveal that *lin-42* acts in parallel with *lin-28* and opposition to *daf-12*, two genes that regulate the expression of *let-7*-family miRNAs. We show by Taqman qRT-PCR that *lin-42* also regulates the levels of heterochronic miRNAs, including Let-7 and miR-48. In *lin-42* mutants, these miRNAs accumulate earlier than in wild-type animals. miRNAs are generated through multiple processing events, and there are several steps at which their levels can be regulated. LIN-42 is nuclear, and PERIOD functions as a negative transcriptional regulator; suggesting that *lin-42* could regulate (pri)mary-miRNA transcription. To determine if *lin-42(lf)* affects pri-miRNA expression, we measured pri-miRNAs during larval development in wild-type and *lin-42(lf)* mutants. We show that primary transcript levels of heterochronic miRNAs oscillate over larval development, as previously reported for *pri-let-7*. *pri-let-7*, *pri-mir-48*, *pri-mir-241*, *pri-mir-84*, and *pri-lin-4* levels all cycle in wild-type animals. Surprisingly, although LIN-42 levels oscillate, *lin-42* is not required for pri-miRNA cycling. Notably, however, the amounts of several of these transcripts are increased in each stage in *lin-42* mutants; supporting the idea that *lin-42* may act to negatively regulate transcription. In mammals, many miRNAs are subject to regulation by the circadian clock. Investigation of *C. elegans lin-42* will provide further insight into mechanisms of temporal regulation of miRNA expression.

843B. Insulin-regulated nutritional checkpoints in post-dauer *C. elegans* larval development. **Adam J Schindler**, L Ryan Baugh, David R Sherwood. Biology, Duke University, Durham, NC.

C. elegans is an amenable model system to study the connection between nutrition and development. Under favorable nutritional conditions, *C. elegans* develop continuously through four larval stages (L1-L4) before reaching adulthood. Unfavorable conditions induce arrest in L1, dauer, or adult stages. It is currently not known how *C. elegans* respond to nutrient removal at other times in development. Using the stereotyped pattern of vulval formation as a marker for late larval development, we identify two previously uncharacterized arrest points that occur in the early part of the L3 and L4 stages. Starved animals arrest at these times for several days without further development. Using a reporter of the molting cycle, we find that arrest occurs after completion of the molt and prior to synthesis of new cuticle components. Tissues respond differently to starvation: the vulva arrests only at precise times, either prior to cell division (L3 arrest) or after completion of three rounds of cell division (L4 arrest), whereas sex myoblasts and seam cells arrest in a variable and graded manner. The insulin signaling pathway regulates the amount of feeding required to bypass the checkpoints. Animals with mutations affecting the *daf-2* insulin receptor arrest at the early-L3 checkpoint even in the presence of food. In contrast, animals with mutations affecting *daf-16*, a negative effector of insulin signaling, require less feeding than wild-type to bypass L3 arrest. Using tissue-specific expression and RNAi techniques, we find

that neuronal DAF-16 functions upstream of hypodermal DAF-16 to regulate the response to starvation. These results demonstrate that checkpoints exist in the early part of the L3 and L4 stages during which the nutritional status of the organism informs a decision to arrest or continue development.

844C. Transcription factors involved in dauer recovery. **Pei-Yin Shih**, Paul W. Sternberg. Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA.

In an adverse environment, the free-living nematode *Caenorhabditis elegans* becomes a dauer larva, a developmentally arrested stage. When the environment becomes favorable again, dauers recover and resume development. This post dauer is a dynamic period in that there are multiple structural, developmental and behavior modulations that occur. The analogous dauer states in some parasitic nematodes are called infective juveniles (IJ)s and dispersal juveniles (DJ)s. It has been shown that conserved neuron and signaling molecules control dauer/IJ recovery in *C. elegans* and parasitic nematodes *Ancylostoma caninum* and *Heterorhabditis bacteriophora*. However, how perception of the environmental change is converted to developmental changes is not well understood. In this study, we analyzed gene expression changes during dauer and dauer exit in *C. elegans* and found some transcription factors involved in the recovery process. We are now analyzing the transcription factors in more detail, including their roles in other parasitic nematodes. In addition, we are looking at the neural circuits that mediate the process of exiting dauer. Better understanding of the mechanism may help develop an evolutionary framework within the nematode phylum and potential novel treatment strategies by blocking IJ or DJ recovery.

845A. What is *lin-28*'s *let-7* independent mechanism? **Jennifer Tsialikas**, Bhaskar Vadla, Kevin Kemper, Eric Moss. UMDNJ-SOM, NJ.

We are investigating the relationship between the heterochronic genes *lin-28*, which encodes an RNA binding protein, and *hbl-1*, which encodes a transcription factor thought to be the most proximal regulator of the L2 to L3 cell fate transition. *lin-28* has two roles: 1) to promote L2 cell fates and 2) to prevent early execution of late fates. LIN-28 directly blocks accumulation of the miRNA *let-7*. We showed that *let-7* is not required for *lin-28*'s role specifying L2 cell fates. Moreover, *lin-28* does not regulate any other miRNA in the same way it regulates *let-7*. *lin-28*'s other mechanism is still undefined. *lin-28* positively regulates *hbl-1* expression in the hypodermis after the L1 (VADLA et al. 2012). In a strain lacking the three *let-7* sisters (*3let-7s*), *mir-48, mir-84* and *mir-241*, the *hbl-1* GFP reporter is constitutively expressed (ABBOTT et al. 2005). However, when *lin-28* is also removed, *hbl-1* is downregulated. Therefore, *lin-28* might inhibit another repressor of *hbl-1*. There are two putative *lin-4* sites in *hbl-1*'s 3'UTR. I constructed a *lin-28(0); 3let-7s(0); lin-4(0)* mutant. In this strain the *hbl-1* reporter was inappropriately expressed late into development. This suggests that *hbl-1* is a target of *lin-4*, and that *lin-28* blocks this interaction. The relationship between these genes is still complex. I made a line carrying an extrachromosomal array of *hbl-1* lacking its 3' UTR regulatory elements. This line was retarded and lacked adult alae as expected. Surprisingly, however, it showed no increase in seam cell number, indicating the L2 double seam cell division was not reiterated. When *lin-28* was removed by RNAi, the line showed a decreased seam cell number, but it lacked precocious alae formation at the L3 molt. Taken together, these results suggest that *lin-28* does not act solely via *hbl-1* to regulate the L2, and *hbl-1* is not sufficient to drive reiteration of the L2. Future experiments will further establish the relationship between *lin-28* and *hbl-1*, as well as elucidate *lin-4*'s role in *hbl-1* regulation. I am making gain-of-function alleles using TALEN targeted mutation to delete miRNA binding sites in the chromosomal copies of *lin-28* and *hbl-1*.

846B. A Quantitative Approach Reveals the Conditional Role of *elt-7* in the *C. elegans* Intestinal Specification Network. **Allison Wu**[1], Scott Rifkin[2]. 1) Bioinformatics and Systems Biology Graduate Program, University of California, San Diego; 2) Section of Ecology, Behavior and Evolution , Division of Biology, University of California, San Diego.

The *C. elegans* intestinal specification network consists of an interlinked set of feed-forward loops consisting pairs of homologous genes that have similar but not entirely redundant functions. Knocking out one of the genes in a pair usually yields a more severe phenotype than knocking out the other. For example, the *end-3* deletion allele *ok1448* has a 7% penetrance while the *end-1* deletion allele *ok558* has zero penetrance. *elt-7* and *elt-2* are even more divergent. While *elt-2* deletions are lethal, *elt-7* is non-essential. Perhaps as a consequence, *elt-7* remains the least studied gene within the *C. elegans* intestinal specification network.

Gene expression noise has previously been shown to lead to partial penetrance of *skn-1* mutations in this network. We use single-molecule fluorescence *in situ* hybridization (smFISH) to quantitatively measure the levels and variation in expression profiles of each gene within this network under perturbations such as RNAi treatment and gene deletions. This approach reveals a conditional regulatory role of *elt-7* and clarifies the network topology. In wild-type N2 worms, *elt-7* is expressed concurrently with *end-1*, suggesting that it is predominantly activated by *end-3*. We observe delayed onset of *elt-7* and an increase of *elt-2* noise in *end-3* -/- worms, revealing that *elt-7* regulation of *elt-2* expression is compromised when *end-3* is absent. While *elt-7* and *elt-2* expression are similar to wild-type in *end-1* -/- worms, we show that *elt-7* functions to maintain *elt-2* level when *end-1* is present. This role would only be relevant and successful when *elt-7* is expressed at the appropriate time. By knocking down *elt-7* in *end-3* and *end-1* deletion strains, we measure the direct effects of *end-3* and *end-1* on *elt-2*. Finally, we use our high resolution data to construct a mathematical model to explain how these deletions and knockdowns lead to gene expression noise and hence to partial penetrance in this network.

847C. A high-throughput genetic screen for lethargus mutants. **C. Yu**[1], M. Churgin[1], D. Raizen[2], C. Fang-Yen[1,3]. 1) Dept. of Bioengineering, School of Engineering and Applied Science, University of Pennsylvania, Philadelphia, PA; 2) Dept. of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA; 3) Dept. of Neuroscience, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA.

Lethargus is a *C. elegans* sleep-like quiescent state which occurs during the four larval molt transitions and is characterized by cessation of feeding and locomotion. Current methods for assaying quiescence are limited to <10 animals, making large-scale genetic screening impractical. We have developed a high-throughput quiescence assay capable of simultaneously imaging activity in hundreds of uniquely identified animals during development. Our assay is based on the WorMotel, a microfabricated polydimethylsiloxane (PDMS) device containing an array of 240 wells optimized for *C. elegans* cultivation and imaging, each containing one worm on standard agar media. The WorMotels are integrated in a standard 384-well microplate format for compatibility with multichannel pipettes, microplate handling equipment, and the COPAS worm sorter. In our experiments, we transfer synchronized populations of L3 larva into wells containing DA837-seeded NGM agar and perform time-lapse imaging of the array with a single camera over a 24 h period. We use custom

MATLAB programs to quantify the activity and total locomotory quiescence of individual worms. Using our quiescence assay on N2 animals we find an L3 lethargus duration of 1.4 ± 0.4 h and L4 lethargus duration of 2.0 ± 0.4 h, consistent with published data based on assays in standard agar plates. We validate our assay on mutants with known lethargus phenotypes including *egl-4* (increased quiescence), *kin-2* (decreased quiescence), and other wild-type strains of *C. elegans*. We describe efforts toward identifying novel mutants with reduced movement quiescence during lethargus in EMS-mutagenized animals. Our method will also be useful for conducting high-throughput assays for other behavioral and developmental phenotypes.

848A. CYP35A3-GFP induction and reproductive toxicity of environmental samples in *C.elegans*. **A. Abbas**, L. Valek, J. Oehlmann, M. Wagner. Goethe University Frankfurt, Frankfurt am Main, Hessen, Germany.

A novel *Caenorhabditis elegans* (*C.elegans*) assay was designed based on ISO10872 to analyze environmental samples containing complex mixtures of pollutants. Special attention was given to polycyclic aromatic hydrocarbons (PAH) and dioxin-like compounds (DLC), known for their mutagenic, carcinogenic or teratogenic potential (Luch, 2005). Water samples were taken from the Danube River and a municipal wastewater treatment plant (WWTP) effluent near Ulm, Germany and enriched by solid phase extraction (SPE). To probe for reprotoxicity and Cytochrome P450 (CYP) 35A3 induction the *C.elegans* N2 strain and transgenic line CYP35A3::GFP (Menzel et al., 2001) were exposed to these samples. CYP35A3::GFP provided a biomarker for the exposure to PAH, DLC and other toxicants. For their quantification the yeast dioxin screen (YDS) served as an additional *in vitro* system. Concentrations were expressed as b-naphthoflavone (bNF) equivalents (EQ). Up to 1.8 ± 0.5 mg/L bNF-EQs were determined in the WWTP sample. This sample caused 89.6 ± 0.3% reduced N2 brood sizes (96 h, n = 33, $p < 0.001$). The Danube river sample did not induce significant dioxin-like activity in the YDS, but showed 38.4 ± 0.7% reduced N2 brood sizes (96 h, n = 27, $p < 0.001$). CYP35A3::GFP expression was induced up 4.7-fold following 24 h exposure to 1.0 mg/L of the reference substance bNF. An intestinal expression of CYP35A3 (Menzel et al., 2001) was detected by fluorescence microscopy. However, no fluorescence signal was detected in transgenic *C.elegans* exposed to environmental samples. Experiments are under way to further investigate the causes of the significant reprotoxicity in Danube River and WWTP samples.

849B. GLD-1 Expression During Germline Development. **Jennifer R. Aleman**, Sudhir B. Nayak. Department of Biology, The College of New Jersey, Ewing, NJ.

Caenorhabditis elegans (*C. elegans*) GLD-1 (defective in Germ Line Development) is an RNA-binding protein that acts as a translational repressor for multiple mRNA targets in the germline preventing inappropriate expression of their protein products. The mRNAs targeted for repression are responsible for various aspects of germ line development such as oocyte development and cell proliferation. Mutations in *gld-1* can lead to aberrant progression through the cell cycle during oogenesis and the formation of germline tumors. Throughout development, expression of GLD-1 protein is tightly controlled in the germline of hermaphrodites. In wild type adult hermaphrodite, GLD-1 levels are low at the distal tip of germline, increase to maximum levels in the transition zone where germ cells are in meiotic prophase, remain high during distal pachytene, and decrease abruptly in diplotene as oocytes begin to differentiate. We took advantage of a transgenic strain which contained GLD-1 fused to GFP (green fluorescent protein) to visualize the expression of GLD-1 in real-time as animals age. We found that GLD-1 levels change as animals age and that GLD-1 localization to the nuclear periphery becomes more evident. Since oocyte quality decline limits reproductive span, we hypothesize that changes in GLD-1 protein expression as animals age may govern reproductive potential.

850C. Role of autophagy genes in *C.elegans* germline proliferation. **K. Ames[1,2]**, A. Meléndez[1,2]. 1) Biochemistry, The Graduate Center, CUNY NY,USA; 2) Biology Department, Queens College, CUNY, Flushing, NY,USA.

Autophagy is a conserved pathway characterized by the formation of double-membrane vesicles to degrade cellular material and maintain cellular homeostasis. BEC-1, the *C. elegans* ortholog of mammalian Beclin1 and yeast Atg6/Vps30, is essential in the vesicle nucleation step of autophagosome formation. In *C. elegans*, we have demonstrated that BEC-1 is crucial for various aspects of development. Importantly, beclin1 is a haploinsufficient tumor suppressor gene in mice and the beclin1 gene is monoallelically deleted in various human cancers. However, the role of autophagy in tumor growth is not well understood.

To better understand how *bec-1* regulates cell proliferation, we used the *C. elegans* germline model. Proliferation of the germline is determined by GLP-1/Notch signaling. Mutations that increase *glp-1* signaling result in an overproliferation phenotype, whereas, reduced *glp-1* signaling results in the lack of germline proliferation and premature meiotic entry. We found that genetically reducing the function of *bec-1, atg-16.2* or *atg-18* results in decreased number of germline mitotic cells, suggesting that autophagy contributes to the proliferation and/or maintenance of the mitotic pool of germ cells. Moreover, compromising autophagy function in *glp-1(gf)* mutants by genetic removal of *bec-1, atg-16.2* or *atg-18*, significantly decreased mitotic cell proliferation of *glp-1(gf)* mutants, as did RNAi against autophagy genes. We conclude that autophagy genes play a role in mediating the proliferation of undifferentiated cells in the *glp-1(gf)* tumor model. We found that the observed effect of reduced autophagy on proliferation is neither due to an increase in cell death nor due to a premature exit from mitosis. Moreover, is not due to an initial lack of proliferation during development. Thus, we hypothesize that the decrease in autophagy gene function may result in defects in cell cycle progression. Future experiments will test this hypothesis. We expect that our studies will help better understand the role of *bec-1* and autophagy in the germline proliferation and identify the molecular mechanisms by which autophagy genes modulate proliferation and/or maintenance of the germline stem cell population.

851A. Anillin promotes syncytial organization and maintenance of the *C. elegans* germline. **Rana Amini[1]**, Sara Labella[2], Monique Zetka[2], Amy S. Maddox[1], Nicolas T. Chartier[1], Jean-Claude Labbé[1]. 1) Institute of Research in Immunology and Cancer (IRIC) and Dept. of Pathology and Cell Biology, Université de Montréal, Montréal, QC, Canada; 2) Dept. of Biology, McGill University, Montréal, QC, Canada.

Cytokinesis, the last step of cell division, is required for the physical separation of the two daughter cells. During certain developmental stages however, incomplete cytokinesis gives rise to interconnected cells, forming a syncytium. The *C. elegans* adult germline is a syncytial organ enriched in ANI-2, a short homolog of the conserved scaffolding protein anillin, which controls cytokinesis. ANI-2 is present from the onset of germ cell specification and is enriched in intercellular syncytial bridges, suggesting that it might promote germline syncytium formation in *C. elegans*. To investigate this possibility, we developed a light microscopy-based assay to analyze the timing of syncytium formation during gonad development. We found that syncytial organization of the

germline occurs progressively during larval development and completes shortly before animals enter the adult stage. Interestingly, while the germline of *ani-2(-/-)* mutants lacks defined syncytial openings, gonad development proceeds normally until animals reach the adult stage, when cytoplasmic partitions regress and germ cells progressively become polynucleated. This stage of gonad development is characterized by the initiation of cytoplasmic flows that promote oocyte growth, suggesting that ANI-2 is required to stabilize intercellular bridges when these flows initiate. In support of this, we did not observe severe multinucleation in male gonads or in tumorous germlines, which both lack cytoplasmic flows. Furthermore, time-lapse analysis of the proximal gonad at fertilization revealed that ANI-2 could confer elastic-like properties to the gonad, indicating that it can compensate for mechanical stress. We propose a model in which ANI-2 is required to promote syncytial organization of the germline and that its presence becomes essential to compensate for the mechanical stress resulting from cytoplasmic flows at the onset of oogenesis.

852B. Structural characterization of the P-granule protein scaffold. **Scott Takeo Aoki**[1], Judith Kimble[1,2]. 1) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI; 2) Howard Hughes Medical Institute, Madison, WI.

C. elegans P-granules are essential for both the development and maintenance of the germline tissue. Disruption of P-granule formation interferes with proper germ cell proliferation and differentiation, resulting in sterility (1, 2). P-granule assembly requires structural scaffold proteins PGL-1 and PGL-3 (3, 4). These nematode-specific paralogs are sufficient to form granules in cells and multimerize through self-association (5, 6). We aim to understand the structural organization of the P-granule scaffold to better understand how the organelle regulates mRNA trafficking and turnover. We are able to express and purify recombinant PGL-1 and PGL-3. Full-length recombinant protein self-assembles into large soluble aggregates. Protease digestion analyses identify a single domain that dimerizes in solution. We are currently trying to obtain a high-resolution crystal structure of the protease-protected fragment, as well as determine the role of the N- and C- terminal regions in scaffold oligomerization. Several different types of RNA granules are required in eukaryotes for cell homeostasis, differentiation, and response to stress. The fundamental mechanisms involved in P-granule organization will undoubtedly shed light on other granules involved in RNA regulation.

References:

1. Updike, D., Strome, S. (2010) *J Androl* 31: 53-60.

2. Voronina, E., Paix, A., Seydoux, G. (2012) *Development* 139: 3732-3740.

3. Kawasaki, I., et al. (1998) *Cell* 94: 635-645.

4. Kawasaki, I., et al. (2004) *Genetics* 167: 645-661.

5. Updike, D.L., Hachey, S.J., Kreher, J., Strome, S. (2011) *J Cell Biol* 192: 939-948.

6. Hanazawa, M., Yonetani, M., Sugimoto, A. (2011) *J Cell Biol* 192: 929-937.

853C. FOG-1 and FOG-3, their mRNA targets and the sperm/oocyte fate decision. Daniel Noble[1], Marco Ortiz[1], **Scott Aoki**[1], Kyung Won Kim[1,2], Judith Kimble[1,3]. 1) Dept of Biochemistry, University of Wisconsin-Madison, Madison, WI; 2) Current address: Dept of Biological Sciences, University of California San Diego, San Diego, CA; 3) Howard Hughes Medical Institute, Madison, WI.

A major unsolved question in development is how germ cells are specified as sperm or oocyte. The *C. elegans* terminal regulators of sperm fate specification are FOG-1, a CPEB family member, and FOG-3, a Tob/BTG protein (1). Vertebrate CPEB and Tob/BTG proteins can function as post-transcriptional regulators and have recently been shown to interact (2). We provide evidence that FOG-1 and FOG-3 proteins physically bind to each other and likely work together to specify sperm fate. Epitope-tagged FOG-1 and FOG-3 co-immunoprecipitate in a mammalian cell culture expression system and do so via the FOG-1 RNA binding region and FOG-3 Tob domain. In addition, we have found that FOG-1 and FOG-3 may regulate a common battery of mRNAs. We used a rescuing epitope-tagged *fog-3::3xFLAG* mosSCI transgene to immunoprecipitate FOG-3 associated mRNAs from spermatogenic L4 larvae, and identified 1616 transcripts by microarray analysis. Most of these putative FOG-3 mRNA targets were germline enriched, with 37% annotated as spermatogenic and 29% as oogenic. We performed similar immunoprecipitation and microarray analyses with a rescuing bombarded *fog-1::3xFLAG* transgene to determine 133 likely FOG-1 bound mRNAs. Remarkably, 109 of these FOG-1 mRNAs were also associated with FOG-3. Moreover, these common target mRNAs were enriched for genes involved in oogenesis or embryogenesis. Our complementary lines of evidence strongly suggest that FOG-1 and FOG-3 work together to regulate a common battery of mRNAs involved in the oogenic program. Our working model supports a conserved function of Tob proteins as CPEB protein adaptors to inhibit target transcripts. For sperm fate specification, we suggest that FOG-1 and FOG-3 work together to inhibit a battery of oogenesis mRNAs.

(1) Ellis, R. and T. Schedl (2007) in *WormBook*, doi:10.1895/wormbook.1.82.2.

(2) Hosoda, N. et al. (2011) *EMBO J* 30: 1311-1323.

854A. Homeodomain interacting protein kinase (HPK-1) is required in the soma for robust germline proliferation in *C. elegans*. **S. Berber**[1], E. Llamosas[1], P. Boag[2], M. Crossley[3], H. Nicholas[1]. 1) School of Molecular Bioscience, The University of Sydney, Sydney, NSW 2006, Australia; 2) Department of Biochemistry and Molecular Biology, Monash University, VIC 3800, Australia; 3) School of Biotechnology and Biomolecular Sciences, University of New South Wales, NSW 2052, Australia.

hpk-1 encodes the sole *C. elegans* member of a family of evolutionarily conserved protein kinases called the homeodomain interacting protein kinases (HIPKs). Mammalian homologues of HPK-1 have been implicated in control of numerous cellular processes including cell survival and proliferation. A *C. elegans* strain carrying a *hpk-1* mutation has previously been studied (Raich *et al*., 2003), but no obvious phenotypes were reported. We decided to use the hpk-1 mutant strain and *hpk-1* RNAi to investigate the role of HPK-1 in the development and maintenance of the *C. elegans* germline. A significant reduction in germline proliferation was observed in the strain carrying the *hpk-1* mutation. The phenotype was characterised by reduced brood size, reduced size of the mitotic region and a decrease in the number of proliferative cells. Knockdown of *hpk-1* by RNAi resulted in a comparable phenotype, confirming that HPK-1 is required for normal germline proliferation. Our results furthermore suggest that HPK-1 is not only required for the maintenance of the mitotic region in adult germlines but that it is also necessary for the establishment of the progenitor pool during development as the reduced proliferation phenotype was also observed at the L4 stage. Interestingly, the brood size and number of proliferative cells were rescued in *hpk-1* mutants

with HPK-1::mCherry-expressing transgenes from which no germline expression had been detected. In addition, knockdown of *hpk-1* in a soma-sensitive RNAi strain resulted in reduced proliferative cell number whereas knockdown in a germline-sensitive RNAi strain had no significant effect. These observations suggest a role for HPK-1 in soma-dependent control of germline proliferation.

855B. Identification of direct targets of the *Caenorhabditis elegans* global sexual regulator TRA-1 by ChIP-seq. **Matt Berkseth**[1], Kohta Ikegami[2], Jason Lieb[2], David Zarkower[1]. 1) Dept. of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN; 2) Dept. of Biology and Carolina Center for Genome Science, University of North Carolina, Chapel Hill, NC.

The nematode *C. elegans* naturally occurs as two highly dimorphic sexes, hermaphrodite and male, with sexual identity determined via a genetic pathway culminating in the transcription factor TRA-1. Null mutations in *tra-1* result in hermaphrodite-to-male sex reversal, indicating that TRA-1 and its downstream targets are responsible for generating all sexual dimorphism in the worm. However only a few direct TRA-1 targets have been described, and additional biologically important targets clearly remain to be identified. We used ChIP-seq to identify 184 high confidence TRA-1 binding sites throughout the genome, including previously described sites near *mab-3*, *xol-1*, *fog-1*, and *fog-3*. Most of these sites harbor the TRA-1 consensus binding motif, but only a small portion of such motifs in the genome show evidence of binding by TRA-1. ChIP-seq in L2, L3, and young adult animals showed that TRA-1 binding at many sites varies across developmental time. In mutant adults lacking a germ line (*glp-4*), a subset of peaks that are present in germ line-containing adults (*spe-11*) were lost, suggesting that TRA-1 binds to some sites only in the germ line. In *C. briggsae*, we identified 48 TRA-1 binding sites, roughly a quarter of which have homologous sites bound by TRA-1 in *C. elegans*. To examine what role putative TRA-1 targets may play in sexual development, we generated reporters for many of these new TRA-1 binding sites and identified several sites that direct male-specific expression in a TRA-1 binding motif dependent manner. Surprisingly, we identified TRA-1 binding sites adjacent to several genes known to function in the global sex determination pathway, including *tra-1*, *xol-1*, *fem-3*, and *sup-26*, suggesting that TRA-1 feeds back onto this pathway to reinforce the sex determination decision. Additionally, several TRA-1 binding sites lie adjacent to members of the heterochronic pathway, suggesting that TRA-1 may impose sexual identity on developmental timing. Further analysis of TRA-1 targets should help reveal the regulatory circuit controlling sexual differentiation.

856C. The Role of Sperm Specific PP1 Phosphatase GSP-3/4 in Kinetochore Localization and Function During Spermatogenesis. **Joseph Beyene**, Diana Chu, PhD. Biology, SFSU, san francisco, CA.

Male infertility affects millions of couples within the US. Male fertility and sperm function depend upon proper chromosome segregation during meiosis. However, little is known about the molecular components required for chromosome segregation during sperm meiosis. Our lab examines the role of the PP1 phosphatases GSP-3 and GSP-4 (GSP-3/4) in *C. elegans* sperm meiosis. GSP-3/4 are 98% identical, sperm-specific, and localize to sperm chromosomes. Deletion of the *gsp-3/4* genes causes failure of sperm chromosome segregation during meiosis. Though we have found that GSP-3/4 colocalize with components of the kinetochore, which attach microtubules to chromosomes, the specific role of GSP-3/4 in chromosome segregation remains unknown. Thus, we hypothesize that GSP-3/4 are required for the correct localization and function of kinetochore components during spermatogenesis. Consistent with this, we have found that kinetochore components HCP-2 and KNL-1 mislocalize in male *gsp-3/4* mutants, suggesting a dependency upon GSP-3/4 for kinetochore localization.To visualize kinetochore localization and microtubule dynamics during the male meiotic divisions, we used immunolocalization of wild-type and *gsp-3/4* mutants. In wild-type animals, HCP-2 surrounds dividing chromosomes then concentrates in the spindle midzone at the end of anaphase 2. In contrast, in *gsp-3/4* mutants HCP-2 improperly concentrates in the spindle midzone at anaphase 1. Subsequent division failure in meiosis II results in the formation of incompletely separated chromosome “rods”. Furthermore, KNL-1, which surrounds dividing chromosomes and localizes around the “lagging x” during anaphase 1, is also mislocalized in *gsp-3/4* mutants. This also results in chromosome rod formation due to meiosis II division failure. Taken together, this data suggests a kinetochore component dependency upon GSP-3/4 for proper localization and function.Studies examining the role of GSP-3/4 in regulating additional kinetochore components are underway. Overall, this work indicates that kinetochore components likely have sperm-specific localization and dynamics that depend upon GSP-3/4.

857A. Exploring miRNA function in the proliferation versus meiosis decision in the *C. elegans* germline. **John L. Brenner**, Gavriel Y. Matt, Tim Schedl. Department of Genetics, Washington University School of Medicine, Saint Louis, MO.

Post-transcriptional regulation of gene expression plays a critical role in the proliferation versus meiosis decision in the *Caenorhabditis elegans* germline. While many RNA-binding proteins are known to have important functions in this process, the role of miRNAs in the proliferation versus meiosis decision remains poorly understood. Loss of miRNA biogenesis components, including *dcr-1*, *drsh-1*, or *alg-1/2*, result in reduced numbers of proliferative zone cells, but it remains unclear that this is due to premature meiotic entry. In addition, it was postulated that miRNAs impact the decision to enter meiosis through regulation of *gld-1*. *gld-1* is normally post-transcriptionally repressed in the stem cell region to prevent premature meiotic entry. While there is some data that suggests that miRNAs negatively regulate *gld-1*, it is not clear that this regulation occurs in the proliferative zone of the germline. We tested this by examining GLD-1 levels and counting the number of proliferative zone nuclei in worms that lack the miRNA biogenesis components, *dcr-1* and *drsh-1*, in sensitized genetic backgrounds where both GLD-1 regulation and the decision to enter meiosis is compromised. Loss of either *dcr-1* or *drsh-1* fails to substantially enhance the premature meiotic entry phenotype of *glp-1(bn18)* mutant worms, and their loss does not substantially alter GLD-1 expression. In addition to this, we further examined the role of the *mir-35* family, which in a previously published report was implicated in regulation of *gld-1*. However, we fail to see a substantial change in GLD-1 levels in the proliferative zone of worms completely lacking the *mir-35* family, nor does complete loss of this family enhance the premature meiotic entry phenotype of *glp-1(bn18)* mutant worms. Our current data is inconsistent with miRNAs acting to negatively regulate *gld-1*, or acting in the proliferation versus meiosis decision in the germline of *C. elegans*.

858B. Distal tip cell processes provide extensive contact between the germline stem cell pool and its cellular niche. **Dana T. Byrd**[1,3], Karla Knobel[3], Katharyn Schmitt[3], Sarah L. Crittenden[2], Judith Kimble[2,3,4,5]. 1) Department of Biology, San Francisco State Univ, San Francisco, CA; 2) Howard Hughes Medical Institute, Univ of Wisconsin-Madison, Madison, WI; 3) Department of Biochemistry, Univ of Wisconsin-Madison, Madison, WI; 4) Laboratory of Molecular Biology, Univ of Wisconsin-Madison, Madison, WI; 5) Department of Medical Genetics, Univ of WI-Madison, Madison, WI.

The germline stem cell niche in *C. elegans* includes the distal tip cell (DTC). The DTC has a complex cellular architecture; there is a cap that covers germ cells with a sheet of membrane, and long external processes that extend along the outside of the germline syncytium, contacting a subset of germ cells. The role of this architecture in germline stem cell biology, stem cell maintenance and the pattern of mitosis and differentiation in the germ line remains an open question. We have identified an additional region of DTC to germline contact: a plexus of membranous processes that surround and intercalate between adjacent germ cells in the distal germ line. We find that the end of the plexus region corresponds with the start of expression of a meiosis-promoting protein marker, GLD-1::GFP. Thus, germ cells within the cap and plexus regions have a high level of niche contact and low levels of a meiosis-promoting factor. One model is that increased niche contact leads to increased Notch signaling in this region and is important for maintaining the "stem cell-like" state of germ cells in the distal germ line. In addition, we find that formation and maintenance of the DTC processes depends on continued germ cell divisions. Thus signals from mitotic germ cells appear to influence niche architecture. This work lays a foundation for studying the complex cellular architecture of a niche, its role in regulating the self-renewal versus differentiation decision of stem cells and two-way communication between niche and stem cells.

859C. PUF-8 controls mitochondrial biogenesis and apoptosis in the germline. **A. Chaturbedi**, G. Anil Kumar, M. Ariz, K. Subramaniam. Biological Sciences & Bioenigneering, Indian Institute of Technology, Kanpur.

Germline apoptosis is essential for oogenesis in organisms as diverse as nematodes and human. However, molecular mechanisms that control the expression of core apoptotic machinery components in the germline have been poorly understood. Here we present evidence that the conserved RNA-binding protein PUF-8 functions redundantly with TOCA-2, a protein involved in endocytosis, to control physiological germline apoptosis. Worms lacking both PUF-8 and TOCA-2 are sterile; their germlines contain considerably less germ cells and show increased apoptosis. An RNAi screen on *puf-8; toca-2* double mutant background revealed that the depletion of the mitochondrial Rho GTPase MIRO-1, which is involved in mitochondrial biogenesis, or the dynamin-related protein DRP-1, which promotes mitochondrial fission, restores fertility in *puf-8; toca-2* double mutant worms. In transgenic worms expressing the *drp-1::gfp* transgene, depletion of both PUF-8 and TOCA-2 dramatically increases mitochondrial and cytoplasmic levels of DRP-1::GFP. Examination of *drp-1::gfp* expression in the *puf-8* and *toca-2* single mutants reveals that PUF-8 negatively regulates DRP-1 expression, whereas TOCA-2 suppresses its mitochondrial localization.

Earlier work has shown that PUF-8 suppresses the translation of *pal-1* mRNA, which encodes a somatic transcription factor, in the distal germline. In somatic cells, PAL-1 activates the transcription of *ced-3*, a core component of the apoptotic machinery. Our in situ hybridization and immunostaining results show that CED-3 is misexpressed in the *puf-8* germline in a PAL-1-dependent manner. Further, depletion of PAL-1 reduces the number of apoptotic nuclei in the *puf-8* mutant germline. Together, our results indicate that PUF-8 regulates germline physiological apoptosis by two ways: 1. It suppresses DRP-1 expression, which is known to promote mitochondrial fission and 2. through its effect on *pal-1* mRNA translation, PUF-8 suppresses *ced-3* transcription.

860A. Timing is everything: dissecting the male sperm activation pathway. **Daniela Chavez**, Joseph Smith, Angela Snow, Gillian Stanfield. Human Genetics, Univ of Utah, Salt Lake City, UT.

C. elegans sperm undergo rapid morphological changes to develop into polarized cells capable of migrating to and fertilizing an oocyte. During this process, termed sperm activation, the cells reorganize their cytoskeleton to generate a pseudopod, allowing them to crawl. Sperm activation is regulated by a serine protease, TRY-5, and a serine protease inhibitor, SWM-1. *swm-1* mutant males contain prematurely activated spermatozoa, which are transferred inefficiently to hermaphrodites. Thus, SWM-1 is required for the temporal regulation of sperm activation and is essential to male fertility. TRY-5 is transferred in seminal fluid to a hermaphrodite and has the characteristics of a male sperm activation signal. Therefore, our model for male sperm activation is that SWM-1 directly or indirectly inhibits TRY-5 from activating spermatids within the male. Examination of the relationship between TRY-5 and SWM-1 is key to testing this model and understanding how sperm activation is regulated. To gain insight into the male sperm activation pathway, we are performing experiments to identify the location of SWM-1 expression and to analyze the functional domains of SWM-1 and TRY-5. We have previously shown TRY-5 to be expressed in the male somatic gonad in cells of the seminal vesicle, valve, and vas deferens. Similarly, we have found HA-tagged SWM-1 to be expressed in and likely secreted from a subset of vas deferens cuboidal cells, near the site of TRY-5 expression. In *swm-1* mutants, TRY-5 becomes localized to the seminal vesicle. Therefore, SWM-1 and TRY-5 localization is consistent with our model in which SWM-1 inhibits TRY-5 from prematurely activating sperm. SWM-1 has two trypsin inhibitor-like (TIL) domains. We are genetically testing the necessity and sufficiency of each TIL domain for sperm activation. Preliminary results suggest that the C-terminal TIL domain plays a greater role in inhibiting sperm activation than the N-terminal TIL domain. Additionally, we are testing necessity of the catalytic domain of TRY-5 and we have preliminary evidence that it is required for activity *in vivo*. In the future, we will further investigate the function of SWM-1 and TRY-5 using biochemical analysis.

861B. The Nucleosome Remodeling Factor complex controls germ cell fates in *C. briggsae*. **Xiangmei Chen**[1,2], Ronald E Ellis[1]. 1) Department of Molecular Biology, Univ of Medicine & Dentistry of New Jersey-SOM, Stratford, NJ; 2) Graduate School of Biomedical Sciences, Univ of Medicine & Dentistry of New Jersey-SOM, Stratford, NJ.

In *Caenorhabditis* nematodes, the transcription factor TRA-1 controls the sperm/oocyte decision by regulating *fog-1* and *fog-3*, which are needed for spermatogenesis. Recent work from our lab indicates that the Tip60 Histone Acetyl Transferase complex works with TRA-1 to control *fog-3*. Thus, we have been investigating the role of other chromatin remodelers in the sperm/oocyte decision.

In *C. briggsae*, knocking down the activity of NURF-1.1 or ISW-1, the core components of the Nucleosome Remodeling Factor (NURF) complex, caused germ cells in both sexes to differentiate as oocytes rather than as sperm. This phenotype was confirmed using deletion mutants that were isolated with TALENs. Molecular tests revealed that these NURF genes are predominantly expressed in the germ line, and that they control the transcript levels of *fog-1* and *fog-3*. Furthermore, the analysis of double mutants showed that the NURF complex acts downstream of TRA-1 to control this decision. We propose that the NURF complex alters chromatin structure in the germ line of L3 larvae, which allows the transcription factor TRA-1 to access the *fog-1* and *fog-3* promoters.

These results imply that the NURF complex acts downstream of the Tip60 complex to control germ cell fates in *C. briggsae*, just as it does to regulate vulval cell fates in *C. elegans*. However, the Tip60 and NURF complexes cooperate in *C. briggsae*, but oppose each other in *C. elegans*. Furthermore, knocking down the activity of the NURF complex did not alter the sperm/oocyte decision in *C. elegans*, *C. remanei* or *C. sp. 9*. Hence, the role that the NURF complex plays in sex determination appears to have changed dramatically during recent evolution.

862C. An importin b controls the sperm/oocyte decision in *C. briggsae*. **Xiangmei Chen**[1,2], Greg Minevich[3], Yongquan Shen[1], Alexander Boyanov[3], Oliver Hobert[3], Ronald E Ellis[1]. 1) Dept Molecular Biol, Univ Med & Dentistry NJ--SOM, Stratford, NJ; 2) Graduate School of Biomedical Sciences, Univ Med & Dentistry NJ-SOM, Stratford, NJ; 3) Biochemistry & Molecular Biophysics, Columbia Univ, New York, NY.

The phylogeny indicates that *C. briggsae* evolved hermaphroditic reproduction independently from *C. elegans*. Moreover, self-fertility in *C. briggsae XX* animals is promoted by a novel protein, SHE-1. Thus, we screened for suppressors of *she-1* to identify additional members of this pathway.

One of the new mutations, *v92*, not only suppressed *she-1*, but also suppressed the feminization caused by *trr-1(RNAi)* or *mys-1(RNAi)*. These genes encode components of the Tip60 Histone Acetyl Transferase complex. Surprisingly, *v92* also caused synthetic lethality with Tip60 mutations.

We used SNP mapping to define a 600 kb interval on LG*II* that contains *v92*. Although the incomplete nature of the *C. briggsae* genome complicated our efforts, we were able to identify a single lesion at the center of this interval by whole genome sequencing. Thus, this method could aid in cloning other new genes in *C. briggsae*. The lesion alters the first intron of *imb-2*, a homolog of human importin b. Using RNAi to knock down *imb-2a* produced Fog mutants, which shows that an importin b regulates the sperm/oocyte decision in *C. briggsae*. This result implies that the nucleocytoplasmic trafficking of key regulatory proteins influences sexual fate in germ cells.

Although the *v92* strain contains a lesion in *imb-2*, it is not yet certain that this lesion is *v92*, nor that it alters *imb-2* activity. Thus, we used TALENs to generate an *imb-2a* frameshift mutation. We are now testing *v92* for complementation with this *imb-2a* null allele, and measuring the levels of *imb-2* transcripts in *v92* mutants.

863A. Control of cell cycling speed to minimize mutation accumulation. Michael Chiang, Amanda Cinquin, **Olivier Cinquin**. UC Irvine, Irvine, CA., California.

Stem cells are commonly thought to play a critical role in minimizing the accumulation of mutations. But much remains to be addressed about the strategies stem cells use to fulfill that performance objective. Slow cycling of stem cells provides in principle a simple cell cycle control strategy that efficiently compromises between conflicting performance objectives. We quantified the speed of cell cycling along the distal-proximal axis of the *C. elegans* gonadal arm, and found that cells at the distal end cycle more slowly. The experimentally-measured magnitude of speed differences along the distal-proximal axis is consistent with predictions derived from computational simulations of mutation accumulation. Although the results are derived within the context of the *C. elegans* germ line, they are of broad relevance to stem cells from other systems and to their role in delaying aging.

864B. Progress on developing the Q system to study GSCs and their control. **Sarah L. Crittenden**[1], Ipsita Mohanty[2], Judith Kimble[1,2]. 1) Howard Hughes Medical Institute, Madison, WI; 2) Department of Biochemistry, Univ of Wisconsin-Madison, Madison, WI.

The Q system (Potter et al., 2010) permits inducible gene expression in *C. elegans* (Wei et al., 2012) and is similar in principal to the widely used GAL4-UAS system. The QF transcriptional activator directs transcription via an upstream activating sequence (QUAS). But in addition, another protein, QS, can inhibit QF activation and QS repression can be relieved by a small molecule, quinic acid or QA. QA is non-toxic and can be fed to worms (Wei et al., 2012). This inducible system has the potential to control target genes in both space and time.

A chemically inducible method to control gene expression in specific cells at specific times would be tremendously useful for analyses of germline stem cells (GSCs) and their niche, the somatic distal tip cell (DTC). To this end, we are generating mosSCI insertion transgenes that rely on DTC-specific and GSC-specific regulatory sequences to drive QF expression. We are also generating a QUAS driven nuclear GFP (fused to H2B). Once we have the QF/QUAS pair working well for spatial regulation, we will add the QS/QA pair for temporal regulation. Preliminary experiments are promising and results will be shared at the meeting.

865C. Giving Light to Sperm-Specific Phosphatases. **Tyler S Curran**, Leslie Mateo, Diana Chu. San Francisco State Univ, San Francisco, CA.

Despite affecting millions in the U.S. alone, male infertility at the molecular level is poorly understood. Phenotypes of male infertility include improperly segregated sperm chromosomes and sperm immotility. For example, in mice these same phenotypes have been linked to defects in a sperm-specific isoform of protein phosphatase one (PP1) isoform, PP1g2. Our research in Caenorhabditis elegans has identified two sperm-specific PP1s GSP-3 and GSP-4, which are 98%; identical. We have shown that GSP-3/4 are functional homologues of PP1g2; targeted deletion of *gsp-3/4* genes causes aberrant chromosome segregation during meiosis and immotility in mature sperm. PP1g2 and GSP-3/4 likely do not act alone, as PP1s rely on interacting proteins for catalytic activity and substrate specificity. Thus, many studies focus on individual PP1-protein complexes. While some sperm-specific PP1-protein complexes are starting to be identified, many of the interacting proteins and their functions remain unknown. To that end, we aim to identify the interacting proteins of GSP-3/4 in C. elegans. Previous work has given us candidate interacting proteins of GSP-3/4. Targeted deletions of gsp-3/4 disrupt major sperm protein (MSP) localization, a key protein in sperm motility. In addition, we have shown that GSP-3/4 localization is similar to that of kinetochores during sperm meiotic divisions; thus we expect GSP-3/4 may interact with kinetochore proteins. Our study is generating worms expressing green fluorescent protein (GFP) tagged GSP-3/4 (GSP-3/4::GFP). GFP will allow for live imaging of the enzymes and will also be a target for co-immunoprecipitation (co-IP) of GSP-3/4::GFP and their interacting proteins. Thus far, we have used Gateway Cloning (Invitrogen) to create GSP-3/4::GFP constructs in Mos1 single copy insert (MosSCI) compatible vectors. Using microinjection into MosSCI compatible EG6699 worms we have integrated single copies *gsp-4::gfp* into genomic DNA. We are currently working to integrate *gsp-3::gfp* constructs and validate fusion protein function. Our future studies will utilize these strains for the identification of interacting proteins by co-IP, as well as live imaging of GSP-3/4::GFP during spermatogenesis.

866A. Signaling pathways that mediate the deleterious effects of dietary fatty acids in the germ line. **Marshall Deline**, Jennifer L. Watts. School of Molecular Biosciences, Washington State Univ, Pullman, WA.

Omega-6 and omega-3 fatty acids are essential to the diets of mammals. However, over-consumption of certain omega-6 fatty acids, which are plentiful in the Western diet, is associated with increased risk of cancer and inflammatory diseases. The mechanism that relates these health issues to omega-6 fatty acids remains elusive. Unlike mammals, the nematode *Caenorhabditis elegans* possesses the enzymatic means to synthesize omega-3 and omega-6 fatty acids *de novo*. In addition to these endogenous fatty acids, *C. elegans* will readily take up and incorporate dietary polyunsaturated fatty acids from supplemented *E. coli* food sources. This provides an excellent genetic model to investigate endogenous and dietary lipid metabolism.

We have previously shown that supplementing the *C. elegans* diet with the omega-6 fatty acid dihomo-gamma linolenic acid (DGLA) leads to destruction of proliferating germ cells. While lipids are known to exert diverse developmental and physiological effects, little is known about the downstream signaling components. Here we use our DGLA-induced germline phenotype as a model to elucidate the signaling pathways that underlie the effects of specific lipids on physiology and development. Using transcriptional analyses, reverse genetic approaches, and gas chromatography mass spectrometry techniques, we have identified a specific G-protein coupled receptor signaling pathway as well as oxidative cytochrome P450 activities that mediate the deleterious effects of DGLA in the germ line. These findings highlight the usefulness of the *C. elegans* germ line as a model to determine lipid signaling pathways and how they affect physiology and development.

867B. A Quality Control Mechanism Coordinates Meiotic Prophase Events. **Alison J. Deshong**, Alice L. Ye, Piero Lamelza, Needhi Bhalla. MCD Biology, Univ of California Santa Cruz, Santa Cruz, CA.

To achieve proper meiotic chromosome segregation, homologous chromosomes are physically linked by chiasmata so that they can effectively biorient on the meiosis I spindle. These linkages are established during meiotic prophase through a series of progressively intimate interactions between homologs (pairing and synapsis) to culminate in meiotic recombination. However, how pairing, synapsis and recombination are coordinated during meiotic prophase is poorly understood. The pch-2 gene has been implicated in various organisms in a wide array of meiotic functions including checkpoint signaling, chromosome axis structure, crossover control and interhomolog bias. Despite these findings, a common conserved function for PCH-2 has remained elusive. We present data that suggests PCH-2 restrains meiotic prophase events. In pch-2 mutants, pairing, synapsis and recombination all occur more rapidly than wildtype. We speculate that PCH-2 inhibits these events to coordinate them and maintain quality control of meiotic products.

868C. GLD-4, a cytoplasmic poly(A) polymerase, is part of a translational feedback loop regulating stem cell pool size and meiotic entry in the adult *C. elegans* germ line. Sophia Millonigg, Ryuji Minasaki, Marco Nousch, **Christian R. Eckmann**. MPI-CBG, Dresden, Germany.

To maintain organ size, it is essential for tissues to keep the right balance between the number of stem cells that replenish the tissue and differentiating cells that are eventually turned over. Equally pivotal is this problem for the adult reproductive tissue. Adult animals of many species remain fertile over a very long period of their lifetime. Thus, the germ line must constantly renew itself by replenishing a stem cell pool and by replacing differentiated cells lost to damage and gamete production. Therefore, a healthy balance between stem cell self-renewal and differentiation is essential for lifetime reproductive capacity. By studying the adult *C. elegans* germ line as a paradigm of this balance, we genetically and molecularly dissected the underlying gene expression circuitry and find that translational activation of mRNAs is an important mechanism to maintain both cell fates. At the molecular level, this mechanism is based on poly(A) tail metabolism via two conserved but distinct cytoplasmic poly(A) polymerases (cytoPAP), *i.e.* GLD-4 and GLD-2. These cytoPAP enzymes catalyze the addition of adenosines to the mRNA's 3'end to stabilize and enhance the translational competence of mRNAs. To prevent stem cell loss in the adult, we find that GLD-4-mediated translational activation of the self-renewing factor GLP-1/Notch operates in parallel to FBF-mediated translational repression of the differentiation factor GLD-2. For the onset of differentiation, we find that translational activation of differentiation factors via GLD-4 and GLD-2 cytoPAP is paired with translational repression of self-renewing factors. Our findings expose two modules of translational activation/repression in germ cells, one specific for stem cell renewal and a second one for differentiation. Moreover, both modules are interconnected via their mRNA targets. This leads to a reciprocal expression of either self-renewal of differentiation factors, which establishes a molecular rheostat that finely bridles the size of the adult stem cell pool.

869A. Gamma Secretase Function During Germline Development. **Cassandra Farnow**, Ipsita Agarwal, Caitlin Greskovich, Caroline Goutte. Amherst College, Amherst, MA.

The canonical Notch signal transduction pathway relies on proteolytic events that release the intracellular portion of the Notch receptor from its transmembrane domain. The final step in this ligand-triggered Notch activation is the intramembranous cleavage event carried out by the g secretase membrane complex. g secretase is composed of four subunits, Presenilin, APH-1, APH-2/Nicastrin, and PEN-2. The presenilin protein contains the catalytic core of the complex, while the other three subunits are thought to have critical roles in assembly and stability of the complex. In *C. elegans* two different presenilin genes, *sel-12* and *hop-1*, function in the context of Notch signaling events and are redundant for most such events: single mutants are viable, fertile, and yield live progeny, while double mutants display Notch loss of function phenotypes (sterility, maternal-effect lethality, Pvl, and 2AC)[1,2]. Given that SEL-12 and HOP-1 share only 35.4% a.a. identity[1], and have different expression patterns, we considered whether functional differences might exist between these presenilin proteins. Although much has been learned about the biochemical details of g secretase activity, little is known about its function over the course of development. Germ cell proliferation in the *C. elegans* gonad requires continuous GLP-1 Notch activation throughout larval and adult life[3]. We compared the need for *hop-1* and *sel-12* over the course of germline development. While *sel-12* and *hop-1* are redundant for mediating germline proliferation during larval development, we demonstrate that germline proliferation in the adult is mediated predominantly by *hop-1*. In order to confirm that the required role of *hop-1* in the adult is to activate GLP-1, we used the hyperactive *glp-1(ar202)*[4] allele that causes late-onset germ cell overproliferation (tumors), and we demonstrate that *hop-1(ar179)* is epistatic to *glp-1(ar202)* late tumor formation. Our analysis identifies the first unique role for *hop-1* in the context of Notch signaling, and we are now analyzing additional requirements for this role during adult germline proliferation. *1.* Li and Greenwald '97; *2.* Westlund et al. '99; *3.* Austin and Kimble'87; *4.* Pepper et al. '03.

870B. Nuclear Envelope Components and Dynein act Coordinately with MEL-28 to Promote Post-Embryonic Development. **Anita G. Fernandez**[1,2], Allison Lai[1], Carly Bock[1], Angela Quental[1], Mike Mauro[1], Emily Mis[1], Fabio Piano[2]. 1) Dept Biol, Fairfield Univ, Fairfield, CT; 2) NYU Center for Genomics and Systems Biology, New York, NY.

MEL-28 is required for fundamental cellular processes such as nuclear envelope integrity and chromosome segregation. Even though MEL-28 has essential roles in basic cellular processes, the *mel-28* gene is a strict maternal-effect embryonic lethal, suggesting that its activity is required only during embryonic development. To find genes that act cooperatively with *mel-28* during post-embryonic development, we performed an RNAi-based genetic interaction screen in which we sought genes that cause phenotypes in *mel-28* homozygous adults but not wild-type adults. We identified over 30 genes that act with *mel-28*, and have further characterized some of the genes by generating double mutants and studying the synthetic phenotypes in more detail. Mutations in genes that encode nucleoporins cause severely reduced lifespan and infertility in a *mel-28* genetic background. In contrast defects in dynein components affect brood size but not viability in *mel-28* mutants. These studies reveal critical new roles for *mel-28* in adult fitness and reproduction and suggest that these processes enjoy multiple genetic redundancies to buffer the system against perturbation.

871C. SPCH-1/2/3 localize to mature sperm chromatin and may play a role in fertility and genome stability. **Jennifer Gilbert**, Dana Byrd, Jordan Berry, Diana Chu. San Francisco State Univ, San Francisco, CA.

During spermatogenesis, chromatin becomes highly compacted to ensure efficient delivery of DNA to the oocyte. Compaction of sperm chromatin in most animals is facilitated by deposition of small nuclear basic proteins called protamines. While protamines share molecular features, their sequence variability makes identification across species challenging. From a proteomic screen, we identified three nearly identical *C. elegans* proteins, SPCH-1/2/3, that share molecular features of protamines and are highly enriched in sperm chromatin. Shared molecular features include low molecular weight, high isoelectric point, and a high percent of arginine and serine residues. Using proteomic analysis of acid solubilized sperm chromatin, we also found that SPCH-1/2/3 are highly phosphorylated. Based on the feature similarity to protamines, we hypothesize that SPCH-1/2/3 may play a role in fertility.

We first examined the localization of SPCH-1/2/3 by immunofluorescence to assess where SPCH-1/2/3 may function. Consistent with a role in male fertility, SPCH-1/2/3 localizes to the compact chromatin of mature sperm. Immediately after fertilization, SPCH-1/2/3 mark the paternal pronuclei and then are removed as the sperm pronucleus decondenses. In order to examine localization of SPCH-1, 2 and 3 independently in live animals and in real time during fertilization, we are currently generating transgenic animals expressing SPCH-1/2/3::GFP.

To test whether loss of SPCH-1/2/3 function results in declined fertility, we counted viable progeny from *spch* deletion mutant animals. Surprisingly, disruption in a single *spch* gene, *spch-2*, results in a statistically significant reduction in the number of viable progeny compared to wild type. We have also found that *spch-2* mutants have a higher percentage of male progeny than wild type, suggesting that SPCH proteins, and perhaps protamines more generally, may play a role in both fertility and genome stability.

872A. Differential expression of germline genes in the presence/absence of H3K9me2. **Yiqing Guo**, Eleanor Maine. Biology, Syracuse Univ, Syracuse, NY.

In *C. elegans*, indirect immunofluorescence experiments indicate a high level of histone H3 lysine 9 dimethylation (H3K9me2) on any unpaired/unsynapsed chromosomes during first meiotic prophase, particularly in pachytene stage nuclei (Kelly et al 2002; Bean et al 2004). In contrast, H3K9me2 marks are relatively low on paired chromosomes at this time. H3K9me2 marks are typically associated with transcriptional repression. Thus, the elevated level of H3K9me2 on unpaired chromosomes may play a role in meiotic silencing of unpaired chromosomes (MSUC). Germline H3K9me2 marks require activity of MET-2 (Bessler et al 2010), a SET domain protein that is the only *C. elegans* ortholog of human SETDB1 H3K9 methyltransferase (Schultz et al 2002).

To begin to investigate the importance of H3K9me2 marks on unpaired chromosomes, we performed RNA-seq on mRNA isolated from *him-8* vs *met-2;him-8* adult hermaphrodite gonads. Our data identify genes that are expressed in the gonads and that differentially expressed in *met-2(0)* and *met 2(+)*. In most cases, the differentially expressed transcripts are elevated in *met-2(0)*, consistent with a role for H3K9me2 in limiting transcription. Although elevated transcripts are not preferentially X-linked, we observe a greater average fold-change for X-linked transcripts than for autosomal transcripts. To evaluate whether the unpaired status of the X contributes to this pattern, we are evaluating additional genotypes. As a complementary approach, we are also investigating the distribution of H3K9me2 on paired vs unpaired chromosomes using a ChIP-seq approach.

873B. **MRG-1 and RFP-1 regulate proliferation in the germline**. **Pratyush Gupta**, Lindsay Leahul, Katie Jasper, David Hansen. Department of Biological Sciences, Univ of Calgary, Calgary, Alberta, Canada - T2N 1N4.

We previously demonstrated that the proteasome inhibits the proliferative fate in the *C. elegans* germ line. Reduced proteasomal function enhances over-proliferation in a sensitized genetic background (Macdonald, Knox et al. 2008). Presumably, the proteasome is involved in degrading proteins that either promote the proliferative fate, or inhibit meiotic entry. We refer to these as proliferation promoting proteins (PPPs). To identify these PPPs, we first sought to identify the substrate recognition subunits (SRS) of E3 ubiquitin ligases that may target the PPPs for degradation by the proteasome. We screened 826 SRSs by RNAi in four sensitized genetic backgrounds and found five that enhance over-proliferation. One of these, RFP-1, specifically enhances *glp-1(ar202gf)* to form a germline tumor, albeit incomplete. Large-scale 2-hybrid screens identified potential binding partners for RFP-1 (Crowe and Candido 2004; Zhong and Sternberg 2006), which could be PPPs that are targeted for proteasomal degradation by RFP-1. One of these, *mrg-1*, partially suppresses the over-proliferation phenotype of *glp-1(ar202gf); rfp-1(ok572)*, suggesting that it may function as a PPP. We found that MRG-1 levels do increase in an *rfp-1* mutant, as well as when proteasomal function is decreased through genetic mutation or chemical inhibition. Therefore, MRG-1 protein levels are likely regulated through proteasomal-mediated degradation. We are currently determining how *mrg-1* may participate in controlling the proliferative fate in the germline.

874C. The RNA binding protein TIAR-1 is essential for *C. elegans* fertility. **Gabriela Huelgas Morales**, Carlos G. Silva García, Rosa E. Navarro. Instituto de Fisiología Celular, UNAM, Mexico City, Mexico.

RNA binding proteins, such as TIA-1 and TIAR, regulate RNA at different levels in a variety of organisms. In the nucleus, these proteins participate in

alternative splicing while in the cytoplasm they regulate mRNA stability and/or translation. Along with these functions, under stress conditions, TIA-1/TIAR aggregate to form stress granules in a reversible manner to protect mRNAs.

In *C. elegans*, there are three TIA-1/TIAR homologs: TIAR-1, -2 and -3. TIAR-1 is the most similar to its mammalian orthologues at both sequence and function level. *tiar-1(tm361)* animals do not respond normally to stress therefore they cannot induce germ cell apoptosis, nor can they form "stress-granules" under these conditions. Under normal circumstances, these mutants are temperature-sensitive sterile and have embryonic lethality. These phenotypes are similar to those shown by the up- or down-regulation of its mammalian counterparts.

Our goal is to understand TIAR-1's role in *C. elegans* oogenesis and ovulation. Also, we would like to determine if TIAR-2 and TIAR-3 act redundantly with TIAR-1 during these processes. So far we have found that mutations on TIAR-1, -2 and -3, lead to a decreased fertility although *tiar-1(tm361)* animals exhibit the more severe phenotype.

tiar-1(tm361) animals have smaller gonads with fewer mitotic nuclei. Oocytes from *tiar-1(tm361)* mutant animals seem to go through all maturation steps, but later on they show an Emo phenotype. The Emo phenotype in the *tiar-1(tm361)* animals is suppressed when *oma-1* and *oma-2* genes are silenced suggesting mutant oocytes do mature. *tiar-1(tm361)* mutants have a wild type MSP expression but an abnormal MAPK activation pattern, in which phosphorylated MPK-1 localizes in fewer proximal oocytes than the wild type and it is usually absent in the pachytene region. This suggests that a component of the MAPK pathway could be a TIAR-1 target.

875A. Exploring the contribution of chromosomal context in shaping the *C. elegans* high-resolution recombination rate landscape. **Taniya Kaur**, Matthew Rockman. NYU, New York, NY.

Variable rates of meiotic recombination in a genome can have a profound effect on the creation of new combinations of alleles and on the efficacy of natural selection acting in different parts of a genome. A number of factors are known to shape recombination rate variability including sex, genetic background effects and specific DNA sequences. The *C. elegans* chromosomes (five autosomes and X chromosome) exhibit a striking pattern of a central domain of low meiotic recombination rates complemented by high recombination rate domains in the chromosome arms, separated by pronounced boundary regions. The factors underlying this consistent domain structure are unknown. The central low recombination rate domains are not physically centered on the chromosome. It is as yet unknown if the chromosomal context (arm vs. center of a chromosome) of the recombination rate boundaries encodes any positional information driving the chromosome-wide recombination rate domain structure. We generated sex-specific high-resolution recombination rate maps for the chromosome II center-right arm boundary region. In order to change the relative chromosomal position of this boundary region, we use end-to-end fusion chromosome strains derived from YA873 (IIL; XR) and YA929 (IIR; XR). Using fusion chromosome strains introgressed into the appropriate genetic background, recombinants between the two markers (*unc-4* and *rol-1)* that flank this chromosome II boundary were selected and genotyped at 183 SNPs spanning this ~ 2Mbp region. The recombination rate maps were then generated after identifying crossover breakpoints from the Illumina GoldenGate genotyping data. We also measured the genetic map distance between *unc-4* and *rol-1* in these strains by scoring phenotypes in the F2 progeny. We have generated high-resolution recombination rate maps for a boundary region in two contrasting chromosomal contexts. Our data indicate that the meiotic recombination frequency in the chromosome II center-right arm boundary region is not comprehensively explained by the chromosomal position of the boundary. We also could not detect an effect of genetic background on recombination frequency in this region for the fusion chromosome strains examined.

876B. RNA recognition by OMA-1, a *C. elegans* oocyte maturation determinant. **Ebru Kaymak**, Sean P. Ryder. Univ of Massachusetts Medical School, Worcester, MA.

Post-transcriptional regulatory mechanisms guide early development during *Caenorhabditis elegans* embryogenesis. Maternally supplied mRNAs encode for necessary developmental regulators until zygotic transcription is activated. These maternal transcripts remain in a translationally silenced state until fertilization. RNA-binding proteins regulate these maternally supplied mRNAs during oogenesis, the oocyte to embryo transition, and early embryogenesis. Identifying the target specificity of these RNA-binding proteins will reveal their contribution to patterning of the embryo. We are studying post-transcriptional regulation of maternal mRNAs during oocyte maturation. Maturation is an essential part of meiosis that prepares oocytes for fertilization. Although the physiological events taking place during oocyte maturation have been well studied, the molecular mechanisms that regulate oocyte maturation are not well understood. OMA-1 and OMA-2 are essential proteins that function redundantly during oocyte maturation. Both OMA-1/2 have CCCH-type tandem zinc finger (TZF) RNA-binding domains suggesting that they may be post-transcriptional regulators of oocyte maturation. However, the RNA-binding and the mRNA target specificity of OMA-1/2 are not known. To determine the RNA-binding specificity of OMA-1 we performed *in vitro* selection. The selected sequences demonstrate that OMA-1 binds UAA and UAU repeats in a cooperative fashion. Additionally, we identified the *glp-1* mRNA as a target of OMA-1. Multiple RNA-binding proteins regulate translation of GLP-1 protein, a homolog of Notch receptor. Two translational regulators of *glp-1* are POS-1 and GLD-1 that bind to a conserved region within the *glp-1* 3'-UTR. Interestingly, OMA-1 also binds to multiple fragments of the *glp-1* 3'-UTR *in vitro*. These fragments include those containing previously identified POS-1 and GLD-1 binding elements. Additionally, OMA-1 and OMA-2 repress *glp-1* expression in oocytes. Mapping the OMA-1 dependent regulatory sites in the *glp-1* mRNA and characterizing the interplay between OMA-1 and other factors will help reveal how multiple regulatory signals are coordinated from oocyte to embryo.

877C. Characterization of non-SMC elements of the SMC-5/6 complex in *C. elegans*. **Jayshree Khanikar**, Jaclyn Fingerhut, Jeremy Bickel, Raymond Chan. Department of Human Genetics, Univ of Michigan, Ann Arbor, MI.

Meiosis is a specialized cell cycle that produces haploid gametes from diploid germ cells. In preparation for meiotic chromosome segregation, DNA double-strand breaks (DSB) are produced and repaired via homologous recombination to generate inter-homolog crossovers, which in turn promote accurate chromosome segregation and increased genetic diversity in the offspring. Given the importance of inter-homolog crossovers, an excess of DSB are produced in meiotic prophase to ensure that the obligate crossovers are generated. Repair of the excess DSB that do not result in inter-homolog crossovers is nonetheless important for successful meiosis, but our understanding of this type of repair is poor compared to inter-homolog repair. We showed previously that the Structural Maintenance of Chromosomes (SMC) 5 and 6 proteins are important for successful repair of these excess DSB by

homolog-independent mechanism(s). The SMC-5/6 proteins are known to associate with multiple subunits, four of which are conserved in human and yeast cells, termed the non-SMC elements (NSE). Three of the NSE subunits promote post-translational modification by the ligation of ubiquitin (Ub) and SUMO to targeted proteins. The mechanism by which SMC-5/6 protein complexes regulated DSB repair has not been defined, however the Ub and SUMO ligase activities are postulated to be involved in this process. Our goal is to identify and characterize the NSE subunits for the *C. elegans* SMC-5/6 protein complex in order to test and understand their relevance in meiotic DSB repair. We have identified putative homologs to NSE-1, -2, -3 and -4 based on protein sequence homology. Using RNAi and genetic mutants, we have observed aberrant localization of SMC-5/6 complex subunits. We will present the on-going characterization of these putative homologs in terms of their subcellular location, protein interactions and functional impact on SMC-5/6-related roles in meiosis.

878A. Novel spermatogenesis-defective gene candidates. **Takashi Koyama**[1], Megumi Endo[2], Yusuke Hokii[3], Chisato Ushida[1,2,3,4]. 1) Graduate School of Agriculture and Life Science, Hirosaki Univ; 2) Department of Biochemistry and Molecular Biology, Hirosaki Univ; 3) Functional Genomics and Technology, United Graduate School of Agricultural Science, Iwate Univ; 4) RNA Research Center, Hirosaki Univ, Hirosaki, Japan.

MT16939 is a *C. elegans* mutant that has a 616-bp deletion on chromosome IV. This region encodes a small ncRNA gene *cer-2a*. Previously we demonstrated some characteristics of *cer-2a* product, CeR-2a RNA: CeR-2 RNA expresses in almost all cells from early embryo to adult, it localizes in the nucleolus, the nucleolar localization depends on the existence of a nucleolar protein NOP56, and CeR-2a RNA has a TMG-cap. These features indicated that CeR-2a RNA is a small nucleolar RNA which functions in the rRNA processing. MT16939 accumulated the precursors of LSU rRNAs. Recently, we found that MT16939 hermaphrodites produce only unfertilized eggs at high temperature. The phenotype was rescued when MT16939 hermaphrodites were mated to N2 males. This suggests that MT16939 is a spermatogenesis-defective mutant. MT16939 worms were stained with DAPI and their sperms were counted. DAPI signals observed in adult MT16939 spermatheca were less numerous and their sizes were larger than those of N2. These suggest that the cells in MT16939 spermatheca are not matured sperms but are spermatocytes, and the spermatogenesis is arrested in MT16939 at high temperature. It is still unclear whether the deletion of *cer-2a* caused the spermatogenesis-defective phenotype of MT16939 or not, because the deleted region on chromosome IV includes a part of 3' UTRs of neighboring protein genes. However, these genes, together with *cer-2a*, have not been reported as spermatogenesis-defective genes and are novel gene candidates which functions in spermatogenesis.

879B. Identifying regulators of sex-specific gonadal development in *C. elegans* by cell-specific RNA-seq. **Mary B. Kroetz**, David Zarkower. Genetics, Cell Biology & Development, Univ of Minnesota, Minneapolis, MN.

The *C. elegans* gonad originates in the embryo with the coalescence of a four-cell primordium composed of two somatic precursor cells (Z1/Z4) that flank two germ line precursor cells (Z2/Z3). The gonadal primordium is morphologically identical in the two sexes until the division of Z1/Z4. With the division of these cells, the gonadal primordium begins to develop via one of two distinct sex-specific programs of organogenesis. Despite the extensive sexual dimorphism and previously defined cell lineages of the gonad, the genetic pathways that direct the development of this organ, including its sex-specific development, remain largely unknown. To define the genetic networks that regulate gonadal development in both sexes, we employed cell-specific transcriptional profiling of Z1/Z4 using RNA-seq to identify the early gonadal regulators. Single sex populations of animals were generated by employing sex determination pathway mutants. A Z1/Z4-specific gfp reporter was used to isolate these cells by FACS from dissociated mid-L1 larval cells, just prior to the division of Z1/Z4 when the first sex-specific differences of the gonad arise. A few hundred Z1/Z4-enriched transcripts were identified for both hermaphrodites and fully masculinized XX-pseudomales, and a much smaller number of sex-specific Z1/Z4-enriched transcripts were also identified. The majority of transcripts with known Z1/Z4-enriched expression were identified by this method, including *fkh-6*, *gem-4*, and *pes-1*, and a number of novel Z1/Z4-enriched transcripts, including previously unannotated isoforms, have subsequently been validated by reporter analysis, confirming the effectiveness of this approach. Loss of function phenotypes are being determined by RNAi depletion and mutant analysis, with emphasis on transcripts that are sex-specifically Z1/Z4-enriched. Taking advantage of the recent innovations in larval dissociation and cell-specific RNA-seq, this work, which focuses on identifying the genetic networks responsible for the sex-specific developmental of the *C. elegans* gonad from a biopotential promordium, should advance our understanding of the regulatory logic and mechanisms underlying sexually dimorphic organogenesis and cell fate determination.

880C. *Y23H5A.4* is a sperm gene that encodes a mitochondrially-associated protein involved in spermatid activation. **Craig W. LaMunyon**, Ubaydah Nasri, Nicholas Sullivan, Jessica Clark. Biological Sciences, Cal Poly Pomona, Pomona, CA.

A mutation in the gene *Y23H5A.4 I* was recovered from a suppressor screen of *spe-27(it132ts)IV*. The *spe-27* gene is a member of the *spe-8* group, which encodes a signal transduction pathway for spermatid activation; *spe-27* mutants are therefore sterile and accumulate inactive spermatids. The *Y23H5A.4(hc198)* mutation suppresses not only mutations in *spe-27*, but also mutations in other genes *spe-8* group genes. Taken together with the fact that mutant males harbor prematurely activated sperm, these observations suggest that the *Y23H5A.4(hc198)* mutation bypasses the need for an activation signal altogether. Mutant hermaphrodites have reduced fecundity at 25 C and experience excess sperm loss as they lay eggs, suggesting the sperm cannot maintain their position in the hermaphrodite reproductive tract. RT-PCR shows the *Y23H5A.4* transcript is sperm specific. The C-terminus of the protein contains an MSP domain, the a2 beta strand of which is altered by the *hc198* mutation. The a2 beta strand is critical for MSP-MSP dimerization, suggesting that the Y23H5A.4 protein has an MSP binding function. GFP translational fusions show colocalization with the mitochondria, a novel location for a sperm encoded protein. The association of potential MSP binding with mitochondrial specificity and sperm function suggests hypotheses for the overall function of the Y23H5A.4 protein in sperm. We also report on the paralog *Y48B6A.5*, which is sperm expressed and bears a C-terminal MSP domain.

881A. DNA damage response and spindle assembly checkpoint collaborate to elicit cell cycle arrest in response to replication defects in the *C. elegans* male germ line. **Kate Lawrence**, JoAnne Engebrecht. Univ of California Davis, CA.

Persistent DNA damage in germline stem cells leads to embryonic lethality, progeny inviability or germline tumors. Consequently, cells closely monitor genomic integrity and delay progression through the cell cycle so repair precedes division. In *C. elegans*, genotoxic stress activates checkpoints that initiate cell cycle arrest in proliferating germ cells. Arrest in response to stalled replication forks induced by hydroxyurea (HU) results in enlarged nuclei and

accumulation of S-phase markers. HU damage is sensed by the *C. elegans* homolog of ATR, a PI3-related protein kinase, which initiates a signaling cascade to induce arrest and repair. The signal transducers and downstream effectors of this DNA-damage-response (DDR) have been extensively studied in hermaphrodites, but not in males. While RNAi knockdown of several of these genes disrupts checkpoint output and thus prevents arrest in hermaphrodites, the same treatment only partially prevents HU-induced arrest in males. We took a candidate approach to identify compensatory pathways that contribute to HU-induced arrest in males. One well-characterized pathway that elicits cell cycle arrest is the spindle assembly checkpoint (SAC). The SAC is most often associated with monitoring kinetochore attachment to spindles during prometaphase/metaphase of mitosis and meiosis. We found that RNAi knockdown of several SAC components alone did not affect HU-induced cell-cycle arrest in males; however, knockdown of both ATR and SAC components resulted in a failure to arrest in the presence of HU, indicating that the DDR and SAC both function to elicit arrest in the presence of stalled forks. Consistent with this, SAC components become enriched at the nuclear periphery in an ATR-dependent manner in response to HU. Our data reveal that the SAC has a novel role in S-phase arrest, which is independent of *fzy-1*/cdc20, an APC activator and key regulator of SAC-induced metaphase arrest. Preliminary studies suggest that the other APC activator, *fzr-1*/cdh1 facilitates SAC-induced S-phase arrest. We hypothesize that FZR-1 forms an S-phase inhibiting complex with SAC subunits analogous to the MCC complex that mediates APC inhibition during SAC-induced M-phase arrest.

882B. Elucidating how TRA-1 promotes spermatogenesis in *C. briggsae*. **Shin-Yi Lin**[1], Yiqing Guo[1,2], Ronald E. Ellis[1]. 1) Molecular Biology, UMDNJ-SOM, Stratford, NJ; 2) Biology, Syracuse Univ, Syracuse, NY.

The Gli family transcription factor TRA-1 plays a conserved role during sex determination in *C. elegans* and *C. briggsae*. TRA-1 is a negative regulator of male fates in hermaphrodites, an activity carried out by a cleaved form that contains only the amino terminal half of the protein. Consistent with this activity, most mutations in *tra-1* transform *XX* hermaphrodites into males. But TRA-1 is also a positive regulator of spermatogenesis in both sexes. This second activity appears to be carried out by the carboxy terminal half of the full-length protein. In *C. elegans*, mutations that block an interaction between TRA-1 and TRA-2 cause *XX* animals to develop as females. In addition, recent work from our lab suggests that TRR-1 and other members of the Tip60 Histone Acetyl Transferase Complex are required for TRA-1 to promote spermatogenesis. This activity results in expression of *fog-3*, which is needed for germ cells to adopt male fates.

To understand how TRA-1 works with other proteins to promote spermatogenesis, we are characterizing the new *C. briggsae tra-1* mutation *v48*. This allele is a semi-dominant mutation with a missense in the carboxyl terminus of TRA-1 that was isolated in a screen for feminizing mutations; it is the first *C. briggsae tra-1* mutation that produces perfect *XX* females. We are also conducting a suppressor screen for mutations that restore spermatogenesis in *tra-1(v48) XX* animals, to identify factors that influence this activity of TRA-1. Finally, we are investigating whether the *v48* mutation affects TRA-1 interactions with TRA-2 or the Tip60 Histone Acetyl Transferase Complex.

883C. Molecular analysis of *ego-3*, an enhancer of *glp-1*. **Jim Lissemore**[1], Elyse Connors[2], Ying Liu[2], Eleanor Maine[2]. 1) John Carroll Univ, Univ Heights, OH; 2) Syracuse Univ, Syracuse, NY.

glp-1 encodes a Notch family transmembrane receptor required for mitotic proliferation of the *C. elegans* germline. Mutations in *glp-1* lead to reduced germline proliferation, with strong loss-of-function mutations rendering animals sterile. *ego-3* was identified in a screen for genetic enhancers of a weak *glp-1* allele, *glp-1(bn18)*. The best studied allele of *ego-3*, *ego-3(om40)*, has a complex recessive phenotype that includes reduced and delayed germline proliferation, slow development characterized by extended larval period, proximal germline proliferation, delayed and abnormal gamete formation, and a severe mobility defect that improves to nearly wildtype mobility in adults. Three-factor and SNP mapping have placed *ego-3* on chromosome VR, to the left of *unc-61* and close to *daf-21*, in a region containing 14 known protein-coding genes. To identify the molecular lesion in *ego-3*, we are using RNAi-mediated knockdown on each of these 14 genes to test for enhancement of *glp-1(bn18)*. In addition, we are testing RNAi for each gene in a variety of genetic backgrounds to see if we can phenocopy the *ego-3(om40)* developmental phenotype.

884A. DAF-2 and ERK regulate C. elegans oogenesis as a physiological adaptive response to nutrient availability. **Andrew Lubin Lopez**[1], Jessica Chen[1,2], Hyoe-Jin Joo[1], Melanie Drake[1], Miri Shidate[1,3], Cedric Kseib[1,4], Swathi Arur[1]. 1) Genetics, UT MD Anderson, Houston, TX; 2) Biology, Washington U., St. Louis, MO; 3) Biology, Rice U., Houston, TX; 4) Medicine, Lebanese American U., Beirut, Lebanon.

To survive and propagate, individuals must adapt to changing environments. Coupling the production of mature gametes and fertilized progeny to favorable nutritional conditions provides an evolutionary advantage to species survival. In invertebrates, the proliferation of female germline stem cells is regulated by nutritional status. But, in many animals, the number of female germline stem cells and oocytes is set early in development, with oocytes progressing through meiosis later in life. Mechanisms that couple later steps of oogenesis to environmental conditions remain unexplored. Using Caeonorhabditis elegans female germline as a model system we show that insulin-like signaling pathway couples nutrient availability to progression through meiosis I and subsequent oogenesis steps. This ensures that oocytes are produced only under conditions that are favorable for the survival of the resulting zygotes. In the presence of food, active insulin signaling acts through the RAS-ERK pathway to drive progression of meiotic prophase I and oogenesis; in the absence of food, the resultant inactivation of insulin signaling leads to the down-regulation of RAS-ERK pathway activity, and oogenesis is stalled. Thus, in the C. elegans germline nutrient status is coupled to meiotic progression via the insulin-like receptor DAF-2 mediated regulation of the RAS-ERK pathway.

885B. C. elegans p53/p63 protein CEP-1 promotes meiotic recombinational repair. **Abigail Rachele Mateo**[1], Kristine Jolliffe[1], Alissa Nicolucci[1], Bin Yu[1], Olivia McGovern[2], Zebulin Kessler[2], Judith Yanowitz[2], W. Brent Derry[1]. 1) Developmental and Stem Cell Biology Program, The Hospital for Sick Children, Toronto, ON; 2) Magee-Womens Research Institute, Univ of Pittsburgh School of Medicine, Pittsburgh, PA.

The C. elegans p53 homologue CEP-1 embodies structural and functional properties similar to all three vertebrate p53 family members. The roles of CEP-1 in cell cycle arrest and apoptosis have been previously characterized, however, its role in DNA repair is not known. Loss of cep-1 results in a mild X chromosome non-disjunction phenotype and increased sensitivity to replication inhibitors, suggesting a potential role in meiotic chromosome segregation

and DNA repair. To determine how CEP-1 regulates these processes, we examined its genetic interaction with mutants that have chromosome segregation defects. Ablation of *cep-1* significantly enhanced lethality in the meiotic mutant *him-5* (high incidence of males-5). *cep-1; him-5* mutants also have abnormal chromosome morphology that is strikingly similar to that observed when *brc-2* and *rad-51* are mutated. We also observed reduced recombination frequencies in *cep-1; him-5* mutants in comparison to wild-type worms, *cep-1* or *him-5* mutants. These results suggest a cooperative role for CEP-1 and HIM-5 in promoting meiotic recombination. We are currently determining the mechanism by which CEP-1 promotes meiotic recombinational repair and hypothesize that CEP-1 regulates the expression or function of one or more DNA repair proteins. Similar to *cep-1*, the mild meiotic defects observed when the Brca1 homologue *brc-1* is mutated are significantly enhanced with the combined loss of *him-5*.We observed 60-80% lethality in the progeny of *brc-1; him-5* mutants but loss of *cep-1* did not enhance the lethality of *brc-1* mutants.There is also no significant difference between the lethality of *cep-1; brc-1; him-5* triple mutants and *brc-1; him-5* double mutants, suggesting that *brc-1* and *cep-1* likely function in the same genetic pathway to promote meiotic recombination. In addition, *brc-1; him-5* mutants also have chromosomal abnormalities. Our data suggest that CEP-1 and BRC-1 cooperate with HIM-5 to promote meiotic recombinational repair.

886C. Acetylation of H2AK5 and genome instability in *xnd-1* mutants. **Brooke McClendon**[1,3], Judith Yanowitz[1,2,3]. 1) Microbiology and Molecular Genetics, Univ of Pittsburgh, Pittsburgh, PA; 2) Dept of Obstetrics, Gynecology, and Reproductive Services, Univ of Pittsburgh, PIttsburgh, PA; 3) Magee-Womens Research Institute.

Maintenance of genome integrity is important for both the survival of the individual and the propagation of the species. Homologous recombination (HR) is a conserved process that is required for the error-free repair of double-strand breaks (DSB) and the restarting of stalled replication forks. During meiosis, DSBs are purposefully created and must be repaired by HR to form crossovers (CO) between homologous chromosomes, linking them together and ensuring faithful separation during meiosis I. Failure to create COs results in nondisjunction and subsequent aneuploidy. In humans, chromosomal aneuploidies account for over half of spontaneous abortions during the first trimester of pregnancy. Thus, competent DNA repair by HR is essential for the formation of viable eggs and sperm. In a screen for meiotic recombination regulatory proteins in *C. elegans*, we identified *X chromosome nondisjunction-1* (*xnd-1*) and found that it is required for programmed DSB formation on the X chromosome, the initial step of CO recombination. Additionally, *xnd-1* mutants exhibit low survival, low brood size, progressive sterility, and the appearance of spontaneous mutations - phenotypes indicative of genome instability. Indeed, *xnd-1* mutants display sensitivity to ionizing radiation (IR) compared to wild type animals. Microarray analyses of *xnd-1* mutant germlines do not implicate misregulation of genes involved in DSB repair, suggesting XND-1 may regulate genome stability either directly or through its effect on chromatin structure. *xnd-1* mutant germlines have increased levels of acetylated H2AK5 (H2AK5ac); RNAi against *mys-1* reduced H2AK5ac and the incidence of seven DAPI-staining bodies at diakinesis. We are now exploring if/how increased H2AK5ac contributes to other *xnd-1* phenotypes. To this end we have been characterizing *xnd-1;mys-1(n3681)* double mutants. Preliminary results suggest that knockdown of *mys-1* in *xnd-1* mutants may partially rescue the IR sensitivity. Further studies are ongoing to determine how XND-1 regulates H2AK5ac through MYS-1 and the developmental consequences of this regulation.

887A. Mechanism of Germ Cell Loss by Ionizing Radiation in a *C. elegans* Tumor Model. **David Michaelson**[1], Xinzhu Deng[2], Diana Rothenstein[2], Regina Feldman[2], Simon Powell[3], Zvi Fuks[3], E. Jane Albert Hubbard[1], Richard Kolesnick[2]. 1) Developmental Genetics, NYU Medical Center, New York, NY; 2) Molecular Pharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY; 3) 3Radiation Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY.

Ionizing radiation (IR) contributes to approximately 50% of cancer treatment protocols in the United States. It is well established that the main effect of ionizing radiation relevant to cancer treatment is the creation of double stranded breaks in DNA. The cellular response to DNA damage is well conserved across evolution and involves a cascade of proteins that sense and respond to DNA damage, whatever the source. Depending on the nature of the DNA damage as well as the cellular and genetic context of the damage, this conserved DNA damage pathway can trigger cell cycle arrest, DNA repair pathways, and, in some circumstances, apoptosis. The exact combination of these responses downstream of DNA damage largely determines the outcome of cancer treatment. We are using *C. elegans* to study the mechanisms of IR-induced effects on a germline “tumor” driven by hyperactive GLP-1/Notch signaling, using *C. elegans* genetics to better understand what determines the response of these cells to IR. Using this model we show that IR induces both G2 arrest and cell death, though the cell death mechanism is not dependent on classical apoptotic caspases. We show that the Homologous Recombination (HR), but not Non-Homologous End Joining (NHEJ) pathway of DNA repair partially protects these cells from the effects of ionizing radiation. We also compare the effects of IR in our worm tumor model to the effects on a human leukemia cell line driven by hyperactive Notch signaling and find that they both show a similar dependence on HR for surviving IR treatment.

888B. Temperature Sensitive Fertility of *lin-35* Mutants. **Brian P. Mikeworth**, Lisa N. Petrella. Dept of Biological Sciences, Marquette Univ, Milwaukee, WI.

Temperature plays a critical role in the ability for an organism to become fertile. High environmental temperatures cause infertility in organisms from nematodes to mammals. One mechanism that may play an important role in high temperature infertility is improper buffering of gene expression levels during germline development, spermatogenesis, or oogenesis. SynMuv B proteins are transcriptional repressors that act to suppress expression of germ line-specific genes within somatic cells. Loss of synMuv B proteins cause high temperature larval arrest (HTA) phenotype resulting in synMuv B mutants that arrest at the L1 stage of development at 26°C. While investigating the HTA phenotype we found that *lin-35* mutant L1s display defects in P-granule morphology and changes in histone modifications in primordial germ cells (PGCs). It has been shown that *lin-35* mutants have decreased fertility at moderate temperatures although the mechanism is not understood. We are investigating temperature sensitivity of the germ line of *lin-35* mutant. In order to test the temperature sensitivity of fertility in *lin-35* mutants we are taking advantage of a strain that rescues the HTA phenotype by expressing LIN-35(+) in the intestine but leaves the germ line deficient for LIN-35. Initial experiments demonstrate that *lin-35* mutant L1s raised at 26°C displayed a range of abnormal P-granule protein localization when compared with mutants raised at 20°C or wild type PGCs at either temperature. To determine the degree of this phenotype, we are scoring the frequency of P-granule defects in the PGCs of *lin-35* mutant L1s raised at 20°C and 26°C. Additionally, to determine if *lin-35* mutants show high temperature loss of fertility, we are scoring the fecundity of *lin-35* mutants raised at 20°C and 26°C. If a high

penetrance of P-granule defects and low fecundity are scored and correlated in *lin-35* mutants raised at 26°C, it could suggest that defects in P-granules in the PGCs could lead to a defective germ line in adult mutants and cause infertility at high temperatures. Finally, we are assessing all stages of germline development to determine at what stages germline defects begin and if they extend throughout larval development.

889C. Spermiogenesis regulation involves multiple sperm cell compartments as revealed through a suppressor screen of *spe-27*. **Ubaydah Nasri**, Misa Austin, Nicholas Sullivan, Craig LaMunyon. Biological Sciences, Cal Poly Pomona, Pomona, CA.

Spermiogenesis is the process by which spermatids differentiate to become mature spermatozoa. During spermiogenesis in *C. elegans*, pseudopods extend from the spermatids and enable the sperm to crawl to the fertilization site within the hermaphrodite reproductive tract. A signal transduction pathway that activates spermiogenesis involves genes in the *spe-8* group (*spe-8, spe-12, spe-19, spe-27,* and *spe-29*). Mutations in any of the *spe-8* group genes disrupt spermiogenesis. A suppressor screen of *spe-27(it132ts)I* identified numerous mutations that bypass the need for an activation signal altogether. Two genes previously identified from this suppressor screen were *spe-4* and *spe-6*. SPE-4 is a presinilin 1 homolog localized to the membrane of the membranous organelle, while SPE-6 is a predicted cytosolic serine threonine protein kinase. Here we identify and characterize three additional genes from the *spe-27* suppressor screen: *spe-46* (*W06D4.2*) and *Y23H5A.4* on Chromosome I, and *K01D12.7* on Chromosome V. The *spe-46(hc197)* mutation causes defects in spermatogenesis in addition to premature sperm activation, and the null phenotype is sterility. SPE-46 has a predicted transmembrane domain and is expressed solely in sperm. The *Y23H5A.4(hc198)* mutation results in excessive loss of sperm that appear normal, lowering fecundity. The Y23H5A.4 protein is sperm expressed and has an MSP domain, which is disrupted by the *hc198* suppressor mutation. This protein localizes to the mitochondria (see our poster on *Y23H5A.4* for additional information). Finally, the *K01D12.7(hc201)* mutation causes a sperm defect in addition to premature spermatid activation. The K01D12.7 protein is very small and has a predicted signaling function and potential eri-1 interaction. We have also mapped and studied several additional suppressor mutations that are all on Chromosome I. While it has been known that membranous organelle fusion with the plasma membrane occurs during spermiogenesis, our results now show the involvement of a novel putative signaling molecule (K01D12.7) and the mitochondria (Y23H5A.4).

890A. Deletion of ccm-3 in C. elegans promotes increased accumulation of reactive oxygen species resulting in apoptosis of germline cells. **SWATI PAL**, BIN YU, W. BRENT DERRY. DEVELOPMENTAL AND STEM CELL BIOLOGY DEPARTMENT, THE HOSPITAL FOR SICK CHILDREN, TORONTO, ONTARIO, M5G 1X8 Canada.

Cerebral Cavernous malformations (CCMs) are the disorders of capillaries in the central nervous system that result from a loss of integrity in endothelial cells. CCMs affect approximately 1 in 500 individuals and presents in patients a variety of pathophysiological symptoms that range from mild headaches to severe hemorrhagic stroke. Till date, mutations in three genes have been identified that account for approximately 90% of patients with familial disease, *ccm-1*, *ccm-2* and *ccm-3*. The most severe prognosis is associated with *ccm-3* mutations, but the CCM3 signalling pathway has not been resolved. In contrast to the familial form, sporadic CCMs have not been linked with any genetic loci. The varying degrees of symptoms and severity of disease in patients with the same mutation in either of the familial CCM genes suggests the existence of modifier genes. We have shown that *ccm-3* (or C14A4.11) affects multiple tissues in *C. elegans*. For example, *ccm-3* homozygote mutants have truncated excretory canals as well as defective oocyte and spermatocyte development that renders them sterile. The oocytes do not grow to the proper size and undergo massive waves of apoptosis, which can be rescued by expression of wild-type *ccm-3* gene. Because mammalian CCM genes have been shown to affect reactive oxygen species (ROS), we cultivated *ccm-3* mutants in presence of N-acetyl cysteine (NAC), a ROS scavenger, and observed partial rescue of the germline phenotype. NAC also partially protected germlines of wild-type animals from radiation-induced apoptosis. Because the Ras/MAPK pathway has been shown to promote apoptosis in the germline, we are now examining the status of activated MPK-1/ERK in ccm-3 mutant germlines. CCM-3 physically interacts with the germinal center kinase III (GCK-III) family of proteins to negatively regulate ERK activation and apoptosis in mammals. Ablation of the sole GCK-III gene in the worm, *gck-1*, also results in defective oocyte development that resembles *ccm-3* mutants. We hypothesize that CCM-3 and its binding partner GCK-1 are required for proper growth and survival of oocytes.

891B. Selective elimination of male-producing sperm by apoptosis in a nematode. **Manish Parihar**, Sarah Smith, Andre Pires da Silva. Biology, Univ of Texas at Arlington, Arlington, TX.

Sexually reproducing animals where the male genotype is XY/XO and the female is XX are expected to produce an equal proportion of XY/XO and XX progeny after crossing. However, in the free-living soil nematode *Rhabditis sp*. SB347, the XO males produce <10% males in cross-progeny. Our previous studies on SB347 have shown that during spermatogenesis in males the major sperm protein (MSP) is segregated only to the X-bearing spermatid and thus, the non-X bearing (nullo-X) spermatid becomes incapable of fertilizing the egg. The objective of this research is to understand the genetic mechanism of this developmental abnormality. Our preliminary observation suggested that the nullo-X spermatids may be undergoing programmed cell death called apoptosis, which is usually triggered in response to stress, DNA damage, and to control tissue growth. We tested the expression of known conserved proteins that regulate apoptotic pathway in *C. elegans* (p53 homolog CEP-1 and caspase CED-3) and saw that they are expressed only in the nullo-X spermatid. Furthermore, the distribution pattern of cytochrome *c* in the dividing secondary spermatocytes suggests that apoptosis of the nullo-X spermatids might be mediated by cytochrome *c*. We also generated a mutant that gave higher proportion of males after crossing and showed equal segregation of the cytoplasmic components. Comparison between the mutant and the wild-type SB347 is underway to precisely identify the genes involved. Our results so far indicate that the nullo-X spermatid in SB347 undergoes programmed cell death through a pathway apparently similar to DNA damage induced apoptosis in *C. elegans*. In addition to further understanding apoptosis, this research will also uncover a novel mechanism of regulating sex ratio by selective elimination of male-producing sperm.

892C. Visualizing dynamics of meiotic prophase chromosome structures. **Divya Pattabiraman**, Baptiste Roelens, Marc Presler, Grace Chen, Anne Villeneuve. Stanford Univ, Stanford, CA.

The synaptonemal complex (SC) is a highly-ordered proteinaceous structure that assembles at the interface between aligned homologous chromosome

pairs during meiotic prophase. Although EM images of SCs give the impression of a rigid, scaffold-like structure, recent studies suggest that the SC may be much more dynamic than previously appreciated. We are investigating the dynamics of the SC structure by using FRAP (Fluorescence Recovery After Photobleaching) to visualize the exchange of SC components within fully assembled SCs in pachytene nuclei of intact worms expressing a functional GFP-tagged version of SYP-3, a component of the SC central region.

This approach has revealed a previously hidden dynamics of the SC, demonstrating that the SC is a malleable structure rather than a fixed scaffold. We observe half-maximal FRAP for GFP::SYP-3 by 10-15 minutes post-bleach, and recovery approaches a maximum by 1-1.5 h. Although this recovery time scale is slower than those observed for nucleoplasmic proteins (seconds) or microtubules in the first mitotic spindle (a few minutes), it is short relative to the length of the pachytene stage when full-length SCs are present (18-24 h). Thus, the observed dynamics indicate that the SC has the potential to undergo substantial remodeling and reorganization in response to different ongoing events of meiotic prophase.

Based on our analyses, we can draw several conclusions: 1)Recovery occurs primarily by redistribution/exchange of SC subunits within the nucleus, and minimally by import of new SC subunits 2)The extent of SC dynamics decreases during pachytene progression and 3)SC dynamics characteristic of wild-type early pachytene were observed in mid and late pachytene nuclei in a *zhp-3* mutant, wherein chromatin and nuclear envelope features that are normally limited to early pachytene are prolonged. Together our data suggest that the dynamic state of the SC structure is an actively regulated feature of the meiotic program.

893A. Live imaging reveals active infiltration of mitotic zone by its stem cell niche. **Adrian Paz**, Brandon Wong, Amanda Cinquin, Elliot Hui, Olivier Cinquin. UC Irvine, Irvine, CA.

Stem cells niches are increasingly recognized as dynamic environments that play a key role in transducing signals that allow an organism to exert control on its stem cells. Live imaging of stem cell niches in their in vivo setting is thus of high interest to dissect stem cell controls. Here we report a new microfluidic design that is highly amenable to dissemination in biology laboratories that have no microfluidics expertise. This design has allowed us to perform the first time lapse imaging of the *C. elegans* germline stem cell niche. Our results show that this niche is strikingly dynamic, and that morphological changes that take place during development are the result of a highly active process. These results lay the foundation for future studies to dissect molecular mechanisms by which stem cell niche morphology is modulated, and by which niche morphology controls stem cell behavior.

894B. Regulation of TGFb signaling in germline stem cell development in *C. elegans*. **O. Pekar**, E.J.A. Hubbard. Developmental Genetics Dept, Skirball Institute, NYU School of Medicine, New York, NY.

Stem and progenitor cell populations are sensitive to different environmental and hormonal cues. This sensitivity likely enables organisms to adjust to new conditions. We are using the *C. elegans* germ line as a model to investigate this control.

In *C. elegans* germ line, progenitors accumulate during larval development to form an adult pool from which gametes are produced. We found that *daf-7* modulates the balance of proliferation versus differentiation in the larval *C. elegans* germ line in response to sensory cues, independent of previously defined roles in the dauer decision and lifespan. *daf-7* encodes a TGFb-like ligand and its reporter expression is robust in ASI. ASI sensory neurons are required for *daf-7*-mediated germ cell accumulation, and the rest of the TGFb signaling complex (TGFb receptor, DAF-1, and the downstream transcription complex, DAF-3/DAF-5) act in the distal tip cell, the germline stem cell niche (Dalfó et al., 2012).

To begin to explore the transcriptional regulation of *daf-7* relevant to germline development, we created reporters containing different regulatory regions of the gene. Using a larger upstream regulatory region than previously reported, we were surprised to identify additional neurons expressing *daf-7*, which were verified by *daf-7*-specific FISH analysis. We delineate a segment of the upstream regulatory region that drives non-ASI expression. We plan to identify the relevant regulators of *daf-7* with respect to the germline phenotype.

895C. Localization dynamics of SPE-6, a sperm-specific CK1 in *C. elegans*. **Jackson Peterson**, Brianna Waller, Diane Shakes. College of William and Mary, Williamsburg, VA.

During spermatogenesis, diploid germ cells develop into small, haploid spermatozoa which only acquire full motility during the final step of sperm activation. In *C. elegans*, sperm activation occurs in response to an external signal which converts sessile round spermatids into bipolar, crawling spermatozoa. Notably, this final conversion occurs in the absence of either transcription or translation, suggesting that these changes are driven solely by post-translational modifications. In fact, since *C. elegans* spermatocytes cease transcription prior to the meiotic divisions and cease translation immediately after the meiotic divisions, many of the key developmental changes occur in the absence of transcription and translation. Therefore, key motility proteins such as the major sperm protein (MSP) must be translated early in spermatogenesis and sequestered within fibrous body membranous organelles (FB-MOs). Here we describe the dynamic localization of a sperm-specific casein kinase 1 (SPE-6). In wild type spermatocytes, SPE-6 is present in both the cytoplasm and in association with FB-MOs. During the post-meiotic budding division, SPE-6 segregates to the spermatids and away from residual bodies, eventually localizing to the sperm chromatin. Then during sperm activation, SPE-6 re-localizes to the pseudopod. In several non-null alleles of *spe-6*, sperm activation occurs in the absence of an activation signal. Our analysis of *spe-6(hc163)* reveals that this precocious activation phenotype is accompanied by dramatic alterations in SPE-6 localization patterns. In developing spermatocytes, the mutant protein is mostly cytosolic. During the budding division, it segregates aberrantly to the residual body; any SPE-6 that does end up in spermatids fails to localize to the chromatin. We present a model of how this aberrant pattern of protein localization may explain the precocious activation phenotype and provide clues regarding the function of SPE-6 in sperm activation.

896A. Ascaroside-mediated sex determination in a nematode with three genders. Vikas Kache[1], Stephan H. von Reuss[2,3], Joshua Yim[3], Jyotiska Chaudhuri[1], Christine Bateson[1], Frank Schroeder[3], **Andre Pires da Silva[1]**. 1) Univ of Arlington, Arlington, TX; 2) Max Planck Institute for Chemical Ecology, Jena, Germany; 3) Cornell Univ, Ithaca, NY.

Nematodes evolved several life history traits that allow them to adapt to unpredictable environments. When encountering high population densities, for example, nematodes enter an arrested dauer stage that allows them to survive in the absence of food and other unfavorable growth conditions. Small

secreted chemicals named ascarosides serve as population-sensing molecules that can induce dauer formation and other signalling functions. In a recently discovered clade of nematodes that produce populations of males, females and hermaphrodites, we found that the germline sex determination correlates with dauer formation: in the species Rhabditidae Gen. 1. sp. 1 strain SB347, female-fated larvae that are forced into dauer formation develop into hermaphrodite adults; if dauer formation is inhibited in hermaphrodite-fated larvae, they become female adults. We are now focusing on the species Rhabditidae Gen. 1. sp. 2 strain SB372, in which dauer formation and sex determination pathways are also linked. In this species, however, ascarosides mediate dauer formation and sex determination across generations. SB372 larvae that are exposed to ascarosides do not develop into dauers and thus become females. In contrast, if the mother senses the ascaroside signals, most of her progeny develop into dauers and hence into hermaphrodites. Given that hermaphrodites and females are genetically identical, our results provide experimental evidence for non-genetic, cross-generational inheritance in the regulation of reproductive mode. This type of life history is predicted to evolve when local environments can be anticipated, thus providing the means to adjust the phenotype of the offspring to enhance their fitness.

897B. Effect of Synapsis Challenges on Meiotic Progression. **Baptiste Roelens**, Susanna Mlynarczyk-Evans, Anne Villeneuve. Department of Developmental Biology, Stanford Univ, Stanford CA.

Meiosis is the complex multi-step program that allows reduction of ploidy to generate haploid gametes during sexual reproduction. Pairing between homologous chromosomes and assembly of a highly ordered proteinaceous structure called the synaptonemal complex at their interface (synapsis) represent intermediate steps in the meiotic program that are essential to form crossover recombination-based linkages between homologs, which in turn enable segregation of the homologs to opposite poles at the meiosis I division. Proper coordination of these events of the meiotic program is essential, and recent research has highlighted the importance of quality control mechanisms to ensure a successful meiotic outcome. Here, we combine karyotype manipulation with immunofluorescence imaging to interrogate how disrupting the normal 1:1 correspondence between homologs affects meiotic progression.

We show that increasing the numbers of homologs leads to persistence of markers associated with active chromosome mobilization and/or increases germline apoptosis, consistent with triggering quality control mechanisms that promote resolution of synapsis problems and/or eliminate meiocytes containing synapsis defects. We also uncover evidence for the existence of mechanisms that “mask” synapsis imperfections, thereby allowing cells with minor synapsis defects to resume their progression and successfully complete the meiotic program. Interestingly, accumulation of the MET-2-dependent H3K9Me2 chromatin mark coincides during meiosis with activation of masking mechanisms, and loss of met-2 function leads to an increase in germline apoptosis, suggesting that this histone methyl-transferase may play a role in this process. We are now analyzing the effects of karyotype manipulation on markers of meiotic recombination to determine whether chromosome mobilization, progression of recombination and chromatin states are similarly affected by synapsis challenges, consistent with coordinate regulation of multiple distinct aspects of the meiotic program.

898C. Elucidating the role of S6K-Notch interactions in cell fate specification in the *C. elegans* germ line. **Debasmita Roy**, E. Jane Albert Hubbard. Skirball Institute of Biomolecular Medicine, New York Univ School of Medicine, New York, NY.

Proper balance of differentiation vs. proliferation in the germline stem/progenitor cell pool is crucial for gamete production and reproductive fitness. We are using *C. elegans* larval germline development as a model to study the developmental and physiological control of this balance.

We showed that RSKS-1, the C. elegans ortholog of the Target of Rapamycin (TOR) substrate p70-S6-Kinase (S6K), promotes accumulation of progenitor cells germline-autonomously. In this role, *rsks-1* both promotes larval germline cell cycle and inhibits differentiation, together with GLP-1/Notch. Loss (lf) of *rsks-1* both enhances the progenitor maintenance defect in reduced (rf) *glp-1* mutants and suppresses tumor formation in elevated (gf) *glp-1* (Korta et al. 2012). More recently, we showed that the cell fate role of *rsks-1* is germline-automomous: germline *rsks-1(+)* rescues progenitor loss in *glp-1(rf) rsks-1(lf)*.

TOR also positively regulates translation initiation factor-4E (eIF4E). We found that *ife-1* (eIF4E ortholog in the germline) is required for optimal expansion of the larval progenitor pool. *rsks-1(lf) ife-1(lf)* produce fewer progenitors than either single mutant (Korta et al., 2012). To assess a possible cell fate role for *ife-1*, we examined *glp-1(rf) ife-1(lf)* double mutants. Unlike *glp-1(rf) rsks-1(lf),* the *ife-1* double retains a progenitor pool, albeit with fewer cells than either single mutant. Similar results are seen in *glp-1(rf); let-363/TOR* (RNAi), or upon food reduction, suggesting a unique role for S6K in progenitor maintenance.

Reducing *cye-1*/Cyclin-E by RNAi causes a loss of the progenitor pool in *glp-1(rf)* (Fox et al. 2011), similar to *rsks-1(lf)*. We are investigating the interactions between *rsks-1*, *glp-1* and *cye-1*. Loss of RSKS-1 does not affect the level or pattern of CYE-1 protein expression, arguing against a general translational control mechanism. Our data suggest a model whereby RSKS-1 acts together with CYE-1, but in parallel with GLP-1, to inhibit differentiation.

899A. Assembly of RNP granules in *C. elegans* oocytes promotes oocyte quality and is regulated by the cytoskeleton. Megan Wood[1], Angela Hollis[1], Kevin Gorman[1], Joseph Patterson[1], Ashley Severance[1], Gregory Davis[2], Peter Boag[2], **Jennifer Schisa[1]**. 1) Dept Biol, Central Michigan Univ, Mount Pleasant, MI; 2) Dept Biochem and Mol Biol, Monash Univ, Victoria, Australia.

In many animal species, oocytes arrest in meiosis until they are fertilized. It is well established that fertility diminishes as oocytes age. Our goal is to better understand the regulation and function of large ribonucleoprotein (RNP) granules that assemble in the germ lines of *Caenorhabditis* nematodes that are either stressed or in which ovulation is arrested. The RNP granules are hypothesized to maintain oocyte quality by regulating mRNA stability or translation in arrested or stressed oocytes (Jud et al., 2008). Their assembly is influenced by nuclear pore proteins, and we have hypothesized that nuclear blebs trafficking from the nuclear envelope to the cortex may promote the formation of the cortical RNP granules (Patterson et al., 2011). We have performed a targeted, functional RNAi screen to identify genes that are required for the assembly of RNP granules in arrested oocytes and identified 143 genes that are necessary. Among the gene classes of our positives are several cytoskeleton proteins including KCA-1 (kinesin cargo adaptor), several beta-tubulins, and WSP-1 (involved in actin polymerization). To gain insight into the mechanism of action of RNP granule regulators we are dissecting defined protein complexes; e.g. we are determining if KLC-1 (kinesin light chain) and UNC-116 (kinesin 1 heavy chain) which function with KCA-1 to position the meiotic spindle (Yang et al., 2005), also contribute to RNP granule assembly. The discovery of these novel regulators of RNP granule assembly allows for

direct testing of our hypothesis for their function. When the normal assembly of RNP granules is prevented, we observe fertility is decreased, supporting the hypothesis that RNP granules maintain the quality of oocytes when fertilization is delayed. On-going studies are testing if RNA stability is diminished or translation of maternal mRNAs is de-repressed when RNP granule assembly is defective. These results have provided insight into novel regulators of RNP dynamics that likely apply to RNPs important for fertility and stress responses in many species.

900B. Nutritional Control of Germline Stem Cells in *Caenorhabditis elegans*. **Hannah S. Seidel**[1,2], Judith Kimble[1,3]. 1) Dept. of Biochemistry, Univ of Wisconsin-Madison, Madison, WI; 2) Ellison Medical Foundation Fellow of the Life Sciences Research Foundation; 3) Howard Hughes Medical Institute.

The germline of the nematode *C. elegans* provides a tractable model for studying how nutritional cues regulate stem cell behavior. Previous work has shown that food availability influences the proliferation of undifferentiated germ cells in the larval germline; this response is mediated in part by insulin/IGF-like signaling (Dev. 2010 137:671-80, Curr. Biol. 2006 16:773-779), TGF-b signaling (Curr. Biol. 2012 22:712-9), and the AMPK pathway (Dev. 2006 133:611-9). Likewise, in oogenic hermaphrodites, nutrient deprivation causes germlines to shrink, and this shrinkage is reversible upon re-feeding (Science 2009 326:954-8, PLoS ONE 2011 6: e28074). Here we show that proliferation of adult germline stem cells (GSCs) requires food: Under fed conditions, GSCs divide continuously, only ceasing division as they enter the meiotic cell cycle; upon food removal, however, GSCs stop dividing and become quiescent. Quiescence can last for several days, with cell division only resuming upon the reintroduction of food. We have tested whether factors required for the larval germline's response to nutritional cues also mediate quiescence of adult GSCs. We find that quiescence of adult GSCs does not require *daf-16/FOXO*, *daf-18/PTEN*, *daf-3*, *daf-5*, *par-4/LKB1*, or *aak-2*. Similarly, quiescence in adults is not affected by mutations that globally disrupt neuropeptide release, nor by exogenous addition of the endocrine signal serotonin; thus, quiescence occurs independently of factors affecting behavioral responses to food. Finally, we find that quiescence influences germ cell differentiation. In particular, our data suggest that constant GLP-1/Notch signaling may be dispensable for maintenance of quiescent GSCs. We are currently investigating possible regulators of starvation-induced quiescence and stem cell maintenance.

901C. IFET-1 an eIF4E-binding protein is required for normal P-granules formation and translational regulation of mRNAs. **Madhu S. Sengupta**, Peter R. Boag. Department of Biochemistry and Molecular Biology, Monash Univ, Melbourne, Australia.

During oogenesis many germ cell mRNAs are produced and stored in a translationally repressed state and are subsequently activated at specific times during oocyte and early embryonic development. Some of these translationally repressed mRNAs are stored in perinuclear cytoplasmic granules called P-granules, which are key sites of mRNA storage and post-transcriptional gene regulation. One mechanism to inhibit cap-dependent translation is to prevent the formation of eIF4E-eIF4G complex by eIF4E-binding proteins displacing eIF4G from the complex. We are studying the conserved eIF4E-binding protein IFET-1 that is required for normal germline development, P-granules formation and translation of some mRNAs. IFET-1 localizes to P-granules in the gonad and the embryos and is required for normal localization of some P-granule components (CGH-1 and CAR-1). Ultrastructure studies of P-granules reveal that substructure of P-granules is abnormal in the absence of IFET-1 suggesting that mRNAs transiting through these granules may be defective.

To understand how IFET-1 localizes to P-granules, we have commenced structure and function experiments. We have ectopically expressed IFET-1 in intestinal cells, which demonstrates that it contains functional NLS and NES domains, and is likely to act as nucleo-cytoplasmic shuttling protein. Interestingly, IFET-1 contains a C-terminal Q-rich domain, which often function as aggregation domains; therefore we are investigating if this domain is required for IFET-1 aggregation and localization to P-granules. Structural and functional analysis of P-granules components in the germline will provide critical insights into the mechanisms of protein and RNA trafficking through these key sights of post-transcriptional gene regulation.

902A. PQN-94 regulates hermaphrodite development by interacting with SHE-1. **Yongquan Shen**, Ronald E Ellis. Molecular Biology, UMDNJ-SOM, Stratford, NJ.

F-box proteins play critical roles promoting hermaphrodite development in two different species of Caenorhabditis. In *C. elegans,* the F-box protein FOG-2 interacts with GLD-1 to repress the translation of *tra-2* mRNAs. Low TRA-2 activity allows the *XX* hermaphrodites to make sperm. By contrast, *C. briggsae* lacks an ortholog of FOG-2, but the F-box protein SHE-1 down-regulates TRA-2; and does so without help from GLD-1. We want to learn how SHE-1 alters the sex-determination process.

Because a missense mutation in the F-box inactivates SHE-1, it might act as a classical F-box protein, bringing a target to the E3 ubiquitin ligase complex to be marked for degradation. To identify potential SHE-1 targets, we used the yeast two-hybrid system. From a screen of about 340,000 cDNAs, we identified three genes that that were represented by multiple, independent clones. The *pqn-94* gene passed two further tests. First, PQN-94 also interacts with SHE-1 when used as bait. Second, *pqn-94(RNAi)* partially suppresses *she-1*. At 25° C, *she-1(v49); control(RNAi)* mothers did not produce hermaphrodites, but *she-1(v49); pqn-94(RNAi)* mothers produced 7% hermaphrodites. Tests with *she-1(v35)* showed the same pattern of suppression, whereas knocking down *pqn-94* on its own had no phenotype.

To confirm that SHE-1 controls germ cell fates by interacting with PQN-94, we used TALEN technology to generate a *pqn-94* null mutant with a frame-shifting deletion. These *pqn-94(v203)* animals are healthy, but *she-1(v35)*; *pqn-94(v203)* mothers produced 12% hermaphrodites at 25°C, whereas *she-1(v35)* controls were all female. Since experiments with the null allele resembled those done with RNAi, we conclude that the absence of PQN-94 partially compensates for a loss of SHE-1. This result supports models in which SHE-1 targets PQN-94 for degradation. However, PQN-94 cannot be the sole target of SHE-1, since *pqn-94(v203)* is not completely epistatic to *she-1*.

903B. Characterization of SYGL-1, a novel regulator of germline stem cells. **Heaji J. Shin**[1], Kimberly Haupt[1], Aaron M. Kershner[2], Judith Kimble[1,2]. 1) Department of Biochemistry, Univ of Wisconsin-Madison, Madison, WI; 2) Howard Hughes Medical Institute, Univ of Wisconsin-Madison, Madison, WI.

C. elegans germline stem cells (GSCs) are maintained by GLP-1/Notch signaling from the stem cell niche. Two GLP-1/Notch target genes, *lst-1* (lateral signaling target) and *sygl-1* (synthetic germline proliferation defective), act redundantly to maintain GSCs throughout development and in both sexes (Kershner, Shin, and Kimble, manuscript in preparation). Here we focus on our characterization of the *sygl-1* gene, which had not been analyzed

previously. The *sygl-1* locus encodes a single transcript (T27F6.4) that is predicted to generate a novel protein with no folded domains or motifs. To begin to investigate *sygl-1* function, we have characterized a strong loss of function *sygl-1(tm5040)* mutant and generated a rescuing epitope-tagged SYGL-1 transgene. The *sygl-1(tm5040)* homozygote is viable, but it possesses a smaller than normal mitotic zone. The *sygl-1* mRNA is restricted to the distal mitotic zone and is dependent on GLP-1/Notch signaling. The SYGL-1 protein is similarly restricted to the distal mitotic zone and is cytoplasmic. Our characterization so far is therefore consistent with a role for SYGL-1 in GSC maintenance, but we still know very little about this enigmatic locus. Experiments to explore *sygl-1* regulation and function are in progress, and results will be reported.

904C. The Role of Condensin I during Meiosis. **M. Sifuentes**, K. Colette, G. Csankovszki. MCDB Dept, Univ of Michigan, Ann Arbor, MI.

Condensin complexes are key determinants of higher-order chromatin structure, and are required for mitotic and meiotic chromosome segregation and compaction. Previously we showed that condensins I and II localize to distinct domains and play non-redundant roles to promote mitosis and meiosis. However, the different roles of condensins I and II in meiosis are poorly understood. By analyzing condensin I-depletions, we found novel meiotic functions for this complex in cohesin regulation and chromosome segregation. We show condensin I promotes the establishment of sister chromatid cohesion during early meiosis. Cohesin complexes localize along the entire length of synapsed chromosomes during pachytene and contain either REC-8, or its paralogs, COH-3 or COH-4 (COH-3/4). We found that in *dpy-28* mutants, a subunit of condensin I, cohesin localization to chromosomes is reduced, suggesting condensin I promotes cohesin loading and/or maintenance between sister chromatids. Consistent with a role in promoting cohesion, condensin I RNA interference (RNAi) in *coh-3/4* and *rec-8* mutants leads to increased dissociation of sister chromatids and the appearance of chromosome fragments in diakinesis. Later in meiosis, condensin I localizes to a ring shaped domain (RSD) between homologs (metaphase I) and sister chromatids (metaphase II) and to the meiotic spindle between separating chromosomes during anaphase. The Aurora B kinase, AIR-2, also localizes to this domain and recruits other proteins to orient and separate chromosomes. We found condensin I is dispensable for AIR-2 recruitment but is required for the recruitment of BUB-1, KLP-19, HCP-1/2, and CLS-2 to the ring-shaped domain. Furthermore, BUB-1 RNAi leads to a reduction of condensin I at the midbivalent, indicating condensin I and BUB-1 are interdependent. Condensin I depletion also causes gross meiotic spindle defects and abnormal chromosome organization, suggesting condensin I promotes proper congression and orientation of chromosomes. Additionally, condensin I depletion prevents successful chromosome resolution at anaphase. Overall, our results indicate that condensin I plays a role in both coordinating cohesin regulation and chromosome congression and separation.

905A. UBC-25 promotes Ras/MAPK signaling to regulate oocyte growth and embryonic morphogenesis in *C. elegans*. **Mideum Song**[1], Kevin Cullison[2], Phil Cheng[1], Meera Sundaram[2], Christian Rocheleau[1]. 1) Departments of Medicine and Anatomy and Cell Biology, McGill Univ, Montreal, Quebec, Canada; 2) Department of Genetics, Univ of Pennsylvania School of Medicine, Philadelphia, PA.

A highly conserved Ras/Mitogen Activated Protein Kinase (MAPK) signaling pathway is used repeatedly during *C. elegans* development to regulate cell differentiation as well as multiple aspects of germline development including oocyte growth. We previously identified UBC-25, a putative ubiquitin-conjugating enzyme as promoting Ras/MAPK signaling during specification of the excretory duct cell in both a genome-wide RNAi screen and a traditional mutagenesis screen. Interestingly, loss of *ubc-25* in conjunction with loss of a Ras/MAPK signaling scaffold protein, *cnk-1*, also causes an embryonic lethal phenotype and a dramatic increase in oocyte and embryo size. We do not see a correlation between embryo size and embryonic lethality suggesting that lethality is not due to an increase in embryo size. Embryos die with a morphogenesis defect not previously linked to Ras/MAPK signaling. Loss of Ras/MAPK signaling results in a potent sterile phenotype and hence precludes analysis of oocyte growth. However, increased oocyte size has been noted in a conditional allele of *mpk-1 Erk* and decreased oocyte size is associated with loss of the MAPK phosphatase, *lip-1*, or a gain-of-function mutation in *let-60 Ras*. Therefore UBC-25 may function with Ras/MAPK signaling to regulate both oocyte growth and embryonic morphogenesis in addition to specification of the excretory duct cell fate. We hypothesize that UBC-25 promotes Ras/MAPK through ubiquitination and degradation of a negative regulator of the pathway. We find that *ubc-25*, but not *cnk-1*, is epistatic to the *lip-1* small oocyte and disorganized germline phenotype suggesting that LIP-1 is not a target of UBC-25 activity, but that UBC-25 functions downstream of LIP-1 closely with MPK-1 Erk.

906B. GLP-1/Notch signaling in germline stem cell maintenance. **Erika B Sorensen**, Amy C Groth, Judith Kimble. Howard Hughes Medical Institute and Department of Biochemistry, Univ of Wisconsin-Madison, Madison, WI.

C. elegans germline stem cells (GSCs) are maintained by GLP-1/Notch signaling. Loss-of-function mutants of the Notch pathway cannot maintain GSCs (1) and gain-of-function mutants cause germline tumors (2). All metazoan Notch signaling relies on cleavage of the Notch receptor to generate the Notch intracellular domain (NICD), which moves into the nucleus and forms a ternary complex with DNA-binding protein LAG-1 and transcriptional co-activator LAG-3/SEL-8. Available antibodies detect the GLP-1 receptor at the plasma membrane but not the NICD in nuclei (3). Moreover only four direct transcriptional targets of GLP-1/Notch signaling have been identified: *fbf-2* (4), *lip-1* (5), *lst-1* and *sygl-1* (Kimble lab, unpublished). To visualize active GLP-1/Notch signaling and to identify additional target genes by chromatin immunoprecipitation (ChIP), we generated Mos-mediated transgenic lines expressing epitope-tagged versions of the GLP-1 receptor and LAG-1 DNA-binding protein. The *glp-1* transgene encodes the entire glp-1 genomic locus plus a C-terminal 6xmyc6HIS tag. GLP-1::6xmyc6HIS rescues *glp-1* null alleles to fertility and enables visualization of intracellular GLP-1. The *lag-1* transgene is driven by the germline-specific *mex-5* promoter and contains an N-terminal 6xFLAG tag. Preliminary ChIP experiments with the LAG-1 transgene reveal enrichment for the *sygl-1* and *lst-1* promoters, but not for control promoters that lack LAG-1 binding sites. Our progress will be reported.

References: (1) Austin and Kimble (1987) Cell 51: 589-599; (2) Berry et al. (1997) Develop 124: 925-936; (3) Crittenden et al. (1994) Develop 120: 2901-2911; (4) Lamont et al. (2004) Dev Cell 7: 697-707; (5) Lee et al. (2006) EMBO J 25: 88-96.

907C. Loss of UNC-84 in the C. elegans germ line activates the recombination and synapsis checkpoints. **Erin Tapley**, Kate Lawrence, K.C. Hart, JoAnne Engebrecht, Daniel Starr. Dept Molecular Cell Biol, Univ California, Davis, Davis, CA.

SUN and KASH proteins form bridges across the nuclear envelope, connecting the cytoskeleton to the nucleoskeleton. The inner nuclear membrane SUN protein UNC-84 interacts with outer nuclear membrane KASH proteins to position nuclei in somatic cells. In the C. elegans germ line, the SUN protein SUN-1 and the KASH protein ZYG-12 are essential for chromosome pairing. We discovered UNC-84 localizes to the nuclear envelope in mid-prophase of meiosis,

suggesting UNC-84 plays uncharacterized roles in the germ line. In support of our hypothesis, unc-84 null mutants have elevated levels of germline apoptosis, indicative of aberrant meiotic progression. Depletion of the checkpoint protein CHK-1 reduces apoptosis to physiological levels, indicating that elevated levels of apoptosis are checkpoint dependent. unc-84 null animals depleted of CED-3 have increased levels of embryonic lethality, suggesting that defective nuclei are culled by apoptosis. unc-84 point mutants, P91S that disrupt the UNC-84 lamin interaction and C994Y that prevents the SUN KASH interaction, also have elevated levels of apoptosis and activated phospho-CHK-1. These data suggest that a functional nuclear envelope SUN KASH bridge is important for UNC-84's meiotic role. Two checkpoints respond to meiotic problems: the recombination checkpoint, which senses unrepaired double strand breaks, and the synapsis checkpoint, which senses unpaired chromosomes. Both checkpoints activate CHK-1. To test which checkpoint is activated in response to a loss of UNC-84, we generated double mutants: unc-84; spo-11 and unc-84; pch-2. Loss of SPO-11 or PCH-2 prevents signaling through recombination and synapsis pathways, respectively. Both double mutants exhibited activated CHK-1, indicating persistent activation of both checkpoints. Both unc-84; spo-11 and unc-84; pch-2 double mutants had intermediate levels of germline apoptosis when compared to either single mutant. These data suggest that loss of UNC-84 in the germ line impairs meiosis and results in signaling through the recombination and synapsis checkpoints. Interestingly, unc-84; chk-1(RNAi) double mutants have severe mitotic defects in the germ line, suggesting UNC-84 has both mitotic and meiotic functions.

908A. The SACY-1 DEAD-box RNA helicase genetically interacts with components of the spliceosome. Seongseop Kim, **Tatsuya Tsukamoto**, David Greenstein. GCD Department, Univ of Minnesota, Minneapolis, MN.

In *C. elegans*, major sperm protein triggers oocyte meiotic maturation through a mechanism involving somatic $G\alpha_s$-adenylate cyclase-protein kinase A (PKA) signaling, gap-junctional communication, and translational regulation by OMA-1/2. Previously we identified *sacy-1* as a negative regulator of meiotic maturation, functioning in the germ line downstream of PKA signaling. Genetic analysis established that *sacy-1* also functions in the hermaphrodite sperm-to-oocyte switch and is required for gamete maintenance.

To isolate additional alleles of *sacy-1*, we screened for mutations that fail to complement *sacy-1(tn1385rf)* for the suppression of *fog-2(lf)* self-sterility. From 15,577 mutagenized haploid genomes, we isolated five new *sacy-1* missense alleles affecting conserved residues in the DEAD-box helicase domain. *sacy-1(tn1481)*, which affects the ATP-binding Q-motif, exhibits a masculinization of the germ line phenotype. This result is consistent with the conclusion that *sacy-1* promotes the oocyte fate. *sacy-1(tn1479)* exhibits gamete degeneration and sterility similar to the *sacy-1(tm5503)* likely null allele; however, *sacy-1(tn1479)* differs from *sacy-1(tm5503)* in that the majority of the former, but not the latter, bursts as adults. This result suggests that *sacy-1(tn1479)* may possess a poisoning activity. Finally, *sacy-1(tn1480)* exhibits temperature-sensitive sterility.

We conducted a genome-wide RNAi screen for *sacy-1* enhancers to address its molecular function. This screen identified three enhancer loci, *mog-2*, *emb-4*, and *cacn-1*. RNAi of each of these three loci caused sterility (*emb-4* and *cacn-1*) or embryonic lethality (*mog-2*) in *sacy-1(tn1385rf)* but not in the wild type under the RNAi conditions utilized. Orthologs of *sacy-1* and these enhancer loci were identified biochemically as components of spliceosomal complexes. Whether the multiple *sacy-1* germline phenotypes result from defects in pre-mRNA splicing or translational regulation remains to be determined.

909B. The Torsin Homolog OOC-5 is Required for Normal Nucleoporin Localization. **Michael J.W. VanGompel**, Sumati Hasani, Lesilee S. Rose. Molecular and Cellular Biology Department, Univ of California, Davis, Davis, CA.

OOC-5 is a Torsin family AAA+ ATPAase required for polarity and spindle rotation in the P1 cell of 2-cell embryos. Torsins localize to the lumen of the endoplasmic reticulum and contiguous nuclear envelope (NE), and mutations in human TorsinA lead to a neuromuscular disease. Integral NE proteins have been found to be interacting partners of TorsinA, including the outer nuclear membrane KASH protein Nesprin-3. However, what role Torsins play at the NE is not known. In *C. elegans* it has been shown that depletion of components of nuclear pore complexes (NPCs), called nucleoporins or Nups, leads to an *ooc-5* like phenotype, suggesting a link between Torsins and nuclear pores in the worm. Using antibody staining and GFP reporters, we found abnormal localization of multiple Nups at the NE in early embryos, and in intestine and germ cells in adults. In embryos, clusters of Nups were seen, while in the germline, more dramatic plaques of Nups were observed. Germline defects are first present at the transition zone, where germ cells enter meiosis, and persist through all stages of meiosis. Areas of discontinuous signal of the outer nuclear membrane KASH protein ZYG-12 were seen in an *ooc-5* background, and co-staining suggests that this is not due to steric exclusion by Nup plaques. Interestingly, the localization of lamin and inner nuclear membrane proteins EMR-1 and LEM-2 appear normal in *ooc-5*. Despite the mislocalization of multiple Nups in the germline, nuclear import occurs; furthermore, nuclei can exclude 70kDa molecules in *ooc-5* mutant germlines, indicating that barrier functions of pores remain in tact. Finally, electron microscopy analysis showed blebbing of the NE in *ooc-5* oocytes, reminiscent of ultrastructural defects seen in TorsinA mutant mice. Together our results show that OOC-5 has functions at the nuclear pore and NE, roles that are likely to be broadly conserved among Torsins. We are currently examining the cause and effect relationship of Nup/ZYG-12 mislocalzation in the germline, and whether these phenotypes are separable from embryonic polarity phenotypes.

910C. Cellular machinery promoting FBF-2 regulatory activity. Xiaobo Wang[1], Dominique Rasoloson[2], Elle Johnson[1,3], **Ekaterina Voronina[1]**. 1) DBS, Univ of Montana, Missoula, MT; 2) Johns Hopkins Univ School of Medicine/HHMI, MBG, Baltimore, MD; 3) Big Sky High School, Missoula, MT.

FBF-1 and FBF-2 are translational regulators maintaining germline stem cells in *C. elegans* (Crittenden et al., 2002). We previously reported that the localization and function of FBF-2 depends on the integrity of P granules, perinuclear RNA granules of germ cells (Strome and Wood, 1982; Pitt et al., 2000; Voronina et al., 2012). To understand the role of cofactors in FBF-2 function, we are characterizing FBF-2 interactome by mass-spectroscopy. To select the components of FBF-2 RNP contributing to FBF-2-mediated regulation, we employed a genetic interaction assay. We depleted candidate FBF-2-interacting partners by RNAi in N2 worms, as well as in *fbf-1(lf)* mutant (Lamont et al., 2004), in which the worm relies solely on FBF-2 for stem cell maintenance, translational regulation, and fertility. Cofactors specific for FBF-2 should be required for FBF-dependent regulation in *fbf-1(lf)* mutant, but not in a wild-type strain, where FBF-1 can compensate. The screen identified *C. elegans* homolog of La protein (provisionally named LHP-1) and dynein light chain DLC-1 as candidate FBF-2 cofactors. La protein contributes to multiple steps in RNA biogenesis in the nucleus, and has been recently implicated in the regulated translation of several mRNAs in the cytoplasm (Bayfield et al., 2012). DLC-1 is a cargo-binding component of dynein motor complex required for cell division (Gonczy et al., 1999), meiotic chromosomal synapsis (Sato et al., 2009), and regulation of meiotic entry (Dorsett and Schedl, 2009). Similar to *pgl-*

1(lf), both *lhp-1(RNAi)* and *dlc-1(RNAi)* in the *fbf-1(lf)* background lead to derepression of FBF target reporter in the distal mitotic region and masculinization of germline; the phenotypes not observed after same RNAi treatments of the wild type or *fbf-2(lf)* worms. Localization of FBF-2 to the nuclear periphery is maintained after *lhp-1(RNAi)*, but is lost after *dlc-1(RNAi)*, even when perinuclear P granules are normal. Our data is consistent with DLC-1 affecting FBF-2 subcellular distribution, and LHP-1 contributing to FBF-2 function downstream of its localization to P granules.

911A. Asymmetric segregation of P granules requires granule remodeling by two novel serine-rich proteins. **Jennifer T. Wang**, Geraldine Seydoux. Johns Hopkins Univ SOM/HHMI, Baltimore, MD.

P granules are RNA-rich granules that are found exclusively in the germline. In adult germ cells, P granules are mostly perinuclear and stable. In contrast, in early embryos, P granules are cytoplasmic and highly dynamic. Activation of P granule dynamics in embryos is required to segregate P granules asymmetrically to the nascent germline [1,2]. We have found that activation of P granule dynamics requires P granule remodeling by two serine-rich proteins. We used a GFP::PGL-1 fusion to analyze P granule dynamics during the oocyte-to-embryo transition. Oocyte P granules are disassembled during ovulation and reappear during meiosis throughout the cytoplasm of the newly fertilized zygote. A similar disassembly/reassembly cycle is repeated in the zygote at each mitotic division, except that reassembly occurs preferentially in the cytoplasm destined for the germline blastomere. We have found that depletion of GEI-12 and its paralog C36C9.1 blocks the disassembly/reassembly cycle. In *gei-12+C36C9.1(RNAi)*, oocyte P granules persist through ovulation and the first mitotic division, and do not segregate asymmetrically. Formation of new P granules is also blocked. GEI-12 and C36C9.1 are 70% identical serine-rich proteins with no recognizable motifs. Sequence analyses indicate that these proteins have low-complexity sequence (LCS) throughout their length. LCS domains have recently been proposed to form dynamic fibers that hold RNA granules together [3,4]. Consistent with functioning as a P granule scaffold, GEI-12 associates with P granules after reassembly in embryos. Deconvolution confocal microscopy suggests that, in the reassembled P granules, GEI-12 localizes to a core that joins together several small PGL-1 granules. We conclude that 1) GEI-12 and C36C9.1 remodel P granules during the oocyte-to-embryo transition and 2) P granule remodeling is essential for the asymmetric segregation of P granules in embryos. 1. Brangwynne CP et al (2009). Science 324, 1729 2. Gallo C et al (2010). Science 330(6011) 1685 3. Kato M et al (2012). Cell 149(4), 753-767 4. Han TW et al (2012). Cell 149(4), 768-779.

912B. Investigating the cellular mechanisms of skewed sex ratios in non-*C. elegans* nematodes. **Ethan S. Winter**, Diane C. Shakes. Biology, College of William and Mary, Williamsburg, VA.

During the post-meiotic phase of spermatogenesis, various proteins and cellular components are either retained by the sperm or discarded in a cytoplasmic “waste bag” (residual body). In nematodes, this spermatid-residual body partitioning event occurs unusually early; either during or immediately after anaphase II. In addition, since nematode sperm are non-flagellated, one of the discarded components is tubulin, which is essential for meiotic chromosome segregation but (except for the centriole) superfluous for the subsequent function of either the spermatids or crawling spermatozoa. Instead, the motility of nematode spermatozoa is driven by the major sperm protein (MSP) which forms dynamic filaments within the treadmilling pseudopod. Our lab has recently discovered that a dramatic sex skew in the non-*C. elegans* species, *Rhabditis* sp. SB347 (Felix, 2004) can be explained by an asymmetric partitioning of cellular components during anaphase II of spermatogenesis (Shakes et al, 2011) To investigate the evolution of this trait and rule out that it is merely an isolated curiosity of *R.* sp. SB347, we are analyzing spermatogenesis in other members of this clade. Here we report similar partitioning patterns during spermatogenesis in three species *R.* sp.SB372, *R.* sp. JU1782, and *R.* sp. JU1809. As in the dividing secondary spermatocytes of SB347, MSP is partitioned exclusively to the X-bearing sperm. In contrast, tubulin and other non-essential components are partitioned exclusively to the nullo-X sperm; true residual bodies are never observed. We also find that the two chromatin masses exhibit differential compaction. Thus, these meiotic asymmetries serve as a common mechanism for generating skewed sex ratios within this clade and potentially throughout the phylum. Ultimately, these ongoing studies may provide new insights regarding not only the evolution of reproductive modes and sexual plasticity but also the cellular and biochemical mechanisms of cell polarization.

913C. Regulation of Apoptosis by Meiotic Checkpoint Proteins: New Roles for *egl-1* and *ced-13*. **Alice L. Ye**[1], Matt Ragle[1], Barbara Conradt[2], Needhi Bhalla[1]. 1) Molecular, Cell, Dev Bio, Univ of California, Santa Cruz, Santa Cruz, CA; 2) Ludwig-Maximilians-Univ München Munich, Germany.

Sexual reproduction relies on meiosis, the specialized cell division that produces haploid gametes such as sperm and eggs. In order for chromosomes to properly segregate during meiotic divisions, they must pair, synapse, and recombine. Defects in these processes result in birth defects, infertility, and disorders such as Down Syndrome. Therefore, checkpoints exist to monitor and ensure proper completion of meiotic events. In C. elegans, there are two meiotic checkpoints: the synapsis checkpoint ensures that homologs are correctly synapsed, and the DNA damage checkpoint responds to unresolved recombination intermediates. The proapoptotic proteins EGL-1 and CED-13 have been shown to contribute to germline apoptosis in response to DNA damage. We investigated the role of these proteins during synapsis checkpoint activation. Interestingly, while EGL-1 is required for each checkpoint if activated alone, it is specifically required for the synapsis checkpoint if both checkpoints are simultaneously activated. When both checkpoints are activated, CED-13 contributes to the DNA damage checkpoint-induced apoptosis. Further, EGL-1 induced apoptosis in response to DNA damage checkpoint activation is negatively regulated by a downstream cis-acting locus, which includes the binding site for transcriptional regulator TRA-1. We are currently performing experiments to test whether TRA-1 binding to this regulatory region contributes to regulation of DNA damage-induced apoptosis.

914A. MRX/N Commits Homologous Recombination of Meiotic Double-Strand-Breaks by Promoting Resection, Antagonizing Non-Homologous End Joining, and Stimulating EXO-1 in *Caenorhabditis elegans*. **Yizhi Yin**, Sarit Smolikove. Department of Biology, Univ of Iowa, Iowa City, IO.

Repair of double-strand DNA breaks (DSBs) by the homologous recombination (HR) pathway results in crossovers (COs) required for a successful first meiotic division. Mre11 is one member of the MRX/N (Mre11, Rad50, Xrs2/Nbs1) complex required for meiotic DSB formation and for resection in budding yeast. In *Caenorhabditis elegans*, evidence for the MRX/N’s role in DSB resection is limited. We report the first separation of function allele, *mre-11(iow1)* in *C. elegans*, which is specifically defective in the meiotic DSB resection but not in formation. The *mre-11(iow1)* mutants display chromosomal fragmentation and aggregation in late prophase I. Recombination intermediates and crossover formation is greatly reduced in *mre-11(iow1)* mutants.

Irradiation induced DSBs during meiosis fail to be repaired from early to middle prophase I in *mre-11(iow1)* mutants. In the absence of a functional HR, some DSBs in *mre-11(iow1)* mutants are repaired by the non-homologous end joining (NHEJ) pathway as removing NHEJ partially suppresses the meiotic defects shown by *mre-11(iow1)*. In the absence of NHEJ and a functional MRX/N, meiotic DSBs are channeled to EXO-1 dependent HR repair, which is active in middle to late prophase I. Depletion of EXO-1 in worms defective of a functional MRX/N and NHEJ greatly reduces HR repair (examined by RAD-51 loading and chromosome morphology of the oocytes). Overall, our analysis supports a role for MRE-11 in the resection of DSBs, in blocking NHEJ and in stimulating EXO-1 repair in early to middle prophase I.

915B. If we have children together, will they be less fit? Hybrid incompatibilities in *Caenorhabditis* species. Piero Lamelza, Jerome Cattin, Vanessa Wilson, Irini Topalidou, **Michael Ailion**. Dept Biochemistry, Univ of Washington, Seattle, WA.

Speciation often occurs by the accumulation of genetic incompatibilities between populations. We are studying the genetic basis of hybrid incompatibility in several *Caenorhabditis* species. In *C. elegans*, hybrid incompatibility between the Bristol and Hawaiian strains is mediated by the *peel-1/zeel-1* genetic element. *peel-1* encodes a sperm-expressed toxin and *zeel-1* encodes its zygotically-expressed antidote. We are interested in determining the cellular mechanism of PEEL-1 toxicity. To identify other factors required for PEEL-1 toxicity, we conducted a screen for suppressors of ectopic PEEL-1 driven by a heat-shock promoter. Thus far, we have isolated five suppressors. The strongest suppressor fully suppresses PEEL-1 toxicity and is not allelic to the heat-shock transcription factor gene *hsf-1*. We have also expressed GFP-tagged PEEL-1 under a galactose-inducible promoter in yeast. Preliminary data suggest that PEEL-1 strongly inhibits growth of the yeast when induced.

We have also begun a search for hybrid incompatibility genes in other *Caenorhabditis* species. We performed crosses between two recently isolated *Caenorhabditis* species, C. sp. 17 and C. sp. 29. When C. sp. 17 males were crossed to C. sp. 29 females, all F1 progeny arrested as embryos. However, when C. sp. 29 males were crossed to C. sp. 17 females, a very small percentage of F1 progeny did not arrest as embryos, but instead grew to the adult stage. Thus, as with *peel-1*, a parental effect contributes to the hybrid incompatibility. Almost all of the viable hybrids were female, indicating that the cross obeys Haldane's rule. The few F1 hybrid males are sterile. F1 hybrid females did not give viable progeny when crossed to C. sp. 29 males, but gave large numbers of viable F2 progeny and some arrested embryos when crossed to C. sp. 17 males. Among the surviving F2, there are more females than males, indicating that this cross also obeys Haldane's rule. There is significant variability in the relative proportion of males, females, and arrested embryos in the F2 generation from cross to cross, suggesting that particular combinations of genes in the rare surviving F1 females determine the proportions seen in the F2 crosses.

916C. Gene movement between X and autosomes and its effect on transcription. **Sarah E. Albritton**, Anna-Lena Kranz, Sevinc Ercan. Center for Genomics and Systems Biology, New York Univ, New York, NY.

In Caenorhabditis species, dosage compensation acts to reduce transcriptional output of both female/hermaphrodite X chromosomes by one half. This balances X expression between the sexes, but potentially gives females the same problem faced by males: functional monosomy of the X chromosome. It has been proposed that a mechanism evolved to upregulate X expression to balance X and autosomal transcription, thereby overcoming male monosomy. However, owing to biased gene content and tissue-specific regulation of the X, direct comparison of X and autosomal transcription is difficult. In order to more directly compare X and autosomal transcription we looked at expression of 1:1 orthologs that are differentially located on the X or an autosome between two nematode species. Our work focused on four species: C. elegans, C. briggsae, C. remanei, and Pristionchus pacificus. The C. elegans and C. briggsae genomes are well assembled and annotated. The genomes of C. remanei and P. pacificus have been sequenced, but their genes have not yet been assigned to chromosomal locations. Since our analysis depends on comparing differentially located orthologs, we first needed to map genes to either the X or autosomes. We took a read-depth-variation approach. We performed genomic DNA-seq in males and females/hermaphrodites of C. brenneri, C. remanei and P. pacificus. C. briggsae males and hermaphrodites were also sequenced as controls. Genes located on the X chromosome were expected to have a 1:2 ratio of sequencing coverage between males and hermaphrodites (X:XX) and all autosomal genes a 1:1 ratio. Our analysis yielded a list of X and autosomal genes for each of the four nematode species and allowed the identification of differentially located 1:1 orthologs. Comparison of X and autosomal transcription showed no bias towards male upregulation of X-located orthologs.

917A. Revisiting the effects of spontaneous mutations on the (micro)environmental variance in Caenorhabditis. **Charles F. Baer[1,2]**, Erik C. Andersen[3], Reza Farhadifar[4], Daniel Needleman[4]. 1) Department of Biology, Univ of Florida, Gainesville, FL; 2) Univ of Florida Genetics Institute, Gainesville, FL; 3) Northwestern Univ, Evanston, IL; 4) Harvard Univ, Cambridge, MA.

An obvious feature of living organisms is that their development is robust to variation in the environmental circumstances in which the organism finds itself - within limits. That is, the phenotype is more or less "canalized" (canalized » "robust"). The "more or less" is of considerable interest to evolutionary biologists, for a variety of reasons, i.e., under what environmental or genetic circumstances does development become more or less canalized? Environmental canalization can be quantified as the phenotypic variation among genetically identical individuals raised in a uniform environment - the "(micro)environmental variance" in the lingo of quantitative genetics, V(E). Here we provide quantitative estimates of the effects of spontaneous mutations on V(E) for a variety of phenotypic traits subject to different selective regimes. Three general quantitative trends emerge. First, in almost all cases, mutation accumulation tends to de-canalize the phenotype (i.e., V(E) increases), typically at a rate similar to the rate of change of the trait itself. Second, there is a strong positive association between the rate of increase of V(E) for a trait and the mutational variance, V(M) for the trait itself. Third, and most intriguingly, mutations affecting V(E) for a trait appear to be usually under stronger selection than mutations affecting the trait itself.

918B. Evolution of a Higher Intracellular Oxidizing Environment in Caenorhabditis elegans Under Relaxed Selection. Joanna Joyner-Matos[3], Kiley A. Hicks[4], Dustin Cousins[3], Michelle Keller[3], Dee R. Denver[5], **Charles F. Baer[1,2]**, Suzanne Estes[4]. 1) Dept Biol, Univ Florida, Gainesville, FL; 2) Univ of Florida Genetics Institute, Gainesville, FL; 3) Department of Biology, Eastern Washington Univ, Cheney, WA; 4) Department of Biology, Portland State Univ, Portland, OR; 5) Department of Zoology, Oregon State Univ, Corvallis, OR.

We explored the relationship between relaxed selection, oxidative stress, and spontaneous mutation in a set of mutation-accumulation (MA) lines of the

nematode Caenorhabditis elegans and in their common ancestor. We measured steady-state levels of free radicals and oxidatively damaged guanosine nucleosides in the somatic tissues of five MA lines for which nuclear genome base substitution and G:C®T:A transversion frequencies are known. The two markers of oxidative stress are highly correlated and are elevated in the MA lines relative to the ancestor; point estimates of the per-generation rate of mutational decay (DM) of these measures of oxidative stress are similar to those reported for fitness-related traits. Conversely, there is no significant relationship between either marker of oxidative stress and the per-generation frequencies of base substitution or GC-TA transversion. Although these results provide no direct evidence for a causative relationship between oxidative damage and base substitution mutations, to the extent that oxidative damage may be weakly mutagenic in the germline, the case for condition-dependent mutation is advanced.

919C. Natural variation and sensory biology of *C. elegans* hermaphrodite control of mating. **Adam K. Bahrami**, Yun Zhang. Organismic & Evolutionary Biology, Center for Brain Science, Harvard Univ, Cambridge, MA.

Reproductive behaviors have manifold consequences on evolutionary processes. *C. elegans* hermaphrodites reproduce through self-reproduction and outcrossing with males, yet most studies have focused on male control of mating. We evaluated the hypothesis that hermaphrodites express reproductive choice by studying a wide array of genotypes in a simple mating pair assay that quantifies mating *vs.* selfing outcome. First, we examined variation in hermaphrodite mating frequency among ~40 putative wild isolates representing *C. elegans* global diversity and found extensive, continuous variation in hermaphrodite reproductive outcome. Within this set, N2 was among the strains strongly favoring self-reproduction and CB4856 (HW) was among those favoring outcrossing. Next, we created recombinant inbred lines to map variation and identified two QTL (on chromosomes IV and V) that explain a large portion of N2 x HW variation in hermaphrodite mating frequency. To characterize mechanisms underlying 'low mating' in N2, we tested mutants that disrupt sensory perception in the hermaphrodite nervous system. We observed that N2 hermaphrodites deficient in mechanosensation and/or chemosensation (*e.g. mec-3* and *osm-6*) exhibit high mating frequency, implicating hermaphrodite perception of males (or, possibly, the environment) as a requirement for 'low mating.' Within chemosensory networks, we find opposing roles for neurons expressing the TAX cyclic-gated nucleotide channel, suggesting both positive and negative sensory-mediated regulation of hermaphrodite mating. Our findings demonstrate that *C. elegans* hermaphrodites actively regulate whether they self-reproduce or outcross with males, highlight the existence of natural variation in hermaphrodite choice, and lay the groundwork for molecular dissection of this evolutionarily important trait.

920A. The hunt for quantitative trait nucleotides: a near-isogenic line based approach in *C. elegans*. **Max Bernstein**, Matthew Rockman. Center for Genomics and Systems Biology, NYU, New York, NY.

The individual nucleotides responsible for phenotypic diversity have been very difficult to isolate. The majority of known causal variants that affect traits in wild populations of a species occur within protein-coding regions. These variants are typically of very large effect and may not be typical of the sorts of alleles that drive evolution. There are many confounding effects that can explain the relative lack of causal variants, or quantitative trait nucleotides (QTNs), particularly alleles of small effect, epistasis, and gene-environment interactions. One way to elucidate quantitative trait loci (QTLs) is through the creation of near-isogenic lines (NILs), where the majority of the genome (>95%) is of one genomic background. NILs have previously been created for the nematode C. elegans and identified QTLs across the genome for several phenotypes. C. elegans is a naturally inbred species, which makes it ideal for isolating QTNs. Starting with a NIL that harbors a ~1.5 Mb segment on Chromosome X originating from the Hawaiian isolate CB4856, we have created a series of more than 1000 sub-NILs that break up this segment into smaller regions by recombination. These sub-NILs will be genotyped at 278 SNPs within the 1.5 Mb segment. We are currently piloting high-throughput fitness assays similar to previous work by Elvin et al (2011) and Ramani et al (2012). Additionally, we plan to do gene expression analysis on a subset of the sub-NIL panel to characterize expression QTNs (eQTNs). QTL mapping on simulated sub-NIL datasets shows that for a single QTL model, we can accurately map small effect (h^2<0.1) causal variants. Due to the linkage disequilibrium pattern in the sub-NIL panel, traditional QTL mapping methods are not applicable. We show that pairwise comparisons among groups of similar sub-NIL genotypes provide a systematic method for identifying QTLs. We have also modeled more complex QTL scenarios, including multiple QTLs and epistatic interactions. These simulations have informed us of our potential statistical power for future QTL mapping experiments.

921B. Evolution and plasticity of embryo retention in Caenorhabditis nematodes. Paul Vigne, **Christian Braendle**. Institute of Biology Valrose, CNRS, INSERM, Univ of Nice Sophia Antipolis, Nice, France.

Under stressful environmental conditions, such as starvation, Caenorhabditis hermaphrodites and females increasingly retain developing embryos in the uterus (« bagging »). As a result, embryos will hatch and continue larval development internally, causing the mother's premature death. Retention of embryos and internal hatching potentially represent adaptive strategies to provide offspring with resources provided by the mother in the absence of food in the external environment. To elucidate the evolutionary significance and genetic mechanisms of worm bagging, we study variation in embryo retention in different C. elegans wild isolates and different Caenorhabditis species in response to variable environmental conditions (e.g. temperature, food quantity and quality). Although bagging occurs to some extent in all Caenorhabditis species, isolates and species may strongly differ in their bagging response, revealing significant genotype-by-environment interactions. Moreover, certain isolates may show a very high frequency of bagging, even under food-rich conditions. Making use of an F2 RIL mapping population we have now started to characterize the genetic differences between isolates that show divergent bagging behaviour, which may lead to the identification of genetic factors involved in the regulation of embryo retention and matricide in Caenorhabditis nematodes.

922C. Evolution of ZIM proteins in *Caenorhabditis*. **Victoria Cattani**, Matthew Rockman. Center for Genomics and Systems Biology, New York Univ, New York, NY, 10003.

Meiosis is an essential process for sexual reproduction, allowing diploid organisms to generate haploid gametes. During this process, homolog chromosomes must recognize each other and align along their lengths to ensure accurate segregation. In *Caenorhabditis elegans* chromosomes, specialized regions known as pairing centers interact with four related zinc-finger proteins (HIM-8, ZIM-1, ZIM-2 and ZIM-3) to stabilize homolog interactions and initiate synapsis. Mutations in the *zim* genes cause meiotic defects such as broods containing inviable progeny and an elevated incidence

of males. Genes involved in meiosis may diverge very quickly during evolution, despite the conservation of the meiotic process across species. In particular, certain zinc-finger proteins have undergone massive expansion during the evolution of eukaryotes. Why are zinc-finger proteins evolving rapidly? Is their function conserved in light of this rapid divergence? To investigate what molecular forces have driven the divergence of the ZIM proteins, I first identified orthologous *zim* genes in six species of the elegans group: *C. elegans*, *C. briggsae*, *C. remanei*, *C. brenneri*, *C*. sp. 11 and *C*. sp. 5. Consistent with previous reports, I found that the C-terminal region of these proteins is highly conserved whereas the N-terminal region is highly divergent. To evaluate the importance of this divergence in ZIM proteins I am testing whether ZIM proteins are interchangeable between closely related species. To that end, I am introducing transgenic *zim* alleles from *C. briggsae*, *C. remanei*, and *C*. sp. 11 into *C. elegans zim* mutants and analyzing whether they rescue the mutant phenotype.

923A. Population genomic variation in the outcrossing species *Caenorhabditis remanei*. Cristel G. Thomas[1], Janna L. Fierst[2], John H. Willis[2], Wei Wang[1], Richard Jovelin[1], Patrick C. Phillips[2], **Asher D. Cutter[1]**. 1) Dept Ecology/Evolutionary Biol, Univ Toronto, Toronto, ON, Canada; 2) Institute of Ecology and Evolution, Univ Oregon, Eugene, OR, USA.

Natural populations of *C. elegans* contain orders of magnitude fewer nucleotide polymorphisms and longer haplotype linkage blocks than their outcrossing *Caenorhabditis* relatives. These factors, together with the lack of a close outgroup to *C. elegans*, limit our ability to interpret patterns of within-species variation in *C. elegans*. To better understand how evolutionary forces interact to shape nematode genome evolution, we are investigating population polymorphism and divergence for *C. remanei* and its close relative *C*. sp. 23 by sequencing whole genomes of many wild isolates of these species. We combine two complementary approaches to investigate patterns of diversity in the genome of *C. remanei*. We used next-generation sequencing to gather whole-genome data from dozens of *C. remanei* isofemale inbred strains from multiple populations from Europe and North American and for several *C*. sp. 23 isofemale inbred strains from a single population in China. These strains are maintained in culture and are available to the worm community. We also directly sequenced genomes from single *C. remanei* individuals immediately collected from the field to minimize the effect of lab breeding. Consistent with previous results based on tens of genes, we find high nucleotide diversity for SNPs, indels and microsatellites in both *C. remanei* and *C*. sp. 23. This data provides a framework to quantify demographic and selective forces shaping nucleotide polymorphism across the entire genome and for testing hypotheses about the molecular evolution of focal gene classes. Our ongoing work is using this resource to understand the microevolution of small RNA genes, their downstream targets, and their upstream regulators. In addition, because these two species are partially interfertile, the collection of variants we uncovered makes this species pair an extraordinary system for addressing diverse questions in ecological and speciation genetics.

924B. RhabditinaDB: online database for wild worms. **D. Fitch**, K. Kiontke. New York Univ, New York, NY.

New species of rhabditid nematodes are being discovered at an increasing rate. In *Caenorhabditis* alone, more species have been discovered in the last decade than in the previous 100 years since *C. elegans* was described. Although this discovery provides more candidate species for comparative biology and genomics, it poses a challenge for taxonomists and for keeping track of comparative data. In response to this challenge, we have created RhabditinaDB, a curated, online, open-access database to provide information on all known rhabditid nematodes, including data on taxonomy, phylogeny, distribution, living and non-living resources such as strains and type specimens, images (including DIC stacks, electron micrographs, and camera lucida drawings), species descriptions and relevant literature, phenotypic character data and molecular sequences. Additionally, we have implemented tools for querying the data, such as keyword searching, BLAST, PhenoBLAST (modified from Gunsalus et al., 2004) and a species-comparison page. Currently, the database shows information for all known species in genus *Caenorhabditis*, but will be expanded in the coming years to show data for all rhabditid species. Some problems in rhabditid systematics have arisen from the difficulty of obtaining taxonomic materials, lack of good keys or ways to compare new species to those already described. Specifically, there has been a high rate of synonymous descriptions and the accumulation of newly discovered but undocumented species. Our database should mitigate these problems by providing as much data as possible via one resource, along with a recent taxonomy (Sudhaus 2011), based primarily on our molecular phylogeny (Kiontke et al., 2007, 2012). Not only will RhabditinaDB facilitate taxonomy, but it will also provide data on rhabditid biodiversity. This in turn should facilitate phylogenetically informed taxon choices for comparative studies and genomics. As RhabditinaDB is a work in progress, we welcome suggestions for improvements, and data submissions. RhabditinaDB can be accessed at: http://wormtails.bio.nyu.edu/Databases . Thanks to M. Katari, J. Lorenzana, NYU Bobst Library, W. Sudhaus. References: Gunsalus et al. 2004, Nucl. Acids Res. 32:D406-D410; Sudhaus 2011, J. Nematode Morphol. Syst. 14(2):113-178; Kiontke et al. 2007, Curr. Biology 17(22):1925-1937.

925C. Microevolution and coexistence of Santeuil and Le Blanc viruses in *Caenorhabditis briggsae.* . **Lise Frézal**, Marie-Anne Félix. IBENS, Paris, France.

We recently found three viruses, Orsay, Santeuil and Le Blanc, which naturally infect *Caenorhabditis* nematodes (1,2). These ss(+)RNA viruses cause intestinal cell symptoms and are horizontally transmitted. Whereas *C. elegans* can so far only be infected by the Orsay virus, European *C. briggsae* genotypes are susceptible to both Santeuil and Le Blanc viruses, and both viruses have been found in the same locations. This vulnerability of *C. briggsae* to two viruses enables studies of in vivo viral competition and of the mechanisms driving their short-term evolution, as well as the impact of their competition on worm fitness.

RNA viruses may evolve rapidly through both high mutation rates and recombination events. The impact of recombination widely varies from one viral species to another but in all cases, for recombination to occur, different virus types have to infect the same host cell. The first step is thus to assess whether different virus species can co-infect the same worm population, the same animal and the same cell.

By using quantitative RT-PCR, we demonstrate that the Le Blanc and Santeuil viruses can coexist in a worm population, even when originally introduced at widely different concentrations. The two viruses are jointly maintained over 10 worm generations. We presently investigate the co-infection at the whole organism and single cell levels by tracking the viral RNAs in co-infected worms using Fluorescent In Situ Hybridization.

1- Félix, Ashe, Piffaretti *et al.* 2011 *PloS biology*.

2- Franz *et al.* 2012 *Journal of virology*.

926A. Evolution of avermectin resistance in *C. briggsae*. **Rajarshi Ghosh**[1], Cristel Thomas[2], Wei Wang[2], Richard Jovelin[2], Asher Cutter[2], Leonid Kruglyak[1]. 1) Lewis Sigler Institute for Integrative Genomics, Department of Ecology and Evolutionary biology, Princeton Univ, Princeton, NJ 08540; 2) Department of Ecology & Evolutionary Biology Univ of Toronto 25 Willcocks St. Toronto, ON, M5S 3B2 CANADA.

Several nematode species have evolved resistance to the widely used anthelmintic avermectins (AVM). AVM is produced naturally by *S.avermitilis*, a ubiquitous soil bacterium. As many nematodes spend part of their life cycle in contact with soil, they are likely to encounter *S.avermitilis*. Widespread AVM resistance may be a result of different nematode species' ability to counter a common selective pressure, namely the toxins produced by *S. avermitilis*.

To test this hypothesis we surveyed AVM resistance in diverse nematode species. We found that resistance to AVM and to *S. avermitilis* was prevalent in this phylum. To identify the genetic basis of natural AVM resistance we focused on *C. briggsae*. We found that two divergent isolates of *C. briggsae* differed significantly in their responses to AVM. Using QTL mapping approach with these two strains , we identified a significant locus on Chromosome II underlying responses to AVM.

We also surveyed 50 isolates of *C. briggsae* for responses to AVM and found that they exhibit wide variation. The pattern of variation in responses to AVM correlated significantly with the observed phylogeographic pattern in *C. briggsae*, with temperate isolates being more likely to be resistant than tropical ones. To gain insights into the evolution AVM resistance in *C. briggsae*, we obtained whole genome sequences of these isolates. Using this data we confirmed that *glc-1*, the causative gene for natural AVM resistance in *C. elegans*, is the result of a duplication of another GluCl subunit in the elegans lineage. Thus *glc-1* is absent in *C. briggsae* suggesting that the genetic mechanisms of natural resistance to AVM in *C. briggsae* are likely different from *C. elegans*. The sequence data will help us determine if variation in candidate targets for AVM correlate with the pattern of resistance in *C. briggsae* and map the genetic basis of differences in responses to AVM in *C. briggsae* .

927B. Strong outbreeding depression and low genetic diversity in the selfing *Caenorhabditis* sp. 11. **Clotilde Gimond**[1], Richard Jovelin[2], Shery Han[2], Celine Ferrari[1], Asher D. Cutter[2], Christian Braendle[1]. 1) Institute of Developmental Biology and Cancer, CNRS, INSERM, Univ of Nice Sophia-Antipolis, Nice, France; 2) Department of Ecology and Evolutionary Biology, Univ of Toronto, Toronto, Canada.

Theory and empirical study produce clear links between the evolution of mating systems and the fitness effects of breeding between close relatives in terms of inbreeding depression. The connections between mating systems and outbreeding depression, whereby fitness is reduced in crosses of unrelated individuals, however, are less well defined. Gonochoristic *Caenorhabditis* species are generally characterized by high population genetic variation whereas the selfing Caenorhabditis species, *C. elegans* and *C. briggsae*, show clear signs of outbreeding depression. Our aim was to further investigate the extent of inbreeding and outbreeding depression in the context of selfing, focusing on the third androdioecious species of *Caenorhabditis*, the undescribed, pan-tropical *C.* sp. 11. Analysis of nucleotide diversity in a collection of approximately 50 *C.* sp. 11 wild isolates revealed very little polymorphism compared to other *Caenorhabditis* species, indicating similar or lower levels of genetic diversity than the other two selfing species. Moreover, crosses between geographically separated *C.* sp. 11 isolates uncovered very strong outbreeding depression in F1 hybrid reproductive performance (offspring number, embryonic mortality). Outbreeding depression was significantly higher in F1 hybrids derived from inter-locality crosses compared to intra-locality crosses and highly variable depending on the parental strain combination, ranging from a 10 to > 50% decrease in larval offspring production relative to F1 pure strains. We also show that male mating efficiency is generally lower in *C.* sp. 11 strains than in the reference strains for *C. elegans* (N2) and *C. briggsae* (AF16). Thus, outbreeding depression appears to be a common feature of selfing *Caenorhabditis* species, and most pronounced in *C. sp.* 11. We propose that self-fertilization is a key driver of outbreeding depression, but that outbreeding depression need not evolve as a direct result of local adaptation per se.

928C. Fainting towards Necromeny: Anesthesia caused by a Beetle Pheromone is Mediated by a Lipid-Binding Protein in *Pristionchus pacificus*. **James L Go**[1], Jessica K Cinkornpumin[1], Margarita Valenzuela[1], Roonalika D Wisidagama[1,2], Ray L Hong[1]. 1) Biology Department, Cal State Northridge, Northridge, CA; 2) Department of Biological Chemistry, U. of Utah, Salt Lake City, UT.

Pristionchus pacificus is a necromenic nematode found to associate with several species of beetles around the world. To better characterize the interactions between *P. pacificus* and its host *Exomala orientalis* (Oriental Beetle), we exposed *P. pacificus* to the beetle's sex pheromone ZTDO. The mutant oriental beetle pheromone insensitive 1 (*obi-1*) in the California strain does not chemotax to the pheromone after treatment with cGMP. To further investigate the role of *obi-1* in ZTDO sensing, we exposed *P. pacificus* with the pheromone and determined the percentage of worms that become arrested in embryonic development or immobilized at different developmental stages (egg, J2, dauer, J4, adult). This anesthetic-like effect was species-specific since ZTDO did not alter the embryonic development and locomotion behavior of wild-type *C. elegans*. We found that the *obi-1* mutant strain was hypersensitive to the pheromone and become immobilized at every stage we tested except as adults. ZTDO delivered in the agar media as well as a volatile odor can arrest mutant embryos before gastrulation immediately and induce complete J4 larvae paralysis within an hour of host pheromone exposure. Obi-1::gfp expression is highest during the J4 stage and can be found in multiple cell types, including the chemosensory amphid sheath cells. Interestingly, the *C. elegans* ortholog of *obi-1*, *C06G1.1*, shows a highly conserved expression pattern except in the sheath cells. *Obi-1* encodes for a secreted protein with lipid-binding motif. This unexpected finding that a host insect pheromone can act as a species-specific volatile anesthetic could provide important clues about the genes and signaling pathways that have evolved for *P. pacificus* to coordinate its behavior and development with its host using an inter-species pheromone (kairomone).

929A. Cross Species NILs - A Resource for Speciation, Evolution and Development. **Jeremy C Gray**, Joanna Bundus, Asher D Cutter. Ecology and Evolutionary Biology, Univ of Toronto, Toronto, ON, Canada.

Caenorhabditis sp 9 and *Caenorhabditis briggsae* are capable of forming hybrids at low rates, despite being separate species. Reproductive isolation is of long standing interest in evolutionary biology and the presence of interbreeding species in this genus presents a unique opportunity to probe the genetics of speciation in a well understood background.

Here we detail the construction and genotyping of 200 near isogenic lines, consisting of small introgressions of one genome into the opposite species in both mitochondrial backgrounds. Introgressions were achieved by random mating and subsequent backcrosses. Next generation sequencing will be

undertaken in order to determine which regions are introgressed, and regions which are under or overrepresented. Strains have been phenotyped for reproductive isolation, cytonuclear incompatibility and general developmental defects. The main thrust of the project is to identify and analyse Bateson-Dobzhansky-Muller incompatibilities within the genus, as well as to identify the genetic basis of the mating system differentiation in the two species.

It is anticipated these strain will be made available to the community at large along with genotype information to allow further studies into the divergence of these two species.

930B. Widespread pleiotropic Bateson-Dobzhansky-Muller incompatibilities between *C. elegans* isolates. Helen E. Orbidans[1,3], L. Basten Snoek[2,3], Jana Stastna[1], Jan E. Kammenga[2], **Simon C. Harvey[1]**. 1) Ecology Research Group, Canterbury Christ Church Univ, Canterbury, UK; 2) Laboratory of Nematology, Wageningen Univ, The Netherlands; 3) Contributed equally.

Bateson-Dobzhansky-Muller (BDM) incompatibilities are a result of deleterious interactions between alleles that are neutral or advantageous in their own genetic backgrounds. Both outbreeding depression and a specific incompatibility causing embryonic lethality have been identified within *C. elegans*. We therefore hypothesised that alleles producing BDM incompatibilities would also be present. Identifying the underlying loci producing such negative epistatic effects within a species is important as it will allow comparison to the loci and alleles that generate isolation between species, i.e. it addresses the role of BDM incompatibilities in driving speciation.

To identify genomic regions showing BDM incompatibilities we undertook screens for regions that disrupted the normal process of egg-laying, a complex, highly regulated and coordinated phenotype. Screens were undertaken in recombinant inbred lines (RILs) and a genome-wide panel of nearly isogenic lines (NILs) both produced from the isolates CB4856 and N2. These RIL and NIL analyses identify a number of quantitative trait loci (QTLs) that show synthetic effects on egg-laying, i.e. the disruption in egg-laying is not seen in the parental isolates and is a consequence of negative interactions between CB4856 and N2 alleles. Analysis of these QTLs shows that they also affect other life history traits, affecting lifespan and the internal hatching of progeny (bagging). Further analysis indicates that this approach identifies only a subset of the incompatibilities between CB4856 and N2, that these incompatibilities are a consequence of complex interactions between multiple loci, and that they interact with the stress response.

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931C. Comparative mapping of dauer larvae development in growing populations of *C. elegans* and *C. briggsae*. James W.M. Green, **Simon C. Harvey**. Ecology Research Group, Canterbury Christ Church Univ, Canterbury, UK.

Dauer larvae in *Caenorhabditis elegans* are long-lived and resistant to environmental stress. Outside of growing populations they are also the only life cycle stage that can be routinely isolated from the environment. The appropriate induction of dauer larvae development is therefore likely to be critical to genotype fitness in *C. elegans*. Given this, the extensive variation observed between wild isolates requires explanation. Does it represent adaptation to different environments or does it imply that there are multiple different ways to maximise fitness within the same environment?

To investigate this question we have analysed dauer larvae formation and population growth in growing populations using different recombinant inbred lines (RILs) of *C. elegans*. These analyses allow direct comparisons between: (1) different RIL panels produced from distinct parental isolates analysed for the same dauer development same trait; (2) RILs and nearly isogenic lines (NILs) produced from the same parental isolates and analysed for the same dauer development same trait; and (3) different dauer development traits mapped using the same RIL panel. These results identify common QTL regions and both genotype and trait specific QTLs. Comparison with the results of analysis of variation in dauer larvae development within growing populations of *C. briggsae* RILs allows a more general picture of the control of variation of dauer larvae development in growing populations.

932A. Co-evolution of Mitochondrial and Nuclear Genomes in *Caenorhabditis*. **Emily E. King**, Scott E. Baird. Biological Sciences Dept, WSU, Dayton, OH.

Nuclear and mitochondrial genomes are coadapted and interactions between these genomes are required for mitochondrial functions. The impact of divergence is between *Caenorhabditis briggsae* populations and between *C. briggsae* and its sister species, *C.* sp. 9 on these coadapted interactions was assessed.

Four sets of hybrid strains were constructed. One set of hybrid lines possessed a *C. briggsae* AF16 nuclear background and *C. briggsae* JU1345 mitochondria. A reciprocal set of hybrid lines possessed a JU1345 nuclear background and AF16 mitochondria. The next set of hybrid lines had *C. briggsae* AF16 mitochondria in a *C.* sp. 9 strain EG5268 nuclear background. The last set of hybrid strains had *C. briggsae* JU1345 mitochondria in an EG5268 nuclear background.

Parental and hybrid strains were scored for intrinsic growth rates, embryonic lethality and the size of mature oocytes. The rate of ovulation was determined by counting progeny in 24 hour periods. No significant differences were observed between parental *C. briggsae* strains and *C. briggsae* hybrid lines. *C. briggsae:C. briggsae* hybrids and *C. briggsae* parentals had similar results in embryonic lethality. Abnormal phenotypes were observed in high rates in the *C. briggsae*:*C.* sp. 9 hybrids and in the EG5268 strain, in comparison to the C. briggsae hybrids, AF16 strain and JU1345 strain. Most of the abnormal hybrids have a shorter life span, preventing them from producing their optimal amount of offspring. Both the EG5268 parental strain and *C. briggsae*:*C.* sp. 9 hybrids exhibited larval lethality and embryonic lethality, by the presence of embryos not hatching after 24 hours. However, the *C. briggsae*:*C.* sp. 9 hybrids showed elevated levels of larval lethality and embryonic lethality relative to the parental *C.* sp. 9 EG5268 strain. Also, the *C. briggsae:C.* sp. 9 hybrids had elevated levels of embryonic lethality in contrast to *C. briggsae* hybrids. One of the hybrids exhibited larval lethality at a four-fold increase compared to EG5268.

933B. The latest update on *Caenorhabditis* species, their ecology, phylogeny, and morphology. **Karin C. Kiontke[1]**, Marie-Anne Félix[2], David H. A. Fitch[1]. 1) Dept. Biology, New York Univ, New York, NY; 2) Institute of Biology, Ecole Normale Supérieure, Paris, France.

Since we reported on *Caenorhabditis* biodiversity in 2011, 13 new species have been discovered. The number of species in culture is now 36, and 50 species are known. Most new species were isolated from rotting plant material, but two were found in fresh figs (N. Kanzaki pers. comm.) and one in the hind gut of a millipede (W. Sudhaus pers. comm.). Three of the new species were isolated from temperate regions, the others from tropical regions. Preliminary phylogenetic analyses with molecular data for 36 species confirm the existence of two well-supported large sister clades, the *Elegans* super-

group with now 21 species and the *Drosophilae* super-group with 11 species. *C. plicata*, *C.* sp. 1 and *C. sonorae* as well as a *C.* sp. 21 branch off basally. Still, no sister species of *C. elegans* has been found. Hybridization is now observed in crosses of 5 species pairs (*C. angaria* - *C.* sp. 12, *C. briggsae* - *C.* sp. 9, *C. remanei* - *C.* sp. 23, *C.* sp. 5 - *C.* sp. 26, *C.* sp. 8 - *C.* sp. 24), providing opportunity for studying the evolution of hybrid incompatibility in *Caenorhabditis*. In at least one case, crosses between individuals from some but not all populations show reproductive isolation, suggesting incomplete speciation. Using light and scanning electron microscopy, we are evaluating morphological characters of all cultured species in detail. Across *Caenorhabditis*, the morphological diversity is large, especially in features of the male tail and the stoma. However, no or only subtle differences are found between many species of the *Elegans* super-group. Mapping phenotypic characters onto the phylogeny shows extensive homoplasy (convergent evolution or secondary loss) across all character complexes. Morphological, biogeographical, ecological, sequence, and taxonomic data on all *Caenorhabditis* species is now available through an open-access online database RhabditinaDB (http://wormtails.bio.nyu.edu/Databases). Strains of most species are available through the CGC.

934C. Evidence for at least two ancient duplications of presenilin genes in the nematode phylum. **Bernard Lakowski**. School of Health and Natural Sciences, Mercy College, Dobbs Ferry, NY.

Presenilin proteins are very well conserved across the eukaryotes. However, two of the three *C. elegans* genes are among the most divergent presenilin genes known. Only *sel-12* is highly similar to human PS1 and PS2 while both *spe-4* and *hop-1* are more divergent. I have identified all presenilin genes from many Nematode species (>20) across the phylum from public databases. I have found that in Nematodes, there are three classes of presenilin genes: 1) conserved standard presenilins, 2) divergent standard presenilins and 3) *spe-4* presenilins. The three *C. elegans* presenilins fit into each of these three classes: *sel-12* is a conserved standard presenilin, *hop-1* is a divergent standard presenilin and *spe-4* is a *spe-4* presenilin. In the nematode phylum, all species have at least one, and usually only one, conserved standard presenilin. These genes share high similarity to the human presenilins and have a large number of residues that are absolutely, or very strongly, conserved. All species within clades III, IV and V (according to the classification of Blaxter and colleagues) also contain a *spe-4* gene. SPE-4 proteins have several conserved residues and other features that differ from canonical presenilins. This suggests that *spe-4* genes may have evolved different functions, or interactions, from standard presenilins. The divergent standard presenilin genes encode proteins with highly divergent sequences that do not contain any highly conserved amino acids not found in conserved standard presenilins. These genes are rapidly diverging, indicating that they are under relaxed selection. The divergent standard presenilins arose from one or more ancient gene duplications. In the basal nematode species *Trichuris muris* there is both a conserved and a divergent standard presenilin. This, and the deep differences between SPE-4 proteins and conserved standard Presenilins, makes it likely that SPE-4 proteins evolved from an already divergent presenilin gene rather than a conserved standard presenilin. This means that a divergent presenilin gene was present in the last common ancestor of the Clades, III, IV and V species (about 500-400 million years ago).

935A. The Role of Pharyngeal Glands in Nematode Feeding and Diet. **James W. Lightfoot**, Ralf J. Sommer. MPI Developmental Biology, Tübingen, Germany.

Nematode pharyngeal glands have historically been associated with feeding as well as cuticle moulting, although little is understood of the molecular biology underlying these processes. By utilising two evolutionary divergent nematode species with differing diets and feeding traits we are elucidating the roles of the pharyngeal gland cells and their function in feeding, diet and other cellular processes. The model organisms *C. elegans* and *P. pacificus* provide an apt system with which to analyse the pharyngeal glands, as while both nematode species contain several pharyngeal gland cells, *C. elegans* is predominantly a bacterial feeder, whereas *P. pacificus* is capable of predating other nematode species in addition to its bacterial feeding habits. Accordingly, gland cells display an evolutionary divergence in both quantity and morphology between species. The pharynx of *C. elegans* contains five pharyngeal gland cells, a large dorsal gland (g1D) exiting at the mouth opening, two ventral glands (g1V) emptying into the median bulb and two ventral glands (g2) exiting into the terminal bulb. The pharynx of *P. pacificus* contains three gland cells, lacking the g2 glands, and additionally the g1D is morphologically distinct as the gland duct exits through the predatory dorsal tooth used to eviscerate other nematodes before feeding. Furthermore, substantial divergence in the function of pharyngeal glands between these two nematode species has been predicted. The dorsal gland in the bacterial feeding nematode *C. elegans* is stimulated through inputs from the isthmus while the dorsal gland in the bacterial and predatory feeder, *P. pacificus* receives inputs from the corpus. This dramatic shift in regulation may indicate an alteration in gland cell regulation towards additional predatory functions. Cell ablation experiments removing g1D cells however reveal much functional conservation as animals become starved due to a bacterial plug blocking the pharynx in both species. Therefore, we have begun generating the transcriptome of the pharyngeal gland cells using mRNA tagging followed by RNA-seq with which we will pursue the genetic candidates behind gland cell function and the expanded diet evident in *P. pacificus.*.

936B. Genomic Analysis of Hox Genes in Five Steinernema Genomes. **Marissa Macchietto**[1], Adler Dillman[2,3], Ali Mortazavi[1], Paul Sternberg[2,3]. 1) Univ of California, Irvine, Irvine, CA 92697; 2) California Institute of Technology, Pasadena, CA 91125; 3) Howard Hughes Medical Institute, Pasadena, CA 91125.

Hox genes are evolutionarily conserved transcription factors that regulate the expression of other developmental genes. They are organized into clusters, with the order of genes in each cluster paralleling their expression along the anterior-posterior axis. In the free-living nematode Caenorhabditis elegans, the Hox genes are much more dispersed along the chromosome, and the anterior Hox genes, ceh-13 and lin-39, are reversed, but little is known about the Hox genes in other nematode taxa. We are interested in the Hox gene cluster architecture of insect parasitic nematodes from the genus Steinernema (S. carpocapsae, S. scapterisci, S. feltiae, S. glaseri, and S. monticolum), for which we have assembled genomes and stage-specific transcriptomes. More specifically, we are interested in exploring the presence, order, and dispersal of the Hox genes in these newly sequenced nematodes, to identify the level of conservation within this genus and their conservation with C. elegans. We also investigated the extent of non-coding conservation around Hox genes, looking for candidate regulatory regions. Surprisingly, the Hox gene cluster among steinernematids is very different from what is known in C. elegans. For example, we have found approximately 10 genes between the steinernematid Hox genes ceh-13 and lin-39, whereas the region between the orthologous ceh-13 and lin-39 Hox genes in C. elegans is a gene desert. Interestingly, many of the intervening genes in the steinernematids are conserved and expressed in C. elegans, but are located nowhere near the C. elegans Hox cluster. We explored this in another nematode genome that we have assembled, Panagrellus redivivus, where we find only two intervening genes present between ceh-13 and lin-39 Hox genes. These findings suggest that the organization of the Hox cluster in nematodes has structural plasticity and varies across the phylum.

937C. Interactions of *C. elegans* with its gut microbiota: from sampling in the wild to molecular genetic studies. **Sarah E. Marsh**, Marie-Anne Félix. IBENS, Paris, France.

Symbiosis is the living together of unlike organisms. These interactions vary across a cost-benefit spectrum from those imposing a high cost on the host (parasitism) to those benefitting the host (mutualism). Indeed, some of these associations are critical for normal host development and survival. Yet the role of many commensal (non-host-harming) strains remains unknown. I have begun to examine *Caenorhabditis elegans* and *C. briggsae* for gut-associated microbes. As established genetic model systems, these worms have numerous molecular-genetic tools available. Worms maintained in the laboratory lose their associated microbiota due to frequent bleaching. Thus, I am working with *Caenorhabditis* sampled from their natural environment, leveraging historical Félix lab collections.

First, I will characterize associated gut bacteria by 16S rDNA sequencing to assess microbial diversity. Second, I will isolate strains on appropriate culture media, then re-inoculate germ-free host worms with individual bacterial strains and monitor key features of host fitness under various environmental stresses to determine their effect on survival and fecundity. Third, I will focus on those bacterial strains showing the largest differences between infection and control strains, interrogating them under a wide variety of conditions, probing specificity and details of recognition, acquisition & maintenance using molecular genetic methods.

Host-gut bacterial associations may be particularly complex, with hosts striving to maintain their bacterial populations at optimal levels. In this case, the host has the difficult task of balancing inevitable exposure to bacteria consumed intentionally or inadvertently as a food source (necessary and likely beneficial to the host), against the likelihood of ingested bacteria profiting from the intestinal environment and becoming pathogenic at high loads. This study seeks begin documenting the role of associated gut microbiota in *Caenorhabditis*, establishing them as a genetic model system to study symbiosis.

938A. From locus to nucleotide to phenotype: mapping the genetic architecture of quantitative traits. **Luke M. Noble**, Matthew V. Rockman. Center for Genomics and Systems Biology, NYU, New York, NY.

Although a decade of intensive association and mapping studies has uncovered numerous quantitative trait loci (QTL) in natural populations, these are typically large in physical extent and small in magnitude of effect. The nature of sequence variation underlying quantitative traits is still largely unknown. A powerful approach to this problem is to map the heritable effects of variation on genome-wide gene expression. Such an approach can simultaneously address the genetic basis and evolution of gene regulation, as well as the architecture of quantitative traits. More than two thousand gene expression QTL (eQTL) have been mapped with a panel of recombinant inbred lines derived from Bristol (N2) and Hawaii (CB4856) founders, which are divergent in sequence and biology. Most detected eQTL reside at or near the transcript for which expression is altered - they are local eQTL - and are amenable to experimental analysis. Strong biases are evident in the location of local eQTL across chromosomes, and in the location of sequence variation across genes. We are in the process of identifying quantitative trait nucleotides (QTN) that generate expression differences between strains, after thorough assembly, annotation and comparative analysis of the Hawaii genome, and incorporation of functional and evolutionary information. Variant effects on gene expression - regulatory, coding, or non-coding - are assayed with fluorescent reporters and confocal microscopy. The presence and penetrance of phenotypic effects will be measured for confirmed QTNs throughout ontogeny and in response to environmental perturbation. In so doing we will gain an understanding of the stability of diverse genetic networks, and of the types and consequences of natural variation within species.

939B. C. elegans harbors pervasive cryptic genetic variation for embryogenesis. **Annalise B. Paaby**, Amelia White, Kris Gunsalus, Fabio Piano, Matt Rockman. Center for Genomics & Systems Biology, New York Univ, New York, NY.

To thoroughly explore the genetic architecture of early embryogenesis in C. elegans, we are searching for conditional relationships between embryonic genes. Genome-wide screens have identified genes that affect embryogenesis in a single wild-type background, providing a lot of information about the genetics underlying the process; we are leveraging this information to probe natural genetic variation across many wild C. elegans isolates. We have silenced a suite of 43 critical embryonic genes in 50 wild strains and characterized differences in lethality across strains. We observe pervasive "cryptic genetic variation" (CGV) for embryogenesis---that is, variation within the networks controlling early cell divisions that has functional consequences only under particular conditions. We find that disrupting genes responsible for polarizing the embryo in the first two cell divisions (PAR family members) uncover particularly high levels of CGV. Using association and linkage mapping, we find that very rarely are cryptic variants uncovered by different silenced genes, even among genes that interact closely and produce the same mutant phenotype. These results imply that CGV for embryogenesis has low pleiotropy, and likely point to new components in the embryogenesis gene network.

940C. Natural Variants of C. elegans demonstrate defects in both sperm function and oogenesis at elevated temperatures. **Lisa N. Petrella**[1,2], Thomas Buskuskie[1], Susan Strome[2]. 1) Department of Biological Sciences, Marquette Univ, Milwaukee, WI; 2) Department of MCD Biology, UC Santa Cruz, Santa Cruz, CA.

The temperature sensitivity of the germ line is conserved from nematodes to mammals. Previous studies in *C. briggsae* and *Drosophila* showed that strains originating from temperate latitudes lose fertility at a lower temperature than strains originating from tropical latitudes. Through analysis of 22 different wild-type isolates of *C. elegans* originating from both temperate and tropical locations, we are investigating mechanisms underlying maintenance of fertility at high temperature. We determined the fertility of temperate and tropical strains of *C. elegans* and observed no correlation between latitude of strain origin and fertility at high temperature. We also observed a wide distribution of population fertility among wild-type isolates at 27°C, ranging from 7% to 56%. To better understand the causes of high temperature sterility, temperature shift experiments were performed. Males up-shifted to high temperature as L4/young adults maintain fertility, while males raised at high temperature lose fertility. Sterile animals contain a wild-type-appearing germ line. Down-shifting males raised at high temperature does not restore fertility. This result differs from that observed in *Drosophila* and suggests that in *C. elegans* spermatogeneis is irreversibly impaired in males that develop at high temperature. Mating and down-shift experiments with hermaphrodites were performed to investigate the relative contributions of spermatogenic and oogenic defects to high temperature loss of fertility. We identified isolates that demonstrate predominantly spermatogenic defects, strains that show a mixture of spermatogenic and oogenic defects, and one strain that shows

predominantly oogenic defects. Our studies have uncovered unexpectedly high variation in both the loss of fertility and problems with sperm function in natural variants of *C. elegans* at high temperature. One variant provides a novel example of oogenic defects underlying loss of fertility. These variants can now be used to investigate the molecular mechanisms that underlie the buffering of fertility in the face of environmental change.

941A. The evolution of nematode operons. **Jonathan Pettitt**, Henrike Goth, Debjani Sarkar, Lucas Phillippe, Bernadette Connolly, Berndt Muller. School of Medical Sciences, Univ of Aberdeen, Institute of Medical Sciences, Aberdeen, United Kingdom.

Operons are a means of organising multiple independent coding regions such that they are transcribed into a single, polycistronic RNA. The presence of operons in an organism's genome is strongly correlated with the ability to carry out spliced leader (SL) trans-splicing, consistent with the hypothesis that the processing of polycistronic RNAs in eukaryotes is dependent upon SL trans-splicing.

Operons have been found in *C. elegans* and other nematodes that fall within the Chromodoria, one of the three major nematode clades. We have previously shown that the nematode, *Trichinella spiralis*, which lies in one of the other two main clades, the Dorylaimia, engages in SL trans-splicing, suggesting that it is a nematode-wide trait. An important question is whether the same is true of operons. We reasoned that if there is an intimate relationship between SL trans-splicing and polycistronic RNA processing, then we should also expect to be able to identify genes organised into operons in these nematodes.

We have previously identified a set of *T. spiralis* genes whose mRNAs undergo SL trans-splicing, and using this dataset, we have demonstrated the existence of operons in this nematode. We have confirmed that they produce polycistronic RNAs and that they are present in the closely related *Trichuris muris*. At least two of the operons are conserved between nematodes in the Dorylaimia and the Chromodoria clades, suggesting that these represent operons likely present in the ancestor of the nematode phylum. We find that mRNAs derived from downstream genes in operons are SL trans-spliced, just as is found for other nematode operons, but there is no equivalent to the specialised SL2 found in *C. elegans*. We are currently expanding upon our limited set of data to build a more comprehensive picture of SL trans-splicing and operon organisation in the Dorylaimia, and thereby gain a better understanding of the influence these processes have had on the evolutionary dynamics of the nematode genome.

942B. Elucidating the cellular and genetic basis of hybrid dysfunction between wild isolates of *Caenorhabditis briggsae*. **Kevin Pham**[1,2], Joseph Ross[1]. 1) Department of Biology, California State Univ, Fresno; 2) Professional Science Masters Program in Biotechnology, California State Univ, Fresno.

A major challenge to identifying the genes involved in the formation of new species is that once two populations are evidently different biological species, standard genetic approaches, such as mapping crosses, are not feasible. Thus, cases should be studied in which inter-population hybrids are viable and fertile, but less so than their parents; genetic loci involved in hybrid dysfunction phenotypes can then be mapped. One such case has been identified in the nematode Caenorhabditis briggsae, where genetic data suggest the possibility that favorable mitochondrial-nuclear coadaptations exist within populations and are disrupted in inter-population hybrids. To investigate the role of mitochondria in hybrid dysfunction, mitochondrial-nuclear hybrids have been produced, genotyped, and phenotyped. The genotypes reveal the possibility of male mitochondrial transmission, but otherwise confirm the expected genotypes of the experimental hybrids. A fitness phenotype, the number of self-progeny, also implicates a role of mitochondria in decreased hybrid fitness. Ultimately, C. briggsae mitochondrial-nuclear hybrids provide an opportunity to genetically map the loci involved in what might represent a naturally occurring case of incipient speciation.

943C. Environmental sensitivity and evolution of *Caenorhabditis* germline proliferation and differentiation. **Nausicaa Poullet**, Anne Vielle, Christian Braendle. Institut de Biologie Valrose, CNRS, INSERM, Univ of Nice Sophia-Antipolis, Nice, France.

We are interested in understanding how environmental variation modulates developmental processes and resulting phenotypic variation. We focus on the *C. elegans* germline, a molecularly well-tractable system that shows a high degree of environmental sensitivity. The basic germline organization is conserved among *Caenorhabditis* species, however reproductive schedules and fecundity are very variable between species and isolates. Yet, it remains unclear how evolution of such life history traits is explained by changes in germline properties, e.g. sperm number, oocyte quality or the sperm-oocyte switch. We aim to link environmental and evolutionary variability of the *Caenorhabditis* germline to better understand genotype-by-environment interactions for developmental traits underlying fitness-related characters. Our first aim was to characterize how germline proliferation and differentiation as well as fecundity are modified in animals exposed to experimental conditions mimicking ecologically relevant environments. Here we present how one specific environment -high temperature exposure (27°C)- affects germline and reproductive properties. In addition to previously reported deleterious effects on sperm function, we found that high temperature significantly impacts germline proliferation and differentiation, apparently through modifications of Distal Tip Cell morphology and signalling through the Delta/Notch pathway. Our second aim was to quantify genotype-by-environment interactions by analysing how isolates of the three hermaphroditic species (*C. elegans*, *C. briggsae* and *C*. sp. 11) differ in germline plasticity across different environments. We uncover significant genotype-by-environment interactions for germline and fecundity in response to high temperature. More specifically, we find that the thermal limits of reproduction are species-specific and may be associated with particular germline defects. We are now using this experimental paradigm to explore how environmental factors impact the *Caenorhabditis* reproductive system and how germline integrity is maintained in variable environments.

944A. Survey of *C. elegans* local polymorphism in an apple orchard by RAD-sequencing. **Aurélien Richaud**[1], Marine Stefanutti[2], Marie-Anne Félix[1]. 1) Institut de Biologie de l'ENS, IBENS, 46 rue d'Ulm, 75005 Paris, France; 2) Institut Jacques Monod, 15 rue Hélène Brion, 75205 Paris cedex 13, France.

Despite increased sampling of *Caenorhabditis* species in rotting vegetal substrates in the last years, we still lack ecological and evolutionary data to understand what drives *Caenorhabditis* distribution and diversity at a local scale. We thus sampled during 4 years a spatio-temporal set of rotting apples in an orchard in Orsay (France). We observed that *C. elegans* and *C. briggsae* were found every year and may occur in the same apples. However their temporal distributions differ: while *C. briggsae* is found mainly during summer, *C. elegans* is found in late fall. This temporal occurrence coincides with their temperature preference in the lab (Félix & Duveau, BMC Biol. 2012). In order to study the genetic structure of *C. elegans* at a local scale, we extracted genomic DNA from over 600 wild *C. elegans* "families" (progeny of a wild-caught worm) from the Orsay orchard. Preliminary results using phenotypically

relevant polymorphisms indicate that genetic diversity can be found in the orchard, including within a given apple. For example, a polymorphism in *drh-1*, a gene involved in the defense against the Orsay virus (see abstract by Le Pen et al.) could be detected in the orchard. 87.5 % of sampled animals display the deleted allele. Both genotypes could sometimes be found in the same apple. Polymorphisms in *plg-1* and *tra-3* were also found, but not (so far) at the *zeel-1 peel-1* incompatibility locus. In order to study the genetic structure more systematically, we initiated a RAD-sequencing approach, a method that detects Single Nucleotide Polymorphisms in an unbiased manner by partial Illumina sequencing next to restriction enzyme cutting sites. A first assay with the SbfI enzyme revealed an insufficient level of polymorphism. Therefore, we decided to shift to the 2b-RAD sequencing approach, an easier and less expensive variant method, that makes use of type IIb restriction endonucleases like BcgI (Wang et al. Nat. Meth. 2012). This approach will allow us to study different population genetic parameters such as genetic diversity, outcrossing rate, spatial distribution, migration, etc.

945B. The rate and spectrum of spontaneous mutations in experimental populations of the nematode *Caenorhabditis remanei*. **Matthew P. Salomon**[1], Chikako Matsuba[2], Dejerianne G. Ostrow[3], Charles F. Baer[2]. 1) Molecular and Computational Biology, Univ of Southern California, Los Angeles, CA; 2) Department of Biology, Univ of Florida, Gainesville FL; 3) Molecular Pathology, Genomics Core, Children's Hospital Los Angeles, Los Angeles CA.

To what degree natural selection has shaped the rate of spontaneous mutations among different taxa remains an important unresolved question in evolutionary biology. While mutation rates are known to vary among and within taxa, the relative importance of natural selection versus non-adaptive processes has yet to be determined. Classical theory predicts that the strength of natural selection to reduce the deleterious mutation rate should be stronger in asexual and selfing taxa than in outcrossing sexual taxa, leading to an adaptive decrease in mutation rate in the former. However, other theory predicts (1) that "mutator" alleles can hitchhike to high frequency in asexual/selfing taxa, thereby leading to a (non-adaptive) increase in the mutation rate, and (2) the efficiency of selection to reduce mutation rate may be substantially greater in outcrossing than asexual or selfing taxa due to the larger effective population sizes of the former. Whether general trends in exist in nature is currently unknown. Nematodes in the genus *Caenorhabditis* provide an ideal system to test questions of how mutation rates vary among closely related species with different reproductive strategies. Within the genus the ancestral reproductive state within is outcrossing (gonochorism), however self-fertilization (hermaphroditism) has evolved independently several times. To examine the role of matting system on the evolution of mutation rates we constructed a set of long-term mutation accumulation (MA) lines of the outcrossing species *Caenorhabditis remanei*. MA lines were maintained by transferring a single male-female pair of worm per generation. After 122 generations of MA, five randomly selected MA lines along with the ancestral control were re-sequenced using Illumina sequencing technology.

946C. Evolution of Nematode Spliced Leader *trans*-splicing. **Debjani Sarkar**, Berndt Müller, Bernadette Connolly, Jonathan Pettitt. Institute of Medical Sciences, Univ of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, United Kingdom.

Spliced-leader (SL) *trans*-splicing is a pre-mRNA maturation event that has a pivotal role in processing polycistronic RNA transcripts from operons into mature monocistronic mRNAs. It is known to occur in multiple, widely distributed eukaryotic groups, including nematodes. Most of our knowledge on SL *trans*-splicing in nematodes has come from investigations in *Caenorhabditis elegans* and other nematodes from the Chromadorean clades. The identification of SL *trans*-splicing in nematodes that lie outside of the Chromadorea has led us to conclude that SL *trans*-splicing is likely to be a phylum-wide process and thus a trait found in the last common ancestor of the nematodes. In this project we investigate the nature of SL *trans*-splicing in nematodes outside of the Chromadorean clades. Previous studies have shown that the Dorylaimid *Trichinella spiralis* uses a range of highly polymorphic SL sequences that have only limited similarity to *C. elegans* SL1 and SL2 (Pettitt *et al*, 2008). In contrast, initial searches for SLs in *Prionchulus punctatus* have shown that it possesses clear SL2-like sequences (Harrison *et al*, 2010). In this study we carried out searches for putative SL sequences in *Trichuris muris* to address differences between SL sequences in *T. spiralis* and *P. punctatus*. Searches indicate that SLs in *T. muris* are similar to the SLs found in *P. punctatus* and *C. elegans*, which is unexpected given that *T. muris* shares a "recent" common ancestor with *T. spiralis*. This in turn highlights that the lineage leading to *T. spiralis* is derived in relation to SL *trans*-splicing. Our results provide us with a valuable insight into the likely nature of SL *trans*-splicing in the ancestral nematode and how it has evolved within the nematode phylum.

947A. Embryogenesis of nematodes: traveling through transcriptomes. Christopher Kraus, **Einhard Schierenberg**. Zoological Institute, Biocenter, Univ of Cologne, D-50674 Köln, Germany.

Although it is known for most developmental stages in C. elegans which molecular pathways are involved in the specification of certain cell fates, the knowledge of such pathways remains largely elusive in other nematodes. Hence, we have started to analyze gene expression patterns during embryonic stages in selected representatives of nematodes by employing Illumina RNAseq. Using *C. elegans* (clade 9*) as a reference, our study includes its close relative *Diploscapter coronatus* (clade 9) and other more derived nematodes such as *Panagrolaimus sp.* PS1159 (clade 10), as well as the basal representative *Romanomermis culicivorax* (clade 2). In addition, we also included the nematomorph *Gordius* as closest phylogenetic neighbor of nematodes. For each of these species we have collected selected embryonic stages, ranging from the zygote to morphogenesis stages for transcriptome analysis. We want to gather information about expression patterns during key developmental stages like (1) conserved molecular signaling pathways, (2) maternally inherited determinants, (3) time point of shift from maternal to zygotic expression of selected genes, (4) stage-specific expression of so far undescribed genes. Our studies should help to better understand how changes in embryonic gene expression resulted in the establishment of developmental variants during nematode evolution. * clades after Holterman et al., (2006) Mol. Biol. Evol. 23, 1792.

948B. Major changes in the core developmental pathways of nematodes: *Romanomermis culicivorax* reveals the derived status of the *Caenorhabditis elegans* model. Philipp Schiffer[1], Michael Kroiher[1], Christopher Kraus[1], Georgios Koutsovoulos[2], Sujai Kumar[2], Julia Camps[1], Ndifon Nsah[1], Dominik Stappert[3], Krystalynne Morris[4], Peter Heger[5], Janine Altmüller[6], Peter Frommolt[6], Peter Nürnberg[6], Kelley Thomas[4], Mark Blaxter[2], **Einhard Schierenberg**[1]. 1) Zoological Institute, Universität zu Köln, Cologne, Germany; 2) Institute of Evolutionary Biology, School of Biological Sciences, Univ of Edinburgh, Edinburgh, Scotland, UK; 3) Institut für Entwicklungsbiologie, Universität zu Köln, Cologne, Germany; 4) Hubbard Center for Genome Studies, Univ of New Hampshire, Durham, NH, USA; 5) Insitut für Genetik, Universität zu Köln, Germany; 6) Cologne Center for Genomics, Universität zu Köln, Cologne, Germany.

Nematodes can be subdivided into the more basal Enoplea (including *Romanomermis culicivorax*) and more derived Chromadorea (including *C. elegans*). Compared to *C. elegans*, Enoplea show a markedly different pattern of early cell division and cell fate assignment but the genetic control of development has not been explored. We generated a draft genome of *R. culicivorax* and compared its gene content with that of *Trichinella spiralis* (another Enoplea), *C. elegans* and as an outgroup the beetle *Tribolium castaneum*. This comparison indicates that *R. culicivorax* possesses components of a conserved metazoan developmental toolkit lost in *C. elegans*. In the latter many genes essential for embryogenesis appear to be restricted to its close phylogenetic neighbors or have only extremely divergent homologs in the studied Enoplea. Our data indicate prominent differences in the genetic programs for early cell specification, but also other aspects like vulva formation and sex determination. We conclude that major variations in the molecular control of development evolved within Nematoda probably due to developmental system drift. At least some of the idiosyncracies of *R. culicivorax* embryogenesis can be correlated with our molecular findings, e.g. the role of par genes. Thus, *R. culicivorax* could serve as a contrasting research model to better understand how a conserved phenotype can be generated from a divergent genomic basis.

949C. Assortative Fertilization in the Elegans-Group of *Caenorhabditis*. **Sara R. Seibert**, Blaine E. Bittorf, Scott E. Baird. Biological Science, Wright State Univ, Dayton, OH.

Assortative fertilization refers to species-specific interactions between sperm and oocytes. One type of interaction is sperm chemotaxis. In *Caenorhabditis* fertilization is internal and amoeboid sperm must crawl along the uterine lining to the spermathecae in response to prostaglandin signals. In this project the impact of species-specific chemotaxis on assortative fertilization was assessed. **Methods:** Crosses were performed between *C. remanei* males and *C. species 9*, *C. briggsae* and *C. sp. 9: C.b.* F1 'females'. Females and CMXRos-labeled males were mated overnight. Mated females were scored for cross-fertilization. Labeled sperm were scored for their degree of localization at the spermathecae. Chemotaxis was considered strong if all sperm were localized to the spermathecae, weak if some but not all sperm were localized and absent if sperm were randomly distributed within the uterus. **Results:** Hybrid progeny were obtained from 64% of *C. b.* AF16 and RE771 [*she-1(v51)*] 'females', 71% and 14% of *C. sp. 9* EG5268 and JU1422 females, respectively, *C. b.: C.sp. 9* 96% EG5268 and 63% JU1422 and *C. sp. 9: C. b.* 80% EG5268 and 26% JU1422 female F1 hybrids. An average of 9 fertilized eggs was obtained from *C. b.* AF16 and RE771, 2.81 *C. sp. 9* EG5268 and 0.78 JU1422, *C. b.: C. sp. 9* 21.1 EG5268 and 11.5 JU1422 and *C. sp. 9: C. b.* 2.68 EG5268 and 0.93 JU1422 female F1 hybrids. Strong localization of sperm to the spermathecae were obtained from 67% of *C. b.* AF16 and RE771 'females', 39% and 25% of *C. sp. 9* EG5268 and JU1422 females, respectively, *C. b.: C. sp. 9* 80% EG5268 and 86% JU1422 and *C. sp. 9: C. b.* 92% EG5268 and 80% JU1422 female F1 hybrids. **Conclusions:** Generally, sperm chemotaxis does exhibit a correlation with cross-fertility. Sperm chemotaxis defects are revealed in the varying degrees of sperm localization in hetero-specific crosses. These rates of chemotaxis appear to be higher than rates of cross-fertility in *C. remanei: C. species 9* crosses. Therefore, sperm-egg fusion, another type of assortative fertilization interaction, appears to also impact fertilization between species. Defects in cell-surface protein interactions may justify lower rates of cross-fertility despite the presence of sperm.

950A. Complex regulation of *C. briggsae tra-1*. **Yongquan Shen**, Yiqing Guo, Ronald E Ellis. Molecular Biology, UMDNJ-SOM, Stratford, NJ.

The transcription factor *tra-1* is the sole nematode homolog of the Gli proteins, and is a key regulator of sexual identity from *Caenorhabditis* to *Pristionchus*. In *C. elegans*, *tra-1(lf)* mutations are recessive and cause *XX* animals to develop as males, whereas *gf* mutations are dominant and cause *XO* animals to become females. Understanding how *C. briggsae tra-1* is regulated is critical for elucidating its sex-determination pathway. However, the large size and germline functions of *tra-1* make transgenic analyses difficult. Thus, we are using TALENs to make specific *Cbr-tra-1* mutations.

First, *C. briggsae* and its close relatives make two *tra-1* transcripts that differ only at the 5'-end. The *v181* mutation creates a frameshift in an early exon that eliminates both TRA-1A and TRA-1B, so it defines the null phenotype. As in *C. elegans,* the *XX* and *XO* mutants develop as males, but produce oocytes late in life. By contrast, the *v182* mutation only eliminates TRA-1A. Since it has a similar phenotype, we conclude that *tra-1a* plays the predominant role in sex determination. Second, *C. briggsae* has a small open reading frame in its 5'-UTR that is conserved in *C. elegans*. The *v247* mutation bypasses a stop codon in this open reading frame, causing dominant feminization of the germ line and partial masculinization of the soma. We infer that this ORF regulates TRA-1 activity. Third, *C. elegans* TRA-1 is cleaved to produce a repressor that shuts down male genes. We isolated *v197*, a frameshift near the cleavage site that removes the C-terminus. Surprisingly, it causes *XX* animals to make a male gonad within a hermaphrodite body. However, the nonsense and frameshift mutations *v46*, *v56*, *nm2* and *nm10* from this half of the protein produce a range of phenotypes. Because they might differ in sensitivity to the mRNA surveillance system, we made *Cbr-smg-5* mutants, and are using them to analyze these *tra-1* mutant phenotypes. Comparing the phenotypes caused by proteins truncated at different points could help us map specific TRA-1 activities.

951B. Inferring the order of mutational changes responsible for mechanistic divergence of a functionally constrained promoter. **Mohammad Siddiq**, Antoine Barriere, Ilya Ruvinsky. Ecology & Evolution, Univ of Chicago, Chicago, IL.

Genetic systems diverge during evolution. A well-known consequence of this is inviability and infertility of hybrids from closely related and phenotypically similar species. Among possible causes of this phenomenon is the divergence of gene regulatory mechanisms that is compensated within each lineage, but because this happens in lineage-specific ways, incompatibility is revealed in hybrids. Previously we showed that while the endogenous expression patterns of *unc-47* are conserved between *Caenorhabditis elegans* and *C. briggsae*, the mechanisms controlling this expression have diverged. As a result, a transgene containing *C. briggsae* promoter is expressed incorrectly when in *C. elegans*. We are seeking to understand how this incompatibility arose, even though evidence suggests that the pattern of unc-47 expression remained constant during *Caenorhabditis* evolution. We inferred the order of substitutions in the promoter of unc-47 by analyzing promoter sequences from the monophyletic group of species containing *C. elegans* and *C. briggsae*. By systematically characterizing their expression patterns in *C. elegans*, we precisely mapped the origin of the functional divergence on the *Caenorhabditis* phylogeny. We are currently interrogating the individual and collective effects of single nucleotide substitutions, insertions, and deletions in different trans-regulatory backgrounds. This work provides a detailed mechanistic explanation of how a trait can diverge while being maintained by stabilizing selection.

952C. Gene-environment interactions drive genomic and transcriptomic diversity in wild *Caenorhabditis elegans* populations. R. J. M. Volkers[1], **L. B. Snoek[1]**, C. J. van Hellenberg hubar[1], R. Coopman[2], W. Chen[3], M. G. Sterken[1], H. Schulenburg[3], B. P. Braekman[2], J. E. Kammenga[1]. 1) Laboratory of Nematology, Wageningen Univ, The Netherlands; 2) Department of Biology, Ghent Univ, Belgium; 3) Department of Evolutionary Ecology and Genetics, Zoological Institute, Christian Albrechts-Universitaet zu Kiel, Germany.

RJMV and LBS contributed equally to this work. **Background:** Analyzing and understanding the relationship between genotypes and phenotypes is at the heart of genetics. Research in the nematode *C. elegans* has been instrumental for unravelling many genotype-phenotype relations. But almost all studies, including forward and reverse genetic screens, are dominated by investigations in one canonical single strain. In order to explore the full potential of the natural genetic variation and evolutionary context of the genotype-phenotype map, it is important to study these relations in wild populations. **Results:** We used multiple wild strains freshly isolated from local habitats to investigate the gene sequence polymorphisms and a multitude of phenotypes including the transcriptome, fitness and behavioural traits. These showed a direct link with the original habitat of the strains. The separation between the isolation sites was prevalent on all chromosomes, chr. V contributing the most. These results were supported by a differential food preference of the wild isolates for naturally co-existing bacterial species. Moreover, we show that genomic and transcriptomic diversity was driven by genes involved in gene-environment interactions, like c-type lectins and fbox genes. **Conclusions:** Importantly, where wild *C. elegans* strains show a broad range of genotype-phenotype relations the widely studied canonical genotype N2 covers only a diminutive part of the myriad of genotype-phenotype relations which are present in the wild. **Funding:** RJMV and LBS: NWO-ALW (project 855.01.151) NEMADAPT, RC and BPB: ESF-EEFG (09-EuroEEFG-FP-002 / G.0998.10N) NEMADAPT. JEK: ERASysbio-plus ZonMW project GRAPPLE (project nr. 90201066). MGS: Graduate School Production Ecology & Resource Conservation. WC and HS: NEMADAPT (DFG grant SCHU 1415/11-1).

953A. From *C. elegans* to parasitic nematodes: *Strongyloides* spp. and *Onchocerca* spp. **Adrian Streit**. Dept Evolutionary Biol, MPI Dev Biol, Tuebingen, Germany.

Compared to what we know about *C. elegans*, our understanding of the biology and pathogenicity of its parasitic relatives is highly limited. In my lab we attempt to apply some of the knowledge and methodology worked out in *C. elegans* for the investigation of two very different groups of nematode parasites. A group of species comprising the parasitic genus *Strongyloides* (among them the human parasite *S. stercoralis*) and the facultative parasitic sister genus *Parastrongyloides* represent an attractive model case to study the basic biology and the evolution of parasitic life styles. Members of both genera can be maintained in the laboratory relatively easily. They have short life cycles and, in addition to parasitic adults, they can also form free-living, sexually reproducing males and females. This makes them amenable to genetic analysis. We studied the inheritance of SNP markers to characterize the modes of reproduction and sex determination as well as the evolution of these traits. Furthermore we isolated the first developmental mutations, which influence the switch between the parasitic and the free-living life cycles. Filarial nematodes of the genus *Onchocerca* parasitize mainly even-toed ungulates as main hosts. One of the few exceptions is *O. volvulus*, which causes river blindness in humans. This species has a very close relative in cattle, *O. ochengi*, with which it also shares the intermediate host, the black fly *Simulium damnosum s.l.* It has been hypothesized that *O. volvulus* arose through a host switch from cattle to their keepers only about 10 000 years ago. The approximately one-year generation time makes these worms unattractive for true genetic analysis. We used SNP markers to characterize reproductive strategies and population genetic parameters in *O. ochengi* and compare them to *O. volvulus.* In both projects my lab collaborates closely with human and veterinary field parasitologists who work in regions where our parasites of interest are common. We undertake molecular phylogenetic studies to address the question of to what extent animals serve as a reservoir for human parasites, both in the short term and on evolutionary timescales.

954B. Genetic and developmental mechanisms underlying sperm size variation in Caenorhabditis nematodes. **Anne Vielle**, Nuno Soares, Nicolas Callemeyn-Torre, Nausicaa Poullet, Christian Braendle. Institut de Biologie Valrose, CNRS, INSERM, Univ Nice Sophia-Antipolis, Nice, France.

Sperm competition is a major evolutionary force driving diversity in sperm morphology. Such sperm competition exists in *C. elegans* where larger male sperm consistently outcompetes hermaphrodite sperm, and larger male sperm outcompetes smaller male sperm. Variation in male sperm size among different wild isolates of *C. elegans* may thus have evolved in response to different degrees of sperm competition. To characterize natural genetic variation in *C. elegans* male sperm size, we have quantified this phenotype in a collection of 100 genetically-divergent *C. elegans* wild isolates. This analysis revealed considerable variation in male sperm size, however, a Genome-Wide Association Mapping Study did not allow detection of potential genomic regions underlying this variation. Surprisingly, however, this survey revealed significant differences in male sperm size of different strains derived from the N2 reference strain, pinpointing potential candidate genes involved in sperm size regulation. We are currently exploring how specific candidate genes affect spermatogenesis and sperm maturation, to ultimately define molecular events controlling sperm size variation. In addition, we will present data on the co-evolution of male and hermaphrodite sperm in *C. elegans*, *C. briggsae* and *C. sp.* 11; and we further discuss interspecific evolution of sperm size in gonochoristic *Caenorhabditis* species, which have been shown to exhibit a significantly larger mean sperm size than males of androdioecious species. We have extended these comparative analyses by integrating newly discovered species in the context of a recently established molecular phylogeny. This analysis indicates rapid evolution of sperm size and the repeated, independent evolution of exceptionally large sperm.

955C. Testing for non-Mendelian assortment of chromosomes in *Caenorhabditis*. Son Tho Le, Chia-Yi Kao, **John Wang**. Biodiversity Research Center, Academia Sinica, Taipei, Taiwan.

Eukaryotic genome sizes range over 10,000-fold with a correlation between larger genomes and greater organismal size and complexity. In part, population genetic principles can explain this observation. Smaller organisms typically have larger population sizes which are more efficient at purging weakly deleterious mutations such as transposons and other insertions resulting in smaller genome sizes; larger organisms are the opposite. In contrast to the population genetic predictions, the reverse is observed for the species with known genome sizes within the Elegans group of the *Caenorhabditis* genus. Gonochoristic (male-female) species have larger genomes than hermaphroditic species despite the former predicted to have larger populations sizes than the latter. Why is this? Interestingly, Mendel's law of random chromosome assortment is violated in *C. elegans* males that are heterozygous for autosomal chromosomes of differing sizes whereby sons inherit the longer chromosome while the hermaphrodite daughters inherit the shorter chromosome, in a

phenomenon which we call skew. Because a single hermaphrodite can start a new population, skew could explain how genomes of hermaphroditic species evolve to be smaller than the ancestral gonochoristic species. For this to be true, skew would be predicted to be a general property of the *Caenorhabditis* species. To this end, we are testing for the presence of skew in other *Caenorhabditis* species. We are also interested in understanding the mechanisms underlying skew and have initiated a forward genetic screen for genes that suppress skew.

956A. Using TALENs to create new genetic model systems. **Qing Wei**[1], Yongquan Shen[2], Xiangmei Chen[1], Yelena Shifman[2], Ronald E Ellis[2]. 1) GSBS, UMDNJ-SOM, Stratford, NJ; 2) Molecular Biology, UMDNJ-SOM, Stratford, NJ.

With the advent of genome sequencing and RNA interference, there has been an explosion in the number of model organisms used for evolutionary research. However, most studies are hindered by their exclusive reliance on reverse genetic approaches. The ability to carry out sophisticated forward genetic screens would allow the unbiased detection of new genes in these species, and greatly increase their usefulness. Unfortunately, the sets of genetic markers, balancing chromosomes and tools that make research so rapid with *Drosophila melanogaster* or *Caenorhabditis elegans* took decades to develop. For example, although *C. briggsae* has been studied for almost 20 years, so far random screens have produced useful sets of marker genes for only four of its six chromosomes.

Since TALENs can induce mutations in any gene, we thought they might dramatically speed up this process. For this approach to work, several assumptions had to be tested. First, how frequently do mutations in a *C. briggsae* gene give a phenotype similar to that of *C. elegans*? Second, can the position of nonsense mutations in one species help in the design of target sites in another? Third, can incomplete genome sequences and SNP maps be used in conjunction with synteny to identify good marker genes?

In just a few months, we have created morphological mutants on the remaining *C. briggsae* chromosomes. Most have useful phenotypes that resemble those of *C. elegans* nonsense mutants, such as *unc-40* and *dpy-5* on LGI, *unc-34* and *unc-51* on LG*V* or *unc-1* on *X*. However, some have more severe phenotypes; for example, *C. briggsae unc-54* and *dpy-11* mutants are barely viable, whereas the corresponding *C. elegans* mutants are healthier. We did isolate an inframe deletion of *dpy-11* and missense mutation of *unc-54* for use as markers. We also created *him-8* mutants that produce numerous males by non-disjunction, and *smg-5* mutants that eliminate the nonsense-mediated decay system. Thus, TALENs can be used to adapt model organisms for genetic research.

957B. BAR-1/beta-catenin regulates expression of a subset of collagen genes that are necessary for normal adult cuticle integrity. B. Jackson, **P. Abete Luzi**, D. Eisenmann. Univ of Maryland Baltimore County, Baltimore, MA.

The highly conserved Wnt signaling pathway functions in metazoan development and homeostasis to regulate, cell polarity, cell migration,cell fate specification as well as adult stem cell maintenance and differentiation, and misregulation of this pathway is a major contributor to colon and other cancers. In C. elegans, the Wnt/BAR-1 canonical (WBC) pathway shares homology with the b-catenin-dependent or 'canonical' Wnt signaling pathway in vertebrates and Drosophila. Our lab and others have shown that the WBC pathway functions in cell fate specification and cell migration in several processes during larval life. Although the components and function of the C. elegans WBC pathway are well-studied, the downstream target genes that are effectors of the activated Wnt pathway are mostly unknown. To identify Wnt pathway targets in the worm, we previously screened for differential expression of transcripts in animals in which the Wnt signal was overactivated by the induction of a stable BAR-1/beta-catenin. Microarray analysis identified 108 genes that were upregulated in response to activated BAR-1, of which 39 were validated by qRT-PCR. Five genes, the collagen-encoding genes col-38, col-49, col-71 and bli-1, and the unknown gene dao-4, are co-expressed in hypodermal cells in the mid L4 stage. Expression of these genes is reduced 2 - 10 fold in a bar-1(ga80) loss-of-function mutant, and increased in a loss-of-function mutant for pry-1, which encodes Axin, a negative regulator of the Wnt pathway. Reduction of function for these genes by RNAi leads to a variety of weak morphological phenotypes in adults suggestive of cuticle or hypodermal defects, and these RNAi animals display hypersensitivity in a Hoechst nuclear staining assay, suggesting that they have defects in adult cuticle integrity. Furthermore, bar-1(ga80) mutants display this same Hoechst staining hypersensitivity. Together these results suggest that Wnt signaling through BAR-1/beta-catenin is required in the L4 stage for expression of a subset of collagen and other genes that are necessary for the proper synthesis and function of the adult cuticle.

958C. Long non-coding RNAs have conserved developmental gene expression patterns. **Gal Avital**, Michal Levin, Itai Yanai. Faculty of Biology, Technion - Israel Institute of Technology,Haifa, Israel.

Myriad RNA classes play central roles in cellular processes including catalytic, structural and regulatory functions. One such class is the recently discovered long non-coding RNAs (lincRNAs) that are spliced and polyadenylated but for which our understanding of their non-coding nature is sparse. We hypothesized that, as in the expression of coding-genes, conservation of lincRNA expression across species may be an efficient approach for their functional analysis. We thus examined lincRNAs conservation of sequence across diverse genomes and of gene expression throughout embryonic development. We collected an embryonic timecourse at 10 minute intervals throughout the first half of both *C. elegans* and *C. japonica* embryology using our CEL-Seq method. For the 170 annotated *C. elegans* lincRNAs we found that more than half are expressed throughout the first half of embryogenesis. We next focused on *linc-5* which begins to be highly expressed one hour before embryonic ventral enclosure in *C. elegans*. Searching for the *C. japonica* ortholog, we found that *linc-5* is conserved throughout the *Caenorhabditis* genus, and resides in a conserved syntenic cluster in *C. japonica*. Strikingly, the expression profile of *linc-5* is highly similar in *C. japonica* implicating its possible function during embryonic development. Our results suggest that expression conservation may constitute a useful approach for the delineation of functional lincRNA orthology.

959A. Pervasive cis-regulatory divergence despite functional conservation in Caenorhabditis nematodes. **Antoine Barriere**, Ilya Ruvinsky. Dept Ecology & Evolution, Univ of Chicago, Chicago, IL.

Due to the diffuse nature of functional encoding within regulatory elements, conservation of primary sequence in these regions cannot be used as a proxy for conservation of function. Instead, the functions of *cis*-regulatory sequences have to be assessed *in vivo*. We conducted a survey of *cis*-regulatory conservation and change between *C. elegans* and closely related species *C. briggsae, C. remanei, C. brenneri*, and an outgroup *C. japonica*. We tested

functional conservation of *cis*-regulatory elements from those species by introducing them into *C. elegans* and analyzing the expression patterns they drove. We tested the promoters of eight genes expressed in different neuronal types and muscles, some of them coexpressed and coregulated: *acr-14, gpa-5, kat-1, mod-5, oig-1, unc-25, unc-46, unc-47*. These *cis* elements were chosen based on expression patterns and the degree of primary sequence conservation. Our results support several significant conclusions. Most exogenous promoters drove expression in the same cells as the *C. elegans* orthologs, indicating gross conservation of regulatory mechanisms. We saw no correlation between the extent of primary sequence conservation and conservation of function. However, *cis*-regulatory elements from the more distant species *C. japonica* showed less functional conservation. The majority of exogenous promoters when placed in *C. elegans* drove expression in other cells in addition to the endogenous pattern, revealing pervasive functional divergence of *cis*-regulatory elements. For several genes, this divergence appears to be compensated by changes in *trans*, as evidenced by conservation of the expression pattern in their endogenous environment. Different promoters from different species drove ectopic expression in the same *C. elegans* cells. We think this reflects biases in the directions in which expression patterns can evolve due to shared regulatory logic of coexpressed genes.

960B. Multiple HRG-1 paralogs regulate heme homeostasis in *C. elegans*. **Haifa B Bensaidan**, Iqbal Hamza. Animal and Avian Science, Univ of Maryland,college Park, MD.

Heme is an essential cofactor for various biological processes. Although the synthesis and degradation of heme have been extensively studied, the mechanisms responsible for inter- and intra- cellular heme transport remain poorly understood. By utilizing *Caenorhabditis elegans*, we identified HRG-1 (Heme Responsive Gene-1) as the first eukaryotic heme importer/transporter. *C. elegans* has three additional putative paralogs of *hrg-1*, which we termed *hrg-4*, *hrg-5*, and *hrg-6*. Our hypothesis is that multiple HRG-1 paralogs function together to transport heme into the intestine of *C. elegans*. Although both *hrg-1* and *hrg-4* are highly regulated by heme, microarray and qRT PCR analysis indicate that *hrg-5* and *hrg-6* are not heme responsive. In addition, *Dhrg-1Dhrg-4* double mutant worms show only a mild growth delay in low heme conditions implying that the worm is still capable of importing sufficient heme. Functional assays in *S. cerevisiae* reveal that HRG-6 enhances the heme-dependent growth of the *hem1D* strain and is therefore *bona fide* heme transporter. Using HRG-6::GFP transgenic worms, we show that HRG-6 is expressed at all heme concentrations and localizes to the apical plasma membrane of the intestine, spermathecal uterine valve, and vulval muscle. Interestingly, HRG-6::GFP transgenic worms reveal a growth defect at low heme concentrations, which is fully rescued with the addition of heme. This growth arrest phenotype can be rescued by knocking down *hrg-6*, *gfp*, and *hrg-4*. RNAi-mediated depletion of *hrg-1* paralogs in HRG-6::GFP transgenic worms reveal that knocking down of *hrg-4* results in attenuation of intestinal HRG-6::GFP expression. Together, our results suggest that HRG-6 functions in concert with HRG-4 to ensure heme uptake in worms, and its function is possibly regulated by HRG-4 under low heme conditions.

961C. Antibiotics can modulate the immune system to enhance survival of Caenorhabditis elegans during Yersinia pestis infection. **Yun Cai**[1,2], Alejandro Aballay[1]. 1) Molecular Genetics and Microbiology, Duke Univ Medical Center, Durham, NC; 2) Department of Clinical Pharmacology, the PLA General Hospital, Beijing, China.

Caenorhabditis elegans has been intensely used as a desirable and efficient model for different genetic and chemical screens. To determine drugs that have a beneficial effect on the immune system, we studied the effect of diverse chemical compounds on the expression of gene F35E12.5, which is a reporter of the activation of the p38/PMK-1 signaling pathway. Using strain AY101, in which a green fluorescence protein reporter gene was fused to the promoter of gene F35E12.5, we identified 45 compounds which can strongly up-regulate F35E12.5. Sixteen of them are antimicrobial agents, including Quinolones, Azole antifungal agents, Tetracyclines, and Polymyxins. We found that animals treated with antibiotics were more resistant to Y. pestis compared to the untreated ones. In addition, treated animals were also more resistant to control animals when they were infected with antibiotic resistant Y. pestis, indicating that the resistance to infection was not only due to the bactericidal effect of the drug. Our studies identified a new mechanism by which certain antibiotics confer protection to bacterial infections.

962A. An RNA-Seq based, longitudinal study of root-knot nematode parasitism. **Soyeon Cha**[1,2], Peter DiGennaro[1], Dahlia Nielsen[2], David Mck. Bird[1,2]. 1) Department of Plant Pathology, NC State Univ, Raleigh NC, 27695; 2) Bioinformatics Research Center, NC State Univ, Raleigh NC, 27695.

Root-knot nematode is an obligate parasite of virtually all plant species and causes severe crop loss world-wide. We are interested in studying transcriptional profiles of the developmental changes throughout the nematode lifecycle and the host response. We infected the model legume *Medicago truncatula* with *Meloidogyne hapla* L2 and harvested tissue over a time course. Tissue was collected from uninfected and infected shoots and roots (t = 1, 2, 4, 5, 7 days post inoculation) as well as *M. hapla* eggs and pre-penetration L2. Four biological replicates were collected for each time point. RNA-Seq data was generated from each of the 22 samples yielding 1.03 X 10^9 high quality Illumina reads. Because the complete genome sequences are available for *M. truncatula* and *M. hapla*, the reads could be deconvoluted by mapping to their respective genome. A total of 4.4 X 10^8 reads mapped to *M. truncatula* (86.5%) and 6.8 X 10^7 mapped to *M. hapla* (13.5%). Analysis of these large data sets is in progress, with an emphasis on identifying pathways in both host and parasite, including dauer exit, muscle atrophy, sex determination and reproduction.

963B. Function of APL-1, a protein related to human APP, which has been implicated in Alzheimer's Disease. **Shah Nawaz Chaudhary**[1], Adanna Alexander[1,2], Christine Li[1,2]. 1) Biology Dept, City College of New York, CUNY, New York, NY; 2) The Graduate Center, CUNY, New York, New York, NY.

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by the presence of intracellular tangles and extracellular plaques in the brain of patients. A major component of the plaques is the Ab peptide, a cleavage product of the amyloid precursor protein (APP). Mutations in APP have been linked to familial Alzheimer's Disease. Mammals have three APP-related proteins that are functionally redundant, making it difficult to study the role of APP. *C. elegans* contains only one APP-related gene, *apl-1*. Deleting *apl-1* results in L1 lethality, which can be rescued by expressing the extracellular domain of APL-1 (APL-1EXT). Furthermore, homozygous *apl-1(yn5)* mutants, which produce only APL-1EXT, are viable, indicating the functional significance of APL-1EXT. Knockdown of *apl-1* in wild-type animals by RNAi feeding does not appear to affect development. By contrast, knockdown of *apl-1* in *apl-1(yn5)* mutants results in F2 lethality. To identify genes that suppress the *apl-1(yn5)* lethality, we mutagenized *apl-1(yn5)* animals and screened for viable F2 *apl-1(RNAi)* progeny. Six suppressors have been identified and are being deep sequenced to determine their molecular lesions. We performed pull-

down assays with a tagged APL-1EXT construct. The adenine nucleotide transporter ANT-1.1 was identified as an interacting protein. ANT-1.1 mediates the exchange of ADP/ATP between the cytosol and mitochondria and is also implicated in apoptosis and axon guidance. We performed a yeast-two hybrid assay and found a weak interaction between APL-1 and ANT-1.1. Genetic knockouts of *ant-1.1* are not viable but knockdown of *ant-1.1* by RNAi produced viable progeny. However, *ant-1.1* knockdown in *apl-1(yn5)* mutants caused lethality in F2 progeny, suggesting an interaction between ANT-1.1 and APL-1EXT. To further investigate these two proteins, we are comparing expression patterns *in vivo*, by adding *gfp* and *mCherry* to the promoters of *apl-1* and *ant-1.1*, respectively. Our studies will identify the pathways in which *apl-1* functions and provide insights into the normal function of APP and the pathogenesis of AD.

964C. Reverse genetics and functional analysis of an uncharacterized cysteine rich gene family in Caenorhabditis elegans. **Poulami Chaudhuri**, Dr Stephen Sturzenbaum. Analytical and Environmental Science Division, Kings College London, London, United Kingdom.

Microarray experiments performed with metallothionein (MT) double knockouts revealed that the transcription of a hitherto uncharacterised family of genes is transcriptionally activated upon metal exposure in an MT null background. The four isomers (W08E12.2, W08E12.3, W08E12.4 and W08E12.5) are consecutively aligned on chromosome IV and encode cysteine rich (13%) proteins.W08E12.2 - W08E12.5 are ³ 90% similar in their coding region and the promoters of W08E12.3 and W08E12.4 display an identity of 100%. Cloning and sequencing of the genomic region of the isomers have confirmed the presence and order of W08E12.2, W08E12.3 and W08E12.5 within the worm genome, however the presence of W08E12.4 still needs to be confirmed. Bioinformatic screening of the isomers predicted the presence of putative metal binding sites within the respective promoter regions. Transgenic-GFP tagged worms revealed the constitutive expression of PW08E12.3::GFP in the pharyngeal region which is induced by metals in the following order: Cd>Zn>Cu. The coding regions were subcloned into an expression vector (pTXB) to allow the in-frame expression of the proteins fused to an affinity tag (intein-CBD domain) thus enabling a single-step purification. SDS-PAGE and Western Blot analysis have confirmed the successful expression of the proteins and subsequent characterisation will provide insight into the structure, function and metal binding characteristics. Taken together, we are addressing the role of an uncharacterised family of genes in metal homeostasis or xenobiotic detoxification which in turn will add to ongoing efforts to develop enhanced biomarkers for metal toxicosis.

965A. Deciphering a genetic regulatory network of the ALA neuron. **Elly S. Chow**[1], Erich M. Schwarz[1,2], Cheryl Van Buskirk[3], Paul W. Sternberg[1]. 1) Division of Biology and Howard Hughes Medical Institute, 156-29,California Institute of Technology, Pasadena, CA, 91125, U.S.A; 2) Department of Molecular Biology and Genetics, Biotechnology 351, Cornell Univ, Ithaca, NY, 14853-2703, U.S.A; 3) Department of Biology, California State Univ Northridge, Northridge, CA91330, U.S.A.

The single, unpaired C. elegans ALA neuron responds to EGF-receptor activation and induces sleep-like behavior, including cessation of feeding and locomotion. We previously discovered a regulatory network comprising one LIM homeodomain transcription factor, ceh-14, and two paired-like homeodomain transcription factors, ceh-10 and ceh-17, that co-regulate EGF signaling pathway components, let-23/EGFR and phospholipase C-g, as well as other ALA-expressed genes. We sought to identify crucial cis-regulatory elements and the logic of gene regulation in the ALA neuron. We identified an 11-bp motif (the ALA-motif) from the regulatory regions of eight known ALA-expressing genes. The ALA-motif is necessary and sufficient to drive green fluorescent protein expression in the ALA neuron when attached to a basal promoter in a reporter construct. This motif harbors putative transcription binding sites for LIM homeodomain Lhx3 (an ortholog of ceh-14) and a paired-like homeodomain (like ceh-17 and ceh-10). Genetic analysis showed that the ALA-motif::GFP expression requires ceh-14 and ceh-17. A genome-wide search identified a set of 597 genes that have the ALA-motif in conserved neighboring non-coding DNA sequences, which may include regulatory regions. Of four neuropeptide genes in this set, we found a least one ALA-motif in the intron or in the 5' non-coding region that are sufficient to drive reporter expression in the ALA. Concurrently, we characterized the ALA transcriptomes from wild-type animals and ceh-14 mutants. We detected 8110 genes in the ALA and a set of these genes overlaps with motif-searched genes, which are also ceh-14-dependent. These results demonstrate that cell-specific cis-regulatory motif can identify co-expressed genes and single cell RNA-seq analysis in wild-type and mutant animals can sort out genes that are sharing the same regulatory inputs. Our combined approach may be used to identify the cis-regulatory network of a cell.

966B. Systematic analysis of *cis*-acting elements of a key transcription factor regulating *C. elegans* ray assembly -*tbx-2*. Kelvin K. K. Ip, **King-Lau Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

C. elegans male tail consists of nine lateral sensory organs called rays, embedded in a fan structure. The sensory rays go through a rapid developmental process involving multiple steps - cell fate choice, patterning, ray lineage execution, assembly and morphogenesis. A T-box transcription factor, *tbx-2*, was identified to be required for the ray assembly process. Mutation of *tbx-2* result in a failure of ray constituent cells to assemble into a ray, creating a ray loss phenotype. While *tbx-2* mutant displays ray loss in all rays, *tbx-2* is expressed in the structural cells of ray 1, 5, and 7 only. We are interested in understanding how such expression pattern arises, what factors are regulating *tbx-2* expression pattern and ultimately, how does a regulatory cascade controlling ray lineage and ray patterning work. A systematic deletion mapping was carried out on the transcriptional reporter of *tbx-2* to identify *cis*-acting elements that control the expression of *tbx-2*. A 138bp region region located 3' to the *tbx-2* coding region was identified to be required and sufficient to drive expression in structural cells of ray 1, 5, 7. We identified 2 conserved motifs within this 138bp region that are essential for the expression. In addition, conserved homeobox protein biding sites were identified in each of them with their function verified in mutant analysis. Two homeobox genes, *unc-62* and *ceh-20*, were found to be required for the activation of *tbx-2* transcription reporter. *unc-62* encodes a TALE homeodomain protein known to form heterodimer with PBX (*ceh-20*). We therefore hypothesize that *unc-62* and *ceh-20* regulate *tbx-2* by forming hetero-dimer. Expression pattern of *unc-62* and *ceh-20* in male tail would be examined and the protein-DNA binding of UNC-62/CEH-20 on the identified *tbx-2* enhancer would be tested. [This study is supported by Research Grants Council, Hong Kong.].

967C. Identification of essential genes that alter L1-diapause recovery. **Shu Yi Chua**[1], Jeffrey S.C. Chu[2], Robert Johnsen[1], Ann M. Rose[2], David L. Baillie[1]. 1) Dept of Molecular Biology and Biochemistry,Simon Fraser Univ, Burnaby, BC, Canada; 2) Dept of Medical Genetics, Univ of British Columbia, Vancouver, BC, Canada.

The growth of an organism is the result of a complex and highly-regulated developmental program, and changes in environmental conditions can alter the expression of key genes, hence altering developmental pathways which result in differences in its phenotype. For example, when faced with conditions of over-crowding, high temperatures or starvation, late L2 C. elegans larvae develop into an alternate L3 stage known was as the Dauer-diapause which have altered morphology, prolonged life, increased stress resistance. In addition to dauer formation, the free-living nematode C. elegans utilizes several strategies to deal with conditions of fluctuating food supply in the soil, such as Adult Reproductive Diapause, and L1-diapause. Specifically, L1-diapause is induced when embryos are hatched in the absence of food. These L1 larvae do not appear to undergo any morphogenetic changes, and are capable of re-entering the developmental pathway when placed back on food. In this study, our aim was to identify essential genes that are required for starvation-induced L1-diapause animals to fully recover from starvation and re-enter the developmental pathway. Essential genes are those required for proper growth into a fertile adult, and mutations lead to a range of lethal phenotypes such as emb, larval arrests, sterile adults and mels. Hence, this study was carried out via a genetic screen of lethal mutants to identify those that, after recovery on food after undergoing L1-diapause, (i) had no effect on their arrest stage, (ii) demonstrated an earlier arrest stage indicating premature arrest, or (iii) demonstrated a later arrest stage indicating extended survival. In addition, to further understand their roles in L1-diapause recovery, whole-genome sequencing of the heterozygous, genetically-balanced strains was carried out to identify their molecular identities, and in 59/75 strains, we were able to successfully ascertain their specific molecular lesions.

968A. A prime and boost mechanism drives left/right asymmetric expression of the miRNA *lsy-6* resulting in neuronal functional asymmetry. **Luisa Cochella**. Research Institute of Molecular Pathology (IMP), Vienna, Austria.

microRNAs are expressed with high spatio-temporal specificity during development. Understanding how these expression patterns are established is necessary to place miRNAs in the relevant cellular context and fully understand their function. The miRNA *lsy-6* is required for the functional left/right asymmetry between the two otherwise symmetric ASE sensory neurons. In the right ASE neuron (ASER) a transcription factor, *cog-1*, results in a right-specific gene battery that includes a set of guanylate cyclase receptors (*gcy*). *lsy-6* functions in the left ASE (ASEL) to repress *cog-1* and trigger the left-specific fate, which relies on a distinct subset of *gcy* genes. The asymmetric function of *lsy-6* is supported by transcription of the miRNA only in ASEL. How asymmetric transcription of *lsy-6* is achieved and thus how ASE asymmetry is established during development was unknown. Here we show that the exclusive expression of *lsy-6* in ASEL results from two activation inputs on two separate enhancers flanking the *lsy-6* locus. These two inputs act in a temporally separated combinatorial manner, which we term “prime and boost”. The *lsy-6* locus is primed by a transient input resulting in chromatin decompaction in the precursor of ASEL but not of ASER, 6 cell divisions before the ASEs are born. This early input is mediated by two transcription factors, TBX-37/38, which are transiently and asymmetrically expressed in the lineage that gives rise to ASEL but not ASER, at the AB16 stage - shortly after the lineages that give rise to ASEL and ASER diverge. TBX-37/38-mediated priming maintains the *lsy-6* locus competent for boosting of expression in ASEL 5 cell divisions later by CHE-1, a transcription factor that is bilaterally expressed in both ASEs. Absence of priming by TBX-37/38 in the ASER lineage results in *lsy-6* becoming refractory to further activation despite the presence of CHE-1 in ASER. This represents a novel transcriptional strategy for cell-type specific gene expression in development. In addition, because asymmetric TBX-37/38 expression is directly linked to the first embryonic Notch signal, we provide a mechanism linking one of the earliest embryonic asymmetries to the lateralized function of postmitotic neurons.

969B. Structural and functional analysis of a *daf-16* homolog in the parasitic nematode *Brugia malayi*. **Kirsten Crossgrove**[1], Katherine Stanford[1], Alexius Folk[1], Chris Veldkamp[2,3]. 1) Department of Biological Sciences, Univ Wisconsin, Whitewater, Whitewater, WI; 2) Department of Chemistry, Univ Wisconsin, Whitewater, Whitewater, WI; 3) Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI.

The parasitic nematode *Brugia malayi* is one of the causative agents of lymphatic filariasis. Over 120 million people worldwide are infected with filarial parasites and 40 million exhibit the debilitating and disfiguring effects of lymphatic filariasis. *B. malayi* is transmitted by mosquitoes during a blood meal and transmission to the human host triggers molting from the L3 (infective stage) to the L4 stage. We are interested in understanding the molecular basis for this developmental transition. The dauer larva stage of the free-living nematode *Caenorhabditis elegans* is thought to be analogous to infective stage larvae in parasitic nematodes such as *B. malayi* . For example, in both species an environmental cue is required to trigger the molt to the L4 stage. An insulin/insulin-like signaling (IIS) pathway is known to be important for dauer recovery in *C. elegans*. An active DAF-2 insulin receptor signals worms to recover from dauer. The DAF-16 protein, a FOXO transcription factor, is a major target of the IIS pathway. Insulin signaling results in localization of DAF-16 in the cytoplasm. In the absence of insulin signaling, DAF-16 is localized in the nucleus where it directly regulates expression of target genes. We have identified a homolog of *daf-16* in *B. malayi*. *Bm-daf-16* generates at least two isoforms by alternative splicing at the 5’ end. These isoforms encode two related DNA binding proteins. The *Bm*-DAF-16a DNA binding domain (DBD) exhibits 81% amino acid identity with *C. elegans* DAF-16a, while the *Bm*-DAF-16b DBD is 92% identical to *C. elegans* DAF-16b. We have confirmed that the *Bm*-DAF-16a DBD is able to bind to DAF-16 response elements. We have also determined a preliminary solution structure for this DBD using NMR. A better understanding of the structure and function of parasite DAF-16 proteins will contribute to assessing their viability as drug targets.

970C. Identification of Phorbol Ester Responsive Genes in *C. elegans* Using Genome-wide Expression Analysis. **Ana DePina**[1], Xiugong Gao[1], Piper Hunt[1], Nicholas Olejnik[1], Andriy Tkachenko[2], Renate Reimschuessel[2], Jeffrey Yourick[1], Robert Sprando[1]. 1) Division of Toxicology, Office of Applied Research and Safety Assessment (OARSA), Center for Food Safety and Applied Nutrition (CFSAN); 2) Center for Veterinary Medicine, U.S Food and Drug Administration (FDA), Laurel, MD.

There is the potential that certain plant derived oils, glycerin, and proteins containing phorbol ester impurities may be substituted for traditional food ingredients in the production of human and animal food, medical products and cosmetics. Conventional test methods may not detect the presence of these toxins, therefore, there is a need to develop better screening methods for phorbol ester contamination. Due to ease of propagation and relatively low cost, we used *Caenorhabditis elegans* as an invertebrate model system to conduct genome-wide microarray analysis to identify phorbol ester

responsive genes. Complex Object Parametric Analyze and Sorter (COPAS) analysis revealed that *C. elegans* larva grown in liquid culture treated with phorbol 12-myristate 13-acetate (PMA) underwent growth arrest, and did not develop to adulthood, whereas adult *C. elegans* treated with PMA for 24hrs did not exhibit any abnormal phenotype. Transcriptome analysis using microarrays of adult worms treated with PMA revealed 296 differentially expressed genes. Functional analysis of differentially regulated genes identified more than 20 gene ontology (GO) biological processes to be significantly overrepresented, including fatty acid/lipid metabolism, oxidation reduction, defense response, and aging. KEGG pathway analysis of the regulated genes revealed three biological pathways affected by PMA treatment: (1) lysosome degradation, (2) sphingolipid metabolism, and (3) other glycan degradation. We propose that phorbol ester responsive genes identified in this study could potentially serve as biomarkers for assessing phorbol ester toxicity. Furthermore, *C. elegans* strains harboring transgenes of interest could provide a rapid and cost-effective medium-throughput screen to serve as biosensors for the detection of phorbol ester contamination.

971A. Transcription of the cadmium-responsive genes *numr-1* and *numr-2* is neuronally regulated. **Queying Ding**, Jonathan H. Freedman. Laboratory of Toxicology and Pharmacology, NIEHS, Research Triangle Park, NC.

Exposure to the carcinogenic metal cadmium can activate multiple signaling pathways to affect the expression of hundreds of genes. However, the molecular mechanism by which cadmium affects transcription is not completely understood. Whole genome microarray studies showed nuclear metal-responsive gene-1 (*numr-1*) mRNA levels increased 7-fold following a 24h cadmium exposure. Subsequent genomic analysis identified a second gene, *numr-2*, that is 99% identical to *numr-1* in both the coding and regulatory regions. Both *numr*'s are developmentally regulated and expressed in identical cells: constitutively in a subset of neurons in the head, vulva and tail; and in intestinal and pharyngeal cells following metal exposure. Metals and calcium, but not other environmental stressors, induce *numr-1/-2* transcription. Bioinformatic analysis identified 25 putative, upstream regulatory elements in the numr promoters. The roles of the cognate DNA binding proteins in *numr*'s expression in the absence and presence of cadmium was examined in loss-of-function mutants. Among the tested genes, two alleles of *osm-9* and a *osm-9*;*ocr-2* double mutant showed significant gene-cadmium interactions, suggesting that TRPV channel proteins regulate cadmium-inducible *numr-1/-2* transcription. Five other genes may also be involved in *numr-1/-2* transcriptional regulation: *ceh-20*, *unc-86*, *cnx-1*, *unc-25* and *gem-4*. Interestingly, all these genes except *gem-4* are expressed in neurons, suggesting a link between neuronal activities and cadmium-inducible *numr-1/-2* transcription. OSM-9 and UNC-86 are involved in serotonin synthesis and secretion. We first tested the effects of serotonin on *numr-1/-2* transcription using serotonin agonists and antagonists. Pre-exposure to methiothepin, an antagonist of the serotonin receptor, suppressed cadmium-induced *numr-1/-2* transcription in wild-type C. elegans. In contrast, methiothepin by itself increased the basal mRNA levels of *numr-1/-2*. These results suggest that serotonin pathway(s) is involved in cadmium-inducible *numr-1/-2* transcription. We are further defining the roles of serotonin pathways and neuronal activity in *numr-1/-2* transcriptional regulation.

972B. Functional interplay of two SWI/SNF chromatin-remodeling subunits during *C. elegans* development. **Iris Ertl**[1], Montserrat Porta-de-la-Riva[1,4], David Aristizabal-Corrales[1], Eva Gomez-Orte[2], Laura Fontrodona[1], Eric Cornes[1], Simo Schwartz[3], Juan Cabello[2], Julian Ceron[1]. 1) Cancer and Human Molecular Genetics, Bellvitge Biomedical Research Institute-IDIBELL, Barcelona, Spain; 2) Center for Biomedical Research of La Rioja-CIBIR, Logroño, Spain; 3) CIBBIM-Vall d'Hebron Hospital, Barcelona, Spain; 4) *C. elegans* Core Facility, -IDIBELL, Barcelona, Spain.

The gene *ham-3* (also known as *swsn-2.1*), its paralog *swsn-2.2* and their human counterparts (SMARCD1, SMARCD2, SMARCD3) encode accessory subunits of SWI/SNF chromatin-remodeling complexes, which are involved in various cellular processes such as transcriptional regulation, cell differentiation and DNA repair. Since we observed that inactivation of *ham-3* causes intestinal hyperproliferation in *C. elegans* and its human homologs have been shown to be involved in tumorigenesis in various tissues, we decided to further explore *ham-3* and *swsn-2.2* functions in worms. Due to steric constrains, only one of these two proteins can be incorporated in a given SWI/SNF complex. Therefore, we investigate whether the genes encoding these paralogs have similar or independent functions. We have two mutant alleles for each of the two genes of interest. Employing mutant strains as well as RNAi, we have examined the function of *ham-3* and *swsn-2.2* in various tissues and developmental steps. Our results indicate that these two genes are redundant to control some processes, e.g. vulva development and fertility. However, they present independent functions in other developmental contexts such as embryonic development and regulation of L1 arrest. In addition, we found that *swsn-2.2* is also implicated in cell cycle regulation and through genetic epistasis analysis we are locating *ham-3* and *swsn-2.2* within the pathway that regulate the intestinal cell number. We have generated diverse transgenic reporters for *ham-3* and a specific antibody for SWSN-2.2 that together will aid us to study gene expression and protein distribution of these two genes. Moreover, we are performing RNA-Seq and ChIP-Seq experiments that will provide detailed information about the functional coupling of these two SWI/SNF subunits.

973C. The *C. elegans* ATPase inhibitors MAI-1 and MAI-2 are localized in different cellular compartments. **Laura P. Fernández-Cárdenas**[1], L.S. Salinas-Velázquez[1], L.T. Agredano-Moreno[2], L.F. Jiménez-García[2], M. Tuena de Gómez Puyou[3], R.E. Navarro[1]. 1) Cell and Developmental Biology, IFC, UNAM, Mexico, D.F., Mexico; 2) Science School, UNAM, Mexico D.F., Mexico; 3) Biochemistry and Structural Biology, IFC, UNAM, Mexico, D.F., Mexico.

In mammals, the ATPase inhibitor protein (IF1) regulates the hydrolysis activity of mitochondrial F_oF_1 ATPase to avoid ATP depletion. Besides mitochondria, this protein can also be found in endothelial and hepatic cell surface where its function is not well understood. In *C. elegans* there are two homologs for the mitochondrial IF1, MAI-1 and MAI-2. These have been studied in yeast where they both inhibit the F_oF_1-ATPase. MAI-1 is localized in cytoplasm while MAI-2 in mitochondria. We are studying *mai-1* and *mai-2* expression and function in *C. elegans.* To study their expression, we generated transgenic animals, that carry the constructs *mai-2::gfp* or *mCherry::mai-1*, by bombardment. We found that *mai-2::gfp* is expressed in all tissues during all embryonic and larval stages; where it appears to be associated to the mitochondrial network. This expression pattern correlates with its IF1 identity. Unexpectedly, *mCherry::mai-1* expression is not associated to the mitochondrial network and instead appears to be diffused in the cytoplasm. While *mai-2::gfp* is expressed ubiquiously, *mCherry::mai-1* was only observed in some cells of the nervous system, hypodermis and intestine during late embryonic, all larval stages and adult animals. *mai-1(RNAi)* and *mai-2(RNAi)* animals had normal growth, development and fertility, however have increased physiological apoptosis.

974A. Actively Transcribed Reverse Transcriptases are Correlated with Hookworm Larval Development. **Xin Gao**[1], Sahar Abubucker[1], John Hawdon[2], Makedonka Mitreva[1]. 1) TGI, Washington Univ School of Medicine, St. Louis, MO 63108; 2) Dept. of Microbiology, Immunology and Tropical Medicine, The George Washington Univ, Washington DC 20037.

Hookworm is a parasitic nematode that remains a major public health concern globally, with an estimated 740 million individuals infected mainly in tropical and subtropical regions. Heavy hookworm infections lead to significant morbidity characterized by chronic anemia, malnutrition, and cognitive impairment. Recently completed genome projects on two major hookworm species, Necator americanus and Ancylostoma ceylanicum, have dramatically improved our understanding about the hookworm biology. Here we present hookworm-specific expansion of reverse transcriptase gene family detected for the first time in parasitic nematodes. Reverse transcriptases generally fall into two major classes: i) those originating from retrotransposons and without specific function in the host cells, and ii) telomerases that possess unique cellular functions. A recent report has identified and characterized a novel class (rvt) with different gene architecture. The non-parasitic model nematode C. elegans only has two reverse transcriptases: one telomerase and one rvt gene. Interestingly, there has been a large expansion of the RT gene family in hookworms, with 90 in N americanus and 209 in A. ceylanicum, the majority of which are rvt genes. Gene expression data indicate that all RTs in hookworms are transcriptionally detectable and a small number of them are even actively transcribed. The transcriptional dynamics are correlated with the hookworm development. Phylogenetic analysis provided evidence of independent gene expansion of these reverse transcriptases within each hookworm species. Further functional investigations of the hookworm reverse transcriptases will help to define their specific roles in the living parasites and provide insights into parasite genome evolution and parasite-host interactions.

975B. The role of non-coding RNAs in gene regulation. **Kah Yee Goh**, Takao Inoue. Biochemistry, National Univ of Singapore, Singapore, Singapore.

The non-coding RNAs (ncRNAs) constitute a large proportion of the genome but functions of many ncRNAs are not well-understood. We are interested in a cluster of predicted ncRNAs near *bed-3*, a transcription factor expressed in the hypodermis and vulval cells to regulate molting and cell division. Of the 18 predicted ncRNAs in the cluster, we are focusing our analysis on three (*F25H8.16*, *F25H8.21* and *F25H8.13*). We found that all three predicted ncRNA genes are transcribed. To determine the function of these ncRNAs, we analyzed their expression pattern, RNAi phenotype and deletion mutants. Transcriptional GFP reporters for these ncRNA genes showed tissue-specific expression; *F25H8.16::gfp* is expressed in vulval muscles and *F25H8.21::gfp* is expressed in neuronal support cells, suggesting that they function in different cells. The expression pattern of these ncRNAs also appeared to be different from that of *bed-3*. We also did not observe any obvious phenotypes in the RNAi experiments. However, a homozygous deletion spanning 14 of 18 ncRNA genes in this cluster caused the Egl phenotype, suggesting that some of the ncRNAs regulate the egg-laying process. Together, these results suggest that the ncRNAs do not regulate *bed-3* even though they are in close proximity; instead they may regulate other genes unrelated to *bed-3*.

976C. Characterization of the *Caenorhabditis elegans* REF-1 Family Member, HLH-25. **Raymarie Gomez-Vazquez**, Casonya Johnson. Biology, Georgia State Univ, Atlanta , GA.

Transcription factors are DNA-binding proteins that control gene expression in response to cellular cues. In *Caenorhabditis elegans*, the REF-1 family proteins are dual-domain basic helix-loop hexlix (bHLH) transcription factors that play important roles during embryogenesis. Though the gene encoding HLH-25 is expressed during embryonic development in response to Notch signaling, there is no genetic or molecular data about its role in embryonic or post-embryonic development. Recently, our laboratory completed a microarray analysis of gene expression changes in *hlh-25*. This analysis has uncovered putative roles for HLH-25 in regulating genes required for cell division and cell cycle progression. Here, I report on my efforts to characterize HLH-25 and to determine its role in larval and adult *C. elegans*. I have examined the embryonic expression profile of an P*hlh-25*::GFP transcriptional reporter. I have also validated by RT-qPCR the microarray results showing that HLH-25 regulates genes required for successful cell division, and I am in the process of generating transgenic lines that will allow me to examine *hlh-25* dependent expression of P*daf-18*::GFP, P*ran-1*::GFP,P*akt-2*::GFP and P*thoch-1*::GFP. Finally, I have examined the embryonic lethality phenotype of *hlh-25* mutants, and I have found that this phenotype is enhanced in *hlh-25*;*hlh-27*(RNAi) animals.

977A. Metallothionein transcriptional regulation, ROS and aging. **J Hall**, JH Freedman. BSB, NTP, NIEHS, RTP, NC.

Reactive oxygen species (ROS) cause cellular damage and affect pathways involved in the aging process. Metallothioneins (MT) are conserved, cysteine-rich metal-binding proteins that function in metal homeostasis and detoxification, as well as the scavenging of ROS. To better understand its transcriptional regulation, genetic screens were used to identify regulatory factors and pathways that control the expression of the *C. elegans* MT gene, *mtl-1*. A mutagenesis screen identified a strain, JF99 that harbored a mutation affecting *atf-7*, a negative transcription factor involved in the JNK/p38 pathway. In both JF99 and an *atf-7* deletion mutant, steady-state *mtl-1* mRNA levels were identical but significantly greater than levels in wild type nematodes in the absence of metal. In addition to *atf-7*, mutations in *pmk-1* caused a decrease in *mtl-1* expression. PMK-1 translocated to the nucleus in response to cadmium. This data suggested that PMK-1 was also involved in *mtl-1* regulation. A candidate gene screen identified three insulin signaling pathway genes (PDK-1 and the AKT-1/AKT-2 complex) that functioned independently of this pathway. Further genetic analysis confirmed that these genes acted upstream of PMK-1 and ATF-7 to regulate *mtl-1* transcription. Based on the genetic analyses and previous work, we propose that ATF-7 resides on the promoter of *mtl-1* to inhibit the constitutively active transcription factor ELT-2, which is important for intestinal cell-specific transcription. In the presence of cadmium, upstream factors signal PDK-1 and the AKT-1/2 complex to release PMK-1 causing it to translocate to the nucleus and phosphorylate ATF-7, allowing ELT-2 to initiate transcription. The activation of this pathway results in an increase in MT, which can scavenge cadmium and free radicals. Other genes were identified that affected *mtl-1* transcription: MAPK pathway members *mek-1* and *mek-2*; a transcription factor *fos-1*; a transcriptional regulator *zfp-1*; a channel *tax-4*; and a PMK-1 pathway member, *tir-1*. Further analysis is underway to determine their involvement in *mtl-1* regulation. This new emerging pathway for *mtl-1* transcriptional regulation suggests a more direct role for MT in the response to ROS. It also provides a possible mechanistic link between activation of the insulin signaling pathway, free radical scavenging and longevity.

978B. Function of the *C. elegans* T-box factor TBX-2 depends on SUMOylation. **Paul Huber**, Tanya Crum, Lynn Clary, Tom Ronan, Adelaide Packard, Peter Okkema. Univ of Illinois at Chicago, Chicago, IL.

T-box transcription factors are critical developmental regulators in all multi-cellular animals, and altered T-box factor activity is associated with a variety of human congenital diseases and cancers. Despite the biological significance of T-box factors, their mechanism of action is not well understood. Here we examine whether SUMOylation affects the function of the sole *C. elegans* Tbx2 sub-family T-box factor TBX-2. TBX-2 is required for ABa-derived pharyngeal muscle, and *tbx-2* null mutants arrest as L1 larva. We have previously shown that TBX-2 interacts directly with the E2 SUMO-conjugating enzyme UBC-9, and that loss of TBX-2 or UBC-9 produces identical defects in ABa-derived pharyngeal muscle development. We now show that TBX-2 is SUMOylated in mammalian cell assays, and that both UBC-9 interaction and SUMOylation depends on two SUMO consensus sites located in the T-box DNA binding domain and near the TBX-2 C-terminus, respectively. In co-transfection assays, a TBX-2:GAL4 fusion protein represses expression of a 5xGal4:tk:luciferase construct. However, this activity does not require SUMOylation, indicating SUMO is not generally required for TBX-2 repressor activity. In *C. elegans*, reducing SUMOylation enhances the phenotype of the temperature sensitive *tbx-2(bx59)* mutant and results in ectopic expression of a gene normally repressed by TBX-2, demonstrating that SUMOylation is important for TBX-2 function *in vivo*. Finally, we show mammalian orthologs of TBX-2, Tbx2 and Tbx3, can also be SUMOylated, suggesting SUMOylation is a conserved mechanism controlling T-box factor activity. We are currently examining how SUMOylation affects TBX-2 activity *in vivo*. We expect these studies will provide further insight into the mechanisms regulating T-box factor activity.

979C. The role of *C. elegans* Histone H2A Variants in Transcription during Spermatogenesis. **Londen C Johnson**, Liezl Madrona, Margaret Jow, Diana Chu. San Francisco State Univ , San Francisco , CA.

One key contributor to male fertility is the proper packaging of DNA in sperm. This packaging is facilitated by histones, which compact DNA and manage the accessibility of DNA for transcription. Histone variants are specialized in function and replace canonical histones. For example, the histone H2A variant HTZ-1 is 54% identical to H2A, expressed in all cell types, and present at 23% of all *C. elegans* promoters, which suggests a role in transcription1. HTAS-1 is 48% identical to H2A and incorporated into sperm chroamtin during a period of global transcriptional silencing. Because both variants are expressed during spermatogenesis, our goal is to understand how the incorporation of HTZ-1 and HTAS-1 may influence gene transcription during sperm formation. We hypothesize that while HTZ-1 will be incorporated at active chromatin in the male germ line, HTAS-1 may be incorporated at repressed chromatin. To test this, we have conducted immunlocalization of HTZ-1 and HTAS-1 during spermatogenesis. First, we found that HTAS-1 and HTZ-1 co-localize during later stages of spermatogenesis and both are under-represented on the X transcriptionally-silent chromosome. This suggests, counter to our original hypothesis, that HTZ-1 and HTAS-1 may both be incorporated in transcriptionally active chromatin. To further assess this, we will test whether HTZ-1 and HTAS-1 are located on active or inactive extrachromosomal arrays in males. Preliminary data has shown that HTZ-1 is localized on active arrays in the hermaphrodite germ line; thus we anticipate that HTZ-1 will also colocalize to transcriptionally active arrays in males. Immunolocalization of HTAS-1 to active and inactive arrays is also currently underway. Further, because HTAS-1 and HTZ-1 differ from H2A in their N- and C-termini, we will create chimeras by swapping these domains onto canonical H2A and analyze their localization to transcriptionally active or inactive sites. We expect that this research will correlate HTZ-1 and HTAS-1 incorporation with transcription and define important features of H2A variant structure required for their potential roles in transcriptional regulation.

980A. The homeobox transcription factors, CEH-14 and TTX-1 regulate the AFD neuron specific gene expression of *gcy-8* and *gcy-18* in *C. elegans*. **Hiroshi Kagoshima**[1,2], Yuji Kohara[1]. 1) National Institute of Genetics, Mishima, Shizuoka, Japan; 2) Transdisciplinary Research Integration Center, Tokyo, Japan.

The identities of a wide variety of cells in multicellular organisms are given by the expression of characteristic sets of genes, mainly controlled by cell-specific transcriptional regulation. In particular, to generate different types of neurons, combinatorial expression of multiple transcription factors play pivotal roles for cell-specific gene expression. To understand the mechanism of the cell-specific transcriptional regulation, we used a pair of AFD thermosensory neurons in *C. elegans* as a model system, and analyzed the regulation of guanylyl cyclase genes, *gcy-8* and *gcy-18*, exclusively expressed in AFD. Here we show that the AFD-specific expression of *gcy-8* and *gcy-18* requires the simultaneous expression of homeodomain proteins, CEH-14/LHX3 and TTX-1/OTX1 in AFD neuron. We found the expression of *gcy-8* and *gcy-18* in AFD was downregulated by either mutation of *ttx-1* or *ceh-14*, and completely lost in the double mutants. We showed direct interaction of CEH-14 and TTX-1 proteins with *gcy-8* and *gcy-18* promoters by EMSA, and identified the binding sites of CEH-14 and TTX-1. Transgenic strains carrying *gcy-8* and *gcy-18* reporters with CEH-14 and/or TTX-1 sites mutation revealed that those binding sites were essential for AFD-specific expression of *gcy-8* and *gcy-18*. We also demonstrated that simultaneous forced expression of CEH-14 and TTX-1 in AWB chemosensory neuron could induce ectopic expression of *gcy-8*::GFP and *gcy-18*::GFP in AWB. Finally we showed that the regulation of *gcy-8* and *gcy-18* expression by *ceh-14* and *ttx-1* is evolutionally conserved in five *Caenorhabditis* species. Although thermosensory- and chemosensory-neurons have different functions, those sensory neurons may share potential exchangeability, and *ceh-14* and *ttx-1* confer AFD identity at the final step of the cell specification.

981B. Affecting gene expression through nucleosome positioning. **Colton E. Kempton**, Steven M. Johnson. MMBIO, Brigham Young Univ, Provo, UT.

Chromatin architecture not only facilitates DNA compaction but has a direct effect on genic regulation. The fundamental unit of chromatin compaction and regulation is the nucleosome composed of histone octamers wrapped with 147 bp of DNA. Where nucleosomes form on the genome (nucleosome positioning) and how they bundle the encoded genes in individual cells dictate many of the phenotypic outcomes observed, especially during development.

To study the effects of nucleosome positioning on gene expression, we are engineering non-coding and non-regulatory flanking DNA sequences surrounding important genetic elements. Manipulation is done on constructs with reporter genes injected into C. elegans forming transgenic lines of animals harboring extrachromosomal arrays. Currently we are constructing and analyzing the effects of various combinations of putative nucleosome repelling and attracting sequences on an *unc-54* enhancer *myo-2* promoter construct. Preliminary observations indicate that in certain promoter/enhancer combinations reporter expression decreases over multiple generations. We hope to design constructs that maintain strong reporter expression over multiple generations by using these putative nucleosome repelling and attracting sequences to reposition nucleosomes and or influence their formation.

In parallel we are developing methods to assay nucleosome positioning on extrachromosomal arrays. We are also conducting genetic screens to identify factors involved in somatic transgene silencing.

Long term we expect that these data and observations will contribute to a general understanding of how chromatin architecture influences gene expression. We also expect to identify factors important for transgene silencing i*n vivo* and to clarify how the combined influence of these factors and the DNA sequence contribute to overall chromatin architecture and gene expression.

982C. Hunting for the causes of inter-individual variation in chaperone expression. **Adam Klosin**, Kadri Reis, Ben Lehner. EMBL-CRG Systems Biology Unit, Centre for Genomic Regulation, Barcelona, Spain.

Genetically identical animals reared in the same environment still vary a lot in terms of their physiology. In the nematode Caenorhabditis elegans natural variation in the level of stress response can be used to predict future phenotypes of individual worms. Animals that express high levels of HSP-16.2 chaperone after a mild heat shock are more resistant to future stress, have extended lifespan and are better at buffering mutations. Similarly, worms that express higher levels of a constitutive chaperone DAF-21/HSP-90 are more likely to suppress phenotypic effects of incompletely penetrant mutations carried in the genome. Therefore, the level of chaperone expression can have important consequences for the worm's fitness. However, little is known about the underlying cause of variation in chaperone expression between individuals. In order to better understand the causes of variation in chaperone expression we performed large-scale RNAi screens using fluorescent reporters of induced and constitutive chaperones and found many genes whose knock-down affects the level of expression. We will present the results of this screen.

983A. Genetic analysis of *pry-1/Axin* regulation and Wnt signaling in nematode vulva development. **Jessica Knox**, Philip Cumbo, Bhagwati P Gupta. McMaster Univ, Hamilton, Ontario, Canada.

The Wnt pathway is a conserved signaling pathway vital for such cellular processes as polarity, migration and fate specification. In *Caenorhabditis elegans*, the Wnt pathway converges with the Notch and Ras pathways to induce 3 of 6 Vulval Precursor Cells (VPCs) to divide and form the mature vulva. The robust patterning of the vulva serves as a paradigm for the study of signal transduction and the complex integration of conserved signaling pathways controlling organ formation. Using the vulva model in *C. elegans* and the related species *C. briggsae*, we can analyze Wnt signaling function and conservation during development in an attempt to tease apart these pathway interactions. To this end, a genetic approach is being taken to dissect the regulation and function of the Axin homolog *pry-1*, a potent negative regulator of Wnt signaling.

We have isolated *Cbr-pry-1* mutants that, along with their *C. elegans* counterparts, display a Multivulva phenotype in which loss of PRY-1 results in activated Wnt signaling and formation of ectopic pseudo-vulvae. Using cell fate markers, cell ablation and genetic interaction techniques it was found that these ectopically induced VPCs adopt a 2° fate in both species, independent of inductive and lateral signals. A crucial interaction between the Wnt and Notch pathways in promoting 2° VPC fate has been uncovered in these *pry-1* mutants, in which we observe a P7.p induction failure, caused by altered regulation of LIN-12 signaling and the resulting persistent expression of its transcriptional target *lip-1*. We have further embarked upon a dissection of *pry-1* regulation throughout development. Using qRT-PCR and transcriptional GFP reporter fusions, we have found that *pry-1* expression is responsive to Wnt signaling activity. In both species, *pry-1* mutants exhibit a significant increase in *pry-1* transcript level at all stages. The opposite trend is observed when key components of the Wnt pathway are knocked down (*bar-1*/b-catenin and *pop-1*/TCF/LEF). An analysis of *pry-1* upstream and intronic UTRs indicates the presence of conserved TCF/LEF binding sites. These results together suggest that *pry-1* is itself being positively regulated by Wnt pathway activity.

984B. Towards Unrestricted Direct Reprogramming: A genome-wide RNAi screen to identify cell fate reprogramming-inhibiting factors. **Ena Kolundzic**, Oktay Kaplan, Martina Hajduskova, Andreas Ofenbauer, Alina Schenk, Baris Tursun. Berlin Institute for Medical Systems Biology (BIMSB) at Max Delbrueck Center (MDC), Berlin, Germany.

Direct conversion of specific cell types into new, distinct cells has implications in basic biology as well as biomedicine. However, transcription factors (TFs) required for induction of specific cell differentiation programs are often inefficient in imposing such programs on other differentiated cells. It has recently been shown that inhibitory mechanisms could be involved in preventing these transcription factors from driving their differentiation programs in other cell types (Tursun et al. 2011, Patel et al. 2012). Here, we present a genome-wide RNAi screening approach to identify inhibitory factors of TF-induced direct conversion of different cell types. In order to study conversion into different cell types, we are using a terminal selector, CHE-1, essential for terminal differentiation of glutamatergic ASE neurons, as well as the myogenic TF HLH-1. Transgenic animals containing the ectopically expressed *che-1* or *hlh-1* gene driven by a heat shock inducible-promoter and a GFP reporter driven by the promoter of an ASE neuron or muscle fate marker are being used for the screen. Wild-type animals express GFP in one ASE neuron, or muscle cells under non heat-shocked conditions, respectively. After heat-shock, the fate-inducing TFs are ubiquitously expressed, but GFP signals of the respective fate reporters are only visible in either ASE and a few other neurons (gcy-5::gfp), or in muscle cells (unc-97::gfp). but no conversion of other cells into neuronal or muscle cells is detectable. The screen has been designed to look for factors which allow CHE-1/HLH-1 to induce expression of ASE/muscle marker in other cell types when silenced by RNAi. We present data for some of the candidates obtained from the screen.

985C. X chromosome dosage compensation in the early C. elegans embryo. **Maxwell Kramer**[1], Sevinc Ercan[1,2]. 1) Developmental Genetics Program, New York Univ, New York, NY; 2) Biology Department, New York Univ, New York, NY.

The genetic networks that direct embryonic development rely on specific levels of gene expression and so do not tolerate large changes in gene copy number, also known as gene dose. Despite having different numbers of X chromosomes, male and female embryos develop similarly. This is due to dosage compensation mechanisms that regulate X chromosome transcription during embryogenesis. These are well studied during later development and adulthood but the mechanisms of dosage compensation during early embryogenesis remain unclear. In C. elegans, imprinted silencing of the paternally contributed X chromosome provides a potential mechanism for early embryo dosage compensation. To determine whether chromosome wide dosage compensation occurs prior to activation of the canonical Dosage Compensation Complex (DCC), populations of hermaphrodite and mixed sex of embryos younger than 40-cell stage were collected their transcriptomes were sequenced. Expression levels from the X chromosome are higher in hermaphrodites,

suggesting that dosage compensation does not occur uniformly across the entire X chromosome in the very early embryo. In order to determine whether paternal X inactivation acts selectively on specific genes, we have begun to analyze expression of paternally contributed alleles in hybrid polymorphic embryos. Expression of paternal SNPs in the early embryo will allow us to determine which X linked genes are expressed from the paternal X chromosome and which genes are expressed solely due to maternally contributed mRNA and/or expression from the maternally contributed X chromosome.

986A. A quantitative system to define the role of transcription factor binding affinity in transcriptional activation. **Brett Lancaster**, James McGhee. Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada.

We are developing a quantitative experimental system to investigate, inside a living animal, how the affinity of a transcription factor for its cis-acting binding sites influences the rate of target gene transcription. In C. elegans, the zinc finger transcription factor ELT-2 binds to a core TGATAA DNA sequence to regulate the majority (possibly all) of the genes transcribed in the differentiating intestine. Our objective is to quantitatively measure the binding affinity of ELT-2 to a series of binding site variants (e.g. NTGATAAN), and, at the same time, measure the transcriptional output (mRNA) of the target gene with these variants in the promoter, all inside the living worm. The asp-1 gene encodes the major intestinal-specific aspartic protease of the worm, has two TGATAA sites in its 1kb promoter and is under direct ELT-2 control. We have constructed two versions of the asp-1 gene that differ only in the location of a KpnI site introduced by silent mutation. Reporter “A” is regulated by the wildtype asp-1 promoter; reporter “B” is regulated by a mutant reporter in which the wildtype CTGATAAG site has been replaced by, for example, an ATGATAAT site. Equimolar mixtures of the two constructs are introduced into worms (unc-119(-); asp-1(-); elt-4/7(-)) as multicopy transgenic arrays, which guarantees that each transgene, on average, will be exposed to the same cellular environment. RNA isolation followed by RT-PCR, KpnI digestion, and microfluidic gel electrophoresis (Agilent Bioanalyzer) yields distinct and easily quantitated profiles that allow us to estimate the levels of transcript produced by each reporter. Expression of a GFP reporter driven by the asp-1 1kb promoter requires both TGATAA sequences and I have quantitatively replicated this observation using this new system. In the future, binding affinity of purified ELT-2 protein to each binding site variant will be determined by fluorescence anisotropy. I will present our results on the reproducibility and dynamic range of the experimental system, together with possible limitations.

987B. *C. elegans* CCM-3 may affect excretory canal development through endosome recycling. **Benjamin Lant**, W. Brent Derry. Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, Canada.

Cerebral Cavernous Malformation (CCM) is a vascular disease affecting approximately 1 in 500 individuals. A mutation in one of three genes (CCM1/2/3) can cause seizures, strokes and other neurological defects; CCM3 mutations being the most severe. In *C. elegans* the two CCM homologues CCM1/*kri-1* and CCM3/*ccm-3* (C14A4.11) are expressed in the intestine, with *ccm-3* also present in the excretory cell. Ablation of both genes causes synthetic lethality. The nematode excretory cell body branches into two canals along the length of the worm, and extends throughout worm development. Like multicellular mammalian tubules, such as blood vessels, the canal has apical and basal surfaces, and employs similar developmental signaling. As such, we used the canal as a model of tubulogenesis to elucidate CCM-3 function. Loss of *ccm-3* gene function causes severe canal truncation and frequent surface cyst formation. Orthologs of the STRIPAK complex, which bind mammalian CCM3 in cultured cells, similarly regulate nematode canal extension. Ablation of the CCM-3 interacting kinase GCK-1 and striatin CASH-1 caused truncation and cysts (like *ccm-3*(lf)), supporting mammalian and nematode STRIPAK conservation. Overexpression of GCK-1 restored canal length in both *ccm-3*(lf) and *gck-1*(lf) mutants, suggesting *gck-1* acts downstream of *ccm-3* to promote canal growth. Subsequently, a pilot RNAi screen to find *ccm-3* interactors identified exocyst gene *exoc-8*, and myotonic dystrophy kinase-related Cdc42 binding kinase ortholog *mrck-1*. As with STRIPAK members, ablation of these genes caused canal truncation and cysts, suggesting they also operate in the CCM-3 pathway. Given the importance of Cdc42 and exocyst complexes in vesicle transport we asked whether the CCM-3 pathway regulates transport or recycling to promote canal growth. Using fluorescent markers for endosomes, CDC-42 and the Golgi, we found that reduction of STRIPAK and *mrck-1* decreased CDC-42 and Golgi signals. Preliminary TEM work also suggests that *ccm-3* (lf) mutants show altered vesicular aggregation and positioning. Collectively, these results suggest that a major function of the CCM-3 pathway is to regulate tube growth through vesicle transport and endosomal recycling.

988C. *In silico* identification and functional analysis of genes responsive to dietary restriction in *Caenorhabditis elegans* . **Andreas H. Ludewig[1]**, Meike Bruns[1], Maja Klapper[1], Thomas Meinel[2], Frank Doering[1]. 1) Molecular Prevention, CAU Kiel, Germany; 2) Institute for Physiology, Charité - Univ Medicine Berlin, Germany.

Increasing mounds of data derived from high throughput approaches assaying a huge number of species, tissues and diseases have been generated over the past 2 decades. However, the integration of those data sets to generate useful biological information can be challenging. Here, we introduce two approaches to translate data derived from extensive microarray analysis into a manageable set of candidate genes according to biological relevant questions. In a meta-analysis of microarray based screens for differentially regulated genes under dietary restriction (DR) from 8 independent experimental set ups in *C. elegans*, we extracted 177 strongly overlapping differentially regulated genes. Among those, the sub class of CUB like proteins was significantly enriched (12,4%). Notably, most of those CUB like proteins have not been linked to DR. In another approach we applied a novel developed bioinformatic tool - the Ortho2Express Matrix (Meinel et al., 2011) - to summarize, compare and interpret gene expression data performed in mouse and *C. elegans* under DR and ad libidum feeding conditions. We use this tool to combine those gene expression profiles with complex gene family information, derived from sequence similarity. We end up with a list of 18 mouse genes and 24 assigned putative functional *C. elegans* orthologs that are regulated in both species in the same direction in response to DR. In conclusion, based on data mining we identified a set of genes which likely play a role in the regulation of the DR response and DR related phenomena such as improved stress resistance and long life. We discriminate between genes with already known functions in DR regulation, genes that have been connoted with other functional context and genes of complete unknown function. First functional assays for heat stress resistance under DR with selected pre-evaluated mutant *C. elegans* strains confirmed our in silico analysis.

989A. Altered-function mutations of the U2AF large subunit are protective modifiers of the *C. elegans SMN* mutant defects. X. Gao, **L. Ma**. State Key Laboratory of Medical Genetics, Central South Univ, Changsha, Hunan, China.

Spinal muscular atrophy (SMA) is caused by loss-of-function mutations in the highly conserved and ubiquitously expressed protein SMN. Mutations in *SMN* can lead to defective RNA splicing. However, little is known about the *in vivo* interactions of *SMN* with RNA splicing factor genes. To understand how *SMN* affects RNA splicing, we examined the splicing of the endogenous splicing reporter gene *tos-1* in the *C. elegans smn-1(ok355D)* (*SMN* null) mutants. We found that *smn-1(ok355D)* caused increased *tos-1* intron 1 retention. By analyzing *tos-1* transgenes carrying different substituted 3' splice sites, we found that *smn-1* is required for efficient splicing only at weak 3' splice sites. Surprisingly, altered-function mutations of the U2AF large subunit gene *uaf-1* could rescue the lifespan and locomotion defects of *smn-1(ok355D)* mutants. Our study suggests that in *C. elegans* the splicing at strong 3' splice sites is likely not dependent on SMN-1 and identifies altered-function mutations of the U2AF large subunit gene as novel rescuing modifiers of *SMN* mutant defects.

990B. An *in vivo C. elegans* gene regulatory network unveils post-developmental role of intestinal transcription factors. **Lesley T. MacNeil**, H. Efsun Arda, Lauren D'Elia, A.J. Marian Walhout. Program in Systems Biology, Univ of Massachusetts Medical School, Worcester, MA.

The *C. elegans* intestine must integrate dietary, stress and physiological information and respond with an appropriate gene expression program. To understand how these inputs are coordinated, we examined physical and regulatory interactions between TFs and the promoters of 19 genes expressed in the adult intestine. We selected a set of genes that include stress and diet-responsive genes, as well as genes that are stably expressed through a variety of conditions. The same promoter fragments were used in two different assays to compare physical and regulatory interactions. Physical interactions were identified by enhanced yeast one-hybrid assays. To identify regulatory interactions, we generated an RNAi-feeding library covering 95% of all *C. elegans* TFs and used this library to perform RNAi knockdown using fluorescent transcriptional reporters strains. In addition to screening transgenic animals under standard laboratory conditions, we screened these transgenes under specific stress and dietary conditions. In total, we identified approximately 200 physical interactions and 300 regulatory interactions. We find little overlap between physical and regulatory interactions suggesting that redundancy may mask the effects of physical interactions. Conversely, regulatory interactions that are not associated with physical binding events may occur indirectly as a result of one TF regulating another, or as a result of compensation. By combining our data for all 19 genes, we have identified TFs that are broad regulators of intestinal gene expression, including *elt-2* and *sbp-1*, as well as more gene-specific or stress-specific regulators. In addition, we can begin to predict relationships between TFs based on shared regulatory targets and knowledge of physical interaction data. Our data suggest that the intestinal gene regulatory network is complex and that compensation and redundancy exist to modulate and/or buffer the appropriate levels of gene expression in response to genetic perturbations.

991C. DBL-1 Target Gene Regulation By SMA-2, SMA-3, and SMA-4. **Uday Madaan**[1,2], Jianghua Yin[1,2], Edlira Yzeiraj[1], Cathy Savage-Dunn[1,2]. 1) Biology, Queens College, City Univ of New York, Flushing, NY; 2) The Graduate Center, City Univ of New York, New York, NY.

The DBL-1 ligand, a TGF-b homolog in C. elegans, is secreted by neurons and is necessary for body size regulation, mesodermal patterning, innate immunity, reproductive life span, and male tail sensory ray identity. DBL-1 signals via SMA-6 and DAF-4, the type I and type II receptors respectively. The Smads SMA-2, SMA-3, and SMA-4 form a heterotrimeric complex and are shuttled to the nucleus. In the nucleus, this complex is suspected to bind DNA along with SMA-9 (zinc finger transcription factor) to regulate DBL-1 target genes.The mechanisms of DBL-1 target gene regulation are still relatively undefined. For example, which target genes are directly or indirectly regulated by Smads is currently unknown. Here we propose to look at direct/indirect regulation of col-41, a DBL-1 target gene, which is hypodermis specific in worms. As previously established, the hypodermis is the main tissue responsible for body size regulation via the DBL-1 pathway. Using microarray analysis and tissue specific promoter constructs we have established col-41 as a DBL-1 target gene. Here we explore whether col-41 is a direct or indirect target of the DBL-1 pathway. The hypodermal specific col-41p::2xNLSmcherry construct was used to monitor col-41 expression. Regulatory region analysis of col-41 revealed 6 conserved regions containing multiple putative GTCT Smad binding sites. Upon deletions of the conserved regions changes in col-41 expression were observed. For further analysis of the regulatory region of col-41, we constructed GST fusions with SMA-2, SMA-3, and SMA-4. We aim use these constructs to determine whether the Smads bind directly to probes composed of fragments of the upstream regulatory region of col-41. We have presented strong evidence of regulation of col-41 by the DBL-1 pathway. Elements 1, 2, 3, and 6 in the col-41 upstream region are required for col-41 expression. Future investigation will involve testing the upstream regulatory region for binding with Smads and with ELT-1, a known master transcription factor responsible for hypodermal cell-fate that may be involved in Smad recruitment.

992A. The Receptor Tyrosine Phosphatase MOA-1 shows a temperature-dependent induction and affects *C. elegans* viability and development. **Vanessa Marfil**, Chris Li. Biology, City College of New York, New York, NY.

Alzheimer disease (AD) is the most common type of dementia and the sixth leading death cause in the US. Brain accumulation of the amyloid beta peptide (Ab) is proposed as one of the causative events in AD pathogenesis. The Ab peptide is the result of the proteolytic cleavage of the transmembrane protein amyloid precursor protein (APP). However, the molecular mechanism underlying APP action has been difficult to determine due to the presence of two functionally redundant proteins, which together with APP have an essential function in mammals. *C. elegans* has a unique APP-related gene, *apl-1*, which is essential for viability. The extracellular domain of APL-1 (APL-1-EXT) is necessary and sufficient to rescue the lethality of the *apl-1* null mutant. To determine the cellular function of APL-1-EXT, we examined the *apl-1*(*yn5*) mutant, which is viable and generates high levels of the extracellular fragment. Among other phenotypes, *apl-1*(*yn5*) shows developmental delay and a temperature-dependent lethality. A mutation in R155.2/*moa-1* (modifier of apl-1), which encodes a tyrosine phosphatase receptor, suppresses the *apl-1*(*yn5*) temperature-dependent lethality. By generating transcriptional and translational reporter lines, we determined that *moa-1* is expressed in many cell types, including neurons and pharyngeal, intestinal, and vulval cells, and is co-expressed with *apl-1* in some tissues. Interestingly, high levels of *moa-1* intestinal expression are induced at 27°C. Furthermore, this temperature-dependent induction of *moa-1* requires functional *crh-1*, the C. elegans orthologue of the CREB transcription factor, which has been shown to be important for thermotaxis and learning. Downregulation of *moa-1* by RNAi produces temperature-dependent lethality and developmental delay; this lethality is enhanced in a *crh-1* null mutant background. MOA-1 and APL-1 do not appear to interact in in vitro pull-down and yeast-two-hybrid assays. Our

results suggest that *moa-1* is a novel temperature-dependent regulated gene that may work with *apl-1* to regulate development and viability. Further research now focuses on elucidating how *apl-1* and *moa-1* interact and on determining which downstream pathways are affected.

993B. Inverted repeat sequences required for alternative splicing of the *unc-17 - cha-1* cholinergic locus. **Ellie Mathews**, Greg Mullen, Jim Rand. Gen Models Disease Res Program, Oklahoma Med Res Foundation, Oklahoma City, OK.

A single phylogenetically conserved locus encodes both the acetylcholine biosynthetic enzyme choline acetyltransferase (ChAT; *cha-1*) and the vesicular acetylcholine transporter (VAChT; *unc-17*). The *unc-17* gene lies within the first intron of *cha-1*, and alternative splicing leads to separate *cha-1* and *unc-17* transcripts. We identified two sets of inverted repeat sequences (designated R1 and R2) in the non-coding sequences flanking the *unc-17* coding region; these repeats could potentially form RNA stem-and-loop secondary structures. The repeats are present in other nematode species, and in each species, the repeats are better matches to each other than they are between species. We speculated that these sequences play a role in regulating alternative splicing. We engineered a “dual reporter” in which the *unc-17* coding region was replaced with green fluorescent protein (GFP) and the *cha-1* coding region with a red florescent protein (wCherry). The resulting construct contains all of the conserved non-coding sequences in the regions flanking *unc-17* and *cha-1*, and transgenic animals containing this construct correctly express both GFP and wCherry in the appropriate neurons. We found that both R1 and R2 repeats are necessary for expression of wCherry (*cha-1*). The function of the R1 repeat is not sequence dependent, but merely requires sequence complementarity, while the function of the R2 repeats shows some sequence dependence in addition to sequence complementarity. We conclude that disruption of either pair of sequences significantly decreases the production of the "downstream" spliced product. The R1 and R2 inverted repeats appear to act synergistically to potentiate the 6856-nucleotide *cha-1* splice and/or reduce the efficiency of the 1146-nucleotide *unc-17* splice. Perhaps these repeats help to "level the playing field" when alternate splices are very different sizes (the proximal splice site would otherwise have a significant advantage). We examined two deletion mutants (*md1447* and *p1156*), both of which eliminate the downstream R1 site yet which lead to different levels of the two transcripts; we conclude that there are additional factors regulating *unc-17/cha-1* alternative splicing and transcript levels.

994C. Poising and pausing of Pol II during starvation. **Colin S. Maxwell**[1], William S. Kruesi[2], Nicole Kurhanewicz[1,3], Leighton J. Core[4], Colin T. Waters[4], Caitlin L. Lewarch[1,5], Igor Antoshechkin[6], John T. Lis[4], Barbara J. Meyer[2], L. Ryan Baugh[1]. 1) Department of Biology, Duke Univ, Durham, NC; 2) Howard Hughes Medical Institute, Department of Molecular and Cellular Biology, Univ of California at Berkeley, Berkeley, CA; 3) Current address: 130 Mason Farm Rd, 1125 Bioinformatics Building, CB# 7108, Univ of North Carolina, Chapel Hill, NC; 4) Department of Molecular Biology and Genetics, Cornell Univ, Ithaca, NY; 5) Current address: Department of Molecular and Cellular Biology, Harvard Univ, Cambridge, MA; 6) Division of Biology, California Institute of Technology, Pasadena, CA.

In order to respond appropriately to changing environments, organisms must quickly alter the genes they express. Arrested L1 larvae rapidly alter gene expression in response to feeding, providing an attractive model to study the mechanisms of rapid gene induction. We previously showed that RNA Polymerase II (Pol II) is regulated at a post-recruitment step during L1 arrest. This regulation correlates with genes up-regulated by feeding, suggesting that it promotes rapid gene induction. We hypothesized that this regulation was mechanistically related to Pol II pausing, which has been proposed to allow the rapid induction of genes. To address this, we located elongation complexes genome-wide during starvation by sequencing short nascent RNAs as well as by using global nuclear run-on sequencing. We show here that Pol II is regulated during early elongation (pausing). Analysis of a TFIIS mutant reveals mechanistic similarities to pausing in other systems. However, Pol II pausing is associated with active stress-response genes that are actually down-regulated upon feeding. In addition to pausing, we show that ‘poised’ Pol II accumulates without initiating upstream of repressed growth genes that are up-regulated upon feeding. Poised Pol II and paused Pol II are associated with distinct core promoter architectures, suggesting alternative pathways for pre-initiation complex formation. Both growth and stress genes are regulated post-recruitment during starvation, but during initiation and elongation, respectively. Our work sheds light on the mechanisms organisms use to cope with changing environments.

995A. Alternative 3’UTRs: A Mechanism for Post-transcriptional Regulation in *C. elegans* Germline & Early Embryo. **D. Mecenas**[1], R. Ahmed[1,2], M. Gutwein[1], J. Reboul[1,3], J. Polanowska[1,3], K. Gunsalus[1,4], F. Piano[1,4]. 1) NYU, New York, USA; 2) MDC, Berlin, DEU; 3) Inserm, Marseille, FRA; 4) NYUAD, Abu Dhabi, UAE.

Proper spatio-temporal control of gene activity is vital for animal development. In the *C. elegans* germline and early embryo, this occurs mainly by post-transcriptional regulation (PTR) via the three-prime untranslated region (3’UTR). PTR depends on cis-regulatory sequences and trans-acting factors, including RNA binding proteins (RBPs) and small regulatory RNAs, which influence the stability, translation, and localization of mRNA. Experimental analysis of PTR is technically challenging, and *in vivo* studies of the molecular mechanisms by which 3’ UTRs regulate gene activity are limited. Sequence analysis of the 3’UTR landscape in *C. elegans* indicates that about half of the protein-coding genes are alternatively polyadenylated (Mangone et al., *Science* 2010; Jan et al., *Nature* 2011). Therefore, alternative polyadenylation (APA) can result in mRNA transcripts with 3’UTRs of varying lengths and regulatory potential. In the germline, 3’UTR-mediated PTR is the primary mechanism of gene regulation (Merritt et al., *Curr Biol* 2008). In addition, a number of key RBPs whose activities are crucial for development are expressed in spatio-temporally restricted regions. Our goal is to address the question of correlation between the availability of regulatory elements within the 3’UTR and their cognate trans-acting factors. By combining data on 3’UTR isoforms with iPAR-CLIP of GLD-1 (Jungkamp et al., *Mol Cell* 2011), we found 38 genes that experience APA and contain binding sites for GLD-1 in the variable 3’UTR region. To test the idea that individual transcripts may escape PTR by selective expression and/or degradation of alternative 3’UTRs in the germline and early embryo, we developed assays to detect and quantify them during development. We have cloned a subset of these 3'UTRs into a two-color *in vivo* reporter system that will provide a readout of 3'UTR-mediated PTR. Combining these expression assays with mutational analysis of 3’UTRs will allow us to assay the relative contributions of alternative isoforms and regulatory elements to specific spatio-temporal expression patterns during development.

996B. In vivo spatiotemporal analysis of mRNA alternative splicing during C.elegans neural development. **Jonathan R. M. Millet**[1,2], Denis Dupuy[1,2]. 1) U869 - ARNA, INSERM, Pessac, Gironde, France; 2) INSERM, U869, ARNA laboratory, F-33000 Bordeaux, France.

Neuronal differentiation is a complex process involving fine-tuned gene regulatory network from neuroblast generation to terminal differentiation of

well-defined neurons. Networks are defined by their components (genes) and by the relationship linking those components (regulatory mechanisms). Alternative splicing is a phenomenon that allows a single gene to produce multiple isoforms in a time- or location-dependent manner. Alternative splicing can lead to protein isoforms with altered stability, localization, specificity or activity, and affect neuronal gene regulatory network. At least 15% of C.elegans genes have two or more splicing isoforms. We compare the spatio-temporal expression of mRNA isoforms from a single gene in transgenic lines. We use a frame shift reporter fused to the gene of interest, that allows us to visualize the splicing regulation using fluorescence microscopy. This method was applied to several genes with multiple isoforms. top-1 encodes one embryo-specific and one neuron-specific mRNA splice variants of the DNA topoisomerase I. che-1 is an important transcription factor involved in ASE terminal differentiation. tax-6 encodes three different isoforms of Calcineurin and play an important role in C.elegans sensory neurons adaptation. gpa-13 encodes three mRNA isoforms of a Galpha-subunits involved in sensory neuron signal transduction. We will use EMS mutagenesis followed by COPAS-assisted screening for mutants with altered fluorescence patterns for the identification of alternative splicing regulators. Using this method, in combination with other genetic approaches, we aim to better understand splicing regulatory networks involved in neuron differenciation. We want to validate neuron-specific alternative splicing regulators and their targets and to understand the contribution of alternative splicing to Caenorhabditis elegans neuronal genetic programming. Unravelling the components of gene regulatory network involved in the development of the compact nervous system of C.elegans could lead to a better understanding of neural development.

997C. A high-throughput EMS screen to identify direct reprogramming regulating factors. **Andreas Ofenbauer**, Oktay Kaplan, Martina Hajduskova, Ena Kolundzic, Stefanie Seelk, Selman Bulut, Alina Schenk, Baris Tursun. MDC/BIMSB Berlin, Germany.

Having multiple cell types with distinct functions has been a prerequisite for the evolutionary success of most multicellular organisms. Accordingly, understanding the mechanisms that form the basis of this strategy has always been of big interest and has historically been the topic of developmental biology. Nowadays several other disciplines deal with the question of how one cell type differentiates into another, such as stem cell biology, epigenetics, systems biology, RNA biology, or even biomaterial research. Transdifferentiation is the process by which an already specialized cell is directly converted/reprogrammed into another specialized cell. This naturally less abundant process is the major focus of our lab. We try to identify and characterize genetic factors that play a role in induced transdifferentiation by mis-expressing transcription factors (TFs). Currently there are several selector genes known that can activate specific cell fates such as *hlh-1*, the worm homolog of the myogenic bHLH TF MyoD. When mis-expressed, HLH-1 induces muscle fate in early embryonic cells but terminally differentiated cells in older animals are resistant to *hlh-1*-induced direct reprogramming. In order to identify factors that antagonize selector genes, we apply both forward and reversed genetics. We use transgenic lines allowing ectopic expression of a specific selector gene and the appropriate cell fate reporter. To complement manual microscopic screening, we recently started to setup a semi-automated high-throughput forward genetics screen combining EMS mutagenesis with a system that allows fluorescence-assisted sorting of large particles (Biosorter, Union Biometrica). Using this approach will greatly increase the output of mutant candidates that lack certain selector gene-antagonizing factors. For our investigation we currently focus on the selector genes *hlh-1* (muscle) and *elt-1* (epidermis). Preliminary results from our ongoing screening will be presented at the meeting. Identified factors will help to understand the different mechanisms that restrict induced transdifferentiation, and if evolutionary conserved, will eventually help facilitating reprogramming of cell linages in mammals.

998A. A global genetic screen for the identification of factors involved in *C. elegans* spliced leader *trans*-splicing. **L. Philippe**, B. Connolly, B. Müller, J. Pettitt. School of Medical Sciences, Institute of Medical Sciences, Univ of Aberdeen, Foresterhill, Aberdeen AB25 2ZD.

Spliced-leader (SL) *trans*-splicing is the precise joining of a short exonic sequence onto the 5' end of pre-mRNAs. In *C. elegans*, this splice reaction modifies mRNAs from ~70% of protein coding genes. *C. elegans* has two types of SL RNAs, SL1 and SL2. SL2 plays a crucial role in the resolution of operon transcripts. These polycistronic pre-mRNAs are processed into monocistronic RNAs through a mechanism involving SL2, allowing RNAs to be separated and capped. In contrast, SL1 is added to mRNAs encoded by monocistronic genes and also to the 5' end of operon transcripts. It is known from work in *Ascaris* and *C. elegans* that SL *trans*-splicing involves components of the splicing machinery as well as *trans*-splicing-specific factors. But despite the fact that the basic steps in spliced leader *trans*-splicing have been described and are similar to *cis*-splicing, how these steps are achieved at the molecular level is poorly understood. To identify new genes involved in SL *trans*-splicing, we have recently developed a novel and sensitive screening strategy. This strategy allows us, for the first time in any experimental system, to visualize loss of SL *trans*-splicing *in vivo*, based on a GFP reporter. We have validated our assay by showing that it is able to detect sub-lethal defects in this process, and have used it to show the involvement of three proteins previously implicated in *in vitro* experiments. We are now carrying out genetic screens to identify genes involved in SL *trans*-splicing. To date we have identified two mutant strains that display defects in SL *trans*-splicing and are currently charactering the molecular lesions that cause reduced SL *trans*-splicing in these strains.

999B. LPR-1 facilitates LIN-3/EGF signaling during the development of the excretory system. **Pu Pu**[1], D. Freed[2], M. Lemmon[2], M. Sundaram[1]. 1) Genetics, UPenn School of Medicine, philadelphia, PA; 2) Biochemistry and Biophysics, Upenn School of Medicine, philadelphia, PA.

In the C.elegans excretory system, the EGF/Ras/Erk signaling pathway plays multiple roles in specifying the duct versus the pore cell fate and maintaining the duct organ architecture. LPR-1 belongs to the large lipocalin family which is a group of small secreted proteins functioning as carriers for lipophilic cargos. *lpr-1* loss-of-function mutants showed a highly penetrant L1 lethal excretory phenotype resembling that of *lin-3*/EGF or *let-60*/Ras mutants, whereas this lethality was almost completely suppressed by overexpressing a *lin-3*/EGF genomic fragment or hyperactivating the downstream Ras signaling pathway. Furthermore, *lpr-1* loss could suppress the excretory pore-to-duct fate transformation of *lin-15* SynMuv mutants. These epistasis results suggest that during excretory system development, LPR-1 facilitates efficient signaling when LIN-3/EGF is at endogenous or slightly increased levels, whereas LPR-1 can be bypassed by high level expression of LIN-3/EGF. Based on our epistasis results and the lipocalin research findings of others in other species, we hypothesize that LPR-1 directly binds to LIN-3/EGF or LET-23/EGFR to facilitate signaling between the adjacent excretory canal and excretory duct cells. We are testing for the *in vitro* interactions between LPR-1 and LIN-3/EGF extracellular domain or LET-23/EGFR extracellular domain using surface plasmon resonance with purified proteins from S2 or Sf9 cells. Further, in order to determine which one or more isoforms of LIN-3/EGF are functionally dependent on LPR-1 *in vivo*, we will generate single copy transgenic lines by MOS-SCI technique and then check the rescue ability of each LIN-3 isoform for *lin-3(n1417)* in the absence of LPR-1 or in the presence of LPR-1.

1000C. *In vivo* reporters for spatiotemporal regulation of genes by microRNA. **Cecile A.L. Quere**, Denis Dupuy. INSERM, Pessac, France.

Caenorhabditis elegans development is very tightly regulated, leading to the same number of cells in each individual. Part of this regulation network relies on small single strand RNAs (miRNAs), which can target homologous sequences in the 3' untranslated regions (3'UTR) of mRNAs. We want to investigate the contribution of miRNAs during neurons differentiation. In order to study the miRNA contribution to gene regulation we use double fluorescent reporters that allow us to visualize the post-transcriptional contribution to regulation throughout development. The GFP and the mCherry are expressed under the control of the gene promoter, but followed by either the 3'UTR of interest, or a control 3'UTR. We first chose as a control 3'UTR the 3'UTR of *unc-54*, a gene encoding a myosin class II heavy chain. It is expressed through all larval stages and in the adult worms. The prediction softwares do not predict any target site for miRNA regulation. Our observations showed that some cells were not expressing the control reporter while expressing the reporter associated to the endogenous 3'UTR. This was the case for 6 different genes. Moreover, constructions using the same 3'UTR showed a perfect colocalization of the two reporters excluding the possibility of an artifact linked to the fluorescent reporters. Therefore, we selected the 3'UTRs from *act-1*, *ubc-1* and *his-24*, because of their constitutive expression and the absence of miRNA target site prediction by Pictar. To confirm the potential post-transcriptional regulation of *unc-54*, we will compare the expression patterns associated with these 3'UTRs with that of *unc-54*. We will also use the promoter of *unc-119* to analyze the 3'UTR*unc-54* behavior specifically in neurons. We will perform an EMS mutagenesis screen to identify the regulators involved in this post-transcriptional regulation. With permissive 3'UTRs, we will be able to extend the characterization of post-transcriptional regulation to more genes participating in neurons development. These genes will be selected based on the prediction of a target site for miRNA expressed in neurons, like *lsy-6*, *mir-273* or *mir-81*.

1001A. The transcriptional repressor CTBP-1 functions in the nervous system to regulate gene expression. **Anna E. Reid**, Duygu Yücel, Estelle Llamosas, Sashi Kant, Hannah Nicholas. School of Molecular Bioscience, The Univ of Sydney, Sydney, NSW, Australia.

C-terminal binding proteins (CtBPs) are recruited by a variety of transcription factors to mediate gene repression. The Caenorhabditis elegans genome contains a single CtBP locus that encodes two variants of CtBP; CTBP-1a, which contains a Thanatos-associated protein (THAP) domain and CTBP-1b. We have previously shown that this THAP domain can bind DNA and may, therefore, be involved in recruiting CTBP-1a to the promoter of target genes. To determine where CTBP-1 functions, we tagged the C-terminus of CTBP-1 with mCherry using fosmid recombineering. We observed that CTBP-1 expression begins during embryonic development and continues throughout larval and adult stages. Also, we have found that CTBP-1 is widely expressed in the nervous system and in the hypodermis. In both tissues CTBP-1 localises to the nuclei, consistent with a role for CTBP-1 in regulating gene expression. To identify genes that are potential targets of CTBP-1-mediated repression, we performed microarray analysis on wild type and a ctbp-1 mutant that carries a splice site mutation, ctbp-1(eg613). 362 genes were up-regulated whilst 138 genes were down-regulated by 2-fold or greater in the ctbp-1(eg613) mutants. We have also predicted CTBP-1 target genes using CisOrtho analysis to identify promoters that contain the CTBP-1 THAP domain DNA binding site consensus sequence, which we derived from site selection experiments. By combining these data we have identified putative direct CTBP-1 target genes in both the nervous system and the hypodermis. Chen et al., 2009 previously demonstrated that a ctbp-1 mutant had an extended life span relative to wild-type. We have determined that two additional ctbp-1 mutants have extended life spans relative to wild-type. Re-expression of CTBP-1 solely in the nervous system of a splice site mutant, called ctbp-1(eg613), restored normal lifespan, indicating that CTBP-1 may function in the nervous system to regulate lifespan.

1002B. Complex expression dynamics and robustness in C. elegans insulin networks. **Ashlyn D. Ritter**[1], Yuan Shen[2], Juan Fuxman Bass[1], Sankarganesh Jeyaraj[3], Bart Deplancke[4], Arnab Mukhopadjay[5], Jian Xu[6], Monica Driscoll[6], Heidi Tissenbaum[1], A.J. Marian Walhout[1]. 1) Univ of Massachusetts Medical School, Worcester, MA, USA; 2) Children's Hospital Boston, Boston, MA, USA; 3) Univ of Tübingen, Tübingen, Germany; 4) École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; 5) National Institute of Immunology, New Delhi, India; 6) Rutgers, The State Univ of New Jersey, Piscataway, NJ, USA.

Gene families expand by gene duplication and resulting paralogs diverge through mutation. Functional diversification can include neo-functionalization as well as sub-functionalization of ancestral functions. In addition, redundancy in which multiple genes fulfill overlapping functions is often maintained. Here, we use the family of 40 Caenorhabditis elegans insulins to gain insight into the balance between specificity and redundancy. The insulin/insulin-like growth factor (IIS) pathway comprises a single receptor, DAF-2. To date, no single insulin-like peptide recapitulates all DAF-2-associated phenotypes, likely due to redundancy between insulin-like genes. To provide a first-level annotation of potential patterns of redundancy, we comprehensively delineate the spatiotemporal and conditional expression of all 40 insulins in living animals. We observe extensive dynamics in expression that can explain the lack of simple patterns of pair-wise redundancy. We propose a model in which gene families evolve to attain differential alliances in different tissues and in response to a range of environmental stresses.

1003C. Uncovering genotype specific variation of Wnt signaling in C. elegans. **M Rodriguez**[1], LB Snoek[1], T Schmid[2], N Samadi[1], L van der Bent[1], A Hajnal[2], JE Kammenga[1]. 1) Laboratory of Nematology, Wageningen Univ. Wageningen, The Netherlands; 2) Institute of Molecular Life Sciences, Univ of Zürich. Zürich, Switzerland.

Induced mutant analyses in C. elegans wild type Bristol N2 have been widely studied for understanding signaling pathways. However induced mutations in a single genetic background do not reveal genome-wide allelic effects that segregate in natural populations and contribute to phenotypic variation. For this reason there is a scanty knowledge of what the phenotypic effect of a mutation is in strains other than Bristol N2. This touches upon fundamental aspects if we want to understand the natural genetic architecture of complex phenotypic traits. Furthermore single mutations induced in C. elegans are of limited value in the approach of complex human disease pathways, for which C. elegans is an important model species, due to the complexity of most of disease pathways and their natural variation. To address this question for the Wnt/b-catenin signaling pathway we investigated the phenotypic effects of bar-1(ga80) in a population of different genotypes. Each genotype carries the bar-1 mutation in a genetic mosaic background of N2 and CB4856 alleles. We measured vulva development index, gonad migration and genome-wide gene expression across all genotypes, and identified loci on chromosome I and II associated with these phenotypes. We confirmed these QTLs in bar-1 induced mutant introgression lines (these are Bristol N2 genotypes which carry the

mutation together with a CB4856 locus). Concretely the QTL on chromosome I spanned a region of 300 Kbp harbouring 100 genes approximately. Experiments are ongoing to identify and characterize the causal modifier gene(s). By applying forward genetics in different genotypes we have revealed hidden genetic modifiers affecting Wnt/b-catenin signaling. Importantly we show that natural genetic variation provides means to study the cryptic variation harboring new players in Wnt signaling. This study was funded by EU FP7 contract nr 222936 PANACEA.

1004A. The sudden transcriptional switch to adulthood in L4 stage *C. elegans*. **L. B. Snoek**, M. G. Sterken, R. J. M. Volkers, M. Klatter, K. Bosman, R. P. J. Bevers, J. A. G. Riksen, J. E. Kammenga. Nematology, Wageningen Univ, Wageningen, Netherlands.

In *Caenorhabditis elegans* the most pronounced difference at the organism level between the juvenile/larval stages and the adult worms is the size difference. At the organ level however the large differences are found, mainly in the size and complexity of the gonad but also in nerve cells and their connections. The development of these structures during the juvenile stage is tightly and timely regulated. Before developmental changes during L4 become visually apparent a change in specific transcript levels precedes them. Since the life cycle of worms at 20°C is very short, different stages follow in rapid progression. In the last juvenile stage, L4, the worm is transformed in to a reproducing adult in a matter of hours

Most transcriptome studies in *C. elegans* are carried out at the L4 stage. Here we show that large gene expression differences related to development occur during L4 within very short time intervals. Furthermore, we show how these development specific transcriptional patterns can be used to estimate the age of other transcript profiled worms. Lastly we provide an example experiment to show how these estimated ages can be applied to find developmental differences caused by the type of bacterial food source.

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1005B. Toxicogenomic responses of *Caenorhabditis elegans* to silver nanomaterials. **Daniel L Starnes**[1], C. Starnes[2], J. Smith[3], E. Oostveen[1], J. Unrine[1], B. Collin[1], P. Bertsch[1], O. Tsyusko[1]. 1) Department of Plant and Soil Sciences, Univ of Kentucky, Lexington KY 40502, USA; 2) Center for Clinical and Translational Science, Univ of Kentucky, Lexington KY 40536, USA; 3) Department of Biostatics, Univ of Kentucky, Lexington KY 40502, USA.

There are over 2000 consumer products containing manufactured nanomaterials (MNMs) available today. One of the MNMs of major concern is silver nanoparticles (AgNPs). During wastewater treatment, AgNPs can undergo transformations resulting in partially or fully sulfidized AgNPs. Our objective is to understand the bioavailability and toxicity of polyvinylpyrrolidone (PVP) coated AgNPs (Ag-PVP) and fully sulfidized AgNPs (Ag-S) to a model organism *Caenorhabditis elegans* using a toxicogenomic approach. Since AgNP toxicity can also be determined by dissolution and release of Ag^+, to differentiate between particle and ion-specific toxicity we included $AgNO_3$ as an additional treatment. Our results showed that for AgNPs, Ag-PVP are more toxic to *C. elegans* than Ag-S due in part to a greater bioavailability and uptake as evidenced by synchrotron-based x-ray microscopy. Evidence also suggests that the observed toxicity is partially particle specific for both Ag-PVP and Ag-S because nematodes exposed to particle free supernatants showed very low mortality. Among endpoints screened, reproduction was the most sensitive and was used for the microarray study. The transcriptomic data indicate that each treatment produced a distinct genomic response. Ag^+ had the largest number of differentially expressed genes (312) followed by Ag-S (223), and lastly by Ag-PVP (136). Of the total number of significant differentially expressed genes only 3% were shared among all of the treatments and 86% were uniquely expressed for the respective treatments. The genomic data support the hypothesis that the observed responses are partially particle specific, since both Ag-PVP and Ag-S have a unique set of genes that are not shared with the Ag^+ treatment. In addition, our results demonstrate that *C. elegans* transcriptomic responses to the Ag-S are distinct from those to Ag-PVP resulting in their different toxicities.

1006C. Cadmium exposure affects insulin signaling in *Caenorhabditis Elegans*. **Y. Sun**, J. Freedman. BSB, DNTP, NIEHS, Durham, NC.

Cadmium (Cd) is a toxic metal and a cumulative environmental pollutant. The main route of Cd exposure is diet and cigarette smoke. Cd is associated with many human health problems, including cancer, heart disease and diabetes mellitus (DM). DM and diabetes-related kidney disease are serious, world-wide health problems. Although there is no direct evidence linking Cd to DM, Cd exposure alters blood glucose levels and potentiates diabetic nephropathy. The insulin/insulin-like growth factor signaling (IIS) pathway regulates multiple biological functions including glucose metabolism and longevity. C. elegans, which has an IIS pathway homologous to that of mammals, was used to investigate mechanistic links among Cd, transcription, and insulin signaling. The focus of this investigation was the *C. elegans* Cd-responsive gene *cdr-1*, whose transcription is up-regulated almost 800-fold exclusively by Cd in intestinal cells. Regulatory factors and pathways that control *cdr-1* transcription were identified by using an integrated transgenic strain of C. elegans containing GFP under the control of the 5'-regulatory region of *cdr-1*. In a candidate screen, genes involved in various stress response pathways were tested for their potential role in controlling *cdr-1* expression. Genes were knocked out either by genetic crosses to known loss-of-function mutants or by RNAi. Changes in *cdr-1* transcription were determined by measuring GFP expression or by qRT-PCR. The expression of *cdr-1*, in both the absence and presence of Cd (100 mM, 5 h) was suppressed when genes in the IIS pathway; *daf-2*, *age-1*, *daf-18*, *pdk-1*, *akt-1*, *akt-2*, *sgk-1* and *daf-16*; were knocked down. Knock down of IIS pathway-related genes; *skn-1*, *hsf-1*, *pha-4*, *pop-1*, *lin-14*, *tor-2*, *ras-1*, and *wnk-1*; also inhibited Cd-inducible *cdr-1* expression. Furthermore, knock down of *pdk-1*, *hsf-1* and *skn-1* significantly suppressed Cd-induced *cdr-1* transcript level indicating that these three genes play key roles in regulating Cd-inducible *cdr-1* expression. These results suggest that the IIS pathway mediates Cd-inducible *cdr-1* transcription. In addition, they support a model where Cd exposure could induce elevated blood glucose levels by directly affecting the IIS pathway. The mechanism by-which Cd activates the IIS pathway is currently being investigated.

1007A. Ribosomal Protein L1 regulates alternative splicing of its own pre-mRNA. **Satomi Takei**, Hidehito Kuroyanagi. Medical Research Institute, Tokyo Medical and Dental Univ, Tokyo, Japan.

Alternative splicing of pre-mRNAs regulates expression of some genes by generating non-productive mRNA isoforms that contain premature termination codons and are rapidly degraded by nonsense-mediated mRNA decay (NMD). To search for such genes comprehensively, we compared mRNA-seq data for N2 and *smg-2* mutant deficient for NMD.

Four ribosomal protein genes, *rpl-1*, *-3*, *-7A* and *-12*, encoding 60S subunit proteins have non-productive isoforms (Mitrovich, Genes & development 14 :

2173, 2000). In this study, we further found that among the rp genes, *rpl-26* and *-30* encoding 60S subunit proteins and *rps-22* and *ubl-1* encoding 40S subunit proteins also have non-productive isoforms. As *rpl-12* is known to autoregulate its own splicing in an overexpression experiment, we knocked down the 8 rp genes to reveal the effects on the alternative splicing of its own gene as well as the other rp genes. The knockdown of any of the 8 rp genes resulted in the increase of its own productive isoform, while the decrease of the productive isoforms of the other *rpl* and *rps* genes. These results suggest that these rp genes form a splicing regulatory network.

To reveal the mechanism of this splicing regulation, we focused on *rpl-1*, since a 40-bp stretch between the two alternative 5' splice sites is highly conserved in the corresponding intron in vertebrates. We constructed fluorescence *rpl-1* reporters to visualize the splicing pattern *in vivo*. The productive isoform from the wild type *rpl-1* reporter increased by *rpl-1* knockdown and the non-productive isoform increased by RPL-1 overexpression. A mutant reporter in which the conserved stretch is deleted was unaffected by knockdown and overexpression. These results indicate that the conserved stretch is essential for the splicing regulation of the *rpl-1* gene. Gel shift assays revealed that recombinant RPL-1 protein directly and specifically recognize the conserved stretch.

These results indicate that RPL-1 regulates alternative splicing of its own pre-mRNA.

1008B. High throughput EMS mutagenesis screen for cadmium response genes in *C. elegans*. **Yong-Guang Tong**, Jonathan H. Freedman. Laboratory of Toxicology and Pharmacology, National Institute of Environmental Health Sciences, RTP, NC 27709.

The heavy metal cadmium (Cd) is highly toxic in both *C. elegans* and humans. Using full-genome microarrays, we previously identified 290 Cd -responsive genes following exposure to 100 mM Cd for 4 or 24 h. RNA-mediated interference (RNAi) demonstrated that decreased expression of 50 of these genes resulted in increased Cd sensitivity. However, gene knock down by RNAi may be ineffective for some genes; therefore genetic knockout to inactivate gene function is essential. To identify and understand transcriptional regulatory networks affected by Cd and to extend our insights on genes involved in Cd resistance and hypersensitivity, we are using phenotype-driven (forward) genetics and performed an EMS high-throughput mutagenesis screen. Firstly, we determined the toxicity of Cd starting with L1 nematodes using a 48 h growth assay using COPAS biosorter. The concentration of Cd ranged from 0 to 550 mM in 50 mM intervals. High concentrations of Cd may inhibit nematode growth and arrest them in larvae stages. The median effective concentration (EC50) for Cd was 283±85 mM (±SD, N=7). After EMS treatment, F1 (L1) nematodes were grown in 96-well plates in liquid K+ media. The same stage F1 *C. elegans* were also cultured on 24-well K agar plates to screen for Cd hypersensitivity. Nematodes on all screening plates were allowed to produce F2 larvae. Afterward, Cd was added onto the plates to final concentrations ranging from 300 mM to 500 mM for the resistance screen and from 50 mM to 200 mM for the hypersensitive screen. Survivors from high concentration Cd exposures and strains containing partially paralyzed/"sick" nematodes from low concentration Cd exposures were our primary screen candidates. The effect of Cd on the growth of each candidate was determined by COPAS-based growth assay. We have examined 504,192 EMS-treated F1 nematodes in the Cd resistance screen and 12,744 EMS- treated F1s in the Cd hypersensitivity screen. One confirmed Cd resistant strain and 13 confirmed hypersensitive strains have been isolated. We are continuing our screens for additional candidates. We are also mapping the exact mutations responsible for the resistance or hypersensitivity to Cd phenotype by Next Gen sequencing.

1009C. In search of genes necessary for the identity or specification of the gonadal sheath. **Laura G. Vallier**. Dept Biol, Hofstra Univ, Hempstead, NY.

In the development of a tissue within any organism, a complex of genes must be expressed in the correct temporal and spatial manner. The gonadal sheath is a tissue composed of five pairs of cells that surrounds the proximal two-thirds of each hermaphrodite gonad arm. The gonadal sheath has four crucial functions important for fertility: 1) maintenance of the continually dividing mitotic stem cell pool 2) exit from pachytene 3) ovulation into the spermatheca and 4) promoting male germ cell fate. Gonadal sheath pair 1 (Sh1) is located distally and Sh5 is located proximally, abutted to the spermatheca. Sh3, 4, and 5 are necessary for ovulation into the spermatheca and have a more dense filament network, than do Sh1 and 2; Sh1 is necessary for the establishment of the continually dividing stem cell pool. Loss of the sheath results in sterility. Approximately 200 genes have been localized to the gonadal sheath; however, few of these have given clues as to the signals necessary or important for the establishment of identity or maintenance of these cells.

Therefore we are using the gonadal sheath to identify the gene products that either give rise to the sheath or establish its identity. Using *tnIs6* [*lim-7::GFP*] (gift of David Greenstein) to mark the gonadal sheath, we are utilizing the RNAi feeding library to search for genes whose products are necessary for the presence of the sheath. Candidate genes are those that, after feeding the dsRNA corresponding to the gene, result in hermaphrodites that do not fluoresce (no sheath) and that are sterile or have only a few progeny. After screening chromosomes I and II, we have identified forty candidate genes, of which the largest class comprises those in metabolism, transcription and translation processes; other broad categories recovered were genes necessary for signaling and transport, among others. The usefulness of this approach in uncovering new and relevant candidate genes lies in the fact that of these 40 candidates, only *smgl-1* overlaps with previously defined genes related to the gonadal sheath. We are continuing to screen the other chromosomes for additional candidates. We will report our progress.

1010A. Molecular genetic deciphering of the reproductive pathway in *Caenorhabditis elegans*. **Liesbeth Van Rompay**, Lotte Frooninckx, Isabel Beets, Liesbet Temmerman, Tom Janssen, Liliane Schoofs. KU Leuven, Leuven, Belgium.

In vertebrates, reproduction is mainly controlled by the hypothalamic-pituitary-gonadal (HPG) axis. A number of well studied G protein-coupled receptors (GPCRs) are involved in this process. Kisspeptin/GPR54 signaling is vital to puberal development and vertebrate reproduction by regulating the hypothalamic release of gonadotropin-releasing hormone, which stimulates the release of follicle stimulating hormone and luteinizing hormone from the anterior pituitary. These hormones in turn bind to their specific GPCRs in the gonads leading to the production of sex steroids, the stimulation of gametogenesis and gamete release. Although genome sequencing data show that in Ecdysozoa putative orthologs occur of the components of the vertebrate HPG axis, its counterpart in *C. elegans* has only been scarcely studied. To unravel the *C. elegans* reproductive signaling system in more detail, we search for putative upstream molecular players that influence the process of vitellogenesis by applying both a forward and reverse genetic approach. An EMS mutagenesis screen was conducted using a transgenic strain that is able to express the integrated GFP reporter under the control of the *vit-2* promoter. A handful of promising mutants showing significantly altered expression of *gfp* were identified by means of a single-step SNP mapping strategy

coupled with whole-genome sequencing. A list of possible variants, including a low density lipoprotein receptor-related protein, a glutamate receptor, and an unannotated hypothetical protein, was retrieved by use of the CloudMap pipeline to analyze the mutant genome sequences. To further unveil the involvement of neuropeptidergic signaling, we carried out an additional RNAi screen of candidate genes, including all predicted neuropeptide GPCR and neuropeptide precursor genes. Genes originating from both screens will be thoroughly characterized by use of a range of morphological and physiological assays.

1011B. Exploring Gene Expression and Transcriptional Regulation Data in WormBase. **Xiaodong Wang**, Wen Chen, Daneila Raciti. WormBase Consortium, Biol Div, Caltech, Pasadena, CA.

Gene expression studies not only inform us on potential gene function but also serve as a measure of cellular and organismal phenotype and provide the foundation for building transcriptional regulatory networks. WormBase offers extensive coverage of C. elegans gene expression from small-scale as well as genome-scale studies such as modENCODE. WormBase includes data from expression results based on reporter gene analysis, immunostaining, in situ hybridization, single molecule FISH (smFISH), RNAseq, and microarrays, among others. Recently, we have started to curate gene expression data from other species. Over the past four years WormBase has adapted SPELL as a tool to access large-scale gene expression data. These now include microarray, tiling array, and RNAseq data. The WormBase implementations of SPELL include the following functions: 1. A display of expression levels of individual genes in each experimental dataset. 2. A search capability for genes with similar expression profiles and biological processes. 3. Access to whole experiment datasets, which are available for download. All SPELL microarray, tiling array and RNAseq data are mapped to the current WormBase release, keeping these data current with the latest gene models. Transcriptional regulation information is captured from reported changes in gene expression due to gene mutation, small molecule/chemical, or heat shock/physical treatment. These data can be viewed as part of gene interaction networks using Cytoscape as an interactive browser. In addition, WormBase accommodates a number of canonical transcriptional factor binding sites using Position Weight Matrix (PWM)/Position Frequency Matrix (PFM) data obtained through literature curation. These data can be accessed with the MotifFinder plugin in GBrowse. Large-scale transcriptional regulation data from modEncode, such as ChIp-seq, transcription factor binding sites (enhancers, silencers, promoters), histone modification sites, and DNAseI hypersensitivity sites, etc., are also integrated into WormBase. These data can also be viewed with GBrowse in their own track. In this poster we will give an overview of the gene expression and regulation data and the ways to access and mine these data.

1012C. Determining the time and tissue specific expression of genes during embryogenesis. **Adam D. Warner**, Chau Huynh, Robert H. Waterston. Genome Sciences, Univ of Washington, Seattle, WA.

Embryonic development is a tightly controlled and regulated process in many species, with a complex network of transcription factors regulating gene activity. In *C. elegans*, the process is so tightly controlled that the lineage of cell divisions is invariant and has been fully mapped (Sulston et al, 1983). The set of genetic messages at an embryo's earliest stage is completely maternal, transitioning over time to being generated completely from its own DNA. Determining which genes become active at specific time points provides insight into when key regulatory events occur, and determining the total network of gene expression in each cell type over time will help map out developmental processes unique to each tissue. Previous studies have utilized a combination of FACS, SAGE, and microarrays (Meissner et al, 2009; Spencer et al, 2011) to gain insight into what genes are expressed in late and mixed stage embryonic tissues. More recently, RNA-Seq has been used for expression studies, producing gene expression data with advantages over SAGE and microarrays such as a broader range of expression levels, differentiation between gene isoforms, and information on every transcript expressed (reviewed in Wang et al, 2009). We have previously shown the ability to isolate time synchronized whole embryos at specific time points throughout embryonic development (see abstract from Max Boeck), and we aim to do the same with isolated tissue types by utilizing a collection of GFP and mCherry labeled *C. elegans* strains (Murray et al, 2012; Sarov et al, 2012) that label individual cells and tissues. Using FACS to isolate specific cell subsets at discrete time points and RNA-Seq for transcript identification and quantification, we will be able to create a map of embryonic gene expression not only specific to individual cells and tissues, but specific to those cell and tissue types over time. This will build upon and complement previous data by adding temporal information and added sensitivity. Clustering of genes with similar expression patterns to genes with known roles in development, combined with emerging ChIP-Seq data, will help identify novel genes involved in specific developmental processes.

1013A. Identification of *cis*-regulatory elements that confer zinc-responsive transcription in intestinal cells of *C. elegans*. Hyun Cheol Roh, Ivan Dimitrov, Krupa Deshmukh, Guoyan Zhao, **Kurt Warnhoff**, Daniel Cabrera, Wendy Tsai, Kerry Kornfeld. Washington Univ in Saint Louis, St. Louis, MO.

Zinc is an essential metal involved in a broad range of biological processes; it serves as a structural and/or enzymatic cofactor in many proteins and as a signaling molecule. Because both zinc deficiency and excess adversely affect the health of humans and other animals, sophisticated mechanisms have evolved to mediate zinc homeostasis. One important homeostatic mechanism is regulation of transcription in response to fluctuations of dietary zinc. To use *C. elegans* to characterize zinc-regulated transcription, we identified four genes that are involved in zinc metabolism and displayed transcriptional induction by dietary zinc; *mtl-1* and *mtl-2* encode metallothionein proteins that are predicted to bind and sequester zinc ions, and *cdf-2* and *ttm-1b* encode CDF family zinc transporters. Using a bioinformatic motif search, we identified a conserved DNA element in these four zinc-responsive genes, which we named the *C. elegans* zinc-responsive element (ZRE). The ZRE was conserved in other nematode species and also present in previously described cadmium-responsive genes. Mutagenesis studies in transgenic animals showed that the ZRE was necessary for zinc-mediated transcriptional induction in intestinal cells. Furthermore, the motif was sufficient to confer zinc responsiveness on a basal promoter. ELT-2 is a transcription factor that is critical for intestinal development, and we noticed that the ZREs were closely associated with a predicted binding site for ELT-2. We demonstrated that the predicted ELT-2 binding site was necessary in *cis* and the ELT-2 transcription factor was necessary in *trans* for zinc-mediated transcriptional induction. These findings suggest that the coordinated activity of the ZRE and the ELT-2 binding site mediate zinc-responsive transcriptional activation in intestinal cells. We are using this signature of a ZRE and an ELT-2 binding site to computationally predict novel zinc-responsive genes. These studies advance our understanding of molecular mechanisms of zinc-responsive transcriptional regulation and contribute to understanding zinc metabolism and homeostasis.

1014B. Integration of metabolic and gene regulatory networks governs the transcriptional response to diet in *C. elegans*. **Emma Watson**[1,2], Lesley MacNeil[1,2], H. Efsun Arda[3], Lihua Julie Zhu[4], Albertha J.M. Walhout[1,2]. 1) Program in Systems Biology, Univ of Massachusetts Medical School, Worcester, MA; 2) Program in Molecular Medicine, Univ of Massachusetts Medical School, Worcester, MA; 3) Department of Developmental Biology, Stanford Univ School of Medicine, Stanford, CA; 4) Program in Gene Function and Expression, Univ of Massachusetts Medical School, Worcester, MA.

Diet greatly influences an organism's physiology and metabolism. Dramatic changes in gene expression occur following dietary shifts in C. elegans, yet the regulatory networks that mediate these dietary effects remain largely unknown. We performed complimentary forward and reverse genetic screens in a transgenic C. elegans strain that expresses GFP under the control of a diet-sensitive promoter, *Pacdh-1*. Specifically this strain expresses high levels of GFP when fed E. coli OP50 and low levels of GFP when fed the soil bacterium Comamonas DA1877. *acdh-1* encodes a short-branched chain acyl-CoA dehydrogenase predicted to function within branched chain amino acid (BCAA) breakdown. Interestingly, we uncovered transcriptional feedback within a metabolic subnetwork consisting of BCAA breakdown and methionine recycling pathways, which serves to integrate dietary input with internal metabolic flux to regulate genes that function within the subnetwork. We identify several novel NHRs as candidate members of a gene regulatory network responsible for monitoring and controlling this metabolic subnetwork. When mutated in humans, orthologous BCAA and methionine metabolic genes cause inborn errors of metabolism, which are clinically managed by dietary intervention. Understanding how these metabolic genes are regulated in response to diet may lead to better therapies for these patients. Altogether, our work establishes C. elegans as a powerful Nutrigenomic model that can provide insights into the effects of diet on gene expression and metabolism, and the regulatory networks involved.

1015C. Transcriptional regulation in the intestine. **T. Wiesenfahrt**[1], J. Berg[1], E. Osborne Nishimura[2], J. McGhee[1]. 1) Univ of Calgary; 2) Univ of North Carolina, Chapel Hill.

The GATA type transcription factor ELT-2 is proposed to be the major regulator of transcription in the C. elegans intestine after endoderm specification. Ectopic expression of END-1 and END-3, redundant GATA factors necessary for endoderm specification, or ELT-7 (another intestine specific GATA factor) can initiate ectopic expression of ELT-2, suggesting that END-1, END-3 and ELT-7 can activate elt-2 expression in the earliest endoderm lineage either directly or indirectly. Previous experiments showed that ELT-2 can bind to its own promoter in vivo. To understand the molecular details of how elt-2 transcription is initiated during embryonic development and is maintained thereafter, we are analyzing the promoter region of elt-2 in C. elegans. Comparison of upstream sequences of the elt-2 gene from 4 different Caenorhabditis species revealed three conserved regions (CRI-CRIII). 3' and 5' Deletion series as well as analysis of reporter constructs containing different combinations of the CRs suggested that CRI contains the basal promoter and CRIII contains the main enhancer of elt-2. There are 3, 3 and 4 conserved GATA sites within CRI, CRII and CRIII respectively. Band shift assays showed that END-1 and ELT-2 can bind to at least one and all four GATA sites within CRIII in vitro respectively. Reporter expression was absent after mutating all GATA sites within a CRIII:CRI:gfp construct, indicating that the GATA sites are necessary for elt-2 expression and that elt-2 is mainly regulated by GATA factors. To test if ELT-2 can drive intestinal specification and differentiation in the absence of END-1/-3, we expressed elt-2 under control of the end-1 promoter in the end-1/-3 double mutant. Indeed, the end-1p::elt-2 construct is able to rescue the end-1/-3 double mutant with 50% penetrance, showing that the endoderm differentiation factor ELT-2 can also drive endoderm specification. We engineered an end-1/end-3/elt-4/elt-7 quadruple mutant and the end-1p:elt-2 construct is also able to rescue this mutant showing that ELT-2 is the only GATA type transcription factor needed for intestinal development and further supporting the hypothesis that ELT-2 is involved in the regulation of every gene expressed in the intestine.

1016A. Phenotype analyses and expression of the sphingomyelin synthase genes in *Caenorhabditis elegans*. **Haruka Yamaji**[1], Yukako Tohsato[1], Kenji Suzuki[2], Masahiro Ito[1]. 1) Depart. of Bioinfo, College of Life Sci, Ritsumeikan Univ, Shiga 525-8577, Japan; 2) Depart. of Pharmacy, College of Pharma. Sci, Ritsumeikan Univ, Shiga 525-8577, Japan.

Sphingolipids have previously been considered as merely structural molecules in eukaryotes. However, recent studies have reported that sphingolipids function as signal transduction molecules and are involved in the control of differentiation, proliferation, and apoptosis. Sphingomyelin synthase (SMS) catalyzes the formation of sphingomyelin from ceramide and phosphatidylcholine. Sequence analysis has revealed that *Caenorhabditis elegans* has 3 *sms* genes (*sms-1*, *sms-2*, and *sms-3*). We characterized the mRNA expression levels and phenotypes of *sms-1*, *sms-2*, and *sms-3* in *C. elegans*. For the mRNA expression analysis, the mRNA levels of target genes in the wild type and each mutant strain were measured and compared by quantitative real-time reverse transcription PCR assay and the DDCt method. We found that the expression level of *sms-1* increased in the *sms-3* mutant strain and that of *sms-3* increased in the *sms-1* mutant strain. The expression level of *sms-2* was the lowest in all strains. From these results, we suggested that functional variations exist between *sms-2* and the other 2 genes. For the phenotype analysis, we compared the phenotypes of the wild type and *sms* gene mutants. Fat and short worms were observed in all *sms* gene mutants. Furthermore, in the *sms-1* mutant strain, an intestinal cavity was observed. The accumulation of neutral fat was observed by the Nile Red staining technique. Excessive fat accumulation was observed in the intestines of the mutant worms.

1017B. Investigating the expression and function of a C. elegans chemosensory receptor. **Jinzi Yang**[1,2], Harleen Basrai[1,2], Alisha Anderson[2], Stephen Trowell[2], Carolyn Behm[1]. 1) Australian National Univ, Canberra, ACT, Australia; 2) CSIRO Ecosystem Sciences, Black Mountain Laboratories, Canberra, Australia.

Olfaction is of central importance for most species. All living organisms can use information from the external environment to guide behavioural responses. Our research is focussed on understanding how this information is collected and integrated using the biological model C. elegans. This species has a highly developed chemosensory system that enables it to detect a wide variety of volatile cues associated with food, danger, or other animals. Chemosensory cues can elicit chemotaxis, rapid avoidance, changes in overall motility, and entry into and exit from the alternative, dauer, developmental stage. These behaviours are regulated primarily by the amphid chemosensory organs, which contain eleven pairs of chemosensory neurons. Each amphid sensory neuron expresses a specific set of candidate receptor genes and detects attractants, repellents, or pheromones. Approximately 600 different putative chemosensory G-protein coupled receptors are present in the C. elegans genome, however the ligands are known for only one olfactory receptor, ODR-10. I am interested in characterising the expression and function of a number of nematode odorant receptors in order to understand how their inputs to the chemosensory system may be integrated. So far, I have made transgenic worms expressing a reporter gene under the control of the *str-112*

chemosensory receptor promoter, the receptor most closely related to the olfactory receptor ODR-10. Expression analysis using confocal microscopy has shown that *str-112* is expressed in two amphid neurons. I have used DiI staining and genetic crosses with mcherry-labelled reference worms to rule out *str-112* expression in AWA and AWB and AWC olfactory neurons. This result suggests *str-112* may not have a role in olfaction. Next, I will use another mcherry reference strain to determine the exact cellular location of the *str-112* chemosensory receptor.

1018C. Independent Regulation of Metabolism but Coordinated Control of Tissue Development by Epidermis Specific Proteins in *Caenorhabditis elegans* . Jiaofang Shao[1], Kan He[1], Hao Wang[2], Vincy Ho[1], Xiaoliang Ren[1], Xiaomeng An[1], Ming-Kin Wong[1], Bin Yan[1], Dongying Xie[1], John Stamatoyannopoulos[1], **Zhongying Zhao[1]**. 1) Department of Biology, Hong Kong Baptist Univ, Hong Kong; 2) Department of Genome Sciences, Univ of Washington, Seattle,WA.

Cell fate specification demands a hierarchy of regulatory events. Initial specification is typically achieved by a master regulator which is relayed by tissue-specific regulatory proteins usually transcription factors for further enforcement of cell identities, but how the factors are coordinated between each other to "finish up" the specification remains poorly understood. *C. elegans* epidermis specification is initiated by a master regulator ELT-1 which subsequently activates its targets NHR-25 and ELT-3, two epidermis specific transcription factors, thus providing a superior paradigm for illustrating how the tissue specific regulatory proteins work together to enforce cell fate specification. Here we addressed the question through contrasting genome-wide *in vivo* binding targets between NHR-25 and ELT-3 that are important for epidermis development but not required for its initial specification in *C. elegans*. We first identified *in vivo* binding targets of NHR-25 by ChIP-seq and then compared them with those of ELT-3, the result of which demonstrated apparently differential regulation of metabolism but coordinated control of epidermal development between the two. Functional validation of the targets demonstrated that both activating and inhibitory roles of NHR-25 in regulating its targets. We further showed differential regulation of specification of AB and C lineage derived epidermis. Our results provide insights into how tissue specific regulatory proteins coordinate with one another to enforce cell fate specification initiated by its master regulator. Combined with functional analysis, we assembled a comprehensive gene network underlying *C. elegans* epidermis development and physiology which are likely to be broadly used across species.

1019A. Knock-down of nuclear pore subunit NPP-11 suppresses the germline apoptosis and differentiation defects of *C41G7.3* mutants in *C. elegans*. **Xue Zheng[1,3]**, Ataman Sendoel[1], Deni Subasic[1,3], Anneke Brümmer[2], Shivendra Kishore[2], Mihaela Zavolan[2], Michael Hengartner[1]. 1) Institute of Molecular Life Sciences, Univ of Zurich, Zurich, Switzerland; 2) Bioinformatics Biozentrum, Univ of Basel, Basel, Switzerland; 3) Molecular Life Sciences PhD program, Life Science Zurich Graduate School, ETH / Univ of Zurich, Zurich, Switzerland.

RNA binding proteins (RBPs) play an important role in regulating gene expression by controlling mRNA stability, subcellular localization and translation. In an RNAi-based screen for candidates that regulate germline apoptosis in *C. elegans*, we identified a new KH domain RNA binding protein - C41G7.3 - which controls germ cell apoptosis. Loss of C41G7.3 function increases germline apoptosis. Additionally, *C41G7.3* mutant animals also show other defects including loss of germline integrity, reduced brood size and delayed egg laying. We used high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) to predict the C41G7.3 binding motif sequence and identify its potential target mRNAs. Based on the HITS-CLIP data, we selected 160 candidates genes that contain a strong C41G7.3 consensus binding motif sequence in their 3'-UTR. As RBPs often act as negative regulators of gene expression, we tested whether RNAi knockdown of these 160 candidate targets could reverse any of the C41G7.3 phenotypes. Knock-down of one candidate, *npp-11*, could largely reverse the germline defects in C41G7.3 mutants. *npp-11* codes for a subunit of the nuclear pore complex (NPC). In *C. elegans*, 23 different nuclear pore complex subunits have been identified; however, the exact molecular function for many of them remains unknown. We found that RNAi knock-down of several other nuclear pore subunit genes could also rescue the germline defects of *C41G7.3* mutants, suggesting that interfering with NPC function can largely compensate for the lack of C41G7.3. Our next goals are to characterize in more detail the molecular basis of the defects in C41G7.3 mutants, to determine how this RBP regulates expression of target mRNAs such as *npp-11*, and to understand how modulation of NPC activity can contribute to the regulation of gene expression.

1020B. Heat shock activates a miRNA-dependent response pathway in *Caenorhabditis elegans*. **Antti P. Aalto**, Ian A. Nicastro, Amy E. Pasquinelli. Division of Biological Sciences, Univ of California, San Diego, La Jolla, CA, USA.

MicroRNAs (miRNAs) are small noncoding RNAs that have been shown to be essential for development, cellular homeostasis and disease in most eukaryotic organisms. MiRNAs are also known to regulate gene expression during stress conditions, but a comprehensive view on the extent of miRNA-dependent responses after stress is currently lacking. We have performed high-throughput (small and total) RNA sequencing in *C. elegans* subjected to acute and chronic heat shock conditions. Our analysis takes advantage of a mutant strain of Argonaute-Like Gene 1 (*alg-1*) that is defective in miRNA-dependent gene expression regulation. Comparing the sequencing results of wild-type N2 animals before and after heat shock revealed that distinct miRNA populations are present in these conditions. Our results also suggest that many of the changes that occur on the mRNA level during stress are dependent on a functional miRNA pathway. The mRNAs of several heat shock proteins and glutathione S-transferases are upregulated during heat shock in wild-type, but not in *alg-1* mutant animals. Conversely, Prion-like-(Q/N-rich)-domain-bearing proteins seem to be more highly expressed when the ALG-1 protein is missing. These observations suggest that the miRNA pathway plays a critical role in preventing the accumulation of misfolded and potentially toxic proteins during an episode of heat shock. In addition, we have analyzed the effects of stress on *C. elegans* longevity. We find that a mild heat shock induces a hormetic effect that extends the lifespan of the nematode, and that this effect is missing in the *alg-1* mutant strain. Thus, the miRNA pathway contributes to the extended survival of worms that have previously experienced heat stress. Our studies show that the miRNA pathway plays an important role in the organismal response to heat stress and reveal specific miRNAs and regulatory targets involved in this process.

1021C. Analysis of microRNA regulation of defecation behavior. Benedict J. Kemp[1], Adele Gordon[1], Carmela Rios[1], Megan Mohnen[1], Spencer Agnew[1], Julien Aoyama[2], **Allison L. Abbott[1]**. 1) Department of Biological Sciences, Marquette Univ, Milwaukee, WI; 2) Department of Biology, Amherst College, Amherst, MA.

The defecation motor program is a well studied rhythmic behavior in worms. The defecation motor program occurs every ~50 sec and comprises three sequential muscle contractions: a posterior body contraction (pBoc), and anterior body contraction (aBoc) and an enteric muscle contraction followed by

expulsion (Emc/Exp). The execution of the defecation motor program depends on intercellular calcium waves that initiate in the posterior intestine. Thus, the posterior intestine functions as the pacemaker for this rhythmic behavior. We have found that the microRNA miR-786 is necessary for the supremacy of the posterior cell in the rhythmic initiation of calcium waves. The fatty acid elongase, *elo*-2, is one target for miR-786 in the posterior intestine. However, the mechanism whereby miR-786 repression of *elo-2* acts to regulate calcium wave initiation in the posterior intestine is unclear. The ELO-2 enzyme functions in the elongation of palmitate and reduced *elo-2* activity results in elevated palmitate levels. Therefore, we hypothesize that higher levels of palmitate due to miR-786 repression of *elo*-2 in the posterior intestine results in enhanced protein palmitoylation of critical membrane proteins that regulate calcium. To test this, we are examining the role of the 14 DHHC palmitoyl acyl-transferase genes and the palmitoyl-protein thioesterase gene, *ppt-1* in the regulation of defecation. In addition, we are screening miRNA deletion mutants and miRNA biogenesis mutants to characterize miRNA regulation of defecation behavior.

1022A. The Visual Detection of odr-1 22G RNAs via a MosSCI Sensor System. **Adriel-John Ablaza**[1], Bi-Tzen Juang[2], Noelle L'Etoile[2], Maria Gallegos[1]. 1) Department of Biological Sciences, California State Univ East Bay, 25800 Carlos Bee Blvd., Hayward, California 94542; 2) Department of Cell and Tissue Biology, Univ of California, San Francisco, 513 Parnassus Ave., San Francisco, California 94143.

Caenorhabditis elegans forages for food by distinguishing between various odorants in a dynamic environment. Their sensory neurons have the ability to adapt to persistent odors when the animal is starved (Bargmann, 2006). Adaptation to specific odors takes place in the AWC, a paired olfactory sensory neuron. In the AWC, a transmembrane guanylyl cyclase, odr-1, is required for chemotaxis towards all odorants. Prolonged odor exposure results in decreased chemotaxis, which correlates with a decrease in odr-1 mRNA. Odor adaptation is initiated by the translocation of a protein kinase, EGL-4, into the nucleus of the AWC (L'Etoile et al., 2002; Lee et al., 2010). Nuclear EGL-4 promotes a 22G directed repression of the odr-1 gene thereby initiating long-term odor adaptation (Juang et al., submitted). ChIP indicated that the heterochromatin state at the odr-1 locus increases during AWC odor adaptation and this increase requires 22G RNA synthesis. However, qRT-PCR and ChIP analysis does not offer a dynamic or cell-specific readout of odr-1 22G RNA function. Here we describe our plans to create a fluorescent reporter that is capable of tracking changes in odr-1 22G RNA function in live worms as they adapt to odor. The sensors that detect odr-1 22G RNAs are inserted via Mos Single Copy Insertion (MosSCI). These sensors will have the capability to detect specific odr-1 22G RNA function in the AWC olfactory sensory neuron as well as cells throughout the worm. The creation of a single copy insertion of an odr-1 small RNA sensor will allow us to test our hypothesis that a small RNA-directed pathway is dynamically activated during olfactory adaptation. In addition, this tool will allow us to understand whether odr-1 22G RNA represses transcription of our reporter in other cells in the worm.

1023B. CEY-1 attenuates *let-7* microRNA-mediated silencing in *C. elegans*. **Amelia F. Alessi**[1], Vishal Khivansara[1], Sang Young Chun[1], James J. Moresco[2], John R. Yates III[2], John Kim[1]. 1) Life Sciences Institute, Univ of Michigan, Ann Arbor, MI; 2) The Scripps Research Institute, La Jolla, CA.

MicroRNAs (miRNAs) are an abundant superfamily of small regulatory RNAs that control diverse cellular and developmental processes. MiRNAs function in the miRNA induced silencing complex (miRISC) to trigger translational repression and destabilization of target mRNAs. The miRISC is guided to targets by its bound miRNA, which recognizes partially complementary sequences in target 3'UTRs. Recent studies suggest miRISC activity is modulated by accessory factors. We have identified the RNA binding protein (RBP) CEY-1 as a negative regulator of *let-7* miRISC activity. *let-7* is a broadly conserved miRNA that regulates cellular differentiation in animals. Its dysregulation is also a hallmark of many cancers. *C. elegans let-7* is essential for viability; it controls developmental timing, including the temporal specification of cell fate. *cey-1* mutants potently suppress the hypodermal seam cell hyperplasia and highly penetrant vulval defects of *let-7* hypomorphic mutants. At the molecular level, our data suggest CEY-1 binds RNA to antagonize let-7 miRISC activity: loss of *cey-1* in *let-7* mutants suppresses upregulation of key *let-7* targets including *lin-41*, immunopurification of CEY-1 complexes recovers *lin-41* transcripts, and high-throughput sequencing of RNA isolated by crosslinking and immunoprecipitation (HITS-CLIP) identifies CEY-1 binding sites in the 3'UTR of *let-7* targets. Interestingly, CEY-1 and the *let-7* biogenesis and stability factor LIN-28 are both part of a deeply conserved family of RBPs homologous to prokaryotic cold-shock proteins (CSPs). Our data implicate a novel, non-redundant function of a second CSP-like protein in modulating *let-7* miRNA activity. We are currently working to further define the molecular relationship between CEY-1 and *let-7* miRISC and elucidate if this relationship is functionally conserved in mammals.

1024C. LIN-28-dependent repression of *let-7* miRNA is required for oogenesis. **Yoshiki Andachi**[1,2], Yuji Kohara[1,2]. 1) Genome Biol Lab, National Inst Genetics, Mishima, Japan; 2) Dept Genetics, SOKENDAI, Mishima, Japan.

let-7 microRNA (miRNA) plays important roles in various phases of development. The maturation of *let-7* miRNA is inhibited by binding of LIN-28 protein to precursors of the miRNA at early larval stages. Although *lin-28* mutants show a precocious phenotype at the L2 stage in seam cells and a protruding vulva phenotype, the defects are not suppressed by *let-7* mutation. To elucidate the biological significance of LIN-28-dependent repression of *let-7* miRNA, we examined the gonad because some *lin-28* mutant worms were sterile. While the ventral half of the adult gonad consists of a row of oocytes in wild-type worms, the region occupied by oocytes was shortened proximally and the syncytium containing pachytene-stage nuclei extended to the remaining part in *lin-28* mutants. The gonadal abnormalities was not only rescued by *lin-28* transgenes, but also suppressed by *let-7* mutation. In the syncytium adjacent to oocytes, MPK-1 is activated by phosphorylation in wild-type worms. The activated MPK-1 was reduced in *lin-28* mutants, but not in *lin-28; let-7* double mutants. We searched for target genes of the ectopically expressed *let-7* miRNAs by our screening method, in which endogenous miRNAs binding to mRNAs were used as reverse-transcription primers for the synthesis of cDNAs from the mRNAs, and clones of the cDNAs were sequenced to identify target genes. Several genes were obtained both from *lin-28* mutant worms and *lin-28(RNAi)* worms, but not from worms RNAi-depleted for control. Of them, gonadal defects similar to but weaker than those in *lin-28* mutants were observed in *pqn-47* mutants whose L1 arrest phenotype was rescued by an extra-chromosomal array including the *pqn-47* coding sequence. Triple mutant analysis revealed that *pqn-47* was epistatic to *lin-28; let-7*. *hbl-1* mutants also exhibited the extension of the syncytium to the ventral side at low penetrance, and the mutation enhanced the gonadal defects in *pqn-47* mutants. The activated MPK-1 was reduced in *pqn-47; hbl-1* double mutants. These results suggest that LIN-28-dependent repression of *let-7* miRNA is required for oogenesis, and the gonadal defects in *lin-28* mutants are due to down-regulation of both *pqn-47* and *hbl-1* by ectopically expressed *let-7* miRNAs.

1025A. Cytoplasmic versus nuclear RNAi mechanisms in transgene-induced gene silencing in Caenorhabditis elegans. **Nadeem Asad**, Laticia Rivera, Arthur Ankeney, Raeann Whitney, Lisa Timmons. Molecular Biosciences, Univ of Kansas, Lawrence, KS.

A phenotypic response to experimentally delivered dsRNA involves a complex set of mechanisms, acting in multiple cell compartments and tissues throughout developmental time. There are several methods for effective delivery of dsRNA to Caenorhabditis elegans, and most studies of RNAi mechanisms make use of “feeding” strategies. Transgene-delivery of dsRNA is also an effective strategy to elicit an RNAi phenocopy. In ingestion-based delivery methods, such as “feeding”, the dsRNA molecules enter cells from environmental sources, whereas in transgene-based delivery, dsRNA molecules are born in the nucleus. The cellular mechanisms that are triggered by experimental delivery of dsRNAs also respond to foreign nucleotides, such as viral and transposon sequences, in both the cytoplasm and the nucleus. dsRNAs are delivered into the cytoplasm from opposite directions in “feeding” versus transgene delivery of dsRNAs. We are investigating the RNAi mechanism with respect to the delivery method in order to determine whether the directionality of dsRNA movement into the cytoplasm can involve specific RNAi pathways in gene silencing. In particular, we are interested to know if transgene delivery of dsRNAs can more effectively trigger transcriptional gene silencing mechanisms. Our genetics-based studies indicate that, even though transgene-derived dsRNA is transcribed in the nucleus, this method relies on cytoplasmic RNAi machinery that acts in the cytoplasm. Also, purposeful expression of hairpin dsRNAs was not an effective strategy to induce mut-7-dependent co-suppression. In keeping with this model, NRDE-3, an argonaute protein predicted to shuttle silencing RNAs into the nucleus, had no effect on the RNAi responses from RNAi-proficient transgenes. Surprisingly, transgenes with negligible RNAi activity in wild-type animals regained RNAi activity in nrde-3 mutants, and from these results, we infer that such dsRNA-expressing transgenes are more susceptible to NRDE-3 silencing than the mRNA target. A variety of factors can influence the RNAi competency of transgene, including copy number of dsRNA-expressing cassettes, transgene design, temperature, maternal effects, and inter-compartmental movement of silencing RNAs.

1026B. Identification of endogenous *let-7* miRNA target sites by iCLIP. **James P Broughton**[1], Michael T Lovci[2], Gene W Yeo[2], Amy E Pasquinelli[1]. 1) Division of Biology, UC San Diego, La Jolla, CA; 2) Department of Cellular & Molecular Medicine - Stem Cell Program, UC San Diego, La Jolla, CA.

A class of small RNA molecules, known as microRNAs (miRNAs), provides post-transcriptional control of gene expression through imperfect binding to target RNAs. This flexibility in binding allows a single miRNA to guide the miRNA Induced Silencing Complex (miRISC) to a range of transcripts. The core component of miRISC, an Argonaute protein, is responsible for binding the miRNA and recruiting other miRISC factors. In *C. elegans*, ALG-1 is the primary Argonaute responsible for miRNA function. Past approaches for identifying miRNA targets have relied on *in silico* methods and reporter constructs, which lack biological context. Consequently, defining endogenous targets remains an important challenge in understanding miRNA function. Recent advances in determining RNA-protein interaction sites through the use of UV crosslinking and immunoprecipitation (CLIP) methods have provided comprehensive views of Argonaute mediated miRNA targets in a variety of systems. While these studies provide evidence for miRNA regulation of a particular transcript, they do not allow for unambiguous determination of which miRNA is responsible for that regulation. This is particularly true for families of miRNAs that share identical ‘seed’ sequences - nucleotides 2 through 7. To address this issue, we have adapted individual-nucleotide resolution CLIP (iCLIP) for use in *C. elegans* and used it to identify endogenous *let-7* targets. The *let-7* miRNA has previously been shown to be important in regulating *C. elegans* development and is highly conserved throughout bilateral animals. Additionally, *let-7* has been shown to interact with non-coding RNA. In *let-7(n2853)* animals, mature *let-7* contains a point mutation in the seed sequence, which decreases the binding efficiency of mature *let-7* to its target transcripts. Additionally, *let-7(n2853)* mutants express significantly less mature *let-7*. By comparing miRNA targets identified by iCLIP between wild-type and *let-7(n2853)* animals, we have comprehensively identified endogenous ALG-1 mediated *let-7* targets.

1027C. *Mir-34* and *mir-83* protect *C. elegans* gonad morphogenesis against temperature fluctuations. **Samantha Burke**[1], Molly Hammell[2], Victor Ambros[1]. 1) Molecular Medicine, Univ of Massachusetts Medical School, Worcester, MA; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mir-34 is a well conserved microRNA that has been implicated in senescence and apoptosis in mammals. We examined *C. elegans mir-34* mutants for developmental phenotypes and determined that the mutants display incompletely penetrant gonad migration defects, specifically wandering gonad arms. The incomplete penetrance of this phenotype suggested co-regulation by additional microRNAs, therefore we tested for genetic enhancement of the *mir-34* phenotype by the loss of other microRNAs sharing predicted targets. Deletion of one particular microRNA, *mir-83*, the ortholog of mammalian *mir-29*, causes a similar migration defect. *Mir-34; mir-83* double mutants exhibit the migration defect with increased penetrance, suggesting that *mir-34* and *mir-83* function redundantly in the regulation of targets involved in gonad migration during larval development. The phenotype is further enhanced when worms are subjected to temperature fluctuations - for example, four cycles between 15°C and 25°C over the course of two hours. The sensitive period for temperature fluctuations occurs in the L1 stage concurrent with the birth of the distal tip cells, before the cells begin their migration. This enhancement is independent of both the magnitude and initial direction of temperature change. Epistatic analysis suggests that *mir-34* and *mir-83* help regulate the activity of the integrin and GTPase signaling network responsible for distal tip cell migration by targeting both *pat-3* and *cdc-42*. Knockdown of either *pat-3* or *cdc-42* suppresses the migration defect seen in *mir-34; mir-83* worms suggesting that the error-prone gonadal migration in *mir-34; mir-83* double-mutant animals results at least in part from elevated integrin and GTPase signaling.

1028A. Receptor of Activated C Kinase RACK-1 may regulate the *Caenorhabditis elegans* heterochronic gene pathway at the larva-to-adult transition. **Shih-Peng Chan**[1], Yu-De Chu[1], We-Chieh Wang[1], Shi-An Chen[1], Frank Slack[2]. 1) Graduate Institute of Microbiology, College of Medicine National Taiwan Univ, Taipei, Taiwan; 2) Department of Molecular, Cellular and Developmental Biology, Yale Univ, New Haven, CT 06520, USA.

The *C. elegans* heterochronic gene pathway regulates the development at each larval stage and the terminal differentiation of hypodermal cells at the larva-to-adult transition. The terminal differentiation requires normal expression of the heterochronic *let-7* microRNA at the L3/L4 stage to down regulate its target *lin-41* that represses the direct regulatory gene *lin-29* for adult cell fate. The multi-functional scaffolding protein Receptor of Activated C Kinase (RACK-1), a member of WD-repeat protein family sharing significant homology the b subunit of G-proteins (Gb), has been shown to facilitate miRNA-induced silencing complex (miRISC) binding to the ribosome and hence to benefit the *let-7* miRNA function in the heterochronic gene pathway. In the previous studies, reduction of RACK-1 by RNAi triggered moderate heterochronic phenotypes of *let-7* family miRNA mutants in wild-type worms, including

defects in hypodermal cell and vulva development. Interestingly, in contrast to the notion that RACK-1 is required for miRNA function, we found reduction of RACK-1 by RNAi significantly suppressed the same heterochronic phenotypes at the larva-to-adult transition in the *let-7* temperature sensitive mutant *let-7(n2853)*, which harbors a point mutation in the seed region of the mature *let-7* miRNA that may affect target mRNA recognition. Along with the suppression of mutant phenotypes, we have observed elevated levels of *let-7* miRNA and its precursors upon reduction of RACK-1 by RNAi. These results suggest that, despite the role in the recruitment of *let-7*-containing miRISC to the ribosome, RACK-1 may regulate the developmental events at the larva-to-adult transition via another regulatory pathway. (Support: National Health Research Institutes, Taiwan. NHRI-EX101-10151SI).

1029B. Characterizing the individual roles of CSR-1 isoforms across development in *Caenorhabditis elegans*. **V.H.W. Cheung**, J.M. Claycomb. Molecular Genetics, Univ of Toronto, Toronto, ON, Canada.

The nematode *C. elegans* expresses 26 Argonaute (AGO) proteins which interact with different small RNAs in the worm to regulate of gene expression[1]. Interestingly, only one AGO has been shown to be essential: CSR-1 (*c*hromosome *s*egregation and *R*NAi deficient)[1]. Loss of CSR-1 causes multiple defects in the animal: impaired ability to raise an RNAi response, abnormal germline morphology, chromosome mis-segregation, and embryonic lethality[1,2,3].

Various studies have explored roles for CSR-1 in modulating centromeric chromatin[2], RNAi[1,2], histone mRNA maturation[4], small RNA-independent translational regulation[5] and meiotic silencing of unpaired chromsomes[3]. However, these studies have neglected to address the contributions of two distinct isoforms of CSR-1 to overall CSR-1 function.

Initially identified by Claycomb *et al.*, the two CSR-1 isoforms differ by presence/absence of a 5'exon encoding an RG-rich motif and have differential expression across developmental stages[2]. We are currently using an integrated approach to examine isoform-specific functions across *C. elegans* tissues and development. Epitope tagged transgenes for each isoform will be used to characterize each form of CSR-1. Preliminary immunolocalization studies have revealed a differential localization pattern for the long isoform of CSR-1 in mitotic embryos distinct from the overall pattern of CSR-1 localization which was previously not appreciated. These observations will be further explored and supplemented with analysis of small RNA and protein binding partners of each isoform to determine the subset of isoform-specific cofactors functioning across C. elegans development.

1. Yigit, E. *et al. Cell* **127**, 747-757 (2006). 2. Claycomb, J.M. *et al. Cell* **139**, 123-134. (2009). 3. She, X., Xu, X., Fedotov, A., Kelly, W.G., & Maine, E.M. *PLoS Genet*. **5**, e1000624. (2009). 4. Avgousti, D.C., Palani, S., Sherman, U., & Grishok, A. *EMBO J.* **31**, 3821-3832 (2012). 5. Friend, K. *et al. Nat. Struct. Mol. Biol.* **19**, 176-184 (2012).

1030C. An RNAi-based screen for the DExD/H-box RNA helicases involved in *Caenorhabditis elegans* microRNA function. **Yu-De Chu**, Tao Huang, Guan-Rong Chen, Shin-Kai Chen, Shih-Peng Chan. Graduate Institute of Microbiology, College of Medicine National Taiwan Univ, Taipei, Taiwan.

MicroRNAs (miRNAs) are small non-coding regulatory RNAs that regulate gene expression at the post-transcriptional level and are involved in a broad spectrum of biological processes. Rearrangements of inter- or intra-molecular RNA structures and conformational changes of ribonucleoprotein complexes in the multiple processes of the miRNA pathway have led to the involvement of RNA helicase activities but little is known so far. In eukaryotes, RNA helicases generally belong to the superfamily 2 (SF2) in helicase classification, especially the DExD/H-box helicase family. The DExD/H-box proteins have been shown in association with many cellular processes involving RNA. To better understand the possible roles of DExD/H-box RNA helicases in miRNA function, we employed RNAi screen to identify genetic interaction between *C. elegans* DExD/H-box RNA helicases and the *let-7* miRNA, which controls the timing of cell cycle exit and terminal differentiation. In addition to the RNA helicase p72, a component of Drosha Microprocessor complex, and CGH-1 that has been reported to facilitate the function of miRNA-induced silencing complex (miRISC), we found several DExD/H-box RNA helicases, which are involved in ribosomal RNA processing, pre-mRNA splicing and mRNA surveillance, may also take part in miRNA biogenesis and/or function. (Support: National Science Council, Taiwan. NSC 100-2311-B-002-006-MY3).

1031A. Functional Characterization of the CSR-1 Small RNA Pathway in *C. briggsae*. Monica Wu[1], Jie Wang[2], Shikui Tu[2], Zhiping Weng[2], **Julie M. Claycomb[1]**. 1) Molecular Genetics, Univ of Toronto, Toronto, ON, Canada; 2) Program in Bioinformatics and Integrative Biology, Univ of Massachusetts, Worcester, MA, USA.

When Sydney Brenner chose *C. elegans* as a model, he set the stage for a series of discoveries that have transformed molecular biology: the discovery of RNA interference (RNAi) and endogenous small RNA pathways, including microRNAs. One of Brenner's alternative nematode species, *C. briggsae* has come into common use during the genomics era and affords the ability to perform comparative genomics studies on a range of questions. Recently several studies have delved into small RNA pathways in *C. briggsae*, cataloging classes of small RNAs from sequencing data, and identifying conserved Argonaute proteins by sequence homology. However, the function of any Argonaute/small pathway in *C. briggsae* has yet to detailed. Here we characterize the functions of an essential Argonaute, CSR-1 (Chromosome Segregation and RNAi Deficient) in *C. briggsae*. CbCSR-1 was an attractive candidate for our studies because: 1. An antibody against CeCSR-1 could recognize CbCSR-1 and 2. CeCSR-1 has been shown to target approximately 4200 germline-expressed protein coding genes via 22G-RNAs to regulate chromatin and impact chromosome segregation. We used Illumina sequencing to identify the small RNA complement associated with CbCSR-1 and examined small RNA populations in worms in which *cbcsr-1* was depleted by RNAi. We find that the small RNAs enriched in CSR-1 complexes overlap with those depleted under *cbcsr-1* RNAi conditions, establishing a high-confidence set of CbCSR-1-dependent small RNAs. We have compared the CbCSR-1 and CeCSR-1 target transcripts to define conserved characteristics that contribute to a transcript becoming the target of the CSR-1 pathway. Finally, we have determined that CbCSR-1 associates with germline and embryonic chromatin, and we demonstrate that depletion of *cbcsr-1* leads to chromosome segregation defects, like its *C. elegans* ortholog. In sum, this is the first characterization of an Argonaute/small RNA pathway in *C. briggsae*, whereby we demonstrate a conserved, role for the CSR-1 pathway and emphasize that this pathway plays a role in chromosome segregation in multiple animal species.

1032B. Identification of genes required for RNAi-mediated antiviral immunity by a genome-wide genetic screen in *C. elegans*. **Stephanie R. Coffman[1,2,4]**, Yuanyuan Guo[2,4], Zhihuan Gao[2], Gina Broitman-Maduro[3], Morris Maduro[3], Shou-wei Ding[1,2]. 1) Graduate Program in Genetics, Genomics and Bioinformatics, Univ. of California Riverside; 2) Department of Plant Pathology and Microbiology, Univ. of California Riverside; 3) Biology Department,

Univ. of California Riverside; 4) These authors contributed equally to this work.

We have previously shown that replication of Flock House Virus (FHV), a positive-strand RNA virus, induces an RNAi-mediated antiviral defense in *C. elegans*. This defense is sufficient to block accumulation of FHV that is lacking the viral suppressor of RNAi, B2. Further genetic analysis has revealed that the antiviral RNAi response requires many of the core components in exogenous RNAi, as well as additional factors that are dispensable for exogenous RNAi. Notably, recent studies have demonstrated that this pathway also restricts natural infection by Orsay virus, which is closely related to FHV. In this study, we carried out a genome-wide feeding RNAi screen in *C. elegans* and identified 135 genes that restrict the in vivo accumulation of a FHV replicon that lacks the B2 protein and is thus highly sensitive to antiviral RNAi. These 135 genes are enriched in genes previously identified to function in RNAi and more than 60% are conserved in humans, some of which have known roles regulating innate immunity. We obtained chromosomal genetic mutants available for 42 of the identified genes for further characterization. We found that some of these mutants present with enhanced infection by Orsay virus, whereas others were defective for feeding RNAi. These results indicate that our FHV replicon can effectively identifying genes in antiviral immunity and RNAi pathways.

1033C. NHL-2 Influences 22G RNAs to Maintain Germline Mediated Chromosomal Integrity. **Gregory M. Davis**[1], Wai Y. Low[1], Julie M. Claycomb[2], Peter R. Boag[1]. 1) Department of Biochemistry and Molecular Biology, Monash Univ, Australia; 2) Department of Molecular Genetics, Univ of Toronto, Canada.

The TRIM family protein, NHL-2, functions as a micro-RNA RISC co-factor in somatic tissues. *nhl-2* null mutants display multiple germline defects, including oocyte chromosomal defects, reduced brood size and embryonic lethality. To investigate the function of NHL-2, we conducted a genome-wide RNAi screen to identify genes that lead to synthetic phenotypes when knocked down in *nhl-2* null mutants. Our screen identified 40 candidate genes, including the DEAD box RNA helicase, *drh-3*, which is required for the biogenesis of CSR-1 and WAGO-1 22G small RNAs. Knockdown of *drh-3* in *nhl-2* null mutants leads to marked chromosomal abnormalities in diakinetic oocytes and anaphase embryos, as well as significantly enhanced embryonic lethality. Similar phenotypes were observed when *csr-1* and other 22G RNA pathway co-factors were knocked down in *nhl-2* null mutants, including *cde-1* and *ekl-1*, suggesting that NHL-2 influences CSR-1 22G RNA activity. In addition to this, qRT-PCR data shows that NHL-2 contributes to the downregulation of selected WAGO-1 22G RNA targets, including protein coding genes, transposons and pseudogenes. Moreover, we show that NHL-2 is required for accurate histone modifications, centromere formation and positively regulates histone gene expression. Taken together, our results suggest that NHL-2 contributes to genome maintenance and germline chromosomal integrity by influencing WAGO-1 and CSR-1 22G RNA pathways.

1034A. Identifying Zrt, Irt-like proteins that promote resistance to zinc toxicity in *C. elegans*. **Nicholas K. Dietrich**, Kerry Kornfeld. Washington Univ in Saint Louis, Saint Louis, MO.

Zinc is a trace element that is essential for the structure and function of a wide range of proteins, and disrupted zinc metabolism is implicated in a number of human diseases. Because both zinc deficiency and excess are deleterious, organisms require homeostatic mechanisms to respond to environmental and dietary changes in zinc availability. In biological systems, zinc exists as a divalent cation that cannot passively cross cell membranes. Therefore, transmembrane proteins that transport zinc across the plasma membrane and the membranes of intracellular organelles play a critical role in zinc metabolism. Two families of evolutionarily conserved zinc transporters have been characterized in animals. Cation diffusion facilitator (CDF) proteins reduce the level of cytoplasmic zinc by transport out of cells or into the lumen or intracellular organelles, whereas Zrt, Irt-like proteins (ZIPs) increase the level of cytoplasmic zinc by transport in the opposite direction. *C. elegans* has been used to study the function of multiple CDF proteins, but *C. elegans* ZIP proteins have yet to be characterized. We used bioinformatic approaches to identify predicted ZIP proteins encoded by the *C. elegans* genome. To determine if any of the predicted genes are functional in zinc metabolism, we perfomed RNAi examining sensitivity to supplemental dietary zinc. To increase the sensitivity of the assay, we utilized a *cdf-2;ttm-1* double mutant animal that is hypersensitive to supplemental dietary zinc. We analyzed twelve ZIP genes and showed that reducing the level of C14H10.1 promoted resistance to the growth arrest caused by supplemental dietary zinc. C14H10.1 encodes a predicted protein that is homologous to human ZIP13, and loss-of-function mutations in ZIP13 cause spondylocheiro dysplastic Ehlers-Danlos syndrome. This connective tissue disorder is hypothesized to result from vesicular zinc trapping leading to a deficiency of zinc entering the secretory pathway. These studies have begun to identify a function for C14H10.1 in zinc detoxification and may lead to a genetically tractable model of a human disease.

1035B. Dissecting the role of NAP-1 in small RNA-mediated chromatin modulation. **M. A. Francisco**, J. M. Claycomb. Molecular Genetics, Univ of Toronto, Toronto, Ontario, Canada.

Small RNA-mediated gene-silencing pathways related to RNA interference (RNAi) are critical regulators of gene expression in a myriad of biological processes. Some of the least understood roles for small RNA pathways are in modulating chromatin and organizing the genome. Misregulation of chromatin can lead to aberrant gene expression and genomic instability, which impacts fertility, development, and can lead to oncogenesis.

Recent studies in *C. elegans* have begun to dissect the molecular mechanisms by which several small RNA pathways impact chromatin. One essential small RNA pathway in *C. elegans*, the CSR-1 pathway (Chromosome Segregation and RNAi Deficient), is required for faithful chromosome segregation and is thought to regulate the proper formation of centromeric chromatin, among other functions. Small RNAs, called 22G-RNAs, guide the Argonaute CSR-1 to its target gene chromosomal loci to influence the landscape of chromatin in these regions. Because CSR-1 is an Argonaute and is not likely to directly modulate chromatin, we hypothesize that there are chromatin-modifying factors that function in the CSR-1 pathway.

To identify such chromatin factors, I performed a candidate RNAi screen of chromatin-related factors previously implicated in gene silencing, looking for key phenotypes that are specific to loss of the CSR-1 pathway. I identified the essential gene *nap-1*, which encodes a homolog of the highly conserved human histone chaperone, NAP-1 (Nucleosome Assembly Protein). Loss of *nap-1* phenocopies loss of *csr-1*, and its expression pattern parallels that of CSR-1. Our ongoing experiments reveal mechanistic insights into the role of NAP-1 in small RNA-mediated chromatin modulation, as well as its more general roles in regulating chromatin throughout development. These studies provide the first detailed examination of NAP-1 in *C. elegans* and reveal key insights into a novel role for this highly conserved protein in small RNA-mediated chromatin modulation.

1036C. *C. elegans* as a model for fatty acid oxidation disorders. **Wen Gao**[1,2], Ronald J. Wanders[1], Riekelt H. Houtkooper[1]. 1) Laboratory Genetic Metabolic Diseases, Academic M, Amsterdam, Netherlands; 2) Master program Biomedical Sciences, Univ of Amsterdam, Amsterdam, The Netherlands.

Fat breakdown by means of fatty acid oxidation occurs primarily during periods of prolonged fasting or exercise, when fatty acids are liberated from fat tissue to be used by other tissues, particularly liver, muscle and heart. Dysfunctional fatty acid oxidation is a hallmark of aging, but also the cause of inherited metabolic diseases. These diseases occur in about 1:10,000 births and have a wide spectrum of clinical presentations, ranging from asymptomatic to infant death. Although the genetic and biochemical defect in fatty acid oxidation patients are quite well understood, no therapeutic intervention is available. Compounds that enhance mitochondrial metabolism were suggested as therapeutics for these rare diseases. This was supported by data showing the protective effects of the polyphenol, resveratrol, on cells derived from fatty acid oxidation patients. In order to identify novel therapeutic modulators of fatty acid oxidation, we use the worm *C. elegans* as a model organism. Advantages of using *C. elegans* for this purpose includes the fact that therapeutic effects can be measured at multiple levels in a multi-cellular organism, for instance using mutant strains and reporters. Using gene homology analysis, we identified the fatty acid oxidation genes in *C. elegans*. We confirmed that fatty acid oxidation is highly conserved at the genetic level between worms and mammals, and ascertained that RNAi of these genes was not detrimental for the larval development of the worms. Currently, we are in the process of setting up and optimizing tandem mass spectrometry measurements to measure fat metabolites, acylcarnitines, in *C. elegans* fed with either control bacteria or RNAi targeted against fatty acid oxidation genes. Our preliminary data indeed show a ten-fold accumulation of acylcarnitines in worms treated with RNAi against fatty acid oxidation. Once the model is fully validated, we plan to test various compounds on fatty acid oxidation deficient worms to assess the potential therapeutics to treat the diseases.

1037A. The Virus Sensing Domains of RIG-I Functionally Replace the Corresponding Domains of DRH-1 in Antiviral RNA Silencing in *Caenorhabditis elegans*. **Xunyang Guo**, Rui Zhang, Jeffery Wang, Rui Lu. Department of biological sciences, Louisiana State Univ, Baton Rouge, LA, 70803.

Small interfering RNAs derived from replicating viral genome mediate sequence-specific silencing of virus in fungi, plants and invertebrates. Accumulating evidence suggested that RNAi-directed viral immunity (RDVI) is initiated upon the processing of viral double-stranded RNA into siRNAs, which then guide the destruction of the viral transcripts. In *C.elegans*, RDVI requires a RIG-I-like RNA helicases (RLHs) termed DRH-1 (dicer-related RNA helicase 1). RIG-I is known as a cytosolic virus sensor in mammals. Currently, how DRH-1 contributes to RDVI remains largely unknown.

Here we show that DRH-1 requires both N-terminal and C-terminal domains in RDVI triggered by artificial virus or natural viral pathogen of *C. elegans*. DRH-2, a homologue of DRH-1 that negatively regulates RDVI, can functionally replace the corresponding domains of DRH-1, indicating that DRH-2 regulates RDVI as a competitor of DRH-1. Notably, both the helicase and regulatory domains of RIG-I, can functionally replace the corresponding domains of DRH-1, suggesting that DRH-1 contributes to RDVI as a virus sensor. Further, virus-derived primary siRNAs were produced at a much lower level in drh-1 mutants compared to that in rde-1 mutants which are defective in the function but not the biogenesis of primary siRNAs. Since mutations within the C-terminal domain that have the potential to disrupt RIG-I function abolished the antiviral function of both DRH-1 and the DRH-1/RIG-I recombinant, we believed that similar mechanisms are used by DRH-1 and RIG-I for virus detection. It was also clear that a third worm homologue of RIG-I, DRH-3, plays a pivotal but distinct role in RDVI, demonstrating that the homologous family of RIG-I as key components of innate antiviral immunity is conserved in both mammals and single-dicer invertebrates.

Taken together, our research not only defined a role for DRH-1 in worm RDVI but also suggested an evolutionary origin of RIG-I domains involved in virus detection.

1038B. Analysis of ok2951, a mutation found in F56D2.6, a putative homologue of the yeast PRP43 protein. **Jonathan E. Karpel**, Miranda Roland. Dept Biol, Southern Utah Univ, Cedar City, UT.

F56D2.6 is a putative helicase of the “DEAH box” family that is a homologue of the yeast spliceosomal protein, Prp43p. The Prp43p protein is required for the release of the lariat intron from the spliceosome and also plays a significant role in ribosome biogenesis. RNAi directed toward the F56D2.6 gene in C. elegans shows no overt phenotype, but a balanced strain that produces homozygous F56D2.6 (ok2951) knockout worms shows a sterile phenotype. DAPI staining of these homozygous mutants reveals that their germlines only have undifferentiated germ cells and no sperm was observed. We are especially interested in potential splicing errors involving the sex determination genes or miRNA required for proper regulation of these sex-determination pathway genes caused by the ok2951 mutation. To date, we have not found any splicing errors, but using miRNA analysis and qRT-PCR we have found a marked decrease in miR35-41 expression in ok2951 mutant worms. Potential implications of this data are discussed.

1039C. Direct Reprogramming of Distinct Cell Types in *C. elegans* into GABAergic Motor Neurons. **Marlon Kazmierczak**, Ena Kolundzic, Baris Tursun. Berlin Institute for Medical Systems Biology (BIMSB) at Max Delbrück Center (MDC) - Berlin, Germany.

One major goal of our group is to elucidate mechanisms restricting transcription factor (TF)-induced direct reprogramming (DR) of cell fates. Our ongoing genetic screens use defined cell fate specification programs in order to identify DR-inhibiting factors. To date, mis-expression of the Zn-finger TF CHE-1, inducing glutamatergic ASE neurons, is used as a neuronal specification program for our screens. To extend our neuronal screening system we will use mis-expression of the Pitx-like TF UNC-30 in combination with *unc-47::gfp* as a reporter for GABAergic neurons. Manual as well as an automatic whole-genome RNAi screen using the BioSorter allows for high-throughput rates to discover new DR phenotypes. Identified factors will be further characterized phenotypically by determining the degree of reprogramming based on morphology and expression of cell type specific markers. Clones allowing UNC-30 to induce ectopic GABAergic neuron fate will be also tested for the possibility to induce ASE neuron or muscle cells upon misexpression of the TFs CHE-1 or HLH-1, respectively. We believe that most identified DR-inhibiting factors will be rather ‘general’ inhibitors. However, we cannot exclude that some might be more cell-type specific. Furthermore, we aim to test neurons derived by DR whether they are physiologically functional. Previously, it was shown that mitotic germ cells can be converted into distinct neuron-like cells upon knock-down of PRC2 subunits (Tursun et al. 2011, Patel et al. 2012). As a first case study, we will test whether germ cell-derived neurons are electrophysiologically active. For this, we will establish an *ex vivo* procedure to convert germ cells in extracted gonads to facilitate the electrophysiology. Additionally, physiological activity of DR-derived neurons will be visualized *in vivo* using calcium imaging. Assessing physiological properties of converted cells will allow to determine the functional degree of cell fate reprogramming and thus

offers an invaluable addition to morphology and cell fate markers. Ongoing work and preliminary results will be presented at the meeting.

1040A. Investigating how an autoregulatory loop enhances let-7 biogenesis. **Sarah A. Lima**, Dimitrios G. Zisoulis, Zoya S. Kai, Vanessa Mondol, Amy E. Pasquinelli. Division of Biology, UCSD, La Jolla, CA.

MicroRNAs (miRNAs) are a class of small noncoding RNAs that typically repress the expression of mRNA targets. Canonically, miRNAs are transcribed as long noncoding primary transcripts called pri-miRNAs. These transcripts are processed by the endonuclease Drosha into precursor miRNAs (pre-miRNAs) and then by Dicer into mature miRNAs. Mature miRNAs are loaded onto argonaute proteins (ALG-1 and ALG-2 in worms) to form silencing complexes that recognize their targets via imperfect base pairing. The let-7 miRNA is essential for development and is highly conserved from worms to humans. Previous work from our lab uncovered a novel feedback loop in which mature let-7 enhances processing of its own primary transcript. We have demonstrated that let-7 loaded ALG-1 interacts with let-7 primary transcripts through an ALG-1 binding site downstream of the miRNA hairpin. Worms expressing a primary transcript lacking the ALG-1 binding site accumulate pri-let-7 and have lower levels of mature let-7. This phenomenon is also observed in *alg-1(gk214)* mutants or worms carrying a point mutation in the seed sequence of let-7. We are now working to address two outstanding questions: how general is the regulation of miRNA primary transcript processing by Argonaute and what is the mechanism by which ALG-1 regulates primary let-7 processing. While the interaction of ALG-1 with pri-miRNAs besides let-7 is yet to be detected in worms, we have observed that several of the pri-let-7 miRNAs in human cells associate with Argonaute, raising the possibility of a conserved regulatory mechanism. One hypothesis is that the Argonaute interaction enhances association of Drosha with the primary transcripts. To test this in *C. elegans*, we have created a worm strain containing a single copy insertion of a rescuing FLAG-Drosha construct. RNA immunoprecipitation assays will reveal if transcripts that are bound by ALG-1 interact more strongly with Drosha or if the regulation is downstream of this interaction. Determining the mechanism of this novel autoregulatory loop will further our understanding of miRNA biogenesis and illuminate new roles for Argonaute.

1041B. *C. elegans* RNA Helicase A genetically interacts with genes involved in two different germline RNAi pathways. **Penelope L. Lindsay**[1], Megan K. Gautier[1], Karen J. Muschler[2], Sarah K. O'Connor[3], Katherine M. Walstrom[1]. 1) Div. Natural Sciences, New College of Florida, Sarasota, FL; 2) Nova SE Univ., Ft. Lauderdale, FL; 3) Dept. Mol. Micro., NIAID, Bethesda, MD.

RNA helicase A (RHA) is a conserved protein with roles in transcription regulation and histone modification in a variety of organisms including flies and humans. In *C. elegans*, the null mutant *rha-1(tm329)* has a temperature-sensitive sterile phenotype in hermaphrodite and male worms (Walstrom et al. (2005) Mech. Dev. 122, 707). Others showed that *rha-1* is required for RNA interference in the germline (Robert et al. (2005) Genes Dev. 19, 782). We tested for interactions between *rha-1* and genes involved in two germline RNAi pathways. The first was the 26G RNAi pathway (Han et al. (2009) PNAS 106, 18674) that requires *eri-1* and *rrf-3*, and the second was the 22G endo-siRNA pathway, downstream of the *prg-1*-dependent 21U piRNA pathway, that requires *mut-7* and *rde-2* (Bagijn et al. (2012) Science 337, 574; Shirayama et al. (2012) Cell 150, 65). We found that *rha-1(tm329);eri-1(mg366)* mutant hermaphrodites produced significantly fewer offspring at 20°C than *rha-1* or *eri-1* hermaphrodites. In extensive real-time RT-PCR assays, *rha-1* hermaphrodites and males did not have significantly increased expression of 26G-regulated genes or 21U/22G-regulated transposons. However, the expression of 26G-regulated sperm-enriched genes in *rha-1;eri-1* males was slightly reduced compared to *eri-1* males. We also found that *rha-1;mut-7(pk204)* hermaphrodites produced fewer offspring at 20°C than *mut-7* or *rha-1* hermaphrodites because they made fewer sperm. Preliminary real-time RT-PCR with 21U/22G-regulated transposons showed that some transposons were overexpressed in *rde-2* or *mut-7* hermaphrodites as expected and that *rha-1;rde-2(ne221)* and *rha-1;mut-7* hermaphrodites often had expression levels restored to near wild-type levels. These results indicate that the germline defects in *rha-1(tm329)* worms are not due to major alterations in the expression of genes controlled by the 26G or 21U/22G RNAi pathways. However, RHA-1 appears to be required for the overexpression of some 21U/22G-regulated transposons in *rde-2* and *mut-7* worms.

1042C. The mir-35 family of microRNAs regulates hermaphrodite fecundity, male development, and genetically interacts with the sex determination pathway. **Katherine McJunkin**, Victor Ambros. Univ of Massachusetts Medical School, Worcester, MA.

The *mir-35-42* family is one of three known essential microRNA families in *C. elegans*. Deletion of all eight microRNAs in the family results in embryonic lethality. To better understand the function of the *mir-35* family, we have examined a viable strain in which seven of eight family members are deleted (*mir-35-41*), resulting in temperature-dependent embryonic lethality. First, we further characterized the *mir-35-41* phenotype, and observed that *mir-35-41* also exhibits a temperature-dependent defect in hermaphrodite fecundity, and males display abnormal tail morphology and reduced mating efficiency. We hypothesized that proteins that bind specifically to the *mir-35*-RNA-induced silencing complex (Wu, et al.) may be modulators of *mir-35* family activity in the context of these phenotypes. Thus we examined genetic interactions between these candidate genes and the sensitized *mir-35-41* background. In particular, inactivation of *sup-26* strongly enhanced *mir-35-41* lethality, suggesting that SUP-26 may act as a positive co-factor for the remaining *mir-35* family member (*mir-42*). Paradoxically, *sup-26* mRNA bears a conserved *mir-35* family target site. If sup-26 is aberrantly up-regulated in *mir-35* family mutants, then *sup-26* inactivation should suppress the *mir-35-41* phenotype. Indeed, a weak *sup-26* allele partially suppresses the temperature-sensitive defect in fecundity of *mir-35-41* (while enhancing embryonic lethality). Thus, *sup-26* may have a dual role as a positive *mir-35* family regulator and a *mir-35* target gene. SUP-26 is an RNA-binding protein that plays a masculinizing role in both hermaphrodites and males through translational inhibition of *tra-2* mRNA. *tra-2* does not contain a conserved *mir-35* binding site, and *tra-2* loss of function does not suppress *mir-35-41* embryonic lethality. However, in addition to *sup-26(lf)*, other feminizing mutations, such as *tra-2(gf)* or *fem-3(lf)* also enhance lethality of *mir-35-41*. Our future work aims to elucidate whether SUP-26 indeed regulates the activity of *mir-35-42* or other microRNA families, and to gain a clearer understanding of how the *mir-35* family functionally interacts with the sex determination pathway.

1043A. Orsay virus replication kinetics in *C. elegans* strain Bristol N2. **Mark G. Sterken**[1,2], Kobus J. Bosman[1], L. Basten Snoek[1], Jikke Daamen[1], Joost A.G. Riksen[1], Jaap Bakker[1], Gorben P. Pijlman[2], Jan E. Kammenga[1]. 1) Nematology, Wageningen Univ, Wageningen, Netherlands; 2) Virology, Wageningen Univ, Wageningen, Netherlands.

The recently discovered Orsay virus (OrV) is the first virus able to complete a full infection cycle in *C. elegans*. This discovery creates the opportunity to

study host-virus interactions in a genetically tractable host system. OrV can be transmitted horizontally (from one individual worm to the next) and the infection process is affected by the RNAi response machinery. OrV gives us the opportunity to investigate viral replication dynamics and anti-viral mechanisms in the host, including RNAi.

By infecting worm cohorts at different time points, and monitoring through qPCR, the influence of worm age and genotype on viral replication was determined. Next, several subsequent generations exposed to virus were re-infected to determine if trans-generational RNAi plays a role in infections in *C. elegans* populations.

We found an age-related resistance to OrV infection and faster replication in the isolate JU1580 (in which OrV was discovered) than in Bristol N2. Nonetheless, N2 is perfectly able to sustain viral replication. However, an inherited anti-viral response makes offspring of infected N2 less susceptible to viral replication. Consequently, N2 populations can lose the infection after a limited number of generations, whereas JU1580 populations remain infected. Experiments with two RNAi deficient mutants confirm that the RNAi response is responsible for this inherited anti-viral response.

We present a quantitative and reproducible method for studying OrV infection in synchronized populations and consider some important parameters for experimental settings. Using this method, we found that the canonical Bristol N2 strain can be used to study OrV, which enables the use of all the tools that are available for this genotype. The presence of a heritable RNAi response establishes the importance of this pathway in clearance of viral infections in *C. elegans*.

1044B. Nicotine Exposed Chronically During the Post-embryonic Stages Systematically Altered the MicroRNA Expression Profiles in *C. elegans*. **Faten A. Taki**, Baohong Zhang. Biology, East Carolina Univ, Greenville, NC.

Tobacco smoking is associated with many diseases including addiction, which is of the most notorious. The tobacco dependence is majorly attributed to nicotine, which is considered one of the most addictive chemicals. In our study, we chose *C. elegans* as a biological model to systemically investigate the effect of chronic nicotine exposure and their regulated biochemical pathway. Nicotine treatment (20mM and 20mM) was limited to the post-embryonic stage from L1-L4 (~31 hours) period after which worms were collected for genome-wide miRNA profiling. Our results show that nicotine significantly altered the expression patterns of 40 miRNAs. The effect was proportional to the nicotine dose and was expected to have an additive, more robust response. Based on pathway enrichment analysis coupled with nicotine-induced miRNA patterns, we inferred that miRNAs as a system mediate "regulatory hormesis", manifested in biphasic behavioral and physiological phenotypes. We proposed a model where nicotine addiction is mediated by miRNAs' regulation of fos-1 and is maintained by epigenetic factors. Thus, our study offers new insights for a better understanding of the sensitivity of early developmental stages to nicotine.

1045C. TEG-1 regulates the stability of miRISC components and the levels of microRNAs. **Chris Wang**, Dave Hansen. Department of Biological Sciences, Univ of Calgary, Calgary, AB, Canada.

TEG-1 (Tumorous Enhancer of Glp-1(gf))-related proteins are found in organisms from yeast to humans. We have previously reported roles for TEG-1 in germline development, including regulating the mitosis/meiosis decision and germline sex determination (Wang et. al. 2012. Dev. Dyn. 241:505-521). Recently, we found that *teg-1* mutants display heterochronic defect phenotypes, including: (1) reiteration of the L2 stage resulting in an increased number of seam cells; (2) defective seam cell fusion at the L4 molt; and (3) retarded alae formation at the L4-adult transition. Since developmental timing defects are often controlled by microRNAs, we sought to determine if TEG-1 also plays a role in microRNA function. We found that mature let-7, mir-58, and mir-62 levels are significantly reduced in *teg-1* mutants. To gain insight into the cause of reduced microRNA levels in *teg-1* mutants, we performed immunoprecipitation (IP) and found that TEG-1 interacts with VIG-1, a *Drosophila* Vasa Intronic Gene ortholog, whose activity is required for proper let-7 function and is believed to be a component of the microRNA RNA-induced silencing complex (RISC) (Caudy et. al. 2003. Nature 425:411-414; Chan et. al. 2008. RNA 14:2104-2114). This interaction was confirmed by co-IP experiments using wild-type extracts. Interestingly, we also found that TEG-1 levels are lowered in *vig-1* mutants, and both VIG-1 and ALG-1 (an Argonaute component of the RISC complex) levels are reduced in *teg-1* mutants. This finding led us to propose that VIG-1 and ALG-1 levels are regulated post-translationally. Consistent with this hypothesis, we found that the mRNA levels of *vig-1* and *alg-1* remain unchanged in *teg-1* mutants, whereas elevated VIG-1 and ALG-1 protein levels are detected in *teg-1* mutants and wild-type animals treated with lactacystin, a specific inhibitor of the 26S proteasome. Together, these data suggest that TEG-1 regulates a broad range of microRNAs through stabilizing the microRISC components, potentially by protecting them from proteasomal degradation.

1046A. 3'LIFE: A functional assay to detect *C. elegans* miRNA targets in high-throughput. **JM Wolter**[1,3], K Kotagama[2,3], AC Pierre-Bez[3], M Firago[3], M Tennant[3], M Mangone[1,3]. 1) Molecular and Cellular Biology Graduate Program, School of Life Sciences, Arizona State Univ, Tempe, AZ; 2) Barrett Honors College, Arizona State Univ, 751 E Lemon Mall, Tempe, AZ; 3) The Biodesign Institute at Arizona State Univ 1001 S McAllister Ave, Tempe, AZ.

MicroRNAs (miRNAs) have emerged as central regulators of diverse biological processes, primarily by binding to the 3' UnTranslated Region (3'UTR) of target genes and repressing their translation. In *C. elegans* each miRNA is bioinformatically predicted to target dozens, if not hundreds of genes, yet relatively few of these predictions have been experimentally validated. Furthermore, our understanding of miRNA targeting is increasingly being challenged by unpredicted interactions that contradict canonical targeting principles. Currently there are no high-throughput tools to identify and characterize miRNA targets. To fill this gap, we have developed a high-throughput platform that allows the unbiased detection and characterization of miRNA targets in worms. This technology, named Luminescence-Based Identification of Functional Elements in 3'UTRs (3'LIFE), is based on the dual-luciferase, and MS2-AGO HITS-CLIP assays. The 3'LIFE library uses clones from the worm 3'UTRome, which includes 3'UTRs for ~18K protein-coding genes. The 3'LIFE assay detects the binding of test 3'UTRs by miRNAs in a high-throughput screen where interactions are individually queried. We transfect human cell lines with luciferase::3'UTR and a query miRNA in 96-well plates, and subject the lysate to a dual-luciferase assay to identify *bona-fide* interactions. The 3'LIFE vector includes multiple MS2 repeats that facilitates the isolation of exogenous mRNA, and the mapping of miRNA binding sites through a conjunction of AGO HITS-CLIP assays. We demonstrate the feasibility of the assay testing a pilot dataset of 384 3'UTRs and study the targeting requirements of two query miRNAs (*lin-4* and *let-7*). The 3'LIFE assay has the potential to advance the miRNA field by providing an unbiased and robust high-throughput platform for miRNA target discovery.

1047B. Analysis of PUF-9 and miRNA interactions in *C. elegans*. **Danny Yang**[1,4,6], Sang Chun[2,4,6], Ting Han[3,4], James Moresco[5], John Yates III[5], John Kim[1,4]. 1) Department of Human Genetics; 2) Department of Computational Medicine & Bioinformatics; 3) Department of Cellular & Developmental Biology; 4) Life Sciences Institute, Univ of Michigan, Ann Arbor, Michigan; 5) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA; 6) Equal contribution.

The Pumilio family of RNA binding proteins is conserved from yeast to humans. They bind specific sequences in the 3'UTRs of target transcripts to direct translational repression, deadenylation, and degradation of RNAs. Recent studies suggest that Pumilio proteins interact with microRNAs in unexpected ways. For example, human PUM1 binds a highly structured region of the *p27* 3'UTR to expose *miR-221* targets sites and regulate cell cycle progression (Kedde, *Nat. Cell. Biol.* 2010). Using the *C. elegans* Pumilio ortholog PUF-9 as a model, we seek to characterize conserved mechanisms by which Pumilio proteins interact with the microRNA pathway for gene silencing. During larval development, PUF-9 cooperates with *let-7* family microRNAs to promote the larval to adult transition. Loss of *puf-9* was previously shown to enhance *let-7* hypomorphic phenotypes, including vulval bursting (Nolde, *Dev. Biol.* 2007). RNA-IP analysis indicates that PUF-9 binds *hbl-1* mRNA, a known target of *let-7*. In addition, PUF-9 associates with the ALG-1 Argonaute miRNA-induced silencing complex (miRISC) in an RNA-dependent manner. Our data suggest that PUF-9 and microRNAs regulate shared targets. Using high-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP), we have mapped PUF-9 and ALG-1 binding sites on mRNAs in embryo, L1, and adult stages; this approach successfully captured PUF-9 and ALG-1 binding sites in the *hbl-1* 3'UTR. Currently, we are mapping PUF-9 and ALG-1 binding sites throughout development to elucidate mechanisms by which expression of common targets bound by particular microRNAs and PUF-9 are regulated.

1048C. The SNARE protein SEC-22 is a negative regulator of RNAi. **Y. Zhao**, B. Holmgren, A. Hinas. Dept. of Medical Biochemistry and Microbiology, Uppsala Univ, Uppsala, Sweden.

In plants and some animals, including *C. elegans*, RNA interference (RNAi) systemically silences gene expression in tissues distal to the initially affected cells. One of the proteins required for efficient cell-cell transport of RNAi silencing signals is SID-5 (systemic RNAi defective). SID-5 likely functions in RNA export and co-localizes primarily with multivesicular bodies (MVBs)/late endosomes. Studies in *Drosophila* and mammalian cells have shown that MVBs are required for efficient cell autonomous RNAi whereas intact lysosomes seem to limit the RNAi efficiency. The co-localization of SID-5 and MVBs indicates that the intercellular RNA transport pathway also relies on endocytosis. As very little is known about the mechanism of function of SID-5, we carried out a yeast-two-hybrid (Y2H) assay to identify SID-5 interacting proteins.

We found that the SNARE protein SEC-22 interacted with SID-5 in a Y2H screen. SNARE proteins are required for vesicle fusion and the yeast homolog of SEC-22 functions in transport between the endoplasmic reticulum (ER) and Golgi complex. In addition, the mammalian homolog has recently been demonstrated to localize to the ER-Golgi intermediate compartment (ERGIC) in dendritic cells, where it recruits ER proteins to phagosomes. Immunohistochemistry of *C. elegans* intestines demonstrated co-localization between SEC-22::GFP and an antibody marking ERGIC. Furthermore, we found that a *C. elegans* strain with a deletion in the *sec-22* gene shows an enhanced response to feeding RNAi targeting *dpy-13*, *unc-22* and *gfp*. The enhanced RNAi (Eri) phenotype can be rescued by injection of a *sec-22* genomic fragment. A *sec-22; sid-5* double mutant displays an intermediate phenotype. One possible explanation for our data is that SEC-22 affects the localization of SID-5 and other, yet unidentified, RNAi factors. This altered localization may in turn affect the balance between MVBs and lysosomes, and thereby RNAi efficiency. It should be noted that SID-5 is known to affect RNA transport but we do not know whether SEC-22 is involved in cell autonomous RNAi and/or in RNA transport. We are currently addressing this question by tissue-specific rescue of *sec-22*.

1049A. The first 100 nematode genomes: towards a genomic biology of Nematoda. **Mark L Blaxter**[1], Georgios Koutsovoulos[1], Sujai Kumar[1], Michael Clarke[1], Martin Jones[1], Alex Marshall[1], Benjamin Makepeace[2], Philipp Schiffer[3], Einhardt Schierenberg[3], Simon Babayan[4], Nick Gray[4]. 1) Institute of Evolutionary Biology, Univ of Edinburgh, EH9 3JT, UK; 2) Department of Infection Biology, Institute of Infection & Global Health, Univ of Liverpool, L3 5RF, UK; 3) Zoologisches Institut, Universität zu Koln, Cologne, NRW, Germany; 4) CIIE, School of Biological Sciences, Univ of Edinburgh EH9 3JT, UK.

Caenorhabditis elegans is but one species among thousands in the phylum Nematoda. To understand the evolutionary processes that generated C. elegans, and facilitate the transfer of C. elegans insights to other species of health and economic importance, we have started an initiative to generate (at least) 959 nematode genomes. With colleagues worldwide, we have built a resource (nematodegenomes.org) to promote sharing and analysis of these genome data. The roster of genomes underway is approaching 100. Just as the lineage of the 959 adult cells was key to unlocking the C. elegans developmental programme, 959 genomes will illuminate the biology of this most abundant of phyla. [eg1] The deep phylogeny of Nematoda has been based on nSSU (18S rDNA). We have used genomes from the three major clades to generate a multi-gene phylogeny, rooted with Nematomorpha and other ecdysozoan genomes. We affirm the general outlines of nSSU trees. We robustly place Enoplia (Enoplus and relatives) as arising basally, and find Dorylaimia (Trichinella and relatives) and Chromadorea (Caenorhabditis and relatives) to be sister taxa. The new genome data emphasise the derived status of C. elegans, as other genomes encode components of universal animal developmental toolkits lost in the model nematode. [eg2] Many filarial nematodes, causing river blindness and elephantiasis in humans, carry Wolbachia symbionts that may play an important role in infection. We have sequenced a series of genomes from across the filarial nematodes, including species that have never had Wolbachia or that have secondarily lost infection. Metabolic reconstructions exclude some hypotheses of symbiosis, and instead support an energy provisioning model. The Wolbachia leave a signal of pseudogenes in the nuclear genomes of these species, which reveals the dynamics of horizontal transfer.

1050B. 959.nematodegenomes.org. **Mark L. Blaxter**[1], Georgios Koutsovoulos[1], Sujai Kumar[1], Philipp Schiffer[2]. 1) Institute of Evolutionary Biology, Univ of Edinburgh, Edinburgh, United Kingdom; 2) Zoologisches Institut, Universität zu Köln, Cologne, NRW, Germany.

The sequencing of the genome of Caenorhabditis elegans was a milestone in biology, and changed nematology for ever. The complete genome has made research projects previously unimagined possible. While very few species will ever have the range and depth of enquiry lavished on C. elegans, genomic data can underpin investigations of evolutionary mechanisms, ecological adaptation, parasitic phenotypes and methods for control of pests. With the ongoing revolution in sequencing and computing, determining the genome sequence of a target species is no longer a task only achievable by large centres

with unlimited funding: small laboratories have commissioned data and performed assembly, gene finding and annotation independently. There are already a dozen nematode genomes published. These distributed efforts need to be supported and used as exemplars for other groups who otherwise might not consider genomics part of their repertoire. In order to build the community of nematode genome scientists and to make the rapid change in this field evident, we founded the 959 Nematode Genomes initiative (959 being the number of somatic cells in adult female C. elegans). The core of the initiative is the 959.nematodegenomes.org website, a wiki built on the semanticMediaWiki platform. This platform has allowed us to build an agile system that offers different views on core data (most importantly lists of species, their relationships, and user-contributed data on genomics efforts). We encourage colleagues to announce when they are starting genomic efforts on a species and to register interests in unsequenced species, so that collaborators can be found to support funding applications or share analytic load. We also encourage the development and application of best practice methods for assembly and analysis. At the 19th C. elegans meeting we will be presenting the wiki and the current state of the effort to sequence and understand nematode genomes. We will assist new and established users in exploring the resource, adding data, and suggesting ways to expand and improve the initiative.

1051C. Advancing and Refining the *C. elegans* 3'UTRome. **SM Blazie[1,2]**, AC Pierre-Bez[2], CE Otto[3], CA Lynch[3], M Mangone[1,2]. 1) Molecular and Cellular Biology Graduate Program, Arizona State Univ, Tempe, AZ; 2) The Biodesign Institute at Arizona State Univ 1001 S McAllister Ave, Tempe, AZ; 3) Barrett Honors College, Arizona State Univ, 751 E Lemon Mall, Tempe, AZ.

Cleavage and polyadenylation determine the length and sequence content of the 3'Untranslated Regions (3'UTRs) of mature mRNAs. Alternative polyadenylation (APA), a mechanism whereby the same gene is processed with multiple 3' UTR isoforms, is pervasive in eukaryotes and was recently found in ~40% of *C. elegans* protein coding genes. The mechanism used to induce APA and its biological significance is still poorly understood, but recent findings point to largely unexplored post-transcriptional regulatory mechanisms, where genes with longer 3'UTR isoforms are likely to be targeted by repressive factors such as miRNAs, while genes with shorter 3'UTR isoforms lacking cis-acting elements may escape this regulation. The worm 3'UTRome was recently proposed by several groups, but further work has to be done to improve its annotation. Establishing a gold standard annotation of 3'UTRs in *C. elegans* is key to improve our understanding of APA and the overall contribution of 3'UTRs in the regulation of gene expression levels. We have taken a multi-pronged genomic approach to refine the 3'UTRome by detecting tissue-specific 3'UTR isoform dynamics and their mechanisms of production. We are generating tissue-specific mRNA libraries from worm tissues coupling the mRNA tagging method with deep sequencing. We prepared, and are currently sequencing, transcriptomes from MosSCI-derived stable transgenic worm lines driving expression of 3xFLAG-tagged Poly-A Binding Protein (*pab-1*) in eight tissues: muscle (*myo-2*, *myo-3*), nervous (*unc-47*, *nmr-1*), hypodermal (*dpy-7*, *grd-10*), and epithelial (*ges-1*, *ajm-1*). We also generated two transgenic worm lines expressing 3xFLAG-tagged CPSF-160 (*cpsf-1*) and CstF-64 (*cpf-2*) and will use the HITS-CLIP approach to map their RNA binding sites and study their contribution to APA. Results from these analyses represent the next step in improving the already unparalleled worm 3'UTRome annotation and will advance our insight of 3'UTR-mediated gene regulation.

1052A. A pair of RNA binding proteins shape alternative splicing regulatory networks in distinct neuronal subtypes. Adam D. Norris[1], Mei Zhen[2], **John A. Calarco[1]**. 1) FAS Center for Systems Biology, Harvard Univ, Cambridge, MA; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Univ of Toronto, Toronto, Canada.

Alternative splicing provides an important mechanism for increasing transcriptomic diversity in metazoans. This increased complexity has likely played a particularly important role in the cellular diversification of the nervous system, where different classes of neurons have evolved to perform distinct functions. However, it has remained a challenge to study the mechanisms and physiological impact of alternative splicing regulation at the level of individual neuronal subtypes. In order to study neuronal subtype-specific regulation of alternative splicing, we created fluorescent two-color reporters to observe alternative splicing in vivo and at single neuron resolution. Our results reveal a remarkable diversity of alternative splicing patterns among individual neuron types. One striking example involved differential inclusion of an alternative exon between GABAergic and cholinergic motor neurons. We conducted a forward genetic screen for regulators of this neuron-type specific splicing pattern and identified two conserved RNA binding proteins, UNC-75/CELF and EXC-7/ELAV. Analysis of splicing patterns in mutant animals showed that UNC-75 and EXC-7 act combinatorially to achieve neuron subtype specificity through partially non-overlapping expression. mRNA-Seq and CLIP-Seq experiments found hundreds of differentially regulated alternative splicing events when either or both factors are absent, and targeted alternative splicing events are enriched in genes associated with synaptic transmission and locomotion. Initial results indicate that the splicing regulatory network can be utilized to implicate both known and previously uncharacterized genes in locomotory behavior and synaptic transmission, and that the impact of individual targeted isoforms on neuronal phenotypes can be teased apart in vivo. Taken together, our findings suggest that partially overlapping expression patterns of multiple alternative splicing factors can create specialized post-transcriptional regulatory networks that contribute to the identity and function of distinct cell types in the nervous system.

1053B. A genome-wide network of genetic interactions in embryonic development. **Patricia G. Cipriani**, Amelia White, Huey-Ling Kao, Eliana Munarriz, Katherine Erickson, Jessica Lucas, Indrani Chatterjee, Jerome Reboul, Kristin Gunsalus, Fabio Piano. Department of Biology, Center for Genomics and Systems Biology, New York Univ, New York, NY.

The phenotypes manifested by genetic alleles are influenced by the genetic background in which they reside. Yet, we still have a very limited understanding of how genetic interactions (GIs) influence animal development. The goal of our project is to use genome-wide screens to identify all enhancing and suppressing GIs for a set of strains harboring temperature sensitive (ts) mutations in 24 essential embryonic genes. We have completed over three million primary GI assays and secondary screening of putative suppressors, and we have archived in a database all experimental metadata and images, along with quantitative scoring results from an automated phenotypic scoring algorithm we developed (DevStaR). DevStaR combines computer vision and machine learning methods to count different developmental stages in mixed populations of animals. Using these results we have developed a quantitative phenotypic "GI score" based on the multiplicative model of independence: if the effects of perturbing two genes are independent, then their combined effects should not deviate from the product of their individual effects. GI scores for individual experimental replicates correlate positively with semi-quantitative manual estimates of interaction strength. Using manual inspection as a reference, we devised criteria to combine GI scores across

replicates that reliably detect suppressing interactions. We then generated final interaction scores that reflect both strength and reproducibility, which we used to define ~800 high-confidence and ~750 intermediate-confidence suppressing interactions. Based on comparisons with manual scoring, we estimate the false discovery rates in these two sets as 2% and 10%, respectively. The resulting GI network provides the first genome-wide map of suppressing genetic interactions for the embryo based on quantitative phenotypic analysis of viability.

1054C. A draft genome assembly of *Caenorhabditis* sp. 9. and its use in characterizing genome shrinkage in self-fertile nematodes. Da Yin[1], Erich M. Schwarz[2], Caitlin M. Schartner[3], Edward J. Ralston[3], Barbara J. Meyer[3], **Eric S. Haag[1]**. 1) Dept. of Biology, Univ. of Maryland, College Park, MD; 2) Dept. of Molecular Biology & Genetics, Cornell Univ, Ithaca, NY; 3) HHMI, Dept. of Molecular & Cell Biology, UC Berkeley, CA.

Sexual mode evolves rapidly in some eukaryotic lineages. This is expected to have pronounced consequences for population genetics, sexual differentiation and the nature and intensity of sexual selection, all of which may be reflected in the genome. *C. elegans* is a self-fertile species, derived recently from an obligately outcrossing male-female ancestor. This trait has evolved in at least two other species of the Elegans sub-genus, *C. briggsae* and *C.* sp. 11. Previous studies indicate that selfing species have smaller genomes and several thousand fewer protein-coding genes than their outcrossing ancestors. This reproducibility may be stimulated by an interaction between partial selfing and segregation distortion affecting large indels in male meiosis. However, the size, location, and gene content of specific deletions remain unknown for any natural system. To characterize the process of genome shrinkage, we have produced a genome assembly from the closest known outcrossing relative of *C. briggsae, C.* sp.9. The *C.* sp. 9 genome is roughly 20 Mb (20%) larger than that of *C. briggsae*. By comparing *C.* sp.9 contigs with the chromosome-level assembly for *C. briggsae*, we created an approximation of the *C.* sp.9 physical map. This allowed us to examine the size and location of *C.* sp.9-specific sequences with respect to chromosome, and to relate them to known domains of gene density and recombination within a chromosome. Using an outgroup, we are also able to infer the identity of specific genes recently lost in the *C. briggsae* lineage. In this poster we present details of these analyses, along with some of their implications.

1055A. Mapping transcriptional regulatory networks in the nematode Caenorhabditis elegans. **Margaret Ho**, Paul Sternberg. Division of Biology, California Institute of Technology, Pasadena, CA.

The nematode *Caenorhabditis elegans* has a well-annotated genome, well-studied development and many genetic tools available. *C. elegans* provides an excellent case to study transcriptional regulation within a multicellular organism over its entire lifecycle, especially as it is easy to collect synchronized populations of worms in distinct developmental stages. In eukaryotes, transcriptional regulation of gene expression is controlled by cis-regulatory modules (CRMs) located within non-coding regions of the genome. These CRMs include enhancers, repressors and insulators. Conventional methods in this nematode to identify CRMs frequently focus on evolutionarily conserved sequences surrounding the promoter of the gene of interest, which are tested for functional regulatory activity using transgenic reporter assays. This technique is effective for individual genes but will often miss elements located away from the promoter. Other methods such as ChIP-seq measure transcription factor (TF) binding genome-wide produce data that can be mined to identify candidate CRMs. However, ChIP-seq requires prior knowledge of relevant TFs and availability of ChIP-grade antibodies. Deep sequencing of DNaseI treated chromatin can be used to map individual TF DNA binding sites with high resolution and this data can be mined to identify de novo active CRMs in the genome. We are adapting DNase-seq to nuclei isolated from *C. elegans*. We expect to generate high-resolution maps of TF binding in the worm at different developmental stages and will use this data to discover candidate active CRMs.

1056B. Analysis of developmental RNA-Seq libraries reveals signature profile for cilia-related genes. **Victor L. Jensen**, Tiffany A. Timbers, Chunmei Li, Ryan D. Morin, Michel R. Leroux. Molecular Biology and Biochemistry, Simon Fraser Univ, Burnaby, BC, Canada.

Ciliogenesis in *C. elegans* hermaphrodites occurs during late embryogenesis, with most cilia being formed within hours of each other. This makes *C. elegans* well suited to expression analyses to identify novel cilia genes. Using the available RNA-Seq libraries we identify a signature expression pattern specific to cilia-related genes, which includes a large spike in expression at late embryo with a reduction at mid-L1 (first larval stage) and low levels of expression at all other stages. P-values for Pearson correlation for each gene in the genome are compared to 41 known ciliary genes and each gene is then assigned the minimum calculated p-value. Using a 1E-5 cutoff and only including genes with human orthologues, we identify 635 genes with the specific pattern. Comparisons to CilDb (an online database of previously published ciliary genomics/proteomics experiments) reveals an 15-fold enrichment of genes with greater than seven or more cilia references. Further clustering of this list based on individual expression patterns increases the enrichment by greater than 40 times. Extending this expression analysis method, we also find a shared—but different from the above—expression pattern among serpentine olfactory G-protein-coupled receptors, many of which are known to be targeted to cilia. Using this approach, we identify evolutionarily-conserved proteins that function within three distinct ciliary regions or modules. One, a recently-characterized protein linked to retinitis pigmentosa, is found in the proximal-most segment of the axoneme, which is implicated as a ciliary gate needed for effective signalling. Another protein is present at the basal body, which is required for the formation of cilia. Finally, a third protein represents a previously undescribed component of the intraflagellar transport (IFT) machinery, which plays a critical role in ciliogenesis and delivering cargo (e.g. signalling proteins) to cilia.

1057C. Characterisation of genomic instability and interstrand crosslink sensitivity associated with mutation of *dog-1*, the functional ortholog of human Fanconi Anemia protein *FancJ*. **Martin R. Jones**, Jeffrey S. Chu, Ann M. Rose. Dept. of Medical Genetics, Univ of British Columbia, Vancouver, Canada.

C. elegans has a simplified FA pathway and as such has proven to be an invaluable model for the discovery and investigation of conserved FA-associated genes. We have previously identified DOG-1 as the functional ortholog of the human FANCJ helicase. Animals mutant for a null allele, *dog-1(gk10)*, share many of the hallmarks of FA cells such as increased genomic instability and sensitivity to interstrand crosslinks (ICLs). Although our understanding of DOG-1/FANCJ is growing, its precise function in ICL repair remains elusive. We are exploiting *C. elegans* genetics in combination with whole-genome sequencing (WGS) to investigate the relationship between genome stability and ICL sensitivity in DOG-1/FANCJ deficient animals. We have sequenced genomes derived from a sequential line of animals that have accrued mutations over a defined number of generations under standard laboratory conditions. From this analysis we are able to identify copy number variations (CNVs), including small deletions, and single nucleotide variations (SNVs). In the *dog-1(gk10)* genomes we find an accumulation of deletions in g-rich DNA as expected. We are currently expanding on this analysis to assess the genomes of *dog-1*

mutants that have been treated with a variety of mutagenic agents including those known to induce ICLs. We have demonstrated the feasibility of using WGS to assess genomic instability in *dog-1* genomes to a high resolution. Assessing the extent and type of genomic damage in ICL treated genomes will lead to a better understanding of how *dog-1/FANCJ's* function in maintaining g-rich DNA is correlated with its ability to prevent sensitivity to ICL inducing agents. Our findings have potential significance for the informed use of chemotherapeutic agents against cancers arising in FANCJ patients.

1058A. Genome-wide binding characteristics of the dosage compensation complex in *C. elegans*. **Anna-Lena Kranz**, Chen-Yu Jiao, Lara Winterkorn, Sarah Albritton, Sevinç Ercan. Department of Biology, Center for Genomics and Systems Biology, New York Univ, New York, NY.

In *C. elegans*, the dosage compensation complex is targeted specifically to both of the hermaphroditic X chromosomes to decrease X-linked transcription by half. This complex is homologous to subunits of the evolutionarily conserved condensins that play key roles in chromosome condensation. The dosage compensation complex condensin I^{DC} has been shown to initially bind to specific recruitment sites on the X and then spread along the chromosome. These recruitment sites are enriched for a previously reported DNA sequence motif. Performing ChIP-seq analyses, we identified a core part of the condensin I^{DC} motif enriched at binding sites of the canonical condensin II. Both the condensin I^{DC} and condensin II motif are not sufficient to explain all condensin binding specificity. Approximately 12% and 25% of the condensin I^{DC} and condensin II binding sites contain the motif, respectively. In addition, at a moderate motif match cutoff, 8% of the motifs across the genome are actually bound by each condensin. To identify additional factors that specify condensin I^{DC} binding to the X specific recruitment sites, we analyzed the chromatin context of bound and unbound motif sites by comparing different histone modifications from the modENCODE project. Employing a machine learning approach, we found that condensins favor an active chromatin environment and that specific chromatin marks are associated with each condensin type. Our results provide a first step into the identification of distinct factors that facilitate specific binding of condensins to the DNA, supporting a model in which active chromatin marks have a high predictive value for condensin binding.

1059B. The use of C.elegans to identify novel mutations that confer benzimidazole resistance. **Sharmilah L.J. Latheef**, Susan S. Stasiuk, John S. Gilleard. Univ of Calgary, Calgary, Canada.

Parasitic nematodes are a huge threat to the livestock industry as well as human health. Livestock infections cause billions of dollars in production loss per annum whilst infections in humans are a leading cause of disability and poverty especially in the developing world. Intensive use of the limited number of anthelmintics available for the treatment of parasitic nematode infections in livestock has resulted in the widespread development of resistance in many regions of the world. The recent increase in community-wide anthelmintic treatment programs in humans in the developing world-using the same drug classes used in livestock- is raising concerns of similar emergence of resistance in human parasites. Hence, there is an urgent need to understand the causal mechanisms of resistance and develop new diagnostic tests and control measures. Benzimidazoles (BZ) are an important drug class for both human and animal parasite control. It is known that amino acid substitutions in the isotype-1 b-tubulin drug target at positions 167, 198 and 200 can prevent drug binding and lead to drug resistance in several parasitic livestock species. The major objective of this project is to identify additional novel causal mutations of BZ resistance using the natural genetic variation found in wild *C.elegans* populations. The effect of albendazole on the motility of 16 natural *C.elegans* isolates was tested and two strains were identified that had a high level of resistance compared to the N2 strain. The *ben-1* locus was sequenced in these two strains and a novel mutation resulting in an Alanine to Proline substitution at amino acid position 185 was found in one strain and a frame-shift in the other. The functional significance of the identified non-synonymous polymorphisms was confirmed by genetic complementation studies with the *ben-1(e1880)* reference strain. Hence, we have identified naturally occurring strains with a high level of resistance to BZ drugs which leads to questions regarding the role of environmental pollution as a selective force. We've also identified a previously unreported amino acid substitution in the *ben-1* locus that is capable of conferring resistance which has yet to be reported in parasitic nematodes.

1060C. The involvement of the Caenorhabditis elegans EPE1 homolog in DNA interstrand crosslink repair. Sang-Yong An, **Changrim Lee**, Hyeon-Sook Koo. Biochemistry, Yonsei Univ, Seoul, South Korea.

DNA interstrand crosslink (ICL) repair involves the core Fanconi anemia (FA) pathway with a coordination of multiple DNA repair processes including nucleotide excision repair, translesion synthesis and homologous recombination. EPE1, JmjC domain-containing protein with no known enzymatic activity in Schizosaccharomyces pombe, acts as an antisilencing factor by preventing the spread of heterochromatin domains into euchromatin domains. Loss of EPE1 leads to the failure of specific heterochromatin boundary formation, which causes a defect in the maintenance of genome integrity. In Caenorhabditis elegans, JHDM-1 encoded by the open reading frame T26A5.5 is most similar to EPE1 in the amino acid sequence. We studied on JHDM-1, C. elegans EPE1 homolog, to find its novel functional role in DNA damage repair. Of interest, jhdm-1 mutant worms showed hypersensitivity to ICLs induced by photoactivated psoralen rather than to DNA damages formed by ionizing radiation or ultraviolet light. The recruitment of FCD-2, which is the major step of the FA pathway, to DNA damaged sites was maintained in jhdm-1 mutant worms. However, nuclear foci of the single-strand binding protein RPA-1 and the RAD-51 in response to ICLs disappeared more rapidly in jhdm-1 mutant worms compared with the wild type. The relaxation of heterochromatin structure for ICL repair was delayed in jhdm-1 mutant worms as probed for trimethylated histone H3 lysine 9. Therefore, inefficient ICL repair in the absence of JHDM-1 is probably due to strengthened heterochromatin structure affecting homologous recombination.

1061A. Using Next-Generation Sequencing to Determine Gene Identity in Temperature-Sensitive, Embryonic Lethal Mutants. **Josh Lowry**, Amy Connolly, Bruce Bowerman. Institute of Molecular Biology, Univ of Oregon, Eugene, OR.

While ~2500 genes in C. elegans are essential, temperature-sensitive alleles are available for only 100-200 of these genes. A major hurdle following any genetic screen has been the positional cloning of causal mutations. This process can be difficult and time consuming, and in the past most research has focused on narrow sub-classes of mutant phenotypes. To more rapidly determine gene identity in conditionally lethal mutants, we are employing simultaneous whole-genome sequencing and SNP mapping (Doitsidou et al, 2010). Briefly, the mutant of interest is outcrossed to the polymorphic strain CB4856.Then populations from ~50 F2 homozygous mutant progeny are pooled and genomic DNA extracted and sequenced on an Illumina HiSeq 2000. SNP mapping data is extracted from the resulting sequencing data using the CloudMap tool (Minevich et al, 2012), defining a chromosomal region in which

the mutation lies. The same sequencing data is then used to generate a list of candidate mutations in that region. Our goal is to map and identify the causal mutations in hundreds of conditionally lethal mutants, sampling a wide range of mutant phenotypes in an effort to explore new areas of essential gene function research. One class of mutants we are now exploring exhibit lethal defects at multiple stages of development. Mutants shifted to the restrictive temperature at the L4 stage produce embryos with eggshell defects. Shifting up at the L1 stage produces sterile adults with gonad development defects. We hypothesize that this class of mutants, the Osm/Ste class, may prove useful for studying the role of membrane trafficking during gonad morphogenesis. To date, we have obtained WGS-SNP mapping data for 16 mutants, with another set of 8 soon to follow. A short list of candidate mutations has been determined for each of them, with complementation tests and transgenic rescue to follow where appropriate. Current candidates include *rpl-7, gip-2, atx-2, aco-2, abtm-1* and *drp-1*. This high-throughput approach will allow us to clone more mutants far more quickly than has previously been possible.

1062B. Genomic analysis of Steinernema: Insights into insect parasitism, intragenus and intergenus evolution. **Ali Mortazavi[1]**, Marissa Macchietto[1], Adler Dillman[2,3], Alicia Rogers[2], Brian Williams[2], Igor Antoshechkin[2], Camille Finlinson[4], Zane Goodwin[5], Xiaojun Lu[6], Patricia Stock[7], Edwin Lewis[8], Heidi Goodrich-Blair[6], Byron Adams[4], Paul Sternberg[2,3]. 1) Developmental and Cell Biology, Univ of California, Irvine, Irvine, CA; 2) Division of Biology, California Institute of Technology, Pasadena, CA 91125; 3) Howard Hughes Medical Institute, Pasadena, CA 91125; 4) Department of Biology and Evolutionary Ecology Laboratories, Brigham Young Univ, Provo, UT 84602; 5) Division of Biology and Biomedical Sciences, Washington Univ, St. Louis, MO 63110; 6) Department of Bacteriology, Univ of Wisconson-Madison, Madison, WI 53706; 7) Department of Entomology, Univ of Arizona, Tucson, AZ 85721; 8) 8Department of Nematology, Univ of California, Davis, CA 95616.

Numerous nematode genera are major parasites of plants, animals, and humans, despite sharing a conserved body plan. Steinernema comprise over 70 characterized species that are lethal parasites of insects with differing foraging strategies and host ranges. We have sequenced the genomes and transcriptomes of five key members of Steinernema (S. carpocapsae, S. scapterisci, S. monticolum, S. glaseri, and S. feltiae) for comparative analysis. We find 20 Mb of conserved sequence, which represents about 23% of the S. carpocapsae assembly. This includes 127,282 non-coding elements accounting for about 5 Mb. We explore genomic differences likely to be involved in insect parasitism. We find gene family evolution of proteases, protease inhibitors, proteolytic cascade proteins, and GPCRs, many of which correlate with known differences in host range and specificity. Steinernema RNA-seq data allows for powerful comparisons to Caenorhabditis gene expression at defined stages, which show surprising plasticity of timing across one-to-one orthologous genes when compared to C. elegans. Our analysis of the conserved non-coding regions reveals that a limited number of motifs are associated with conservation of stage-specific ortholog expression, which suggests that key underlying gene regulatory relationships that control development are similar in the two genera.

1063C. Selenocysteine incorporation in metazoa: the peculiar case of the nematode lineage. **Lucía Otero[1]**, Laura Romanelli[1], Vadim N Gladyshev[2], Antonio Miranda-Vizuete[3], Gustavo Salinas[1]. 1) Universidad de la República, Montevideo, Uruguay; 2) Harvard Medical School, Boston, MA; 3) Instituto de Biomedicina de Sevilla, Spain.

Selenocysteine (Sec) incorporation into proteins occurs through a non-canonical mechanism: a Sec incorporation sequence (SECIS) present in the selenoprotein mRNA binds to a SECIS-binding protein allowing a UGA codon to be reprogrammed for Sec. Using *Caenorhabditis elegans* to study Sec incorporation, we found that its SECIS-binding protein (K04G2.11) is significantly shorter than any known SECIS-binding protein and lacks the putative Sec incorporation domain (SID). We amplified the full-length mRNA and confirmed the annotated sequence. A comparative analysis with other SECIS-binding proteins showed extended amino acid homology in the annotated 5'UTR, upstream the first in-frame AUG codon. This suggests that K04G2.11 would have a non-AUG translation initiation. Moreover, the extension of the ORF completes the functional L7Ae domain. Initiation of translation would occur at an AUU codon in an adequate context for translation initiation (GAAAAUU). We generated transgenic strains that overexpress K04G2.11 fused to a polyHis tail. By sequential metal and SECIS affinity chromatography, we isolated from transgenic strains, but not from N2, a 20kDa protein, which corresponds to the expected mass from the proposed AUU start codon. By ^{75}Se metabolic incorporation we demonstrated that this gene is functional and essential for Sec incorporation. The absence of the SID domain in the genome is remarkable and suggests that Sec incorporation mechanism in *C. elegans* is peculiar. We investigate Sec incorporation in the nematode lineage. The analysis of nematode genomes indicates: i) the SID domain is absent, ii) SECIS-binding proteins consist of the L7Ae domain only, iii) the existence of non-AUG initiation of translation in nematodes SECIS-binding proteins, and iv) the absence of Sec incorporation in plant nematode parasites. Our results indicate that nematoda is an interesting lineage to study the dynamics of the evolution of Sec incorporation. They also point out the occurrence of non-AUG translation initiation in *C. elegans*, not described before, a finding that needs to be further investigated.

1064A. Molecular characterization of mitocycin C-induced lethal mutations in *Caenorhabditis elegans*. **Annie Tam**, Jeffrey SC. Chu, Ann M. Rose. Department of Medical Genetics, Univ of British Columbia, Vancouver, British Columbia, Canada.

Mitomycin C (MMC) is a DNA crosslinking agent used clinically as a diagnostic for the genome instability syndrome Fanconi anemia, and also as a chemotherapeutic agent to treat a wide range of cancers. MMC covalently interacts with guanines in GC-rich segments of DNA, resulting in inter- and intrastrand DNA crosslinks, as well as DNA adducts. These interactions can cause DNA lesions that inhibit critical cellular processes such as replication, and if unrepaired lead to a range of chromosomal lesions. Although widely used, the prevalence and range of genomic instability caused by MMC has not been characterized. The nematode *Caenorhabditis elegans* is a well characterized genetic model in which to study genomic damage generated by exposure to crosslinking agents. In this context, we have performed forward genetic screens, determined the forward mutation frequency at different doses of MMC, and have isolated a number of lethal mutations for study.The lethal mutations were isolated and maintained as heterozygotes using *hT2*, a genetic balancer involving a reciprocal translocation between chromosomes I and III. We used three-factor mapping to narrow down the location of over 60 MMC-induced lethal mutations on chromosomes I and III. DNA from the genetic strains carrying the MMC-induced mutations has been sequenced using next-generation sequencing. As a consequence of the precise mapping of the lethal mutations, we can efficiently identify the physical basis of the lesions. We are in the process of characterizing the spectrum of mutagenic lesions caused by MMC, examining both their nature and frequency. Identification of the

types of DNA lesions caused by MMC not only furthers our understanding of this widely used drug, but may also lend insight into the molecular mechanisms required for their repair.

1065B. Combining genomic approaches to characterize alternative splicing events in *C. elegans*. **June Tan**[1,2], Arun Ramani[2], Hong Na[2], Debashish Ray[2], Timothy Hughes[1,2], Andrew Fraser[1,2]. 1) Department of Molecular Genetics, Univ of Toronto, Toronto, ON, Canada; 2) Donnelly Centre, Univ of Toronto, Toronto, ON, Canada.

Alternative splicing (AS) is a highly regulated process that contributes to proteome complexity. With recent advances in sequencing and other high-throughput technologies, our knowledge of the many AS events in *C. elegans* has rapidly increased. However, for many of these events, it is still not known which splicing factors are involved, and how they function to regulate each splicing decision. We have generated RNA-Seq, RNA binding, and genetic interaction data for several splicing factors to model how these factors regulate different AS events- whether individually, or combinatorially with other splicing factors.

We have identified AS events that are perturbed in several splicing factor-defective backgrounds- including loss-of-function of *asd-1*, *fox-1*, *mec-8*, *sym-2* and *exc-7*. Combining this data with RNA-binding specificities for these splicing factors, we have identified genes that are likely direct targets of these splicing factors, and are in the process of validating these splicing targets *in vivo*. In addition, we systematically probed for other factors that genetically interact with these splicing factors by screening an RNAi library targeting ~400 genes containing known or predicted RNA-binding domains. Scoring for enhanced population fitness defects, we have identified interactions with components of the spliceosome, regulatory splicing factors, as well as other factors involved in mRNA-processing. We are using this data as an added resource to predict factors that may function in the same splicing regulatory pathways, to identify instances of combinatorial splicing regulation.

1066C. Determining Fragile Nucleosome Distribution Bias Within the Chromosome. **Ashley Wright**, Steven Johnson. MMBIO, Brigham Young Univ, Provo, UT.

Nucleosomes are the fundamental units of chromatin compaction and play a major role in many molecular phenomena including gene regulation. We recently published an analysis comparing nucleosome occupancy between *in vivo* isolated nucleosomes and *in vitro* nucleosome reconstitutions on *C. elegans* genomic DNA[1]. This analysis revealed nucleosome depletion on the arms of worm chromosomes *in vivo*. This result can be explained by two possibilities. Either the ends of chromosomes, in general, have a lower frequency of nucleosomes or this distribution may be the result of a higher frequency of nucleosomes sensitive to Micrococcal nuclease digestion (Fragile Nucleosomes[2]) localized to the chromosome ends. In order to understand the cause of this nucleosome depletion we are doing experiments to establish if fragile nucleosomes are unevenly distributed and depleted towards the chromosome ends. Therefore, we have isolated mononucleosome cores from a series of Micrococcal nuclease digestions of increasing concentration and digestion time. Extensive Sanger sequencing has been performed to confirm the integrity and diversity of our nucleosome core library. Our current efforts are concentrating on high-throughput sequencing of our cores and genomic analysis. Upon successful completion of sequencing, we will map the reads back to the worm genome in order to determine the overall distribution of each digestion condition. The result can then be correlated to our *in vivo* data. An uneven distribution of the lightly digested cores, favoring the ends would suggest the general presence of fragile nucleosomes. At the end of this investigation, we hope to continue to push forward our understanding of the global positions of nucleosomes and their effect on cellular processes.

[1]Locke, G. et al. (2013) Global remodeling of nucleosome positions in C. elegans. BMC Genomics.

[2] Xi, Y. et al. (2011) Nucleosome fragility reveals novel functional states of chromatin and poises genes for activation. Genome Res. 21 (5):718-724.

1067A. Identification of Molecular Targets of the Antidiabetic Drug Metformin in C. elegans. **Lianfeng Wu**[1,2], Alexander Soukas[1,2]. 1) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; 2) Department of Medicine, Harvard Medical School, Boston, MA, USA.

Metformin is a most widely prescribed drug to treat type II diabetes. It has been in use to treat diabetes for more than 60 years, and yet the molecular mode for their action remains poorly characterized. Unbiased functional screening for metformin targets in vertebrates remains difficult for technical reasons. However, metformin has effects on growth and metabolism of C. elegans, thus enabling its use as a gene discovery platform. Metformin induces multiple beneficial effects such as lowering blood glucose and free fatty acid levels in humans. C. elegans has highly conserved metabolic pathways with higher eukaryotes, making it an ideal system to study the molecular and cellular response to metformin. In contrast to recently published effects of metformin on bacterial metabolism, we find that metformin has dose-dependent effects directly on C. elegans growth. In this study, we screened for genes involved in the action of metformin in C. elegans using a custom RNAi library of 1058 genes. Those genes are annotated to play a role in metabolism based upon description or gene-ontology (GO) term. We screened for genes, which, when knocked down by RNAi, made C. elegans resistant or sensitive to the effects of metformin on slowing growth rate. After quadruplicate repeat screening, 16 metformin response genes were identified. All 16 genes have human orthologs, and 14 have been associated with metabolic traits such as fasting glucose, insulin, body mass index, or insulin resistance in humans. One of our most confident hits is a gene with unknown function (mrg-1, metformin response gene 1), having a human ortholog annotated to play a role in fatty acid oxidation. Metformin increases the expression of mrg-1 at both of the mRNA and protein levels, independent of the effect on the bacterial food source. We have identified 12 genetic mutants by whole genome sequencing upstream of mrg-1, which play a role in the response to metformin. Our ongoing work will uncover the role of mrg-1 and its related pathways in the regulation of metformin action.

1068B. Analysis of Histone methylation in germ cells using C. elegans as model system. **Pier Giorgio Amendola**, Toshia Myers, Anna Elisabetta Salcini. Biotech Research and Innovation Centre, Copenhagen, Ole Maaløes Vej 5, Denmark.

Background. DNA is packaged in the cellular nucleus as chromatin, which consists of repeating units of DNA wrapped around core histone proteins. Histone tails undergo a number of modifications such as methylation at arginine and lysine residues, which regulate gene activation and repression by influencing chromatin compaction. The addition and the removal of methyl groups from histone tails are carried out by histone methyltransferases (HMTs) and histone demethylases (HDMs), respectively. Both these classes of enzymes are evolutionarily conserved across diverse species ranging from C. elegans to humans. The C. elegans germ line represents an excellent system to investigate the dynamic nature of chromatin modifications and the roles of

chromatin factors in establishing and maintaining chromatin marks. **Results**. In this project I propose to unravel the 1) in vivo role(s) of the histone demethylase JMJD-1.1, both under physiological and stress conditions, 2) its catalytic activity and 3) its crosstalk with other histone modifiers using C. elegans as model system. JMJD-1.1 is a putative H3K9/K27me2 demethylase and shows co-linearity and homology to the members of the mammalian KDM7/PHF family. We identified the specific expression of jmjd-1.1 in the germ line by quantitative PCR (qPCR) analysis performed in wild-type animals and in animals lacking the germ line. Accordingly, we found a misregulation of both the histone marks H3K9/K27me2 in the germ line of mutant male animals. First evidences suggest that jmjd-1.1 may be involved in the process of spermatogenesis. Two mutant alleles for jmjd-1.1 are available. We are currently caracterizing phenotypes associated to loss of jmjd-1.1. First results indicate that these phenotypes are mostly male-related, further suggesting that jmjd-1.1 may have a role in spermatogenesis. **Conclusions**. JMJD-1.1 is the first histone demethylase identified to be exclusively express during spermatogenesis. Initial characterization of mutant alleles indicates that JMJD-1.1 has a role during spermatogenesis, that we are further characterizing using male population. These and additional results will be reported at the meeting.

1069C. Functional analysis of H3K79 methylation. **Fanelie Bauer**, Alex Appert, Julie Ahringer. The Gurdon Institute, Univ of Cambridge, UK.

H3K79 methylation has been linked to transcriptional activation however the mechanism of H3K79 targeting and function are not well understood. H3K79 methylation is carried out by the Dot1 family of histone methyltransferases, which are characterized by a class I SAM-dependent methyltransferase domain. In all studied organisms, DOT1 is solely responsible for all H3K79 methylation states as the knockout of this gene results in a total loss of mono- di- and tri- methylation of H3K79 methylation. In yeast, non-methylated H3K79 is found at silenced telomeres, whereas H3K79 methylation at telomeres disrupts transcriptional silencing. In humans, leukaemia causing MLL fusions to AF9 recruit Dot1, and inactivation of Dot1 reduces expression of MLL-AF9 targets and inhibits progression of the disease. Dot1 has also been implicated in DNA damage checkpoint control and repair, the cell cycle, and is required for early embryonic development in both mice and flies. We are carrying out a functional analysis of H3K79 methylation in *C. elegans*. The worm genome has six DOT1L homologs, of which only Y39G10AR.18 is highly expressed across all developmental stages. As in other organisms, H3K79me2 and H3K79me3 are present on the bodies of transcriptionally active genes in early embryos and L3 larvae (Liu et al 2011). However, the pattern of H3K79me1 differs in early embryos and L3 larvae. Regions of high H3K79me1 in early embryos often lack this modification in L3 larvae. We are assessing which genes carry out H3K79 methylation and studying the function and developmental regulation of H3K79 methylation.

1070A. Roles of histone demethylation in germline maintenance. **Sara E Beese-Sims**, Monica P Colaiacovo. Department of Genetics, Harvard Medical School, Boston, MA.

Histone tails are the targets of various covalent but reversible modifications that affect chromatin structure and direct the activities of protein complexes to sites along the chromosome for specialized functions. The methylation of histone lysines has roles in transcription and DNA damage response, where defects can result in cancers, and also plays a prominent role in crossover formation during meiosis, where defects can result in birth abnormalities, infertility and miscarriages.

Three putative LSD1/2 (lysine specific demethylase) homologs exist in *C. elegans* (SPR-5, LSD-1, and AMX-1). SPR-5 demethylates H3K4me2[1,2], an *spr-5* mutant has a progressive sterility phenotype[1], and early generation *spr-5* mutant germlines experience a defect in DNA double strand break repair (DSBR)[2]. Microarray analysis of *spr-5* mutants revealed increased *amx-1*[1,2] expression, suggesting a functional overlap between SPR-5 and AMX-1. To understand the interplay between the LSD1/2-like proteins in *C. elegans* meiosis, we investigated their germline functions by comparing single, double, and triple mutants.

We found that early generation *spr-5;lsd-1* and *spr-5;amx-1;lsd-1* mutants have reduced brood sizes and elevated embryonic lethality relative to wild type, and that the *lsd-1*, *spr-5;amx-1*, and *spr-5;amx-1;lsd-1* mutants have an increased frequency of males. These phenotypes are progressive and are indicative of impaired meiotic chromosome segregation. All of the early generation homozygous mutants experience a defect in meiotic DSBR, as evidenced by elevated levels of RAD-51 (a DSBR protein) foci and the activation of a pachytene DNA damage checkpoint. Additionally, SPR-5[2] and LSD-1 relocalize to chromosomes in response to DSB induction, suggesting a physical role for these proteins in DNA damage response. Taken together, our analysis indicates that all three LSD1/2-like proteins play a role in DSBR that is important for proper maintenance of the *C. elegans* germline, and that SPR-5 and LSD-1 may play a direct role in DNA damage repair.

1.Katz, et al., 2009. *Cell*. 2.Nottke, et al., 2011. *PNAS*.

1071B. Forward Genetic Screen for Induced Conversion of Germ Cells. **Idris Selman Bulut**, Oktay Ismail Kaplan, Baris Tursun. Max Delbrück Center (MDC), Berlin, Germany.

The ability to induce conversion of cell identities could become very important for future therapeutic approaches. Understanding mechanisms, which regulate or counteract conversion of cell types, is relevant for such medical goals. It is poorly understood whether similar mechanisms restrict cell fate plasticity during cell differentiation in development, in mature cells or in cells that need to maintain undifferentiated states such as stem cells or the germline. There are different ways for changing cell identities by reprogramming. One involves direct reprogramming (DR) of a cell type into a defined cell fate by a specific cell defining transcription factor (TF). The source and identity of cells matters for DR, suggesting not all cells are equally easy to be reprogrammed. For example, ectopic expression of the Zn-finger TF CHE-1 (required for specification of ASE neurons) in *C. elegans* could convert only a few cells into ASE-like neurons, raising the question why most other cells do not respond. There are two possibilities to explain such restriction in cell reprogramming: the absence of co-activators or the presence of inhibitory mechanisms. The latter has been addressed in *C. elegans*, where RNAi mediated knock-down of chromatin-associated factors revealed that LIN-53 (Rbbp4/7 in mammals) is a barrier for TF-induced conversion of germ cells into specific neurons or muscle-like cells (Tursun et al. 2011, Patel et al. 2012). Using transgenic worms, which ectopically express *che-1* along with a fluorescence tagged ASE neuron marker (*gcy-5::gfp*), we have performed a forward genetic screen to identify factors inhibiting conversion of germ cells into ASE-like neurons. Our screen has generated over 50 mutants phenotypically resembling the conversion of germ cell into ASE-like neurons upon CHE-1 induction when LIN-53 is knocked down. Initial analysis revealed that upon induction of ectopic che-1 expression germ cells in a number of these mutants acquire neuron like morphology (axo-dendritic-like extension and neuron like nuclei). We will report our progress in characterizing these mutants at the meeting.

1072C. O-GlcNAcylation of a conserved chromatin factor, ZFP-1(AF10), as a possible glucose-sensing mechanism. **Ainhoa Ceballos**, Germano Cecere, Daphne Avgousti, Grishok Alla. Columbia Univ, New York, NY.

The hallmark of Type 2 Diabetes is a defect in the glucose-sensing mechanism inducing insulin resistance. Here, we propose a novel epigenetic regulation of the insulin signaling pathway that involves a chromatin-associated protein and O-GlcNAcylation. Recently, we have described a role for C. elegans ZFP-1 protein, a conserved chromatin-binding factor, in negatively modulating transcription of the pdk-1 gene, which encodes a kinase critical for insulin signaling (Mansisidor et al., 2011). In addition, preliminary results from our laboratory show evidence of post-translational modifications of ZFP-1 and we have noticed almost identical genome localization profiles of ZFP-1 (Mansisidor et al., 2011) and O-GlcNAc modification (Love et al., 2010). O-GlcNAcylation is one of the most abundant eukaryotic post-translational modifications, which participates in the regulation of insulin production and modulates systems responsive to insulin. Based on these multiple observations, we are investigating whether ZFP-1 is modified by O-GlcNacylation, whether glucose stress promotes this modification and whether this leads to enhanced negative modulation of pdk-1 transcription. Finally, we are also interested in determining whether the mammalian homolog of ZFP-1, AF-10, is similarly involved in this new mechanism of insulin signaling inhibition.

1073A. "Who wants to live forever?"- chromatinome RNAi screen for longevity in *C. elegans*. **Karolina Chocian[1]**, Hayley Lees[1], Helena Cantwell[1], Gino Poulin[2], Jane Mellor[1], Alison Woollard[1]. 1) Department of Biochemistry, Univ of Oxford, Oxford , United Kingdom; 2) Univ of Manchester, Manchester, United Kingdom.

To aid our understanding of the ageing process and its connection to epigenetics I used a non-biased approach to uncover novel chromatin factors affecting life span in *C. elegans*. To date, there is some evidence that aged organisms and cells display particular epigenetic marks, however, the mechanism by which chromatin is modified and the biological significance of such modifications as we age is unclear. I performed an RNAi screen for longevity using a chromatinome library and assessed the accumulation of lipofuscin as a biomarker of ageing. Preliminary results are encouraging, with 16 'confident hits' (where lipofuscin accumulation is significantly delayed on both days of screening) and a further 28 genes under investigation. The validity of the screening approach is confirmed by matching several of the hits to factors that have previously been associated with lengthening lifespan in model organisms (e.g. *mes-2*, *T26A5.8*, *F21H12.1*). A further secondary screen with a simple lifespan assay is being undertaken to select genes of particular interest.

1074B. synMuv B regulation of chromatin states at high temperature. **Meghan Elizabeth Costello[1]**, Andreas Rechtsteiner[2], Thea Egelhofer[2], Susan Strome[2], Lisa N Petrella[1,2]. 1) Department of Biological Sciences, Marquette Univ, Milwaukee, WI; 2) Department of MCD Biology, UC Santa Cruz, Santa Cruz, CA.

Most organisms experience variations in temperature throughout their lifespan and thus require mechanisms to buffer gene expression in response to fluctuations in temperature. It has been observed that during development, chromatin states are influenced by temperature. For example, in *Drosophila* heterochromatin formation and spreading is partially compromised at high temperatures. In order to understand the effect of temperature during development, it is critical to understand how chromatin structure affects the buffering of gene expression at elevated temperatures. We are investigating changes in chromatin state at high temperature through study of the *C. elegans* synMuv B chromatin regulators. The synMuv B proteins include Retinoblastoma/LIN-35 and other members of the DRM complex and HPL-2, the worm homolog of HP1. Mutations in many synMuv B genes cause high temperature larval arrest (HTA) due to derepression of germline gene expression in somatic cells, most notably the intestine. Loss of germline chromatin modifiers suppresses somatic expression of germline genes in synMuv B mutants and rescues the HTA phenotype. This suggests that changes in chromatin states in the soma underlie the temperature-sensitive gene misexpression in synMuv B mutants. Consistent with this hypothesis, immunostaining of synMuv B mutants revealed altered levels of specific histone modifications in somatic tissues. To investigate changes in active and repressive chromatin marks at high resolution genome-wide in synMuv B mutants, we have performed ChIP-seq experiments in *lin-35*, *lin-37*, and *lin-15B* mutants at both low and high temperature. Additionally, in order to determine if the absence of wild-type synMuv B proteins causes the loss of a closed chromatin state at elevated temperatures, we are using the lacO/LacI system to visualize changes in chromatin compaction in *lin-15B* and *lin-35* mutants.

1075C. Epigenetic regulation of fertility in *C. elegans* males depends on the gamete source and chromatin history of the X chromosome. **Laura Gaydos[1]**, Andreas Rechtsteiner[1], Wenchao Wang[2], Susan Strome[1,2]. 1) Dept of Molecular, Cell and Developmental Biology, Univ of California Santa Cruz, Santa Cruz, CA, USA; 2) Dept of Biology, Indiana Univ, Bloomington, IN, USA.

Organisms with different numbers of X chromosomes in the two sexes have evolved diverse strategies to regulate X-linked gene expression. Somatic cells compensate for the difference in X dosage by inactivating one X in XX mammals, down-regulating both Xs in XX *C. elegans*, or up-regulating the single X in XY *Drosophila*. Germ cells in *C. elegans* display a more extreme type of regulation, near silencing of both Xs in XX hermaphrodites and the single X in XO males. X repression in the germline is achieved at least in part by the MES proteins, epigenetic regulators that must be maternally provided to progeny to ensure survival of the primordial germ cells. In the adult germline, the MES-2/3/6 complex, which is the worm version of Polycomb Repressive Complex 2, concentrates a repressive histone modification (H3K27me) on the Xs. MES-4 participates in X repression in an indirect manner. MES-4 catalyzes a mark of active chromatin (H3K36me) on germline-expressed genes on autosomes; this repels H3K27me from autosomal regions and helps concentrate H3K27me on the Xs. Recent investigation of whether the MES proteins participate in repression of the single X in XO males has revealed that the answer depends on the gamete source of the X. Maternal MES function is required when the X in males is inherited from the oocyte but not when the X is inherited from the sperm. In the latter case, X repression appears to be mediated by enzymes that generate the repressive histone modification H3K9me. Our studies have shown that the opposing activities of MES-2/3/6 and MES-4 repress transcription from the two Xs in XX germ cells, and that in XO germ cells H3K9me can serve as another mode of repression.

1076A. CEC-4 is a novel chromodomain protein involved in perinuclear chromatin anchoring. **Adriana V. Gonzalez Sandoval[1,2]**, Veronique Kalck[1], Benjamin D. Towbin[1,2], Teddy Yang[3], Kehao Zhao[3], Susan M. Gasser[1,2]. 1) Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland; 2) Univ of Basel, Faculty of Sciences, Klingelbergstrasse 50, CH-4056 Basel, Switzerland; 3) China Novartis Institutes for BioMedical Research Co., Ltd. Building No. 3, Lane 3728 Jinke Road, Pudong New Area, Shanghai 201203. China.

A genome-wide RNAi screen identified two histone H3K9 methyltransferases, MET-2 and SET-25, which deposit histone H3K9 me1, me2 and m3, respectively, as being necessary for both the anchoring and the repression of heterochromatic gene arrays (Towbin et al., 2012). H3K9m1 or me2 are sufficient for tethering, while trimethylated H3K9 was necessary for repression. To identify the proteins involved in heterochromatin anchoring at the nuclear envelope we screened "readers" of these histone marks in a targeted RNAi screen. Neither homologue of HP-1, HPL-1 nor HPL-2 were implicated in the anchoring, although loss of HLP-2 led to derepression. We screened 44 other Chromo-, MBT-, PHD- and Tudor- domain-containing genes in a strain deficient for HPL-1 and LIN-61, for loss of heterochromatin. We identified a previously uncharacterized chromo domain protein, CEC-4, as being essential for maintaining heterochromatin at the nuclear periphery. However, in contrast to the double mutant of *met-2* and *set-25*, deletion of *cec-4* does not cause array derepression. CEC-4 forms a ring around the nucleus, and colocalizes with the anchored array. We show that the chromodomain of CEC-4 binds all three methylated forms of H3K9, but no other methylated lysines in vitro. Using two points mutations within the chromodomain that lose affinity for methylated H3K9 in vitro, we confirm that CEC-4 requires a functional chromo domain to bind and tether heterochromatin, while interaction with the nuclear envelope requires the non-chromo domain portion of CEC-4. By LEM-2 ChIP-seq we determined that deletion of *cec-4* globally reduces the interaction of chromosome arms to the nuclear periphery.

1077B. An extended RNAi sub-library to uncover chromatin factors implicated in direct cell-type conversion. **M. Hajduskova**, M. L. Beato del Rosal, E. Kolundzic, B. Tursun. Max Delbrück Center, Berlin, Germany.

Reprogramming of cellular identities is a strategy of tremendous application potential in regenerative medicine. Scientists seek to convert fully differentiated cells into pluripotent cells or into differentiated cells of another identity. The latter alternative presents a direct cell-type conversion that changes a differentiated cell into another cell without inducing a stem cell-like state. The capacity of cells to be directly reprogrammed may depend on the cellular environment and it may normally be prevented by protective mechanisms. *C. elegans* permits studies of direct cell-type conversion within a range of tissue contexts, yet at a single-cell resolution. In addition, powerful genetic and RNAi screenings allow identification of inhibitors of cell conversion. It has been recently shown that depletion of LIN-53 or other subunits of the Polycomb repressive complex (PRC2) facilitates germ cell conversion into neuron or muscle-like cells upon ectopic expression of a specific fate-inducing transcription factor (TF) [1,2]. The role of *lin-53* emerged from a previously generated RNAi sub-library targeting 560 factors implicated in regulating chromatin structure and modifications. However, this sub-library was incomplete, prompting us to construct an updated RNAi sub-library against *C. elegans* chromatin factors. Candidate genes were selected for conserved protein domains and based on literature. This novel '2.0 Chromatin' RNAi sub-library comprises 665 clones, of which 531 originate from the Ahringer library, 49 from the Ahringer Supplement, and 38 from the Vidal library. Additional 41 clones were newly generated in our lab. We are currently screening this RNAi sub-library for inhibitors of direct cell reprogramming. Upon expression of fate-inducing TFs in the RNAi backgrounds, we look for ectopic activation of specific cell-fate reporters. Resulting positive phenotypes will be confirmed in mutant backgrounds (if available) and the nature of induced cell conversion will be further characterized. We believe to discover molecular mechanisms that normally prevent direct cell conversion *in vivo*. 1] Tursun et al., 2011. Science 331:304-8. 2] Patel et al., 2012. Cell Rep 2:1178-86.

1078C. Nucleosome organization in *C. elegans* gamete chromatin. **Tess E. Jeffers**[1,3], Jason D. Lieb[1,2,3,4]. 1) Curriculum in Bioinformatics and Computational Biology, Univ of North Carolina, Chapel Hill, NC; 2) Department of Biology, Univ of North Carolina, Chapel Hill, NC; 3) Carolina Center for Genome Sciences, Univ of North Carolina, Chapel Hill, NC; 4) Lineberger Comprehensive Cancer Center, Univ of North Carolina, Chapel Hill, NC.

We are interested in how the genome is packaged in gametes (sperm and oocytes), and how differences might contribute to the specification of zygotic gene expression programs. Using *fem-1* and *him-5* mutants, we separately isolated pure populations of *C. elegans* sperm and oocytes and performed RNA-seq and nucleosome mapping through MNase-seq. Preliminary transcriptome analysis reveals ~700 transcripts with higher relative levels in sperm, and ~2000 transcripts with higher relative levels in oocytes, in agreement with quantities reported by previous microarray and proteomics experiments (Reinke et al., Mol. Cell 2000 & Development 2004, Chu et al., Nature 2006). Sperm are enriched in transcripts encoding proteins that function in the cytoskeleton, as chaperones, and in the mitochondria while oocytes are enriched for transcripts that encode proteins functioning in mRNA processing, mitosis and meiosis. By integrating our nucleosome and transcriptome data, we find nucleosome depletion at the promoters of genes highly transcribed in the germline. Future experiments will investigate the use of differential histone isoforms and histone modifications in gamete genome packaging.

1079A. Conversion of epithelial cells into a neuron like cell in *C. elegans*. **Oktay Ismail Kaplan**, Idris Selman Bulut, Baris Tursun. Max Delbrück Center (MDC), Berlin, Berlin, Germany.

Cell reprogramming has been an intensive focus of research in recent years due to its great potential for new medical applications. Different procedures such as converting one cell type to another distinct cell type either through generating stem cell-like states in vitro or by direct reprogramming are continuously being explored. One limitation is that many cells display resistance to be directly reprogrammed probably due to the absence of "co-activators" or presence of "inhibitory factors" required for cell reprogramming. C. elegans has recently emerged as a genetic tool to identify "inhibitory factors" required for cell fate reprogramming. A recent reverse genetic analysis of chromatin factors has successfully revealed a role for the conserved gene lin-53 (mammalian Rbbp4/7) in reprogramming mitotic germ cells into neuron and muscle like-cells, depending on induction of fate-inducing transcription factors such as CHE-1 or HLH-1 (MyoD), respectively. However, forced reprogramming of differentiated somatic cells into neurons has remained to be difficult in C. elegans (Tursun et al. 2011, Patel et al. 2012). Towards uncovering novel inhibitory factors required for conversion of differentiated somatic cells into a neuronal like cell, we have conducted a forward genetic screen. Our screen has identified numerous mutant backgrounds, where ectopic expression of che-1 (induces glutamatergic ASE neuron fate) induces expression of numerous neuronal genes in epidermis, potentially overcoming a barrier for directly reprogramming epidermal cells into neuron-like cells. Our findings suggest that, in addition to germ cells, differentiated somatic cells in C. elegans have the potential to be reprogrammed into neuron like cells. We are in the process of mapping genes responsible for restricting the reprogramming of differentiated somatic cells. **References:** 1)Patel T, Tursun B et al Removal of Polycomb Repressive Complex 2 makes C. elegans germ cells susceptible to direct conversion into specific somatic cell types. Cell Reports 2012; in press (DOI: 10.1016/j.celrep.2012.09.020) 2)Tursun B, Patel T et al Direct conversion of C. elegans germ cells into specific neuron types Science 2011; 331: 304-308.

1080B. Examining the Role of Histone Acetyltransferases in Targeting the *C. elegans* DCC to the X Chromosomes. **Alyssa C. Lau**, Gyorgyi Csankovszki. Department of Molecular, Cellular, and Developmental Biology, Univ of Michigan, Ann Arbor, MI.

Dosage compensation (DC) is the gene regulatory mechanism that equalizes X-linked gene expression between the sexes. Worm DC is achieved by the activity of the dosage compensation complex (DCC). The DCC contains a subcomplex similar to the evolutionarily conserved condensin complexes, which help promote chromosome compaction, organization and segregation during meiosis and mitosis. Therefore, it is believed that worm DC is involved in changes in X chromosome structure; however, the molecular mechanism is not known. How the DCC is able to distinguish the X from the autosomes is also not fully understood, although an X-enriched sequence motif is known to be involved. We aim to uncover the roles of chromatin structure modulation by histone acetyltransferases (HATs) in DCC targeting and function.

HATs are responsible for acetylating the histones within the nucleosome. Using FISH microscopy we noticed that worms depleted of the HATs MYS-1, MYS-4 or CBP-1 exhibit a dispersed X chromosome structure when normally the X chromosome territory is compact. This contradicts the belief that histone acetylation influences a less compact chromatin configuration. Interestingly, the same dispersed X chromosome structure is also seen in DCC mutants. However, the autosomal structure of chromosome 1 appears unchanged when the HATs are depleted. In addition, using immunofluorescence and FISH microscopy, we find that DCC localization is disrupted in HAT depletions. We see mislocalization of the DCC away from the X chromosomes and onto autosomes, suggesting that HATs play a role in targeting the DCC to the X chromosomes, which in turn may be responsible for maintaining a compact X chromosome structure. Therefore, chromatin organization likely plays an important, and as yet incompletely understood, role in *C. elegans* dosage compensation.

1081C. Dao-5/CeNopp140 modulates rDNA chromatin epigenetic status and transcription to sustain oogenesis. **Chi-Chang Lee**[1], Yi-Tzang Tsai[2,3], Li-Wei Lee[2], Chih-Wei Kao[2], Huey-Jen Lai[2], Tien-Hsiang Ma[2,3], Yu-Sun Chang[2,3], Ning-Hsin Yeh[1], Szecheng J. Lo[1,2,3]. 1) Institute of Microbiology and Immunology, National Yang-Ming Univ, Taipei, Taiwan; 2) Department and Gradate Institute of Biomedical Sciences, Chang Gung Univ, Taoyuan, Taiwan; 3) Center of Molecular Medicine Research, Chang Gung Univ, Taoyuan, Taiwan.

Human diseases of impaired ribosome biogenesis resulted from disruption of rRNA biosynthesis or loss of ribosomal components are collectively described as “ribosomopathies”. Treacher-Collin syndrome (TCS), a representative human ribosomopathy with craniofacial abnormalities, is contributed to the mutations of tcof1 gene which has a paralog gene called nopp140. However, the role of Nopp140 in human disease has not been identified yet. Here, we established a *C. elegans* model for studying Nopp140 phenotypes and functions. With the features including conserved protein domains, nucleolar localization and rDNA occupancy, we demonstrated that DAO-5 is *C. elegans* Nopp140 (CeNopp140) implicated in rRNA synthesis. A null *dao-5* mutant (*ok542*) with a phenotype of semi-infertility showed a delayed gonadogenesis as well as a higher incidence of germ-line apoptosis. Both in situ Br-UTP incorporation assay with the derived embryonic cell cultures and the ChIP assay determining RNA pol I occupancy on the rDNA promoter revealed an inefficient rRNA transcription pattern in the absence of DAO-5. Since rRNA transcription is tightly coupled with histone modifications on nucleosomes around the rDNA promoter, we examined whether DAO-5 deficiency is also impinged in this level. Chromatin immunoprecipitation (ChIP) assays showed a lower amount of acetylated histone 4 (H4Ac) and a higher amount of H3K9me2, but not H3K9me3, around the rDNA promoter in dao-5 mutants, suggesting that Dao-5 is crucial for rDNA promoter accessibility. This is the first demonstration that Nopp140 involves in epigenetic control of rRNA transcription and links to oogenesis impairment when its function lost in C. elegans. We thus allege that *C. elegans dao-5* mutant could be a valuable model for studying human Nopp140-associated ribosomopathy at cellular and molecular levels.

1082A. Epigentic regulation of L1 longevity. **Inhwan Lee**, Young-jai You. Biochemistry and Molecular Biology, VCU, Richmond, VA.

Animals encounter food infrequently and starvation is a common physiological state in their natural circumstance [1]. Hatching in the absence of food, worms arrest their development at the L1 stage and survive starvation more than two weeks [2]. It was shown that adult longevity is regulated epigenetically by chromatin alterations and the genes that regulate epigenetics [3]. Here, we investigate whether L1 longevity is also regulated epigenetically. When we measured various histone modifications using Western blot, we found histone 3 lysine 4 tri-methylation, known to be a modification to activate gene transcription, is increased in L1 starvation. Moreover, mutants of set-2, which encodes a histone 3 lysine 4 tri-methyl transferase that functions in adult longevity [3], has reduced L1 longevity. There are several genes essential for normal L1 longevity, such as aak-2 and daf-16. Based on our data, we hypothesize that chromatin remodeling through histone 3 lysine 4 tri-methylation regulates transcription of genes involved in L1 longevity. Currently we are testing this hypothesis by measuring expression levels these genes by ChIP-qPCR during L1 starvation in set-2 mutant. References [1] Brian H. Lee, Plus Genetics, 2008 [2] Inhwan Lee, Plus One, 2012 [3] Eric L. Greer, Nature, 2010.

1083B. Spatial gene positioning in the *Caenorhabditis elegans* embryo. **Darina Majovská**, Christian Lanctôt. Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles Univ in Prague, Prague, Czech Republic.

We have investigated spatial gene positioning in the nuclei of the early C. elegans embryo. The developmental constancy of this model organism allows 1) to compare 3D gene positioning in cells that are equivalent in terms of history, developmental potential and gene expression profile; and 2) to identify changes associated with lineage commitment and cellular differentiation. We have used multi-color 3D FISH to determine whether gene positioning in the nucleus follows reproducible patterns during the development of C. elegans. Centrosomes were immunolabeled prior to FISH and served as extra-nuclear reference points to align embryonic nuclei both in time and space. The spatial distributions of 4 pairs of genomic segments (approx. 30-40kb in length) was assessed in the AB and P1 blastomeres of the 2-cell embryo. Comparisons were made between corresponding nuclei from different embryos. Our initial analysis failed to reveal reproducible geometrical patterns of 3D gene positioning at this early stage. The radial positioning of loci, given by the distance to the nuclear border in the spherical embryonic nuclei of C. elegans, was also analyzed before (2-cell embryo) and after embryonic genome activation (26-cell embryo). At these stages, no significant correlation was observed between nuclear localization and transcriptional activity or chromosomal localization. At later stages (~100-cell embryo), active genes were found to be preferentially localized in the nuclear interior. Altogether, these results suggest that the early worm embryo, despite fixed lineage commitment and gene expression programs, is characterized by a plastic architecture of its cell nuclei.

1084C. Dynamics of nuclear compartments in *C. elegans*. Rahul Sharma[1], Georgina Gomez-Saldivar[2], Jop Kind[3], Bas van Steensel[3], Peter Askjaer[2], **Peter Meister[1]**. 1) Cell Fate and Nuclear Organization, IZB - Univ of Bern, Bern, Switzerland; 2) CABD, CSIC-Universidad Pablo de Olavide, Sevilla, Spain; 3) Division Gene Regulation, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

During development and differentiation, the genome gets progressively organized inside the nuclear space. The function of genome nuclear organization remains elusive, although a number of human diseases are linked to mutations in structural elements of the nucleus. Decreasing mobility of chromatin and chromatin factors moreover correlates with loss of differentiation potential, suggesting chromatin plasticity could be an integral part of pluripotency. Similarly to mammalian cells, the worm genome gets partitioned during cell fate acquisition into active and silent domains inside the nuclear space. In *C. elegans* current data suggest that the nuclear interior is a rather active domain, while the nuclear periphery is a mosaic of active and silent compartments. The dynamics of these domains during physiological challenges, cell fate determination or during organismal aging is however poorly understood. Genomic characterization of subnuclear domains has been achieved using damID, a technique in which a fusion protein between the *E. coli* adenine methyltransferase *dam* is fused to a nuclear protein specific of the nuclear compartment of interest. Adenines in genomic stretches proximal to this protein are methylated and this can be detected using microarrays or sequencing. damID has been successfully used to uncover DAF-16 target genes, but also the nuclear organization of chromosomes relative to the nuclear lamina using a *dam* fusion to LMN-1, the unique worm lamin. Using a newly developed methylated adenine binder, we are now able to track in vivo nuclear compartments in developing and aging worms. We will present this system in the worm as well as the first results on dynamics of subnuclear compartments in aging or physiologically challenged worms.

1085A. Towards understanding the role of histone demethylation in replication-induced DNA damage repair. **Toshia Myers**, Pier Giorgio Amendola, Anna Elisabetta Salcini. BRIC, Copenhagen Univ, Copenhagen, Denmark.

Post-translational modification (PTM) of histones is required to successfully repair DNA damage, which is caused by endogenous (e.g. replication stress) and exogenous (e.g. ionizing radiation) DNA stressors. Histone PTMs facilitate the DNA damage repair (DDR) response by regulating chromatin remodelling and recruitment of repair factors to sites of DNA damage. Although emerging evidence suggests that histone methylation promotes DDR, the role of pre-existing histone methylation and histone demethylation on cellular response to replication stress remains elusive. Therefore, we have taken steps to understand the role of the C. elegans Jumonji C-domain containing histone demethylase homologues in replication stress response. In a survival screen for sensitivity or resistance to hydroxyurea (HU), a chemical that inhibits ribonucleotide reductase leading to replication stress, we identified *jmjd-1.1(hc184)* and *jmjd-1.2(tm3713)* as mutations that confer HU resistance. JMJD-1.1 and JMJD-1.2 are histone demethylases specific for H3K9me2 and H3K27me2, marks associated with heterochromatin and transcriptional repression. These mutants have significantly less embryonic lethality than wild-type controls after treatment with 25mM HU (and other chemicals that cause replication stress induced DNA damage) but are not resistant to ionizing or ultraviolet radiation. Although these mutants have increased germline levels of both H3K9me2 and H3K27me2, they are otherwise phenotypically wild-type for germline morphology, brood size, and embryonic lethality under normal conditions. Similarly, mutants have normal cell cycle arrest, RAD-51 expression, and apoptosis, despite that H3K27me2 levels are diminished in the wild-type germlines but remain high in mutant germlines, after HU treatment. Currently, we are trying to understand the mechanism by which these proteins regulate replication stress response by testing if HU resistance is related to aberrant transcriptional regulation or to chromatin structure.

1086B. The *C. elegans* acetylome identifies genes and molecular pathways involved in dopamine neuron vulnerability. **Richard M. Nass[1]**, Jonathan Trinidad[2], Natalia VanDuyn[1]. 1) Pharmacology & Toxicology, Indiana Univ Sch Med, Indianapolis, IN; 2) Chemistry, Indiana Univ, Bloomington, IN.

Background: Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, and is characterized by the selective loss of dopamine neurons (DA) in the substantia nigra. Recent studies suggest that epigenetic dysregulation may play a significant role in neurodegenerative diseases. Histone deacetylases (HDACs) are a class of enzymes that remove acetyl groups from histone and non-histone proteins that play critical roles in cell regulation, longevity, and neuropathological disorders. Statement of purpose: To generate the first reported genome-wide identification of acetylated proteins in *C. elegans* in WT and acetylation-associated mutants, and determine whether these modulate DA neuron vulnerability to PD-associated proteins or neurotoxicants. Methods: We utilized liquid chromatography-tandem mass spectrometry (LC-MS/MS), biochemical assays, immunofluorescence, reverse genetics, transgenic *C. elegans*, RT-PCR, Western and neuronal morphology analysis to characterize expression, localization and the role that acetylation plays in PD-associated DA neurodegeneration. Results: Our results indicate that lysine acetylation is abundant in a wide variety of proteins including those involved in transcription, translation, metabolism, and stress responses. We also demonstrate that an HDAC mutant inhibits DA neurodegeneration up to 15-fold relative to WT, and acetylation of specific proteins modulates DA neuron vulnerability and induces specific stress response pathways. Conclusion: This study defines the first *C. elegans* acetylome and identifies acetylation-associated proteins involved in neuroprotection that will likely yield novel insight into the molecular basis of DA neuron vulnerability and associated diseases. Support: NIEHS ES014459 and ES003299 to RN; and EPA STAR Graduate Fellowship to NVD.

1087C. Starving chromosome; reshaping the chromatin to survive. **Ehsan Pourkarimi**, Mark Larance, Angus Lamond, Anton Gartner. Dundee Univ , UK.

Caloric restriction is associated to increase in life span in many model systems including C. elegans. Previously many pathways have been identified to be involved in ageing, including Insulin/IGF-1 and TOR signalling. Loss of function mutation in DAF-2, a C. elegans insulin receptor, results in life span extension while depletion of the most down stream component of insulin signalling (IS), DAF-16, results in reduced longevity. In addition to increased lifespan, daf-2 mutants are resistant to stress responses such as heat, hypoxia and oxidative stress. Many of DAF-2/DAF-16 targets have been identified using; genetics approach, transcriptome analysis and chromatin immunoprecipitation. Besides IS, TOR regulates ageing and function as a master switch to regulates cell growth in response to nutrition availability. However despite the intensive research, the exact mechanism as to how cells response to starvation is not fully understood. We present the use of quantitative mass spectrometry of C. elegans to analyse organismal proteome changes upon starvation. We have recently developed a stable isotope labelling of amino acids in cell culture (SILAC) based technique for use in worms. Using SILAC we were able to detect more than 4000 proteins of which nearly 350 proteins were significantly up regulated while 127 were down regulated upon starvation.

Gene ontology analysis has shown that majority of the up regulated proteins are associated to nucleosome assembly, ageing and stress response while a number of down regulated proteins are known to be involved in fatty acid and amino acid metabolism and cell division of which some are known to be a target of TOR. Nearly 10% of the up-regulated proteins that we have detected have previously been reported to be a target of daf-16. Interestingly, we have detected up regulation of some chromatin remodelers and histone variants. The up-regulation of some of the chromatin remodellers observed in our study has been confirmed using transgenic worms expressing these proteins tagged with GFP. A null mutant of one of the remodellers exhibits a dramatic reduction in life span. Currently we are analyzing the protein changes of worms depleted for DAF-2 and AMPK signalling.

1088A. *usp-48* encodes a deubiquitinating enzyme involved in cell fate restriction during development. **Dylan Rahe**, Tulsi Patel, Oliver Hobert. Department of Biochemistry and Molecular Biophysics, Columbia Univ Medical Center, New York, NY 10032.

During development, cells use various mechanisms to orchestrate the choreography of differentiation, resulting in an organism composed of individual cells with discrete identities. Concurrently, however, cells become restricted in their fate, and many differentiated cells are known to be refractory to fate transformations once terminally differentiated. This process of cell fate restriction during development is poorly understood. In order to investigate this process, we screened for mutants that lack the ability to restrict cell fates. In the screen, mutagenized worms are subjected to overexpression of the terminal selector gene *che-1*, which is necessary to induce ASE chemosensory neuron fate, in late-larval and adult stages. In wild-type worms, very few cells are affected by the expression of *che-1*, as assayed by expression of the ASE-specific *gcy-5::gfp* reporter. However, we have recovered mutants that develop to adulthood but lack robust cell fate restriction. In one such mutant, *usp-48(ot674)*, expression of *che-1* results in a conversion of epidermal tissue to an ASE-like fate. USP-48 is a deubiquitinating enzyme that is ubiquitously expressed at all developmental stages and appears to be associated with DNA in the nucleus. Using several fluorescent markers as well as single molecule FISH, we have shown that the conversion is specific to ASE fate, includes expression of pan-neuronal markers, and results in the loss of epidermal-specific fate markers. The mechanistic basis for the conversion, the concomitant loss of epidermal fate, as well as the genome-wide regulation of this event, are currently being explored.

1089B. SET-2, ASH-2 and WDR-5 regulate distinct sets of genes in the *C. elegans* germline. **Valérie J P Robert**, Marine Mercier, Lucie Kozlowski, Cécile Bedet, Diyavarshini Gopaul, Stéphane Janczarski, Francesca Palladino. LBMC, ENS Lyon, Lyon, France.

Methylation of histone H3 lysine 4 (H3K4me), a mark associated with gene activation, is mediated by Set1 and the related mixed lineage leukemia (MLL) histone methyltransferases (HMTs) across species. *C. elegans* contains one Set1 protein, SET-2, and one MLL-like protein, SET-16. Set1/MLL family members act as part of large multisubunit complexes known as COMPASS (COMPlex ASsociated with Set1), which also contain the core subunits ASH-2 and WDR-5, both conserved in *C. elegans*. In a previous study, we demonstrated that SET-2 is required for global H3K4 methylation at all developmental stages and that, surprisingly, SET-2 and ASH-2 are differentially required for H3K4 methylation in embryos and adult germ cells [1]. These results strongly suggested that SET-2 and ASH-2 may act in different regulatory complexes depending on the developmental stage and tissue. To further investigate the respective roles of SET-2, ASH-2 and WDR-5 in the *C. elegans* germline, we performed microarray analysis on dissected gonads. We will present data supporting of distinct functions for SET-2, ASH-2 and WDR-5 in the *C. elegans* germline. 1.Xiao, Y., et al., Caenorhabditis elegans chromatin-associated proteins SET-2 and ASH-2 are differentially required for histone H3 Lys 4 methylation in embryos and adult germ cells. PNAS, 2011. 108(20): p. 8305-10.

1090C. The characterization of the histone-chaperone lin-53. **Stefanie Seelk**, Baris Tursun. Berlin Institute for Medical Systems Biology, Max-Delbrück-Center for Molecular Medcine, Berlin, Germany.

The histone-chaperone lin-53 (Rbbp4/7 in mammals) is a component of several histone remodeling and modifying complexes. It was shown previously (Tursun at el., 2011) that removal of lin-53 by RNAi knock-down leads to conversion of mitotic germ cells into specific neuron types or muscle-like cells when cell fate-inducing transcription factors (TFs) are ectopically expressed. We want to characterize LIN-53 with respect to tissue-specific interaction partners and DNA-binding sites using Mass Spectrometry (MS) and Chromatin-immunoprecipitation (ChIP). For this reason new fosmid recombineering cassettes that provide affinity tags for protein purification procedures were engineered. Those contain different tags (AVI-tag, HA-tag, FLAG-tag) and cleavage sites (TEV, PreScission) for efficient protein purification. The AVI-Tag allows tissue-specific biochemistry by expressing the biotin ligase BirA, which is required for biotinylating the AVI-tag, under the control of promoters that allow expression only in specific cell types. Additionally, we have combined these protein purification tags bi-cistronically (using an SL2 splice signal) with fluorescent tags (GFP, YFP) and nuclear localization signals (NLS or a histone fusion). These purification cassettes were recombineered into lin-53- containing fosmids (Tursun et al., 2009) and either bombarded or microinjected. Expression and biochemical analysis of LIN-53 is ongoing. Furthermore, we are interested in the effect of the lin-53 depletion on the transcriptome- and proteome-level to systematically derive the list of genes that are regulated by lin-53. Proteomes of lin-53-depleted worms and wild-type animals will be compared by SILAC (stable isotope labeling of amino acids in cell culture) combined with mass spectrometry. Additionally, changes of transcript expression levels will be analyzed in a transcriptome-wide manner. For this, deep sequencing of extracted RNA from lin-53 mutant and wildtype animals will be compared. Preliminary results will be presented at the conference.

1091A. HTZ-1/H2A.z maintains cell fates through transcriptional repression in an H3K27me-independent manner. **Yukimasa Shibata**[1], Hitoshi Sawa[2], Kiyoji Nishiwaki[1]. 1) Dept. of Biosci., Kwansei Gakuin Univ., Japan; 2) NIG, Japan.

Epigenetic mechanisms, including histone modifications, are likely to play crucial roles in the cell-fate maintenance. The roles of histone methylation, for example the repression of Hox gene by Polycomb Group proteins, are studied well, in contrast, the roles of histone acetylation remains largely unknown. We recently reported that AcH4-binding protein, BET-1, and MYST HAT, MYS-1, are required for the maintenance of cell fates in multiple cell lineages, indicating that BET-1 and MYS-1 are parts of the fundamental mechanism that maintains cell fates.

To understand how acetylation-dependent mechanism maintains cell fates, we tried to find the component of the bet-1 pathway, and found *htz-1* that encodes a H2A variant, H2A.z. Because of the defect in the maintenance of cell fates, *htz-1 mys-1* double mutants produce extra DTCs that were induced by ectopic expression of *ceh-22*/Hox. We also found that *ceh-22* locus is a target of HTZ-1 in somatic gonadal cells (SGCs). Thus, HTZ-1 and MYS-1 maintain the fates of the SGCs through the repression of *ceh-22*.

We also found that, in *bet-1* and *htz-1 mys-1* mutants, disruption of H3K27 demethylase, UTX-1, suppressed the extra DTCs phenotype and the ectopic *ceh-22* expression. Genome-wide analyses of modENCODE data indicated that stronger signals for HTZ-1 were observed in the region where H3K27 is less methylated. These results suggest that HTZ-1 represses genes in the region where H3K27 is methylated to a lesser extent. Therefore, HTZ-1-dependent repression appears to be independent from H3K27me-dependent repression. It is known that the HTZ-1 occupancy on the genome is co-related with RNA Pol II occupancy, but to a lesser degree with transcriptional activity, suggesting that the HTZ-1 correlates with pausing of RNA Pol II. Thus, we propose that, in the maintenance of cell fates, histone acetylation and H2A.z maintain the paused state of RNA Pol II to repress the genes that induce specific cell fates.

1092B. Turnover of the H3K9me2 Mark During Late Spermatogenesis. **Matthew P. Snyder**, Xia Xu, Eleanor Maine. Syracuse Univ, Syracuse, NY.

Meiotic silencing is a conserved phenomenon targeting unpaired chromosomes and chromosomal regions during prophase of meiosis I. Meiotic silencing in animals typically occurs at the chromatin level and involves accumulation of histone modifications thought to promote a closed chromatin configuration. This chromatin structure may contribute to transcriptional repression and meiotic chromosomal events such as chromosome disjunction (Bean et al 2004, Jaramillo-Lambert and Engebrecht 2010). During meiosis in C. elegans, non-synapsed chromosomes are enriched for H3K9me2 relative to synapsed chromosomes (Kelly et al. 2002; Bean et al. 2004). Such non-synapsed chromosomes include the male X, homologous chromosomes that fail to synapse due to mutation, and chromosomal translocations/duplications. The pattern of H3K9me2 accumulation during meiosis depends on activity of the small RNA machinery, which may have a role in targeting the mark to unpaired chromosomes and ensuring that the mark does not persist abnormally (Maine et al. 2005; She et al. 2009). Taking a combined biochemical/genetic approach, we are identifying additional factors important for regulating H3K9me2 distribution during meiosis. Through this approach, we are identifying genes that appear to be important for turnover of the H3K9me2 mark during late spermatogenesis.

1093C. Molecular Characterization of Epigenetic Inheritance Factors in *C.elegans*. **G. Spracklin**, S. Kennedy. Department of Genetics, Univ of Wisconsin, Madison, WI.

Double-stranded (ds)RNA can silence a gene in a sequence specific manner in *C.elegans*. In some instances, this silencing persists for 6-10 generations in the absence of the initial dsRNA trigger (termed RNAi inheritance) (1,2). Gene expression is eventually restored to wild-type levels demonstrating that RNAi inheritance is an epigenetic phenomenon. To characterize the pathway driving RNAi inheritance, we have conducted a forward genetic screen isolating mutant animals that are defective for RNAi inheritance, which we have named heritable RNAi defective (*hrde*) mutants. The first gene identified by this screen, *hrde-1*, is a nuclear Argonaute protein expressed in germ cells (2). HRDE-1 directs NRDE-1/2/4-mediated nuclear RNAi in germ cells to facilitate RNAi inheritance. The second gene identified is termed *hrde-2*, which is a worm-specific factor with no conserved protein domains. We are in the process of placing *hrde-2* in the Hrde pathway. Our goal is to identify additional *hrde* genes to further elucidate the molecular mechanism of RNAi inheritance. References:(1) Vastenhouw and Plasterk (2006) Nature 442, 882 (2) Buckley and Kennedy (2012) Nature 489, 447-451.

1094A. Expression of MET-2, a H3K9 methyltransferase, in the C. elegans germ line. **Bing Yang**, Xia Xu, Eleanor Maine. Biology, Syracuse Univ, Syracuse, NY.

An important feature of core histones, especially their N-terminal tails, is their subjection to a plethora of epigenetic modifications. One aspect of epigenetic modification involves histone methylation (Liu et al., 2011). Numerous reports have shown the methylation status of certain amino acids correlates with transcriptional status. For example, methylation of histone H3 lysine 4 (H3K4me) serves as a marker of active transcription in euchromatin regions, while methylation of histone H3 lysine 9 (H3K9me) and histone H3 lysine 27 (H3K27me) usually correlates with transcription repression in heterochromatin regions (Fischer et al., 2003, Sims et al., 2006). The C. elegans germ line serves as an ideal system to study the establishment and maintenance of chromatin modifications. Our research focuses on regulation of H3K9me2 deposition. In the adult germ line, H3K9me2 marks are broadly distributed at a relatively low level among all chromosomes as nuclei pass through the various stages of first meiotic prophase. In addition, chromosomes that do not pair and synapse, such as the male X, are highly modified with H3K9me2 (Kelly et al., 2002). MET-2, a member of the SETDB1/ESET family of lysine methyltransferases, is required for germline H3K9me2 (Bessler et al., 2010). We are investigating the mechanism that targets MET-2 activity to unpaired chromosomes. To this end, we generated several single copy, functional met-2::gfp transgenes by using Mos1 single copy insertion (mosSCI), and we also generated anti-MET-2 antibody. These reagents have allowed us to determine that MET-2 preferentially localizes to meiotic nuclei in the adult germ line, an observation consistent with a role for MET-2 as a histone methyltransferase. We are using these reagents to identify MET-2 interactors.

1095B. Using a glo-2 enhancer screen to investigate BLOC-1 function in protein trafficking to gut granules. **Alec Barrett**, Daniel Saxton, Greg Hermann. Lewis & Clark College, Portland, OR.

Caenorhabditis elegans intestinal cells are characterized by the presence of gut granules, lysosome-related storage organelles that contain autofluorescent and birefringent material. Gut granule formation requires the activity of evolutionarily conserved genes that when disrupted result in the loss and/or mislocalization of birefringent material into the embryonic intestinal lumen (the Glo phenotype). We have recently found that one of the genes, *glo-2*, which exhibits a Glo phenotype when mutated, encodes a small cytoplasmically localized protein orthologous to mammalian Pallidin. In C. *elegans*, as seen in other organisms, GLO-2/Pallidin functions as part of the multisubunit BLOC-1 complex in trafficking to lysosome related organelles. Currently, the function of BLOC-1 is poorly understood. To identify genes that function in concert with, or in parallel to, BLOC-1 in trafficking to gut granules we have carried out a genetic screen for EMS induced mutants that enhance a *glo-2* partial loss of function allele. From the primary screen we identified 18 mutants that displayed an enhanced Glo phenotype in the *glo-2(-)* background, which fell into three distinct phenotypic classes. By crossing *glo-2(-)* out of the genetic background in ten strains we found that four were new Glo mutants and that six were enhancers of *glo-2(-)*. We present our genetic characterization of the enhancer mutants and our studies investigating their impact on protein trafficking to gut granules.

1096C. The *Caenorhabditis elegans* UDP-Glc:glycoprotein glucosyltransferase homologue CeUGGT-2 is an essential protein that does not function as a glycoprotein conformation sensor. Lucila Buzzi[3,4], Sergio Simonetta[3,4], Armando Parodi[3,4], **Olga Castro[1,2]**. 1) Department of Biolgical Chemistry, School of Sciences Univ of Buenos Aires, BA, Argentina; 2) Instituto de Química Biológica de la Facultad de Ciencias Exactas y Naturales (IQUIBICEN-CONICET). BA, Argentina; 3) Fundación Instituto Leloir,BA, Argentina; 4) Instituto de Investigaciones Bioquímicas de Buenos Aires (IIBBA-CONICET, BA, Argentina.

The UDP-Glc:glycoprotein glucosyltransferase (UGGT) is the key component of the glycoprotein folding quality control mechanism that takes place in the endoplasmic reticulum (ER). It behaves as a sensor of glycoprotein conformation as it exclusively glucosylates glycoproteins not displaying their native conformations. The addition of this glucose residue enables the interaction of folding intermediates with Calnexin/Calreticulin. An enzymatically active UGGT is encoded by a single gene in *Schizosaccharomyces pombe*, *Drosophila melanogaster*, *Trypanosoma cruzi* and plants. There are two homologues coding for UGGT-like proteins in in the genus *Caenorhabditis*. Both UGGT homologues are expressed in human cells, the former but not the latter displays UGGT activity and is upregulated under ER stress conditions. Bioinformatics analysis showed that in *Chaenorhabditis elegans* there are two genes, *uggt-1* and *uggt-2* coding for UGGT homologues. We had previously reported that *C. elegans* expressed an active UGGT protein localized to the ER encoded by the *uggt-1*.gene and its expression is upregulated under ER stress. Here we report that CeUGGT-2 expression is essential for normal development and this enzyme lacks UGGT activity. We analyzed heterozygous *uggt-2(ok2510)* worms, chromosome balanced with hT2 (I; III), and found that more than 50% of the eggs laid by this strain did not develop to progressive larval stages. A small amount of embryos progressed to L1 stage, looked very sick and had many vacuoles in their intestinal cells. We expressed CeUGGT-2 and two chimeric proteins, one composed by the N-terminal domain of *S.pombe* UGGT and the CeUGGT-2 C-terminal domain; and the other one composed by the CeUGGT-2 N-terminal domain and the *S.pombe* C-terminal domain in *S.pombe*. Both UGGT-2 and the two chimeric protein proved to be fully inactive.

1097A. An RNAi screen for LRP-1 trafficking regulators reveals a role for EPN-1 epsin in endocytosis of LDL receptor superfamily. Yuan-Lin Kang[1], John Yochem[2], Leslie Bell[2], Erica Sorensen[3], **Lihsia Chen[1]**, Sean Conner[1]. 1) Dept. of Genetics, Cell Biology & Development, Univ of Minnesota, Minneapolis, MN; 2) Dept. of Molecular biology, Univ of Wyoming, Laramie, WY;; 3) Dept. of Biochemistry, Univ of Winconsin-Madison, Madison, WI.

Low-density lipoprotein receptor (LDLR) is required for the removal of cholesterol-laden LDL particles from circulation in humans. Defects in LDLR trafficking promote elevated serum cholesterol levels that can lead to atherosclerosis. As our understanding of mechanisms controlling LDLR trafficking is incomplete, we carried out a genome-wide RNAi screen to identify factors that govern LDLR trafficking, using *C. elegans* LRP-1 as a model for LDLR transport. LRP-1 is the megalin orthologue and a member of the LDLR superfamily that is required for molting and animal viability. Our screen is based on the principle that abnormal LRP-1 trafficking will result in molting defects and/or larval lethality. In using this criterion and an *lrp-1::gfp* transgene to track LRP-1 trafficking, we uncovered a role for EPN-1/epsin in LRP-1 internalization. Our analysis of mammalian epsin, an endocytic factor, also revealed a role for epsin in mediating LDLR endocytosis. As a first approach to dissecting how epsin mediates LDLR trafficking, we performed an *in vivo* dissection in *C. elegans* of EPN-1, a modular protein with several conserved protein interacting motifs. We identified the ENTH domain, which can bind PIP2, as essential for epsin's as-yet-uncharacterized role in animal viability, in contrast to the other conserved protein interacting motifs, which are dispensable for animal viability. Of these, only the ubiquitin-interacting motif (UIM) is required for LRP-1 internalization and for biochemical interaction with LDLR. In examining how the epsin UIM engages LDLR for receptor endocytosis, we uncovered a novel and additional endocytic mechanism that is independent of two documented internalization motifs encoded in the LDLR cytoplasmic tail.

1098B. A microtubule minus-end binding protein and minus-end directed transport function in the C. elegans epidermis. **Marian Chuang[1]**, Tiffany I. Hsiao[1], Amy Tong[1], Shaohe Wang[2,3], Karen Oegema[2], Andrew Chisholm[1]. 1) Division of Biological Sciences, UCSD; 2) Ludwig Institute for Cancer Research, UCSD; 3) Biomedical Sciences Program, UCSD.

The *C. elegans* epidermis is a barrier epithelium that functions as the first line of defense against the environment. The epidermis actively defends worms against pathogen infection and repairs wounds. The death-associated protein kinase *dapk-1* negatively regulates epidermal immunity and wound responses. Loss of function *dapk-1* mutants exhibit hyperactive wound responses and excessive secretion of cuticle.To better understand how DAPK-1 regulates these processes we screened for suppressors of *dapk-1* morphological phenotypes. After EMS mutagenesis we identified two suppressor mutations, both of which affect genes implicated in the microtubule (MT) cytoskeleton. The first suppressor mutation causes complete loss of function in *ptrn-1/pqn-34* which encodes the *C. elegans* member of the Patronin/Nezha/CAMSAP family of MT minus-end binding proteins. Recent studies have analyzed how Patronin affects MT minus-end dynamics in cultured cells but their *in vivo* functions are not well understood. Patronin is thought to bind specifically to MT minus ends and protect them against kinesin-13-based depolymerization. We find that loss of *klp-7*/kinesin-13 function suppresses the ptrn-1 suppression phenotype, consistent with the hypothesis that PTRN-1's minus-end protective function is important in the epidermis. *ptrn-1* function is required in the epidermis for *dapk-1*'s hypersecretion phenotype. GFP::PTRN-1 forms puncta in the cytoplasm and at epidermal attachment structures, possibly the locations of MT minus ends in the worm epidermis. Our second suppressor is a phenotypically silent allele of *dhc-1* (dynein heavy chain). A stronger *dhc-1* allele *or195* fully suppresses *dapk-1* epidermal defects. In simple epithelia MTs are usually oriented with minus ends apically. Thus, we hypothesize that MT-based minus-end directed transport in the epidermis is required for apical secretion, and that DAPK-1 somehow antagonizes this process. These findings reveal the roles of MT cytoskeleton in the function of the differentiated epidermis. We are also investigating roles of PTRN-1 in wound healing and axon regeneration.

1099C. Polarity proteins regulate the localization of a spindle-positioning mediator, LET-99. **Eugenel B Espiritu**, Jui-Ching Wu, Kari Messina, Lesilee Rose. Molecular and Cell Biology, Univ of California, Davis, Davis, CA.

Spindle positioning is essential for asymmetric divisions, where the spindle must be aligned with the axis of cell polarity. In many systems, the conserved PAR polarity proteins establish polarization of the cell and regulate spindle movements via a complex including Ga, GPR and LIN-5. LET-99 is a key regulator of GPR asymmetry in C. elegans embryos. LET-99 is asymmetrically localized at the cortex in a lateral-posterior band pattern, where it inhibits GPR localization. Analysis of LET-99 in mutant backgrounds showed that PAR-3 is required to inhibit cortical LET-99 localization at the anterior, while a gradient of PAR-1 inhibits LET-99 at the posterior. In addition, PAR-1, a Ser/Thr kinase, associates with LET-99 in vitro. To gain further insight into the mechanism of

LET-99 localization, we tested LET-99 for association with the 14-3-3 protein, PAR-5. In other systems, phosphorylation of targets by PAR-1 and the PAR-3 associated kinase PKC-3 generates binding sites for 14-3-3 proteins, which alters the targets' localization. We found that PAR-5 bound to His::LET-99 in wild-type embryo extracts, but PAR-5 binding was greatly diminished in extracts from *par-1(RNAi)* embryos. Computer predictions combined with yeast-two hybrid data identified two LET-99 serine residues essential for PAR-5 binding. To determine the *in vivo* relevance of these sites, we introduced S-to-A mutations into an otherwise full-length rescuing LET-99 transgene (LET-99-AA). When transferred into a *let-99* deletion background, the LET-99-AA protein mislocalized to the entire posterior of the one-cell embryo, similar to LET-99 localization in *par-1* mutant embryos. These and other results support a model that PAR-1 phosphorylates LET-99 to create binding sites for PAR-5, which prevents LET-99 association with the posterior-most cortex. To begin to determine how LET-99 localization is restricted from the anterior, we analyzed LET-99 after depletion of anterior PAR components. We found that PAR-3 is not sufficient for LET-99 localization, but rather the PAR-3 associating proteins, PAR-6 and PKC-3, restrict LET-99 localization from the anterior. We are now testing if PAR-1 and PKC-3 directly phosphorylate LET-99 using *in vitro* kinase assays.

1100A. Networks regulating pharyngeal development and morphogenesis. **David S. Fay**, Stanley Polley, Jujiao Kuang, Jon Karpel, Evguenia Karina, Aleksandra Kuzmanov, John Yochem. Dept Molec Biol, Univ Wyoming, Laramie, WY.

Development of the C. elegans pharynx is regulated by a network that includes the pRb ortholog, LIN-35, and PHA-1, a novel cytoplasmic protein. Deletion of pha-1 impairs pharyngeal development and body morphogenesis leading to a completely penetrant embryonic arrest. The lethality of pha-1 mutants is strongly suppressed by loss of function mutations in sup-35/ztf-21 and sup-37/ztf-12, which encode Zn-finger proteins, and by mutations in sup-36. Whereas sup-35 is non-essential, sup-37 is required in a subset of for pharyngeal muscle cells for pumping and also promotes ovulation in the somatic gonad. We have cloned sup-36 and shown it to encode a divergent Skp1 protein family member. Similar to SUP-35, SUP-36 protein levels are negatively regulated by a conserved E2-E3 complex composed of UBC-18/UBCH7 and ARI-1/HHARI. Unlike sup-35, however, sup-36 expression is not regulated by LIN-35-EFL-1 or HCF-1. Our analysis indicates that SUP-35/36/37 act at a single step to antagonize PHA-1 activity and inhibit pha-1 expression. Protein interaction studies show that SUP-35 and SUP-37 bind directly to each other and that SUP-35/37 may also be linked to SUP-36 via microtubule-associated cytoskeletal components. Specifically, SUP-35 binds to microtubules and SUP-36 interacts with the microtubule-associated protein PTL-1/tau. Consistent with these proteins acting within a complex, SUP-35 and SUP-36 display a similar dynamic pattern of expression during embryonic development. Because mutations in sup-35/36/37 also suppress the synthetic lethality of lin-35; ubc-18 double mutants, we sought to expand the pharyngeal regulatory network by identifying additional suppressors of lin-35; ubc-18. A genome-wide RNAi screen identified 39 suppressors, most of which are associated with transcriptional regulation. 23 of the genes that suppress lin-35; ubc-18 also suppress independent lin-35-synthetic phenotypes, consistent with these genes acting via the LIN-35 pathway. In addition, several Skp-1-Cullin-F-Box (SCF) components were identified, further indicating that SCF complexes are part of the greater network controlling pharyngeal development.

1101B. Disparate endocytic recycling of the TGFb signaling receptors, Sma-6 and Daf-4, regulates signaling of the Sma/Mab pathway. **Ryan Gleason**, Adenrele (Dee) Akintobi, Ying Li, Nanci Kane, Barth Grant, Richard Padgett. Waksman Inst, Rutgers Univ, Piscataway, NJ.

Our study aims to delineate the intracellular trafficking pathways that regulate the type I and type II TGFb receptors, Sma-6 and Daf-4 respectively. The TGFb superfamily of ligands regulate a diverse array of developmental processes across metazoan biology. Genetic and genomic analysis in C. elegans has contributed to the delineation of the highly conserved TGFb signal transduction pathway. An unanswered and critical question is how receptor-mediated endocytosis orchestrates the availability and intracellular trafficking (degradation versus recycling) of TGFb signaling receptors. By utilizing C. elegans we have identified a novel role of two distinct endocytic trafficking pathways in regulating both the recycling and signaling of the two TGFb receptors, Sma-6 and Daf-4. To test the receptor trafficking of the Sma/Mab pathway, functional translational fusions of both Sma-6 and Daf-4 were developed to various fluorescent proteins to assess the in vivo localization and availability of the receptors in genetically tractable endocytic mutants. Here we show that the protein sorting complex, the Retromer, is an essential linchpin in the decision to recycle or degrade Sma-6. In the absence of the Retromer complex Sma-6 is not recycled back to the Plasma membrane and is mis-sorted to the Lysosome for degradation. In contrast, Daf-4 recycles through the recycling endosome and is not dependent on the Retromer complex. Co-IP experiments demonstrate a direct interaction between the Retromer complex and Sma-6. Together, this work establishes a novel role for disparate intracellular trafficking in the regulation of TGFb receptor availability and signaling.

1102C. The EXC-1 RAS-Domain Protein Mediates Vesicle Movement in the Excretory Canal. **Kelly A. Grussendorf**, Alexander T. Salem, Christopher J. Trezza, Matthew Buechner. Molecular Biosciences, Univ of Kansas, Lawrence, KS.

Many critical biological structures are single-celled tubes, including mammalian capillaries and glial sheaths surrounding peripheral nerves. The *C. elegans* single excretory canal cell provides a simple model to study these small tubes. The cell extends narrow (<5 mm wide) hollow processes the length of the worm. A set of EXC proteins maintain the narrow diameter of the canal apical surface. Mutations affecting these proteins allow formation of fluid-filled cysts in the lumen of the canal.

The Exc-1 loss-of-function (lof) phenotype shows cysts that are often located at the distal ends of the canals. These cysts vary in size and number, from cysts not much wider than a normal lumen to cysts expanded to the entire diameter of the worm. We have cloned the *exc-1* gene, which encodes a homologue of the RAS GTPase family, specifically the family of Interferon-Inducible GTPases (IIGP), which regulate autophagy and endosome formation in mammalian cells. EXC-1 is expressed in the canals, and also in the amphid sheath, a glial structure surrounding the amphid neuron sensory endings. *exc-1* (lof) mutants show accumulation of early endosome marker EEA-1, and concomitant attenuation of recycling endosome marker RME-1 within the excretory canals, a phenomenon also seen for *exc-5* mutants. Current studies are being carried out to determine the subcellular location of EXC-1.

Overexpression of *exc-1* forms a tubule with a normal apical surface but defective in formation of the basal surface. Epistasis experiments suggest that EXC-1 acts downstream of the EXC-9 LIM domain protein, and upstream of the EXC-5 guanine exchange factor. Activated EXC-1 binds directly to EXC-9 in a yeast two-hybrid assay. Our results suggest that these proteins function together to mediate efficient trafficking from early endosomes to recycling endosomes. Further assays will assess binding of EXC-1 to other likely targets and to determine the regions of EXC-1 that bind to EXC-9.

1103A. A screen for mislocalization of the LET-23 EGF receptor during vulval development. **Andrea Haag**, Juan M. Escobar Restrepo, Alex Hajnal. Institute of Molecular Life Sciences, Univ of Zurich, Switzerland.

In polarized epithelial cells, the apical and basolateral membranes are composed of distinct proteins and lipids that provide specific functions. The mammalian epidermal growth factor receptor (EGFR), a member of the ErbB family of receptors, is mainly localized to the basolateral cell membrane. Mislocalization of mammalian ErB family members to the apical surface can de-regulate signaling by the receptor and result in disease. Similarly, the *C. elegans* EGFR homolog LET-23 is targeted to the basolateral plasma membrane in the vulval precursor cells (VPCs). Vulval development is abnormal if LET-23 is mislocalized. Previously, a ternary complex consisting of LIN-7, LIN-2 and LIN-10 has been shown to play an important role in the retention of LET-23 on the basolateral surface. Nonetheless, the exact mechanism of LET-23 localization and the control of the receptor dynamics remain poorly understood. To identify new regulators of LET-23 localization, we performed an RNAi feeding screen using a functional LET-23::GFP translational reporter. We analyzed over 700 RNAi clones that are known to cause a protruding vulva (Pvl) phenotype. By evaluating LET-23::GFP expression at different developmental stages, we were able to identify several genes regulating LET-23 localization or expression in the VPCs and their descendants. To investigate if receptor mislocalization alters LET-23 signaling, we performed RNAi against selected candidates in a sensitized *let-60 ras(gf)* background. By this approach, we have so far identified three genes that negatively regulate RAS/MAPK signaling and control LET-23 localization. Translational reporter constructs for two of these genes revealed expression in the VPCs, suggesting that they act in a cell autonomous manner to regulate LET-23 localization. To confirm the RNAi results, we analyzed LET-23::GFP localization in the corresponding mutant strains. Currently, we evaluate LIN-12/Notch reporter localization following gene silencing to determine the functional specificity of the identified genes. Further experiments aim at investigating how these proteins interact to control LET-23 localization and/or expression.

1104B. Identification and characterization of conserved factors mediating gut granule protein trafficking. **Greg Hermann**, Jared Delahaye, Olvia Foster, Annalise Vine, Thomas Curtin. Lewis & Clark College, Portland, OR.

Lysosome-related organelles (LROs) comprise a class of cell-type restricted compartments with specialized functions that share functional characteristics with conventional lysosomes. *C. elegans* gut granules are LROs that contain autofluorescent and birefringent material, which are restricted to intestinal cells. Protein trafficking to LROs utilizes endosomal compartments and factors that mediate trafficking between these organelles. These factors include subunits of the AP-3 and HOPS complexes and Rab32/38. At present, it is unclear how HOPS and Rab32/38 mediate trafficking to LROs. We present our studies demonstrating the role of HOPS in protein targeting and transport to gut granules, which represent the first analysis of HOPS activity in trafficking to LROs in any system. HOPS is an effector of RAB-7, a late endosome associated protein, which mediates the fusion of late endosomes and lysosomes during protein trafficking to conventional lysosomes. Interestingly, we find that RAB-7 plays only a minor role in determining the protein composition of gut granules, suggesting that another Rab interfaces with HOPS to mediate trafficking to gut granules. We show that GLO-1, a homologue of Rab32/38, which is required for the formation of gut granules may fulfill this role. For GLO-1 to function in trafficking to gut granules it must be activated by a GEF, which mediates the exchange of GDP for GTP, and results in the recruitment to the organelle where it functions. To identify the GEF for GLO-1 we have screened our collection of mutants defective in gut granule biogenesis for defects in the membrane association of GFP::GLO-1 and identified two candidate genes. We describe results of a genetic bypass experiment that supports the role of these genes as a GEF for GLO-1.

1105C. *C. elegans* Rag genes are involved in endosome / lysosome biogenesis in a TORC1-independent fashion. **K. Iki**, Y. Ito, Y. Shimomura, H. Kajiho, M. Fukuyama, K. Kontani, T. Katada. Graduate School of Pharm. Sci., The Univ of Tokyo, Bunkyo, Tokyo, Japan.

Amino acids are not only act as building blocks for proteins, but also as signaling cues that have been known to affect cell growth and autophagy via protein complex called as Target of Rapamycin Complex 1 (TORC1). Recent intensive studies using mammalian cultured cells established that two subtypes of Rag proteins form a heterodimer to mediate intracellular signaling from amino acids to TORC1. However, whether physiological significance of Rag solely ascribes to activation of TORC1 or not remains to be thoroughly investigated *in vivo*. We found that a subset of late endosomes / lysosomes in the intestine are abnormally enlarged in the *C. elegans* Rag mutant animals. This enlargement requires activity of *arl-8* and *rab-7*, both of which play an important role in fusion of late endosomes and lysosomes. Further characterization of the enlarged vesicles indicated that they are not autofluorescent gut granules, but often display a double membrane structure with inner and outer lumens showing slight acidification and localization of a lysosomal enzyme, respectively. Since similar, but smaller vesicles also exist in wild type animals, loss of Rag genes likely impairs normal fusion, scission or other appropriate biogenesis of this class of vesicles, leading to their enlargement. It has been reported that animals whose TORC1 activity is reduced show excessive accumulation of lipid droplets in the intestine. We found that loss of Rag genes also results in similar defects. This suggests that as mammalian cultured cells, Rag is required for full activation of TORC1 in *C. elegans*. However, despite the presence of excess lipid, knockdown of genes encoding components of TORC1 does not cause enlargement of late endosomes / lysosomes in the intestine. Thus, these findings suggest that Rag plays a critical role in proper biogenesis of a subclass of late endosomes and lysosomes in a TORC1-independent manner. To clarify the mechanisms of this vesicle enlargement might lead to discovery of a novel cellular event which couples amino acid signaling to endosome / lysosome biogenesis.

1106A. Identification of genes important for excretory system function and maintenance using Whole Genome Sequencing. **Michelle Kanther**, Jennifer Cohen, Jean Parry, Meera Sundaram. The Univ of Pennsylvania, Philadelphia, PA.

Tubes form the building blocks of organ development in invertebrates and vertebrates. Defects in tube development and maintenance are associated with several human diseases. The critical steps of tube development, including cell polarization and epithelial junction formation, have been well studied; however, the genes regulating these steps remain poorly defined.

The *C. elegans* excretory system provides a simple and genetically tractable system for studying unicellular tube formation and maintenance. This tubular network is required for osmoregulation and its integrity is necessary for development and survival. It is composed of three tandem unicellular tubes: the canal, duct, and pore. These cells are connected by apicolateral junctions and form a continuous lumen that opens to the outside environment via the pore cell. Both the duct and pore cells form by a poorly-understood cell wrapping mechanism. The pore retains a characteristic autojunction, while the duct becomes a seamless tube. Defects in this system result in fluid accumulation and death of “rod-like” L1 larvae.

To discover novel genes involved in the formation and maintenance of unicellular tubes, we performed an EMS mutagenesis screen. From our screen we isolated 85 recessive, rod-like lethal mutants. Phenotypic analysis of these mutants reveals three distinct classifications. Class A mutants initially display normal junctions within and between all excretory cells; mutants in this class include alleles of *let-653*, *lpr-1*, *egg-6*, *let-4*, and *rdy-2,3,4*. Class B mutants exhibit two pore cell autojunctions (similar to Ras loss-of-function), while Class C mutants lack a pore cell autojunction (similar to Ras gain-of-function or Notch loss-of-function). To identify the causative mutations underlying our observed Class A phenotypes, we used balancers to map 23 mutations to chromosomal regions. After testing known candidates by complementation, we performed SNP mapping and whole genome sequencing using a scheme adapted from Doitsidou et. al. (2010) and Minevich et. al. (2012). We are now testing which variants are causative and extending our approach to the other mutant classes. Note: Kanther & Cohen co-authors.

1107B. Starvation-responsive behavioral plasticity is tuned by tubulin polyglutamylation in sensory cilia. **Yoshishige Kimura**, Alu Konno, Koji Tsutsumi, Saira Hameed, Mitsutoshi Setou. Dept. Cell Biol. and Anat., Hamamatsu Univ. Sch. of Med., Hamamatsu, Shizuoka.

Caenorhabditis elegans shows various well-characterized sensory behaviors. We have identified that an osmotic avoidance was partially suppressed by starvation, that is dependent on *ttll-4* gene encoding a tubulin glutamate-ligase essential for tubulin polyglutamylation of axonemal microtubules (MTs) in sensory cilia. Tubulin polyglutamylation is a post-translational modification (PTM) that generates lateral glutamate chains on target proteins, and changes the interaction between MT and associated proteins. Measurement of the velocity of intraflagellar transport (IFT) particles has revealed that starvation accelerates IFT, which is also dependent on the level of tubulin modification. Additionally, *ttll-4* is activated under the control of p38 MAPK. Tubulin polyglutamylation level in sensory cilia is also enhanced by various environmental stimuli including starvation. Amino acid substitution of TTLL-4 has specified a threonine residue, which is a putative MAPK-phosphorylation site, is required for enhancement of both IFT and tubulin polyglutamylation. Similar elevation of tubulin polyglutamylation is also observed in mammalian photoreceptor cells in retina responding to light exposure. These results indicate that environmental responsiveness of tubulin polyglutamylation in cilia is regulated by evolutionary-conserved molecular mechanism.

1108C. Instructive polarization of early embryonic cells by the cadherin-catenin complex and the RhoGAP PAC-1. **Diana Klompstra**, Dorian Anderson, Jeremy Nance. Skirball Institute, NYU School of Medicine, New York, NY.

Early embryonic cells in many species polarize radially by distinguishing their contacted and contact-free surfaces. In *C. elegans*, radial polarity begins at the four-cell stage, when cell contacts restrict the PAR polarity proteins to contact-free surfaces. During the subsequent cell movements of gastrulation, radial polarity is required for cytoskeletal asymmetries that promote timely cell ingression. The goal of my project is to determine how cell contacts induce the PAR protein asymmetries that polarize early embryonic cells, preparing them for gastrulation.

We previously identified the RhoGAP PAC-1 as an upstream regulator that is required to exclude PAR proteins from contacted surfaces of early embryonic cells. PAC-1 is recruited specifically to sites of cell contact and directs PAR protein asymmetries by inhibiting the Rho GTPase CDC-42. How PAC-1 is able to sense where contacts are located and localize to these sites is unknown. We identified an N-terminal fragment of PAC-1 that is sufficient for localization to cell contacts and showed that localization of this fragment depends on HMR-1/E-cadherin. HMP-1/a-catenin and JAC-1/p120-catenin, which interact with the HMR-1/E-cadherin cytoplasmic tail, function redundantly to recruit the PAC-1 N-terminus. We identified a conserved adaptor protein that acts to physically link the PAC-1 N-terminus to the cadherin-catenin complex. HMR-1 functions redundantly with another cue to localize PAC-1, since in contrast to the PAC-1 N-terminus, full-length PAC-1 can localize to cell contacts when HMR-1 is removed. E-cadherin has a conserved role in promoting contact-induced cell polarization, but there has been debate as to whether its role is instructive (defining contact sites) or permissive (promoting adhesion for other polarity regulators to operate). We show that mislocalizing the intracellular domain of HMR-1 to contact-free surfaces of cells recruits full-length PAC-1 to these sites and depolarizes cells, indicating that HMR-1 plays an instructive role in polarization by recruiting a symmetry-breaking polarity regulator to cell contacts.

1109A. Unraveling the interactome underlying cell polarity. **Thijs Koorman**, Monique van der Voet, Sander van den Heuvel, Mike Boxem. Utrecht Univ, Utrecht, Netherlands.

Polarity is a near universal trait of life, and guides many aspects of animal development: from the creation of cells with different developmental potentials through asymmetric divisions, to proper functioning and morphogenesis of cells in multicellular organisms. Although a number of key polarity regulators have been identified, many interactions with proteins acting downstream likely remain to be elucidated. In addition, there is evidence for extensive crosstalk between different polarity pathways, but the mechanistic details underlying this crosstalk are not well known. To identify novel proteins or new links between polarity regulators, we are combining high-throughput yeast two-hybrid (Y2H) screens with in-vivo studies using existing models of polarity in *C. elegans*. We used 68 polarity-related proteins, which touch on multiple aspects of polarity in *C. elegans*, as baits to identify the polarity-protein interaction network. For each bait protein, several fragments based on gene size and protein domain composition were used to screen two *C. elegans* Y2H libraries. Using multiple constructs per bait protein enabled us to identify a total of 527 interactions with 61/68 bait proteins (compared to 117 interactions with 21/68 bait proteins using full-length baits alone), and added additional detail to the network in the form of protein-protein interaction domains. We are able to confirm published interactions and identified many new ones. To complement the Y2H approach and identify candidate polarity regulators, we are performing systematic RNAi screens for polarity specific phenotypes in 5 post-embryonic polarity marker strains. We observed a phenotype in at least one of these strains for 34 bait proteins, and are currently pursuing the interacting preys. Furthermore, we are using double-RNA between bait-prey pairs to identify functional interactions. Taken together, we have identified numerous novel interactions with a high-throughput Y2H interaction mapping approach and are currently following up protein-pairs in-vivo by RNAi . We hope to obtain more in-depth knowledge how the polarized state of a cell or tissue is established, maintained and controlled.

1110B. An ABC transporter regulates systemic heme homeostasis in *C. elegans*. **Tamara Korolnek**, Iqbal Hamza. Animal and Avian Sciences, Univ of Maryland, College Park, MD.

Hemes are metalloporphyrins used by nearly all organisms as protein cofactors for energy production, binding and sensing gases, and as a catalyst for various reactions. While heme biosynthesis has been well-characterized, the pathways for transporting heme between cells and within a cell remain poorly understood. *C. elegans* serves as a unique animal model for uncovering these pathways, as it is unable to synthesize its own heme and depends on the uptake of dietary heme for growth and reproduction. Functional RNAi screens implicated *mrp-5* as a potential heme transporter in *C. elegans*. This gene encodes a membrane-bound ABC transporter that is highly expressed in the worm intestine and is transcriptionally regulated by heme. Worms lacking *mrp-5* were unable to lay viable eggs, a phenotype that was fully rescued by dietary heme supplementation. Depletion of *mrp-5* activated a number heme deprivation signals within the worm, including activation of the *hrg-1* and *hrg-2* promoters. Furthermore, *mrp-5* RNAi worms accumulated the fluorescent heme analog, zinc mesoporphyrin, in intestinal cells, indicating a defect in heme export from the intestine. Indeed, GFP-tagged MRP-5 localizes to the basolateral intestinal membrane, and intestinal-specific knockdown of *mrp-5* recapitulates the embryonic lethality of whole animal *mrp-5* RNAi. Functional assays in yeast support the hypothesis that MRP-5 is capable of exporting heme across cell membranes. Expression of MRP-5 in a yeast strain deficient for heme synthesis resulted in decreased growth in the presence of exogenous heme. Consistent with the reduced growth, MRP-5 expression in yeast lowered heme levels in the cytosol with a concomitant increase in heme levels in the secretory pathway, suggesting a role for MRP-5 in transporting heme from the cytosol into the secretory compartment. Altogether, our results implicate MRP-5 as a key regulator of systemic heme homeostasis in *C. elegans* that is required for heme export from the intestine to other tissues.

1111C. Regulation of TBC-2, an endosomal Rab5 GAP. **Fiona Law**, Laëtitia Chotard, Farhad Karbassi, Christian Rocheleau. Department of Anatomy and Cell Biology, Department of Medicine, McGill Univ, Montreal, Quebec, Canada.

Diseases such as cancer, metabolic disorders and neuronal degeneration can originate from endocytic trafficking defects. The proper trafficking of endosomes depends upon regulating proteins such as Rab GTPases that associate with their vesicular membrane. These proteins alternate between active GTP and inactive GDP bound forms through the actions of Guanine nucleotide Exchange Factors (GEFs) and GTPase Activating Proteins (GAPs). Different Rab GTPases function at discrete stages of the endocytic pathway to provide directionality to vesicular traffic and to recruit effectors for mediating downstream processes. During early to late endosome maturation, Rab5 is replaced by the Rab7 GTPase in a process called Rab conversion. Using *C. elegans*, our lab identified TBC-2 as a RAB-5 GAP that regulates RAB-5 to RAB-7 conversion. Loss of *tbc-2* function or expression of constitutively active RAB-5 leads to the formation of large RAB-7 positive endosomes in intestinal cells. Our data suggest that TBC-2 is recruited to endosomes by RAB-7 where it inactivates RAB-5, thus facilitating the RAB-5/RAB-7 conversion. Recently, in collaboration with the lab of Barth Grant, we found that TBC-2 physically interacts with CED-10/Rac1 and that both proteins regulate endosome recycling. In *ced-10* mutants, TBC-2 localization is strongly reduced, yet these mutants do not display the *tbc-2* mutant phenotype. Therefore, is membrane localization important for TBC-2 function? To answer this question, I am conducting transgenic rescue experiments to identify which domains of TBC-2 are required for its localization and/or function. If there exists a TBC-2 domain that is required for both of these roles, I will test whether targeting the catalytic domain of TBC-2 alone to late endosomes is sufficient for rescue of TBC-2 function. Aside from CED-10/Rac1, TBC-2 has also been found to bind to two other *C. elegans* Rac GTPases: RAC-2 and MIG-2. Future experiments involve testing for genetic redundancy among these Rac proteins in early to late endosome trafficking. This work is funded by CIHR.

1112A. Depletion of *mboa-7*, an enzyme that incorporates polyunsaturated fatty acids into phosphatidylinositol (PI), impairs PI 3-phosphate signaling. **Hyeon-Cheol Lee**[1], Takuya Kubo[1], Nozomu Kono[1,2], Eriko Kage-Nakadai[2,3], Keiko Gengyo-Ando[2,3], Shohei Mitani[2,3], Takao Inoue[1,2], Hiroyuki Arai[1,2]. 1) Graduate School of Pharmaceutical Sciences, Univ of Tokyo, Tokyo 113-0033, Japan; 2) CREST, Japan Science and Technology Agency (JST), Kawaguchi, Saitama 332-0012, Japan; 3) Department of Physiology, Tokyo Women's Medical Univ School of Medicine, Tokyo 162-8666, Japan.

Phosphatidylinositol (PI) is a constituent of biomembranes and a precursor of all phosphoinositides (PIPs), which participate in various types of signal transduction. A prominent characteristic of PI is that its *sn*-2 position is highly enriched in polyunsaturated fatty acids (PUFAs), such as arachidonic acid or eicosapentaenoic acid (EPA). We previously identified the hitherto unannotated enzyme *mboa-7*, as an acyltransferase that incorporates PUFAs into the *sn*-2 position of PI, and showed that *mboa-7* mutants had a reduced EPA content in PI. However, the biological significance of PUFA-containing PI remains unknown, in part due to the absence of overt phenotypes in *mboa-7* mutants. To investigate which PIPs signaling pathway is affected by the reduction of PUFA in PI, we performed an RNAi enhancer screen against PI kinases and phosphatases using *mboa-7* mutants. We found that severe growth defects were caused in *mboa-7* mutants by knockdown of *vps-34*, a catalytic subunit of class III PI 3-kinase that produces PI 3-phosphate (PI3P) from PI. In both *vps-34* RNAi-treated wild-type worms and *mboa-7* mutants, the size of PI3P-positive early endosomes was significantly decreased. An RNAi enhancer screen against PI3P-related genes revealed that, like knockdown of *vps-34*, knockdown of autophagy-related genes caused severe growth defects in *mboa-7* mutants. Finally, we showed that autophagic clearance of protein aggregates is impaired in *mboa-7* mutants. Taken together, these results indicate the significance of the polyunsaturated fatty acyl moiety of PI in PI3P signaling.

1113B. Suppressors of the *pam-1* aminopeptidase and the role of centrosome-cortical contact in one-cell anteroposterior polarity. Margaret Williams, Ashley Kimble, Zachary Klock, Jessica Meeker, Kevin Kozub, Eva Jaeger, **Rebecca Lyczak**. Biol Dept, Ursinus Col, Collegeville, PA.

Polarity establishment in the single-cell *C. elegans* embryo determines the anteroposterior axis of the organism. It is widely accepted that the sperm-donated centrosome cues polarity establishment by triggering changes in the posterior cortex. The centrosome is able to initiate polarity establishment from a distance from the cortex, but contact with the posterior cortex is subsequently observed. The timing and duration of this contact and the role in polarity establishment are poorly understood. To further understand the importance of centrosome contact timing and duration, we have used time-lapse confocal imaging of wild-type and *pam-1* mutant strains. We have discovered that *pam-1* mutants, which lack the puromycin-sensitive aminopeptidase, have significantly shorter centrosome-cortex interactions than wild-type. In addition, these interactions occur at an earlier cell-cycle stage than WT. *pam-1* mutants also fail to properly establish polarity, a defect that is rescued when centrosome contact is lengthened through inactivation of dynein. By comparing wild-type and *pam-1* mutants with similar contact times, and tracking polarity establishment, we are beginning to discover the relative

importance of timing of contact versus duration of contact. In addition, we took advantage of the embryonic lethal phenotype of *pam-1* mutants to conduct suppressor screens. We have identified four suppressor strains, which increase the *pam-1* hatch rates from 1% to near 50%. Using DIC microscopy we have discovered that the presence of each suppressor often rescues the polarity defects associated with *pam-1* mutants. Genetic and phenotypic characterization of these suppressors as well as further analysis of centrosome movements in the strains should reveal new insights into the role of this aminopeptidase in centrosome positioning and polarity establishment.

1114C. Sequential roles of Atg8 homologs during autophagosome formation. **Marion Manil-Segalen[1]**, C. Lefebvre[1], C. Jenzer[1], C. Boulogne[2], B. Satiat-Jeunemaitre[2], V. Galy[3], R. Legouis[1]. 1) CGM, CNRS UPR3404, Gif-sur-Yvette FRANCE; 2) ISV, CNRS UPR2355, Gif-sur-Yvette FRANCE; 3) UMR 7622 CNRS-UPMC, Paris FRANCE.

Autophagy allows the bulk degradation of cytoplasmic constituents within lysosomes by formation of a double membrane vesicle called autophagosome. Numerous studies have highlighted the large variety of physiological and physiopathological roles of autophagy but it also plays a critical role in the remodeling of tissues during development. We use C. elegans as a genetic model to study the functions of the ubiquitin-like proteins Atg8/LC3. Atg8 and its homologues, which are located at the membrane of the autophagosomes, are essential in the autophagic process, but their exact function is not yet known. The presence in C. elegans of two Atg8/LC3 homologues, LGG-1 and LGG-2, constitutes an intermediate situation between the yeast with a unique gene and the complexity of mammals with seven genes. We have shown that these two homologues act synergistically during development, starvation and aging and that LGG-1/2 autophagy is essential for degradation of paternal mitochondria upon fertilization. Moreover, the depletion of LGG-1 and LGG-2 results in an embryonic lethality with epithelial defects. However, the respective roles of these two proteins during the autophagic process are not yet understood. To gain new insights into the mechanisms of autophagy, we have recently analyzed their functions and localizations during embryogenesis. Using electron microscopy, we have shown for the first time that both LGG-1 and LGG-2 localize at the autophagosomal membrane. Immunolocalization and time lapse experiments indicate that LGG-1 and LGG-2 define three populations of autophagosomes: one positive for LGG-1, one positive for LGG-2 and the third positive for both. We combined genetics and microscopy analyses to characterize the specific roles of LGG-1 and LGG-2. Our results indicate that they have sequential functions during formation and maturation of autophagosomes While LGG-1 is essential for their formation, LGG-2 is involved later in the autophagic process to facilitate their degradation. I will present our working model on LGG-1 and LGG-2 functions in autophagosomal maturation.

1115A. LET-99 regulates G protein signaling and spindle positioning during asymmetric division. **Jennifer A. Milan**, Dae Hwi Park, Lesilee S. Rose. Univ of California Davis, Davis, CA.

Asymmetric divisions that generate cell diversity are required for normal development and stem cell maintenance. During asymmetric division, the mitotic spindle is aligned with the cell polarity axis so that cell fate determinants are differentially segregated to daughter cells, giving them different fates. The conserved PAR polarity proteins establish cell polarity, and regulate spindle movements via a complex involving Ga subunits, GPR, and LIN-5. The Ga/GPR/LIN-5 complex is necessary for the cortical forces that pull on astral microtubules by recruiting regulators of the microtubule motor dynein. In *C. elegans* one-cell embryos, GPR and LIN-5 are present at higher levels in the anterior during prophase, when pulling forces center the nuclear-centrosome complex and rotate it onto the polarity axis. During the subsequent spindle displacement movements, the cortical localization of GPR and LIN-5 becomes bipolar, with more at the posterior cortex. We previously showed that LET-99 acts downstream of the PAR proteins during both nuclear centration/rotation and spindle displacement. LET-99 antagonizes G protein signaling by inhibiting the localization of GPR at the cortex. The highest levels of LET-99 are present in a posterior-lateral cortical band, which results in the lowest levels of GPR in this region; LET-99 is also needed for the overall anterior and posterior enrichment of GPR throughout the cell cycle. Gbg sequesters Ga in an inactive state, and in *gpb-1* mutants GPR is uniform on the cortex, as in *let-99* mutants. Additionally, RIC-8 has been shown biochemically to be needed for Ga/GPR/LIN-5 complex formation. To gain more insight into the mechanism of LET-99 action, we compared the *let-99* and *gpb-1* phenotypes and performed double mutant analysis with *ric-8*. Consistent with previous reports we found that loss of Gb bypasses the need for RIC-8 activity in terms of force generation. In contrast, loss of LET-99 does not bypass RIC-8. This and other genetic analyses suggest that LET-99 action requires Gb, and that LET-99 acts upstream of Ga/GPR/LIN-5 complex formation. To further elucidate the molecular mechanism of LET-99 action, we are testing LET-99 for interactions with the components of the pathway.

1116B. Patched family member PTR-2 is required for permeability barrier formation in the *C. elegans* zygote. Jade P. X. Cheng[1], **Sara K. Olson[2,3]**, Alexander Soloviev[1], Olivier Zugasti[1], Karen Oegema[3], Patricia E. Kuwabara[1]. 1) Univ of Bristol, Bristol, UK; 2) Pomona College, Claremont, CA; 3) Ludwig Institute for Cancer Research, San Diego, CA.

The *C. elegans* zygote is protected by a trilaminar eggshell, consisting of vitelline, chitin and proteoglycan layers, which provides mechanical rigidity and blocks polyspermy. At anaphase I of meiosis, cortical granule exocytosis coincides with the formation of an inner eggshell layer composed of CPG-1/2 proteoglycans. Subsequently, a permeability barrier is established that blocks the passage of small molecules. We show that the *C. elegans* PTR-2 (Patched-related) protein, a homologue of the vertebrate Hedgehog receptor Patched, is required for the formation of an intact permeability barrier. 100% of *ptr-2*(*RNAi*) treated embryos arrest at the 100 cell-stage; by contrast, *ptr-2*(*ok1338*) homozygotes are maternally rescued, but arrest at the hatching/L1 stage because of zygotic *ptr-2* absence. *ptr-2*(*RNAi*) arrested eggs are highly permeable to Hoechst 33258, exhibit osmotic sensitivity, and often display cytokinesis defects, all of which are indicative of eggshell and/or permeability barrier defects. We have rescued *ptr-2* mutants with a chromosomally integrated PTR-2::mRFP reporter and have found that PTR-2 is a cortical granule marker. During exocytosis, PTR-2::mRFP vesicles show significant co-localisation with caveolin-1 (CAV-1) vesicles. Mass spectrometry followed by co-immunoprecipitation provides further evidence that PTR-2 is associated with a CAV-1 complex. We have also observed that *ptr-2* depletion leads to aberrant membrane trafficking, which is highlighted by the accumulation/aggregation of the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2) marker phospholipase C-d1 (PH-PLC(d1)). Taken together, our data indicate that *ptr-2* is likely involved in the synthesis or regulation of cortical granule cargo(s), which is delivered at anaphase I; alternatively, *ptr-2* might have a role in establishing the eggshell permeability barrier after anaphase I. We are also examining the importance of the RND permease domain and the sterol sensing domain in mediating PTR-2 function. Finally, we will report on the identification of other proteins that appear to co-localise with

PTR-2.

1117C. Screen for endocytic genes required for dauer development and autophagy. **Nicholas J. Palmisano**[1,2], David Jimenez[2], Alicia Meléndez[1,2]. 1) Biology, The Graduate Center, CUNY, New York, NY; 2) Biology, Queens College, CUNY, Flushing, NY.

Autophagy and endocytosis are two cellular pathways that are vital to proper development and tissue homeostasis. Autophagy is a dynamic and catabolic process used by eukaryotic cells, involving the de novo formation of a double-membrane vesicle called the autophagosome that engulfs long-lived proteins and organelles. The autophagosome then fuses with the lysosome forming an autolysosome, resulting in the degradation and recycling of the engulfed material. Endocytosis involves the uptake of extracellular material into the cell through the formation of intracellular vesicles termed endosomes. Crosstalk between endocytosis and autophagy is evident by multiple findings. Autophagosomes can directly fuse with lysosomes, or can initially fuse with endosomes to form hybrid organelles called amphisomes, which then fuse with lysosomes. Morphological studies in mammals show that amphisomes consist of early autophagosomes and late endosomes/multivesicular bodies (MVB). Additionally, loss of the Endosomal Sorting Complex Required for Transport (ESCRT), a series of protein complexes required for MVB formation, results in the accumulation of autophagosomes. To further study the relationship between autophagy and endocytosis and identify endocytic genes which function in autophagy, we utilized *daf-2(e1370)* mutants expressing a transgene with an autophagy marker, GFP::LGG-1. In C. elegans, *daf-2* encodes the insulin-like receptor and *daf-2(e1370)* mutants are dauer constitutive at 25°C, long lived at the 15°C, and have increased autophagy levels in both. Furthermore, defects in both autophagy and endocytosis affect the longevity phenotype of *daf-2(e1370)* mutants. Previous data showed that knockdown of autophagy genes in *daf-2(e1370)* mutants results in defects in normal dauer morphology, increased global fluorescent intensity of GFP::LGG-1, and the formation of GFP::LGG-1 positive aggregates. Therefore, to identify endocytic genes that may affect autophagy, we have performed a screen for endocytic genes that when knocked down by RNAi, result in the lack of dauer phenotypes and presence of GFP::LGG-1 aggregates. Here, we present data that identify several endocytic genes important for autophagy function and dauer development.

1118A. Role of fibroblast growth factor receptor in regulation of membrane traffic. **Navin David Rathna Kumar**, Zita Balklava. School of Life & Health Sciences, Aston Univ, Birmingham, United Kingdom.

Several studies show that membrane transport mechanisms are regulated by signalling molecules. Recently, genome-wide screen analyses in *C.elegans* have enabled scientists to identify novel regulators in membrane trafficking and also signalling molecules which are found to couple with this machinery. Fibroblast growth factor (FGF) via binding with fibroblast growth factor receptor (FGFR) mediate signals which are essential in the development of an organism, patterning, cell migration and tissue homeostasis. Impaired FGFR-mediated signalling has been associated with various developmental, neoplastic, metabolic and neurological diseases and cancer. In this study, the potential role of FGFR-mediated signalling pathway as a regulator of membrane trafficking was investigated. We analysed GFP-tagged yolk protein YP170-GFP trafficking in worms where 1) FGFR signalling cascade components were depleted by RNAi and 2) in mutant animals. From these results, it was found that the disruption of the genes *egl-15* (FGFR), *egl-17*(FGF), *let-756*(FGF), *sem-5*, *let-60*, *lin-45*, *mek-2*, and *mpk-1* lead to abnormal localization of YP170-GFP, suggesting that signalling downstream of FGFR via activation of MAP kinase pathway is regulating membrane transport. We further investigated the route of trafficking, to pinpoint which membrane step is regulated by worm FGFR by analysing a number of GFP-tagged intracellular membrane markers in the intestine of WT and FGFR mutant worms. FGFR mutant worms showed a significant difference in the localisation of several endosomal membrane markers, suggesting its regulatory role in early steps of endocytosis. A further research to understand the functional relationship between cell signalling machinery and membrane transport will help us identify the conservation of these regulatory mechanisms between organisms and could pave way to develop novel therapies against multiple developmental diseases and cancers associated with altered FGFR mediated signalling.

1119B. Several ArfGEFs regulate the apoptotic fate in Q neuroblast asymmetric cell divisions. **Jerome Teuliere**, Shaun Cordes, Gian Garriga. Molec & Cell Biol, Univ California, Berkeley, Berkeley, CA.

Arf guanine nucleotide exchange factors (GEFs) can regulate cell adhesion, actin cytoskeletal dynamics, and membrane trafficking through the activation of the Arf small GTPases. We previously found that an Arf GTPase Activating Protein (GAP), CNT-2, was essential in controlling cell size and the cell death fate in the Q neuroblast lineage (Singhvi et al, 2011). We report here that several ArfGEFs control the asymmetric divisions of the Q cell daughters. Q.a and Q.p are both neuroblasts that divide to produce a larger neuronal precursor or neuron and a smaller cell fated to die. Loss of the cytohesin ArfGEF homolog GRP-1 resulted in the production of daughter cells that are more similar in size and in the transformation of the apoptotic daughter into its sister, resulting in the production of extra neurons. GRP-1's GEF activity, mediated by its SEC7 domain, is necessary for its role in the Q.p neuroblast. The loss of GRP-1, however, resulted in a phenotype that was less severe than the loss of CNT-2, suggesting that additional ArfGEFs remained to be identified. By screening for *grp-1* enhancers, we indeed identified two more ArfGEFs, EFA-6 and M02B7.5/Schizo/IQSEC, that are necessary for the Q.p division. Functional GFP-tagged GRP-1 proteins localized primarily to the nucleus, but targeting the GRP-1 SEC7 domain to different cellular compartments suggests that GRP-1 acts at the cell cortex. EFA-6 localized at the cell cortex, suggesting a functions there as well. Surprisingly, loss of GRP-1 and CNT-2 resulted in identical Q.a phenotypes, and neither *efa-6* nor *M02B7.5* appears to function in this division. We will propose models that explain the differential involvement of these ArfGEFs in these two asymmetric divisions. Singhvi et al., Curr Biol. 2011 Jun 7;21(11):948-54.

1120C. Regulatory effect of MAGUK/LIN-2 on kinesin-3-based transport in the neuronal system of C. elegans. **Gong-Her Wu**, Oliver Wagner. Institute of Molecular and Cellular Biology,NTHU 101, Section 2 Kuang Fu Road, Hsinchu, Taiwan 300, Republic of China.

UNC-104, also named KIF1A in mammals, is a well-known neuron-specific kinesin-3 in C. elegans which is mainly involved in rapid axonal transport of synaptic vesicles containing SNB-1 (synaptobrevin-1), as well as other active zone proteins. Although UNC-104/KIF1A plays an important role in axonal transport, the regulatory mechanisms of UNC-104 still remain unclear. Earlier studies have shown that UNC-104 comprises a MAGUK binding site (MBS), targeting CASK and liprin-alpha, while MBS and LBS (liprin-alpha binding sequence) highly overlap. Since we have previously shown that SYD-2/liprin-alpha activates and clusters UNC-104 along axons, we hypothesize that LIN-2/CASK may have similar effects on UNC-104 (based on the overlapping binding sites

on the motor's stalk domain). First, we have shown that LIN-2A isoform reveals equal expression levels throughout C. elegans' life cycle, while LIN-2B expression levels decrease gradually, concluding that the major regulatory isoform in adult animals is likely to be LIN-2A. Immune-fluorescence data show that LIN-2 co-localize with UNC-104 in CAN and HSN neurons, and also co-immunoprecipitation assays reveal interactions between LIN-2 and UNC-104. In addition, yeast two-hybrid analysis provides evidence that a minimum liprin-alpha binding domain (MinLBD) of UNC-104 is able to tightly bind to LIN-2's GUK domain, and that UNC-104's FHA region is capable to interact with LIN's PDZ domain. In situ studies employing LIN-2 knockout worms reveal diminished anterograde motility of UNC-104 and enhanced retrograde movements (while different lin-2 background alleles reveal similar effects). Particularly, UNC-104's retrograde velocity in lin-2 KO worms is increased and depends on the dynein interacting protein dynactin/DNC-1. These data demonstrate that LIN-2 is a novel regulator of UNC-104-based axonal transport and that functional interactions occur between LIN's PDZ and UNC-104's FHA domain.

1121A. VANG-1, one of the PCP core components, is involved in asymmetric divisions of seam cell. **M. Yokoo**, H. Sawa. National Institute of Genetics, Mishima, Japan.

The planar cell polarity (PCP) pathway, one of the Wnt signaling pathways, is involved in the coordination of orientation of cell polarity or organizing convergent extension for correct morphogenesis. In *Drosophila* wing cells, it is known that Van Gogh is asymmetrically localized to the proximal cortex, while Frizzled (Fz) and Dishevelled (Dsh) are localized to the distal cortex. During asymmetric divisions in *C. elegans*, although Fz and Dsh are asymmetrically localize to the posterior cortex as in the PCP pathway, asymmetric division is controlled by the Wnt/b-catenin asymmetry pathway that involves asymmetric localization of WRM-1/b-catenin to the anterior cortex. The involvement of VANG-1, the sole homolog of Van Gogh, in asymmetric divisions has not been clearly demonstrated. To examine whether VANG-1 is involved in asymmetric divisions in *C. elegans*, we analyzed the phenotypes of *vang-1* mutants in seam cells. Seam cells V1-V6 are positioned on each lateral side of the animals and repeatedly undergo self-renewing asymmetric divisions in each larval stage to produce anterior daughter cells that fuse with the hypodermal syncytium called hyp7 and posterior daughter cells that remain as seam cells. In *vang-1* single mutants, we found that asymmetric divisions were abnormal at the L2 stage when each seam cell divides twice; a first proliferative division and a second self-renewing asymmetric division. In *vang-1* mutants, polarity of the second division tended to be reversed specifically in the anterior (Vn.pa) but not in the posterior (Vn.pp) daughters of the first division. At the L1 stage, although seam cell polarity was normal in *vang-1* single mutant, we found that *vang-1* genetically interacted with Wnts. The polarity of seam cells is redundantly controlled by four Wnts (*lin-44*, *cwn-1*, *egl-20* and *cwn-2*) (Yamamoto et al 2011). Although, in triple Wnts (*lin-44*, *cwn-1* and *egl-20*) mutants, polarity of V4 and V6 are almost normal, further mutation of *vang-1* disrupted, polarity of these cells but not V1 or V2. These results suggest that *vang-1* is needed for the long-range signaling of *cwn-2* that is expressed anterior to the V cells. We will further study genetic interactions between vang-1 and Wnt genes.

1122B. Amphiphysin 2 binds nesprin and regulates nuclear positioning. **M. D'Alessandro**[1], K. Hnia[1], C. Koch[1], C. Gavriilidis[1], S. Quintin[1], N.B. Romero[3], Y. Schwab[1,2], M. Labouesse[1], J. Laporte[1]. 1) IGBMC, Illkirch, France; 2) EMBL, Heidelberg, Germany; 3) Institut de Myologie, Paris, France.

Centronuclear myopathies (CNM) are characterized by skeletal muscle weakness and abnormal position of nuclei at the center of myofibers, while they are normally at the periphery. We decided to investigate the genetic and molecular pathway that leads to the CNM nuclear positioning defects looking for protein partners of AMPH2, a membrane curvature sensing protein mutated in the autosomal CNM form. By Y2H we found that human AMPH2 binds to Nesprins that have a pivotal role in nuclear positioning/anchorage. We confirmed this interaction by both GST pull down and Co-IP. To investigate the biological significance of this interaction, we turned to C. elegans and studied the position of nuclei in seam cells. Using a nuclear marker to visualize nuclei we found that RNAi or a null mutant of the C. elegans ortholog of AMPH2 (amph-1) displays abnormal position of the seam cell nuclei that resembles the defects observed in mutants for ANC-1 (homologous to mammalian Nesprin). We characterized at the ultrastructural level these nuclear defects by Correlative Light Electron Microscopy. We created an anc-1;amph-1 double mutant that shows percentage of mis-positioned seam nuclei comparable to each single mutant, suggesting that there is no additive effect and that the two proteins are in the same pathway. Collectively, our data sustain a role for AMPH-1 and ANC-1 in nuclear anchorage during development. There is a cooperation between microtubules and nesprins in regulating nuclear positioning. We used spas-1::mCherry driven by heat-shock promoter to sever microtubules and observed defects on seam nuclear positioning. RNAi screening showed that two microtubule binding proteins lead to seam nuclear mis-positioning like in anc-1 and amph-1 mutants. In eukaryotic cells nesprin promotes perinuclear AMPH2 membran tubules. AMPH2 was also found around the nucleus in murine muscle fibers and human muscle biopsies with AMPH2 mutations had abnormal nuclei aggregation. We propose that defect in the AMPH2/Nesprin interaction affects the correct nuclear positioning in CNM through a potential link with cytoskeleton.

1123C. EXC-2 and Maintenance of excretory canal tube structure. **Hikmat Al-Hashimi**, Robyn Harte, Jenny Hackett, Stuart Macdonald, Matthew Buechner. Molecular Biosciences, Univ of Kansas, Lawrence, KS.

Long, narrow, single-celled tubes are found in many tissues in our body, such as capillaries and nephrons. The excretory canal cell of *C. elegans* provides a model for investigating the formation and maintenance of narrow tubes. Mutations in a series of *exc* genes allow the excretory canals to swell into fluid-filled cysts. The position of all these genes has been narrowed, but a few remain to be cloned. We are currently using whole-genome sequencing methods to identify the sequence of *exc-2*. Preliminary data suggest that EXC-2 may act upstream of other EXC proteins that effect endosomal recycling. We are characterizing the potential role of EXC proteins in the endosomal transport pathway by monitoring several endosomal compartments via FRAP. Recovery of fluorescence of cytosolic molecules of labeled RAB-5 and RAB-11 are not affected by *exc-2* mutation. A difference was seen, however, with RAB-5 and RAB-11 bound to their target compartments (the early and recycling endosomes, respectively). Puncta carrying marked RAB-5 recovered rapidly after bleaching. Puncta of RAB-11 molecules, exhibited a different behavior. In the wild-type background, bleached puncta recovered fluorescence fully within 180 seconds. When *exc-2* was mutated, however, recovery was essentially halted, with no significant increase in fluorescence visible after 280 seconds. This result suggests that EXC-2 function is required for turnover of RAB-11 on the recycling endosome. We are now analyzing other subcellular markers such as EEA-1, RME-1, GRIP, GLO-1, and RAB-7 in order to gain a broader understanding of the role of EXC-2 and other EXC proteins in the endosomal recycling.

1124A. Microtubules and Fertilization: The Meiosis to Mitosis Transition. **Sarah M. Beard**, Ben G. Chan, Paul E. Mains. Biochemistry and Molecular Biology, The Univ of Calgary, Calgary, AB, Canada.

During embryonic development, dramatic changes of the C. elegans cytoskeleton occur in the transition from the meiosis to mitosis requiring precise regulation of molecules specific to each type of spindle. Defects in this transition can result in tissue pathologies, aneuploidy or even cancer. The microtubule severing complex, MEI-1/MEI-2, is required for C. elegans meiotic spindle formation, but MEI-1/MEI-2 must be inactivated to prevent disruption of mitosis. Degradation of MEI-1 during mitosis is dependent on the MEL-26/CUL-3 E3 ubiquitin ligase complex. However, there are other redundant pathways involved in this tightly regulated meiosis to mitosis transition. One pathway, involving the anaphase promoting complex (APC) and the MBK-2/DYRK kinase, promotes mitotic MEI-1 degradation in parallel to MEL-26 mediated degradation. The goal of this project is to measure anti-MEI-1 staining levels in several mutant strains to determine how known genes of these pathways function relative to one another. Using antibody staining of single and double mutants, I have shown that APC and MBK-2 act sequentially relative to one other. We are also interested in deciphering the exact role of CUL-2, another E3 ubiquitin ligase, in mitosis. Double mutants reveal that CUL-2 acts with MBK-2 to mediate MEI-1 degradation in parallel to the MEL-26/CUL-3 complex. Loss of RFL-1, a ubiquitin ligase activator, has been shown to result in ectopic MEI-1 staining. RFL-1 may activate CUL-2, CUL-3 or both. Preliminary results suggest that RFL-1 is acting with MBK-2 to degrade MEI-1. Lastly, HECD-1, another E3 ubiquitin ligase, may also degrade MEI-1 in mitosis. We will test where it is acting relative to the other E3 complexes.This project will assist in decoding the key regulatory molecules of the developmental remodeling.

1125B. The molecular genetics of epithelial cell morphogenesis- functional dissection of *C. elegans* homologue zyxin. **Keliya Bai**, Jonathan Pettitt. School of Medical Sciences, Univ of Aberdeen, Aberdeen, Scotland, United Kingdom.

The zyxin family has been implicated in a wide range of cell behaviours, including cell adhesion and motility, and more recently in the regulation of Hippo signalling. All family members consist of three well-conserved LIM domains and an N-terminal, “pre-LIM” domain. Although highly conserved across animal phyla, the precise mechanisms by which these proteins function are not well understood. We are investigating the function of the sole *C. elegans* family member, ZYX-1. Genetic analysis indicates that ZYX-1 contributes to, but is not required for cadherin-mediated morphogenesis in *C. elegans*. Our data shows that ZYX-1 localises to adhesion junctions and becomes more strongly recruited as those junctions undergo actomysin-dependent tension. We are investigating the roles of these domains in the function and localisation of ZYX-1, as well as determining the mechanism by which ZYX-1 is recruited to adhesion junctions. In addition to its role in epithelia morphogenesis, we have also shown that it collaborates with other regulators of actin dynamics to coordinate the changes in cell shape and adhesion that accompany gastrulation.

1126C. Dissecting the mechanistic insights through which EGL-26 controls *C. elegans* vulva tubulogenesis. **Nagagireesh Bojanala**, Avni Upadhyay, Hongliu Sun, Matt Crook, Ariana Detwiler, Nishat Seraj, Sarah Chang, Jimmy Goncalves, Ryan Fine, Nick Serra, Wendy Hanna-Rose. Biochem and Mol Biol, Penn State Univ, Univ park, PA.

Generation of tissue architecture and biological lumen formation during development occurs through various morphogenetic processes, and defective morphogenesis results in tissue malfunctions and disease. *C. elegans* vulva tubulogenesis involves formation of distinct dorsal and ventral lumens and is a good model for analyzing morphogenesis *in vivo*. Previously we established the role of EGL-26 (Lecithin Retinol Acyl Transferase family member) in vulva dorsal lumen formation. *egl-26(lf)* results in defective morphogenesis of the dorsal most toroid VulF. But expression analyses suggested a non-cell autonomous function for EGL-26 within the neighboring toroid VulE. To shed more insight into the mechanistic processes through which EGL-26 regulates dorsal lumen formation, we are taking a candidate RNAi screen approach to identify additional genes/*egl-26* targets regulating dorsal lumen. So far, our screen identified interesting candidates involved in spindle formation, cytoskeleton, transcriptional, translational, and membrane transport processes. These observations suggest various gene regulatory modules working in conjunction with EGL-26 during morphogenesis. To further analyze cytoskeletal changes during vulva tubulogenesis, we are carrying out gamma tubulin staining in wild type and *egl-26 (lf)* animals. Our preliminary observations in wild type animals identified strong microtubule presence around the dorsal toroidal membrane, and hence providing a cytoskeletal signature during vulva morphogenesis. Any possible changes occurring within this signature in *egl-26 (lf)* situation will identify its potential direct and indirect targets regulating cytoskeleton during lumen formation. In addition our analyses of *cis*-regulatory modules that govern *egl-26* expression within VulE and F lineages identified a 200bp region within *egl-26* promoter that is sufficient and necessary for VulE-specific expression and contains a binding site that negatively regulates VulF expression. Thus, the findings from this study will provide more details about proper maintenance of biological lumens during development.

1127A. Characterizing regulators of the *C. elegans* embryonic elongation pathway. **Benjamin Chan**, Simon Rocheleau, Paul Mains. Biochemistry and Molecular Biology, Univ of Calgary, Calgary, Alberta, Canada.

Eukaryotic organisms begin as a roughly spherical ball of cells, but the final shape of a species is a precise and tightly regulated process. In *Caenorhabditis elegans*, a smooth muscle-like contraction of an actin/myosin network mediates the elongation of a worm embryo from an ellipsoid into a long, thin worm. Here we will continue to characterize a gene known to regulate this process, and discuss a novel gene which may be also involved. Previous work has shown that non-muscle myosin is able to generate contractile force through two redundant pathways in *C. elegans*. Phosphorylation of myosin light chain (MLC-4) activates non-muscle myosins NMY-1/2, which drives contraction. In contrast, dephosphorylation of MLC-4 is regulated by MEL-11/myosin phosphatase. In one pathway, the small GTPase RHO-1 activates LET-502/Rho-binding kinase, which inhibits MEL-11, allowing contraction to proceed. In a second parallel pathway, FEM-2/protein phosphatase 2c and PAK-1 are involved in regulating contraction (Piekny et al., 2000; Galley et al., 2009). In a suppressor screen of *mel-11*, an allele of a Rho GEF (guanine exchange factor) *rhgf-2* was identified. *rhgf-2* genetically acts upstream of *let-502* and in parallel to *fem-2*. In addition, RHGF-2 acts as a GEF for RHO-1 (Lin et al., 2012). The cellular localization of RHGF-2 has yet to be determined, and our current work will use a RHGF-2 antibody to address this question. Using a RHGF-2 antibody, expression is ubiquitous throughout the worm embryo in early embryogenesis. During elongation and contraction, RHGF-2 appears to be at cell membranes, consistent with the localization and function of eukaryotic Rho GEFs. Following elongation, RHGF-2 is prominently expressed in seam cells compared to the dorsal/ventral cells. In our previous suppressor screen of *mel-11*, a novel gene represented by the allele *sb89* was also isolated. Whole genome sequencing and traditional mapping suggests that *sb89* is an allele of

ZK185.1, and the allele from the knockout consortium also suppresses of *mel-11*. *ZK185.1* is largely uncharacterized except for a predicted zinc finger. Future experiments will continue to characterize this gene.

1128B. The RhoGEF ECT-2 is Required for Ventral Enclosure During C. elegans Embryogenesis. **Y. Chen**, A. Marte, G. Stylianopoulos, A. Piekny. Biology, Concordia Univ, Montreal, Quebec, Canada.

Ventral enclosure is the process where ventral epidermal cells migrate to enclose the *C. elegans* embryo in a single layer of epidermal cells. This process is initiated by the migration and adhesion of two pairs of anterior leading cells, followed by the migration and adhesion of eight pairs of posterior pocket cells. The migration of ventral epidermal cells is mediated by F-actin rich filopodia-like protrusions, which are under the control of the Rac - Wave/Scar - Arp2/3 pathway. However, it is not known if nonmuscle myosin also contributes to the migration of these cells by regulating cell shape changes and/or adhesion. Proteins that are part of adhesion junction complexes are also required for ventral enclosure, as they maintain contacts between cells so they can migrate as a unit and form new junctions with contralateral neighbours. RhoA/RHO-1 regulates actin-myosin contractile events for cytokinesis in the early embryo and for elongation of the lateral epidermal cells in late embryogenesis. ECT-2 is the GEF that activates RHO-1 during cytokinesis, and a different GEF, RHGF-2, activates RHO-1 in late embryogenesis. A hypomorphic, maternal ts allele of *ect-2*, *ax751*, is required for polarity in the early embryo, but displays few cytokinesis defects, especially at non-permissive temperatures. Imaging *ect-2 (ax751)*; AJM-1::GFP embryos at non-permissive temperatures revealed a requirement for *ect-2* in ventral enclosure. The ventral epidermal cells in *ect-2 (ax751)* embryos migrated significantly slower compared to cells in wild-type embryos, and often failed to meet their contralateral neighbours. Genetic crosses suggest that *ect-2* functions in parallel to the Rac pathway and may be part of the Rho pathway, supporting a role for myosin in ventral enclosure. Interestingly, *ect-2* may also function in the cadherin/catenin pathway, suggesting that *ect-2* could also regulate the actin-myosin filaments that contribute to adhesion. GFP::ECT-2 and GFP::RHO-1 localize to epidermal cell boundaries during mid-embryogenesis and are basolateral in comparison to junction components. We are further investigating the role of *ect-2* during ventral enclosure, and how it regulates myosin for cell shape changes and/or adhesion.

1129C. The Regulation of Bone Morphogenetic Protein Pathway by LON-1 in Extracellular Matrix using a novel BMP readout. **King-Lau Chow**, Ho-Tsan Wong, Kwok-Hei Wong. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

Bone Morphogenetic Protein (BMP) pathway controls body length in *C. elegans* by repressing the transcription of its downstream target, *lon-1* gene. The *lon-1* product subsequently blocks endoreduplication in hypodermis. In the absence of the BMP signal, *lon-1* is active, resulting in worms of shorter body length. In *lon-1(e185)* mutant, un-repressed endoreduplication leads to longer worms. Meanwhile, the *lon-1* gene product, LON-1, shows physical interaction with a BMP facilitator, CRM-1, in yeast-2-hybrid assay, and it is detected in the hypodermal membrane fraction by specific antibody. When the body length of *dbl-1 (nk3); lon-1 (e185)* double mutant showed an intermediate phenotype, it raises the possibility that *lon-1* might antagonize *dbl-1* activity, which has not been defined. Since *lon-1* is also required to repress endoreduplication, the elimination of *lon-1* function may have a dual and potentially opposite effect acting extracellularly and intracellularly, and the body length could not be used as the sole indicator of BMP signal activity. We dissociate the two different effects by introducing a novel p4k*lon-1::gfp* transcriptional reporter to assess its extracellular regulatory role on BMP signal. The green fluorescence in the hypodermis would change inversely to the BMP signal. Our data shows that BMP signal decreases in *lon-1 (e185)* mutant, with an intestinal BMP transcriptional reporter *pspp-9::gfp* signal as reference, indicating that *lon-1* might be a positive regulator of BMP pathway. In the yeast-2-hybrid system, SCP-CRISP domain of LON-1 protein could interact with Cysteine-Rich (CR) domain of CRM-1 protein. We therefore hypothesize that the membrane bound LON-1 protein might complex with CRM-1 and DBL-1, which augments the presentation of the DBL-1 to its receptor. To demonstrate the complex formation of LON-1, CRM-1 and DBL-1, these three genes will be expressed in the Drosophila S2 cell line and followed by co-immunoprecipitation assay. Using the *lon-1* transcriptional reporter to monitor the BMP signal, we expect to have the *lon-1* overexpressing worms with an enhanced BMP signaling activity will show a weaker green fluorescent signal. (This study is supported by Research Grants Council, Hong Kong).

1130A. A genome-wide RNAi screen to identify new players of a muscle-to-epidermis mechanotransduction pathway essential for embryonic elongation. **Christelle Gally**, Agnès Aubry, Michel Labouesse. IGBMC, Illkirch, France.

The interplay between different layers or tissues adds a level of complexity in the understanding of how organs develop. As an example, loss of muscle activity in the *C. elegans* embryo results in embryonic elongation arrest at the 2-fold stage (Pat phenotype) for a reason that has long remained unclear. We recently unraveled a mechanotransduction pathway between muscles and epidermis that accounts to a large extent for the Pat phenotype (Nature, 471, 99-103, 2011). Specifically, during the second phase of embryonic elongation, muscle contractions activate the serine/threonine p21-activated kinase PAK-1 in the epidermis and trigger the remodeling of hemidesmosomes (HDs) connecting the apical and basal sides of the epidermis through intermediate filaments. PAK-1 controls intermediate filament phosphorylation and their anchoring to HDs. The most upstream known component of this pathway is the adaptor protein GIT-1, whose localization to HDs requires muscle activity. However, since *git-1* mutants do not induce a two-fold arrest, we predict that muscle contractions also trigger a second parallel pathway, probably to activate actomyosin remodelling. In order to identify this putative parallel pathway, we have undertaken a genome-wide RNAi screen in the synthetic *git-1(tm1962)* mutant background, looking for enhancers that lead to an elongation arrest. As an approach, we used 96-well worm liquid cultures of L4 mothers (see Nat Protoc, 1, 1617-20, 2006), looking for late embryonic arrests in their progeny. In the primary screen, we have identified 78 candidates that induce body-morphology defects as well as an elongation arrest in the *git-1* mutant in a more effective way than in the wild-type N2 background. We have confirmed this *git-1* enhancement for 39 candidates in a secondary screen. Among them, four genes encode HD components and six genes are involved in muscle differentiation/function or attachment to the extracellular matrix, hence validating our approach. Now, we are focusing on genes that were not previously linked to embryonic elongation. We are currently testing their involvement in HD organization as well as in actomyosin organization and function, and will present our preliminary results.

1131B. Regulation of *C. elegans* MCAK by Aurora Kinase Phosphorylation. **Xue Han**, Martin Srayko. Biological Sci, Univ Alberta, Edmonton, AB, Canada.

Microtubules are required for multiple cellular processes including mitosis, cytokinesis, and vesicle transportation. Factors that regulate microtubule dynamics in the cell help determine the final form and precise cellular role of many cytoskeletal structures. Among the known modulators of microtubule

dynamics, we are specifically interested in the microtubule-depolymerizing kinesins of the kinesin-13 family, such as KLP-7 (CeMCAK). In mitosis, *C. elegans* KLP-7 locates to the kinetochore and the centrosome, and it functions to regulate spindle checkpoint and microtubule outgrowth at the centrosome. During female meiosis, KLP-7 localizes to chromosomes, the chromosomal passenger complex region and the spindle poles in metaphase, and localizes to chromosomes in anaphase and telophase. KLP-7 depletion resulted in an excessive microtubule network near the meiotic spindle and the cortex. Extensive work on the vertebrate homologues of KLP-7 indicates that they are negatively regulated through phosphorylation by the Aurora kinases. In order to understand how KLP-7 is regulated in worm embryos, we tested the possibility that Aurora kinases are directly involved. 2D gel-electrophoresis revealed an alteration in the ratio of potential KLP-7 phosphorylation variants in lysates from either *air-1(RNAi)* (Aurora A-depleted) or *air-2(RNAi)* (Aurora B-depleted) embryos, compared to wild type. Furthermore, we found that both AIR-1 and AIR-2 kinases phosphorylated KLP-7 *in vitro*. We used a combination of *in vitro* kinase assays and an Aurora kinase phospho-site prediction algorithm to identify potential in vivo phosphorylation sites within KLP-7. Using *in vitro* kinase assays, we found that AIR-1 and AIR-2 kinases phosphorylate N- and C-terminal domains of KLP-7. We are also performing a structure-function analysis to determine which putative Aurora sites are required for KLP-7's intracellular location and/or its depolymerase activity at the centrosome. Results obtained thus far indicate that mutating S546 at the C-terminus from serine to glutamic acid (to mimic constitutive phosphorylation) or alanine (to mimic non-phosphorylation) interferes with KLP-7 function but not its ability to target to centrosomes or kinetochores.

1132C. Mechanisms of Sperm Competition in *C. elegans*. **Jody Hansen**, Daniela Chavez, Gillian Stanfield. Human Genetics, Univ of Utah, Salt Lake City, UT.

In *C. elegans*, male sperm compete with hermaphrodite self sperm, resulting in the preferential use of male sperm. This differential fertilization success, termed male precedence, is nearly absolute. Evidence indicates that male precedence relies on intrinsic differences between male and hermaphrodite sperm. One such difference is that males have larger sperm than that of the hermaphrodite. While larger sperm size is correlated with faster crawling speeds, size does not correlate perfectly with velocity, suggesting that additional factors contribute to male precedence. By identifying sperm-specific gene products required for male precedence, we seek to understand the cellular and molecular mechanisms important for cell competition. Mutations in the kinase gene *F37E3.3* lead to reduced male precedence and migration defects. While wild-type male sperm migrate rapidly to the spermathecae, mutant sperm slowly accumulate in the spermathecae. However, *F37E3.3* defects are dependent on the competitive context, as mutant sperm are functional by several criteria. Critically, mutant males and hermaphrodites have normal fertility. In addition, mutant sperm appear grossly normal with properly polarized sperm structures, and their *in vitro* velocity is indistinguishable from the wild type. The mutant sperm migration defect is ameliorated in *spe-8* hermaphrodites, which lack activated sperm and contain unfertilized oocytes. Thus, the hermaphrodite reproductive environment likely plays a role in the aberrant mutant sperm migration behaviors. Currently, we are testing two models; that unfertilized oocytes might provide a better substrate for adhesion for mutant sperm, or the presence of activated hermaphrodite sperm might have an inhibitory effect on male sperm. F37E3.3 is expressed in and displays a punctate pattern that is restricted to the cell body, but it does not associate with known sperm structures. Notably, mutant spermatids are the same size as wild-type, suggesting that mechanisms in addition to size contribute to male sperm precedence. To date, *F37E3.3* is the only gene identified that specifically affects *C. elegans* male precedence and its characterization likely will reveal a novel mechanism for sperm competition.

1133A. Actin-based cell motility in developing *C. elegans*: dissecting actin assembly factors. **Svitlana Havrylenko**[1,2], Philippe Noguera[1,2], Julie Plastino[1,2]. 1) PhysicoChimie, Institut Curie, Paris, France; 2) CNRS UMR168, Paris, France.

Protrusive cell shape changes powered by actin assembly are one of the bases of cell motility observed in both normal and pathological processes. Actin assembly factors Enabled/Vasodilator-Stimulated Phosphoprotein (Ena/VASP) and the Wiskott-Aldrich Syndrome Protein (WASP) families of proteins are among numerous biochemical factors that affect actin cytoskeleton network architecture and dynamics. Mechanism of action of these multidomain proteins and interplay between them remain poorly understood. Developing *C. elegans*, and in particular ventral enclosure, a collective cell migration event during embryogenesis, offer a system to study the role of Ena/VASP and WASP proteins in mediating protrusive activity of the cells in the complex *in vivo* environment. We are constructing transgenic strains expressing different mutant forms of Ena/VASP and WASP proteins in the corresponding genetic knock-out background, while visualizing actin filaments with LifeAct:GFP expressed under the control of a tissue-specific promoter. In parallel we are testing the same modified forms of the Ena/VASP and WASP proteins in a stripped-down *in vitro* bead system in order to understand their mechanism of action. Our preliminary results indicate that the F-actin binding capacity of Ena/VASP, its tetramerization domain and its recruitment of the actin monomer binding protein, profilin, are necessary for enhanced bead motility *in vitro* and for efficient ventral enclosure *in vivo*. All together these results suggest that Ena/VASP acts during ventral enclosure to increase protrusion via multiple interactions with growing actin filaments.

1134B. A heterogeneous mixture of F-Series prostaglandins promotes sperm guidance in the *Caenorhabditis elegans* reproductive tract. **Hieu D. Hoang**[1], Jeevan K. Prasain[2], Michael A. Miller[1]. 1) Cell, Developmental, and Integrative Biology, Univ of Alabama at Birmingham, Birmingham, AL; 2) Pharmacology and Toxicology, Univ of Alabama at Birmingham, AL.

In the C. elegans reproductive tract, amoeboid sperm crawl around fertilized eggs and across the uterine epithelium to the spermatheca, the site of fertilization. The oocytes that lie adjacent to the spermatheca synthesize sperm guiding cues called F-series prostaglandins (PGFs). These PGFs are derived from polyunsaturated fatty acids (PUFAs) provided in yolk lipoprotein complexes. Here we use genetics and electrospray ionization tandem mass spectrometry to partially delineate PGF metabolism pathways. We show that omega-6 and omega-3 PUFAs, including arachidonic and eicosapentaenoic acids are converted into more than 10 structurally related PGFs, which function collectively and largely redundantly to promote sperm guidance. PGFs are synthesized using a biochemical mechanism that is distinct from mammalian cyclooxygenase-dependent pathways, yet $PGF1_a$ and $PGF2_a$ stereoisomers are still generated. Cyclooxygenase enzymes are the targets of non-steroidal anti-inflammatory drugs, which inhibit PG synthesis. Our data in worms, zebrafish, and mice are consistent with the existence of a conserved cyclooxygenase-independent mechanism for PG synthesis. PGFs have been implicated in regulating human sperm motility via an unconventional interaction with the CatSper Ca^{2+} channel complex (Lishko et al, 2011; Strunker et al, 2011). Our genetic studies in C. elegans support the model that PGF signal transduction requires a sperm GPCR pathway(s). Multiple related GPCR genes that are essential for sperm guidance are physically clustered in the genome. Together, these studies suggest that ovaries of worms and mammals convert dietary fats into chemical cues that affect sperm function.

1135C. Anatomic Expression of the Tubulin Superfamily. **Daryl D. Hurd**. Dept Biol, St John Fisher Col, Rochester, NY.

a- and b-tubulins are globular cytoplasmic GTP-binding proteins that form heterodimers which self-assemble into microtubules. The dynamic microtubule cytoskeleton underlies a broad range of cellular processes including cytoplasmic polarity, mitosis, intracellular transport and cilium structure/function. The *C. elegans* tubulin superfamily consists of nine a-tubulins and six b-tubulins. Study of the phenotypes caused by tubulin mutations has thus far revealed essential roles for tubulin in embryonic development (*tba-1*, *tba-2*, *tbb-1* and *tbb-2*) and neurological function (*tba-1*, *mec-7*, *mec-12*, *ben-1*, *tba-6*, *tba-9* and *tbb-4*). The anatomic expression for many of these tubulin isotypes has been reported. In contrast, mutant phenotypes or expression patterns for some tubulins are unknown. To begin to address the cellular and developmental roles of the remaining tubulins, I fused upstream promoter sequences to fluorescent proteins, created transgenic lines and analyzed the anatomic expression of the unstudied members of the tubulin superfamily (*tba-4*, *tba-7*, *tba-8* and *tbb-6*). Combined with previous data, this analysis supports the grouping of the tubulins into three classes based on tissues in which they are expressed: sensory neurons, blastomeres/motor neurons, and epithelial cells. These data, along with the availability of loss of function mutations for nearly all tubulins, should facilitate a thorough investigation of tubulin biology in nematodes.

1136A. ROL-3, the ortholog of the human proto-oncogene ROS1, is required to orchestrate the morphogenesis and development of the seam syncytium and interacts with the Bicaudal-C homolog *bcc-1*. **Martin R. Jones[1]**, Ann M. Rose[1], David L. Baillie[2]. 1) Dept. of Medical Genetics, Univ of British Columbia, Vancouver, Canada; 2) Dept. of Molecular Biology and Biochemistry, Simon Fraser Univ, Vancouver, Canada.

The orphan receptor ROS1 is a human proto-oncogene, mutations of which are found in an increasing number of cancers. Little is known about the role of ROS1, however in vertebrates it has been implicated in promoting differentiation programs in specialized epithelial tissues. Using a combination of genetic and molecular techniques we show that the *C. elegans* ortholog of ROS1, the receptor tyrosine kinase ROL-3, has an essential role in orchestrating the morphogenesis and development of the seam syncytium, a specialized epidermal tissue, highlighting a potentially conserved function for ROL-3 in coordinating crosstalk between developing epithelial cells. We have also used a deletion biased mutagenesis screen to identify two suppressors of *rol-3*, providing evidence of a direct relationship between ROL-3, the mucin SRAP-1, and BCC-1, the homolog of mRNA regulating protein Bicaudal-C. Our work answers a long standing question as to the developmental function of ROL-3, identifies three new genes that are expressed and function in the developing epithelium of *C. elegans*, and introduces the *C. elegans* as a potentially powerful model system for investigating the increasingly important, yet poorly understood, human oncogene ROS1.

1137B. A novel protein complex required for the collective migration of the male somatic gonad. **Mihoko Kato[1]**, Tsui-Fen Chou[2], Collin Z. Yu[3], Wen Chen[1], Paul W. Sternberg[1]. 1) Div. of Biology, California Inst Technology, Pasadena, CA; 2) Div. of Medical Genetics, Harbor-UCLA Medical Center, Torrance, CA; 3) School of Pharmacy, UCSF, San Francisco, CA.

The morphogenesis of the *C. elegans* male gonad occurs by the migration of the individual linker cell and the adhesion of the proliferating follower cells behind this leader. We identified a conserved, single-pass transmembrane protein, TAG-256, that is required for the gonadal cells to stay connected during their migration; in mutants with a partial *tag-256* deletion, dissociated gonadal cells are left behind during the migration, and this mutant can be rescued with a wild-type genomic copy of *tag-256*. TAG-256::YFP fusion protein expresses in the entire somatic gonad and localizes to the plasma membrane. Antibodies generated to different domains of TAG-256 show additional localization of the extracellular domain to large cytoplasmic vesicles and the intracellular domain to the nucleus, suggesting proteolytic cleavage. Since TAG-256 has no known interactors, we used SILAC mass spectrometry on human 293T cells to identify protein interactors of its human ortholog, ITFG1. Sixty-six proteins were ³ 5-fold enriched in immunoprecipitates with ITFG1, and of these, 46 proteins (70%) were conserved in *C. elegans*. To identify the genes that function together with *tag-256* in *C. elegans*, we performed RNAi of the 46 interactors having worm orthologs in wild-type animals, and looked for a gonad detachment phenotype. *ruvb-1*, *ruvb-2* and *tba-2* yielded the same phenotype as *tag-256*, and we confirmed the physical interaction of their human orthologs by Western blot. We are currently investigating the subcellular localization of this new complex and the role of the AAA+ ATPases, RUVB-1 and RUVB-2, in this process. Based on the membrane localization of TAG-256 and gonad detachment phenotype of the mutant, we propose that TAG-256 has a physical role in cell-cell adhesion.

1138C. The role of SYM-3 and SYM-4 in tissue integrity and organogenesis. **Pushpa Khanal**, John Yochem, Anna Justis, David Fay. Molecular Biology, Univ of Wyoming, Laramie, WY.

sym-3 and sym-4 encode structurally unrelated but highly conserved proteins. Mutations in sym-3 and sym-4 are synthetically lethal with mec-8, which encodes a conserved RNA-binding protein involved in alternative splicing (1-3). mec-8; sym-3/4 double mutants arrest as L1 larvae, whereas sym-3 sym-4 double mutants are viable, suggesting that SYM-3 and SYM-4 act together within a pathway or complex. mec-8; sym-3/4 mutants show defects beginning at the 1.5-fold stage of embryogenesis, at which time the anterior pharynx, having attached to the epithelium, begins to elongate. Whereas wild-type embryos show only a slight ingression at the anterior hypodermis in the region of the future buccal cavity, mec-8; sym-3/4 mutants display a strong deformation of the anterior hypodermis, which resembles a keyhole. Subsequently, the pharynx and buccal cavity of mec-8; sym-3/4 L1 larvae are displaced towards the posterior, preventing feeding and leading to a “bulbous nose” phenotype. Using alleles of pha-1, we find that formation of the keyhole, displacement of the pharynx, and formation of the bulbous nose can be suppressed by preventing early pharyngeal attachment or by severing the connection between the elongating pharynx and the anterior hypodermis later in development. Our results provide evidence for an inward pulling force on the anterior hypodermis during pharyngeal elongation and suggest that the ability of the hypodermis to withstand this force is weakened in mec-8; sym-3/4 mutants. Interestingly, a keyhole defect is also observed in mir-51-family mutants (4), and this phenotype is also suppressed by pha-1 mutants. To understand the roles of SYM-3, SYM-4 and MEC-8 in the maintenance of hypodermal integrity, we have undertaken RNAi-feeding screen for genes that interact with sym-3 and sym-4. We are also seeking to identify SYM-3 and SYM-4 binding partners using a 2-hybrid approach and to characterize the expression of SYM-3 and SYM-4. Finally, we describe a strategy for isolating genetic suppressors of mec-8; sym-3/4 mutants using a counter-selectable marker. 1 1999 Genetics, 15:117-34. 2 2004 Genetics, 3:1293-306. 3 1994 Genetics, 1:83-101. 4 Genetics, 3:897-905.

1139A. Molecular mechanism of *egl-15*/FGFR and *ina-1*/a-integrin in gland cell migration during the development of *Caenorhabditis elegans* pharynx. **S. Kim**, J. Kormish. Biological Sciences, Univ of Manitoba, Winnipeg, MB, Canada.

The pharynx of *Caenorhabditis elegans* is a simple, yet a powerful experimental model for the study of mechanisms regulating cell migration during organ development. There are five gland cells within the mature pharynx.[1,2] All of these cells send cytoplasmic extensions to more anterior locations in the pharynx where they connect with the pharyngeal lumen and release secretions that aid in digestion.[3] During development of the pharynx, the dorsal gland cell, g1p, is born in the anterior aspect of the pharyngeal primordium and undergoes a form of morphogenesis called retrograde extension.[4,5,6] As the cell body migrates to the terminal bulb, a part of the cell body is left anchored in the anterior region of the pharynx. When migration is complete, a long extension is left behind the cell's migratory path. The objective of our research is to elucidate the molecular mechanism of this distinctive mode of gland cell migration. *egl-15*, the single Fibroblast Growth Factor Receptor (FGFR) in *C. elegans*, and *ina-1*, one of two a-integrin receptors, are required for the proper migration of g1p cell.[5] Worms mutant for either *egl-15* or *ina-1* show similar defects where the gland cell bodies migrate past the terminal bulb, and are located in proximity of the intestine. Genetic epistasis and molecular studies will be used to explore the possibility of FGFR and integrin cross-talk, and more precisely determine the mechanism of receptor action during retrograde extension. Currently, *egl-15* null mutant transgenic rescue experiments are being performed with different EGL-15 domain deletions to distinguish the domain(s) that are required for gland cell migration. Transgenic rescue strategies will be used to locate the site of *egl-15* and *ina-1* expression and function. References: [1]Altun ZF & Hall DH. 2009. In WormAtlas. oi:10.3908/wormatlas.1.3.; [2]Albertson, DG & Thomson, JN. 1976. Philos Trans R Soc Lond B Biol Sci. 275: 299-325.; [3]Smit RB, Schnabel R & Gaudet J. 2008. PLoS Genet. 4: e1000222.; [4]Sulston JE, Schierenberg E, White JG & Thomson JN. 1983. Dev Biol. 100:64-119.; [5]Kormish J, Raharjo W, Kim S, Rohs P, Srayko M, & Gaudet J. *in prep*.; [6]Heiman MG & Shaham S. 2009. Cell. 137:344-55.

1140B. Using genetics and proteomics to identify substrates of a PP2A phosphatase required for mitotic spindle assembly. **Karen I. Lange**, Martin Srayko. Biological Sciences, Univ of Alberta, Edmonton, AB, Canada.

Accurate segregation of sister chromatids during mitosis is essential for the maintenance of genome ploidy, and thus, the health and proper development of all multicellular organisms. The mitotic spindle is a complex microtubule-based apparatus that facilitates the accurate segregation of chromosomes. This structure forms through coordinated cell-cycle regulation and precise control of microtubule polymer behaviour, necessitating temporal and spatial modulation of protein activity. This protein modulation occurs primarily through post-translational modifications such as phosphorylation and dephosphorylation. RSA-1 (regulator of spindle assembly) is a PP2A B'' regulatory subunit required for mitotic spindle assembly in *C. elegans*. In *rsa-1(RNAi)* embryos, the mitotic spindle collapses and there is a decrease in the number of astral microtubules that emanate from the centrosomes. In order to identify the targets of this phosphatase, we are using both genetics and proteomics approaches. First, we screened for genetic suppressors of a temperature sensitive embryonic lethal mutation, *rsa-1(or598ts)*. This allele disrupts the protein interaction between RSA-1 and the PP2A structural subunit PAA-1. While most suppressors identified were mutations in subunits of the complex that restored this interaction, three suppressors were recovered that do not correlate with any genes known to encode components of the RSA complex. We are currently identifying these suppressors, which likely represent substrates or novel regulators of the PP2A complex. We are also using 2-dimensional differential gel electrophoresis (DIGE) to identify proteins that are deferentially phosphorylated between control and *rsa-1(RNAi)* embryos. With this proteomics technique, different fluorescent dyes are used to label the samples and protein mobility shifts can be identified as uniquely-coloured protein spots on the 2D gel. Substrates of the RSA-1/PP2A complex are expected to be more phosphorylated in *rsa-1(RNAi)* embryos and these candidates will be identified using mass spectrometry. Microscopy, genetics, and biochemical assays will be used to verify the potential PP2A substrates that are identified in both of these analyses.

1141C. Identification of DMD-3 targets in the *C. elegans* male tail tip. **H. Littleford[1]**, R. A. Herrera[1], K. Kiontke[1], A. Mason[2], J-S. Yang[1], S. Ercan[1], D. Fitch[1]. 1) New York Univ, New York, NY; 2) Siena College, Loudonville, NY.

Morphogenesis is a fundamental developmental process in which cells can change shape and identity, migrate, and fuse to form a variety of structures. Despite its importance in development, little is known about how transcriptional regulation is linked to cytoskeletal processes that facilitate morphogenesis. Morphogenesis occurs in 4 cells of the *C. elegans* male tail tip as it retracts and changes shape during L4. Previous work demonstrated that DMD-3, a conserved, DM-domain transcription factor related to the human male fate-determining gene DMRT1, is necessary and sufficient for male tail tip retraction (Mason et al. 2008). DMD-3 is at the center of a genetic network with a bow-tie structure, regulated by pathways including Wnt signaling and heterochronic regulation, and in turn regulating genes involved in cellular processes such as vesicle trafficking and cytoskeletal rearrangement (Nelson et al 2011). However, while some of the regulatory pathways involved in specifying DMD-3 expression are known, the structure of the network downstream of DMD-3 is uncharacterized. We are using two methods to investigate downstream targets of DMD-3. To identify tail tip-specific targets, we are performing transcriptome profiling of tail tip tissue from worms that undergo tail tip morphogenesis (wild-type males and hermaphrodites ectopically expressing *dmd-3*), and worms that do not (wild-type hermaphrodites and *dmd-3* mutant males). To identify direct DMD-3 transcriptional targets, we are employing chromatin immunoprecipitation and next-generation sequencing (ChIP-Seq), using an epitope-tagged DMD-3::GFP::FLAG transgene. Preliminary microarray transcriptome analysis on tail tip-specific RNA identified 134 significantly differentially-expressed genes (alpha = 0.05) in the *dmd-3* mutant relative to wild-type. Combining the results of both approaches will yield direct targets of DMD-3 that act in the male tail tip. References: Mason et al. 2008. Development 135:2373-2382. Nelson et al. 2011. PLoS Genetics 7(3):e1002010.

1142A. Analysis of tissue-to-tissue signaling and its effects on cytoskeletal polarity during embryonic cell migrations. **Sailaja Mandalapu**, Martha Soto. Department of Pathology and Laboratory Medicine, UMDNJ-RWJMS , Piscataway, NJ.

Cell migration during development requires organization of the actin cytoskeleton. In order to understand how signals from outside cells can reorganize actin, we need to better understand signaling pathways and mechanisms that polarize actin. The WAVE/SCAR complex is a major regulator of branched actin in C.elegans during morphogenesis. We have identified the axonal guidance receptors UNC-40/DCC, SAX-3/Robo and VAB-1/Eph as upstream signals that activate the WAVE/SCAR complex via CED-10/Rac during C.elegans development. Now that signals that regulate actin through WAVE complex have been identified we are interested in identifying the tissue requirements of these signals. Studies in various model organisms have shown that exchange of

signals between different tissues contribute to healthy tissues and proper development. For instance signals from the epidermal cells are important for proper muscle arm termini formation in C. elegans larvae and during the induction of muscle patterning in Drosophila. However, little is known about the mechanisms or signaling pathways involved in these interactions. In C.elegans during the process of epidermal enclosure, the epidermal cells are directly in contact with muscle cells and neuroblasts. Both the muscle and the epidermal cells migrate as tissues and their movements occur during the same dynamic stage of embryonic epidermal enclosure. We hypothesized that the epidermis receives signals from the surrounding tissues to initiate cell migrations and finally enclose the embryo. Our preliminary tissue-specific rescue experiments suggested that expression of morphogenesis components in the muscle tissue could partially rescue the aberrant epidermal cell movements seen in mutants. This led us to ask if the muscle is playing a role in epidermal morphogenesis, and vice-versa. To address this question we made an embryonic muscle F-actin transgenic strain. We are imaging the actin dynamics during embryonic muscle cell migrations in wild type embryos and morphogenesis mutants. Our studies will provide a better understanding of how cells in neighboring tissues can influence the regulation of actin dynamics and cell migration in other tissues.

1143B. pix-1 differential expression along the anterio-posterior axis of the embryos controls early elongation in parallel to mel-11 and let-502 in Caenorhabditis elegans. **Emmanuel Martin**, Sharon Harel, Bernard Nkengfac, Karim Hamiche, Mathieu Neault, Sarah Jenna. Dpt Chimie, UQAM, Montreal, Quebec, Canada.

The C. elegans embryo elongation into the long, thin worm is driven by changes of hypodermis morphology during late embryogenesis. Early elongation involves the contraction of circumferential actin filaments (CAFs) by the phosphorylation of myosin-light chains by the Rho GTPase effectors LET-502, MRCK-1 and PAK-1 in hypodermal cells. These kinases are antagonized by the MLC phosphatase MEL-11, which is active in ventral and dorsal hypodermal cells and inactive in the lateral cells where most of the contraction occurs. The regulators of MLC phosphorylation are organized in two parallel pathways; the let-502/mel-11/mrck-1 and the pak-1 pathways. To date the biological significance of the functional redundancy of these two pathways is unknown. We identified the Rac- and Cdc-42-GEF, pix-1, as a new component of the pak-1 pathway controlling early elongation. We have shown that pix-1 functions in parallel to let-502 and mel-11during early elongation. We have also shown that PIX-1 is expressed in all hypodermal cells and is located in the cytoplasm and partially co-localize with adherens junctions and actin cytoskeleton during early phase of elongation. Importantly, we showed that, at that stage, PIX-1 expression is reduced in dorsal posterior hypodermal cells. We showed that this differential expression of PIX-1 along the anterio-posterior axes of the embryo is required to ensure its appropriate morphology and elongation. We propose a model in which contraction induced by the the mel-11/let-502 pathway may provide most of the contractile forces required to drive the early elongation while the pix-1/pak-1 pathway may fine tunes these contractile forces in order to achieve proper morphogenesis of the embryo along the anterior-posterior axis.

1144C. Three distinct Wnt signaling mechanisms act sequentially to position the migrating QR neuroblasts of C. elegans. **Remco A. Mentink**, Chung Y. Tang, Marco C. Betist, Hendrik C. Korswagen. Korswagen Group, Hubrecht Institute, Utrecht, Utrecht, Netherlands.

The Q neuroblast lineage of C. elegans provides a sensitive system to study the role of Wnt signaling in cell migration. During early larval development, the descendants of the left and right Q neuroblasts migrate in opposite directions along the anteroposterior axis. The anterior migration of the QR.d is mediated by a non-canonical Wnt signaling mechanism. Our aim is to gain further insight into this signaling pathway. In agreement with previous studies, we found that migration of the QR.d is controlled by Wnts EGL-20, CWN-1 and CWN-2. These ligands signal through the receptors CAM-1/Ror and MOM-5/Fz, which are expressed and cell-autonomously required within the Q cell lineage. Live cell imaging showed that signaling of EGL-20 through CAM-1 is required for persistent polarization of the migrating QR.a and QR.p cells, while signaling of CWN-1 through MOM-5 controls migration independently of cell polarity. Interestingly, *cam-1* shares important similarities with the QR.d migration phenotype of *mig-2/Rho* and *mom-5* with *ina-1/integrin* (Ou et al. 2009). Based on these results, we speculate that CAM-1 and MOM-5 act in parallel pathways that control Rho GTPases and integrin mediated adhesion, respectively. We found that termination of QR.d migration requires activation of canonical Wnt/b-catenin signaling. Thus, in bar-1/b-catenin mutants or in transgenic animals expressing dominant negative POP-1/Tcf in the Q cell lineage, the QR.d fail to stop and migrate to more anteriorly located positions. Single molecule mRNA FISH (smFISH) analysis showed that the switch to canonical Wnt signaling is correlated with the specific upregulation of the Fz receptor MIG-1. During the final leg of the migration, the QR descendants QR.paa and QR.pap migrate to specific dorsoventral positions. We found that this part of the migration is dependent on the PCP components vang-1 and prkl-1 and that it coincides with upregulation of prkl-1 in QR.pa. Taken together, we conclude that QR.d migration is mediated by three sequentially acting Wnt pathways that each control a specific aspect of the migration process.

1145A. "Ultrastructure analysis of the sarcomeres in worms that lack Z-line formins". **Lei Mi-Mi**, David Pruyne. Cell and Developmental Biology, SUNY Upstate Medical Univ, Syracuse, NY.

Even with extensive studies that revolve around actin cytoskeleton, the detailed mechanism as to how actin filaments are recruited into sarcomeres, the smallest units of striated muscle contractile lattice, is yet to be elucidated. The barbed ends of actin filaments anchor at the Z-line structures (dense bodies in *C. elegans*) that define the sarcomere ends. Net actin polymerization favorably occurs at the barbed end that is known to have unique association with formins, one of the highly conserved and most widespread families of actin nucleating factors. We have shown in our previous study that two nematode formins, CYK-1 and FHOD-1 are Z-line-associated proteins that work together to organize and maintain sarcomeric organization in *C. elegans* striated muscle — the first evidence in an intact organism. In this study, we have carried out the ultrastructure analysis on sarcomeric structures of worms lacking CYK-1 and/or FHOD-1. Not only do the current electron microscopy results complement our previous conclusions, they also suggest that CYK-1 and/or FHOD-1 may contribute in achieving normal Z-line structures in *C. elegans* sarcomeric organization.

1146B. dpy-19 and mig-21 control the persistent directionality of migrating Q neuroblasts in Caenorhabditis elegans. **Teije C. Middelkoop**[1], Thijs Koorman[2], Mike Boxem[2], Hendrik C. Korswagen[1]. 1) Hubrecht Institute KNAW and Univ Medical Center, Utrecht, Netherlands; 2) Developmental Biology, Utrecht Univ, The Netherlands.

During C. elegans development several cells migrate along the anteroposterior axis. We use the migration of Q neuroblasts as a model to study cell

migration in vivo. At the time of hatching, two Q neuroblasts occupy equivalent left/right positions on the lateral sides of the animal. During larval development both Q neuroblasts polarize and migrate in opposite directions where the left Q neuroblast (QL) migrates posteriorly and the right Q neuroblast (QR) migrates anteriorly. After an initial short-range migration both Q neuroblasts undergo an identical pattern of division, generating Q descendants (Q.d) that migrate further along the anteroposterior axis. While Q.d migration is known to be controlled by Wnt signaling, the initial polarization process is Wnt signaling independent. Previously, two transmembrane proteins, MIG-21 and the conserved DPY-19 protein, were shown to control initial Q cell migration. Using high resolution time lapse imaging we have found that both genes are necessary for the formation of a protruding front with persistent directionality. In the absence of either dpy-19 or mig-21 the main protruding front changes its direction multiple times during migration resulting in a random migration direction. Upon loss of both genes we found that the direction of the main protruding front is persistent but is pointing towards the dorsal direction in both QL and QR. These results indicate that dpy-19 and mig-21 are both necessary for the persistent directionality of initial Q cell migration along the anteroposterior axis. In order to gain a functional understanding of this process we are now examining putative interactions of these genes with components involved in cytoskeletal remodeling. Furthermore, we are performing a genome-wide yeast-two-hybrid interaction screen in order to identify DPY-19 interactors.

1147C. The role of LIN-3 during morphogenesis of the dorsal lumen in the vulva. **Louisa Mueller**, Matthias Morf, Alex Hajnal. IMLS, Zurich, Switzerland.

The hermaphrodite vulva is an excellent organ to identify and study the molecular mechanisms controlling tissue morphogenesis during development. Vulval development is initiated by the anchor cell (AC) in the somatic gonad, which secretes LIN-3 EGF and induces the vulval cell fates in three of the six adjacent vulval precursor cells. After vulval induction, the AC breaches two basal laminae and invades in-between the innermost 1°-fated VPC descendants (the VulF cells). AC invasion is important for proper morphogenesis of the dorsal lumen formed by the VulF toroids [1] and to establish the uterine-vulval connection. During vulval morphogenesis, LIN-3 is secreted from the VulF cells to specify the uv1 fate [2]. Here, we investigated another function of LIN-3 produced by VulF during dorsal lumen morphogenesis. Vulva-specific lin-3 RNAi using an rde-1(lf) mutant expressing rde-1(wt) in the Pn.p cells prevented the expansion of the dorsal lumen by the AC. A similar defect in dorsal lumen morphogenesis was observed in egl-38(lf) mutants that do not express LIN-3 in VulF cells [1,3]. Moreover, egl-38(lf) mutants displayed defects in AC polarization. Based on these and further results, we propose that LIN-3 expressed by the VulF cells controls dorsal lumen morphogenesis by polarizing the AC and thus enabling it to migrate in between the VulF cells and expand the dorsal lumen. [1] Estes, K. A. and Hanna-Rose, W. (2009). The anchor cell initiates dorsal lumen formation during C. elegans vulval tubulogenesis. Dev Biol 328, 297-304 [2] Chang, C., Newman, A. P. and Sternberg, P. W. (1999). Reciprocal EGF signaling back to the uterus from the induced C. elegans vulva coordinates morphogenesis of epithelia. Curr Biol 9, 237-46. [3] Rajakumar, V. and Chamberlin, H. M. (2007). The Pax2/5/8 gene egl-38 coordinates organogenesis of the C. elegans egg-laying system. Dev Biol 301, 240-53.

1148A. Analysis of novel pathways for nuclear migration in C. elegans. **Shaun P. Murphy**, Yu-Tai Chang, Daniel A. Starr. Univ of California. Dept. of Molecular and Cellular Biology, Davis, CA.

Moving the nucleus to an intracellular location facilitates many cell and developmental processes. To investigate the behavior of migrating nuclei *in-vivo*, we utilize live-cell imaging to analyze the behavior of larval P-cell nuclei in. During the mid-L1 stage, P-cell nuclei migrate from the lateral side into the ventral cord through a cytoplasm that is only 150 nm between the cuticle and body wall muscles. Transgenic lines of animals expressing fluorescent nuclear, cytoskeletal, and cell boundary markers driven by a P-cell-specific promoter reveal that cytoskeletal actin, nucleation sites of microtubules, intermediate filaments, and cellular shape are dynamically rearranged within P-cells during nuclear migration. Null mutations in *unc-83* or *unc-84*, which encode KASH and SUN proteins, inhibit nuclear migration by disrupting interactions between the nucleoskeleton and the cytoskeleton at 25°C. However, at 15°C, P-cell nuclear migration in KASH/SUN null animals occurs normally. We hypothesize that additional pathway(s) functions synthetically to the KASH/SUN pathway to move P-cell nuclei at 15°C. Supporting our hypothesis, genetic screens for enhancers of the nuclear migration defect of *unc-83/84 (emu*) mutants have been carried out. Whole-genome sequencing and RNAi approaches have identified *toca-1* and *fln-2* as *emu* genes; here we focus on the divergent filamin *fln-2*. Filamins are large proteins involved in actin regulation. Significantly, no role for filamins has previously been shown in nuclear migration. Both *unc-84(n369); fln-2(RNAi)* and *unc-84(n369); fln-2(tm4687)* animals have severe defects in P-cell nuclear migration. Additionally, we can rescue the P-cell nuclear migration defect of *unc-84(n369); fln-2(tm4687)* animals using a *fln-2::gfp* extrachromosomal array. The organization of actin filaments in P-cells appears normal prior to nuclear migration in *unc-84(n369); fln-2(tm4687)* worms, however, the actin cytoskeleton is disrupted at the time of nuclear migration. We hypothesize that FLN-2 is involved in actin regulation during nuclear migration in *C. elegans* larval P-cells.

1149B. Mitochondria-type GPAT is required for mitochondrial fusion. **Y. Ohba**[1], T. Inoue[1], T. Sakuragi[1], N. H.Tomioka[1], A. Inoue[2], N. Ishihara[3], J. Aoki[2], E. Kage-Nakadai[4], S. Mitani[4], H. Arai[1]. 1) Graduate School of Pharmaceutical Sciences, Univ of Tokyo, Japan; 2) Graduate School of Pharmaceutical Sciences, Tohoku Univ, Japan; 3) Institute of Life Science, Kurume Univ, Japan; 4) Department of Physiology, Tokyo Women's Medical Univ School of Medicine, Japan.

Lysophosphatidic acid (LPA) is a key intermediate in the *de novo* synthesis of glycerolipids such as phospholipids and triacylglycerol. In the first step of this pathway, glycerol-3-phosphate (G3P) is converted to LPA by G3P acyltransferase (GPAT). LPA is further acylated by LPA aycltransferse (LPAAT) to form phosphatidic acid (PA), a precursor of triacylglycerol and a variety of phospholipid classes. To date, four mammalian GPATs have been cloned; GPAT1 and GPAT2 are located at the mitochondria (mitochondrial GPATs), and GPAT3 and GPAT4 are located at the endoplasmic reticulum (ER) (microsomal GPATs). *C. elegans* genome contains one mitochondrial GPAT (*acl-6*) and two microsomal GPATs (*acl-4, acl-5*), and these *C. elegans* GPATs show significant overall homology to mammalian mitochondrial and microsomal GPATs, respectively.Although most of *de novo* synthetic enzymes of glycerolipids are known to localize at the endoplasmic reticulum (ER), GPATs exist in the mitochondria as well as ER, the biological significance of which has not been elucidated. In this study, to clarify the functional differences between ER-GPAT and mitochondrial (Mt)-GPAT, we generated all the deletion mutants of *C.elegans* GPATs and demonstrate that LPA produced by Mt-GPAT is essential for mitochondrial fusion. In Mt-GPAT-depleted cells, mitochondrial fusion was strongly inhibited, which led to fragmented mitochondria. This defect was rescued by inhibition of mitochondrial fission protein, DRP-1 and by overexpression of mitochondrial fusion protein FZO-1. Furthermore, our genetic analysis revealed that the mitochondrial defect of Mt-GPAT-depleted worms was rescued by

inhibition of LPAAT or injection of LPA, both of which led to accumulation of LPA in the cells. These results indicate that LPA produced by Mt-GPAT not only functions as a precursor for glycerolipids synthesis, but also plays an important role in regulating mitochondrial dynamics as a pro-fusogenic lipid.

1150C. Genomic analysis of the duct and pore cells reveals novel effectors and regulators of morphogenesis. **Gregory Osborn**, Travis Walton, Meera Sundaram, John Murray. Univ of Pennsylvania, Philadelphia, PA.

Organogenesis involves complex cellular processes such as migration, morphological changes and differentiation, which are frequently orchestrated by the integration of regulatory networks. To understand how these cellular behaviors are coordinated at single cell resolution, we are studying the C. elegans excretory system, which contains three tubular cells: the duct, pore and canal cell. During embryogenesis, the excretory duct and pore cell first exhibit mesenchymal characteristics as they migrate from disparate locations to meet the canal cell at the ventral midline, where they change shape, differentiate into epithelia, and convert into a contiguous three cell tubular organ. To identify molecular factors involved in these complex processes, we are characterizing the transcriptomes of these cells during development. To isolate the duct and pore, we have taken a fluorescence-activated cell sorting (FACS) strategy, which allows for isolation of cells expressing a fluorescent reporter of interest. Specific early markers for the duct and pore during organogenesis are not known; thus we have taken an intersectional approach, sorting for cells expressing both ceh-6::GFP and hlh-16p::mCherry in C. elegans embryos. The expression of these reporters overlaps exclusively in the duct and pore, as well as their sisters, the DB1/3 motor neurons. RNA-seq of these cells shows an enrichment of factors known to be important in the formation of the excretory system, such as EGF signaling components (let-23, lin-1) and genes involved in proper tube formation and function (aff-1, let-4, lpr-1). Interestingly, novel factors were also enriched, including many HOX transcription factors and proteins predicted to be secreted or membrane-localized. This dataset could be augmented in the future by using additional available markers to separate the duct/pore from the DB1/3 motor neurons by FACS and determining the expression signatures unique and shared between these cells. This work will provide a framework to identify novel candidates to functionally test as regulators and effectors of the complex cellular behaviors of the developing excretory system.

1151A. What is tubulin glutamylation good for? **Nina Peel**[1], Zach Barth[1], Ruchi Shah[1], Jessica Lee[1], Kevin O'Connell[2]. 1) Department of Biology, TCNJ, Ewing, NJ; 2) LBG, NIDDK, NIH, Bethesda, MD.

Microtubules are dynamic polymers that play essential structural roles in cell division, vesicle trafficking, and cilia function. Microtubules grow and shrink by the addition and loss of tubulin monomers and this dynamic behaviour is central to their function. Much is known about the regulation of microtubule dynamics by interacting proteins, but the post-translational modification of tubulin as a regulatory mechanism remains relatively unexplored. Tubulin glutamylation is the covalent attachment of glutamic acid to tubulin in the polymerized microtubule. This modification is enriched on long-lived microtubules, and it is proposed that tubulin glutamylation contributes to centriole stability, cilia motility and axon function. Studying the function of glutamylation is, however, complicated by the presence of a large family of enzymes that participate in glutamylation, and because the glutamylated residue in tubulin can be alternately modified. *C. elegans* has only 5 glutamylating enzymes (TTLLs) and no competing modifications and therefore is the ideal system in which to study the function of tubulin glutamylation. We are using two complimentary approaches to explore the function of tubulin glutamylation. First, we have made worms that express non-glutamylatable tubulin and are testing whether this can substitute for the wildtype tubulin. Second, we have obtained deletion mutantions in all five TTLL enzymes, non of which display overt phenotypes. To test for redundancy we are currently combining the mutations. By taking this multipronged genetic approach we seek to definitively test the role of tubulin glutamylation in *C. elegans*.

1152B. The in vivo Dynamics of IFT Motors and Axoneme Microtubules in Cilia Signaling. **Jay Pieczynski**[1], Patrick Hu[1,2,3], Kristen Verhey[1]. 1) Dept. of Cell and Developmental Biology, Univ of Michigan, Ann Arbor, MI; 2) Life Sciences Institute, Ann Arbor, MI; 3) Dept. of Internal Medicine, Univ of Michigan, Ann Arbor, MI.

Cilia are microtubule-based organelles protruding from the surface of most mammalian cells. The cilium acts as a cellular signaling center and defects in cilia morphology or cilia signaling lead to developmental defects (ciliopathies), cystic and fibrotic diseases, and certain types of cancers. An essential process in building/maintaining cilia structure and delivery of signaling components is the transport of proteins in an anterograde and retrograde manner along the cilium microtubule core (axoneme) by motor-mediated intraflagellar transport (IFT). Anterograde IFT kinesins move cargoes from the base of the cilium towards the tip, while retrograde IFT dynein moves cargo back towards the cilium base. In mammals, loss of either anterograde or retrograde IFT usually results in severe defects or lethality, limiting analysis to in vitro under/overexpression systems. However, in C. elegans ciliated sensory neurons (CSNs), defects in evolutionarily conserved genes necessary for cilia formation/signaling have little to no effect on viability and development allowing for analysis in an intact organism. Since worm cilia protrude through the cuticle to sense the external environment, we can therefore utilize C. elegans cilia to isolate and elucidate the roles of microtubules and microtubule motor-based IFT in ciliary signaling events in response to external stimuli in vivo. To model IFT motor protein dynamics, we have utilized Mos1-transposase mediated single copy insertion (MosSCI) to generate GFP-tagged single copy knock-in animals to mimic expression of ciliary proteins at nearly endogenous levels and specifically expressed these proteins in ciliated sensory neurons. Using the C. elegans homologue of the retrograde IFT dynein intermediate light chain, xbx-1, fused to eGFP, we aim to elucidate how cilia utilize retrograde IFT to transmit external signals into physiological output and cellular changes. In addition, we also are beginning to examine the relationship between axonemal microtubule morphology and motor function and how this relates to cilia formation and signaling in vivo.

1153C. The Secretory Protein Calcium ATPase PMR-1 is essential for cell migration during gastrulation. **V. Praitis**[1], J. Simske[2], S. Kniss[3], R. Mandt[1], L. Imlay[1], C. Feddersen[1], M.B. Miller[1], J. Mushi[1], W. Liszewski[1], R. Weinstein[1], A. Chakravorty[1], D-G Ha[1], A. Schacht Farrell[1], A. Sullivan-Wilson[1], T. Stock[1]. 1) Bio, Grinnell College, Grinnell, IA; 2) Rammelkamp Ctr, Case Western, Cleveland, OH; 3) MGCB, U. Chicago, Chicago, IL.

Lesions in SPCA1, the human homolog of PMR-1, cause Hailey-Hailey disease (MIM#169600), a skin blistering disorder associated with loss of cell adhesion. Previous studies in cell culture and in *C. elegans* larva and adults indicate PMR-1 is important for calcium signaling, stress response, thermotolerance, pathogen resistance and metal homeostasis, but its role in embryogenesis has not been well characterized. Our laboratory has found that PMR-1 is essential for cell migration during embryonic development. *pmr-1(lof)* alleles cause embryonic lethality at 25C, with increased viability at

lower temperatures. While terminal phenotypes include enclosure and morphogenesis defects similar to the cell adhesion phenotypes of Hailey-Hailey disease, temperature-shift experiments indicate *pmr-1* is required earlier, during gastrulation. To characterize the primary defects that lead to embryonic lethality, we examined cell fate, lineage, and positioning in *pmr-1(lof)* lines. These experiments show that cell fate, lineage and division patterns are normal in *pmr-1(lof)* embryos. However, C-derived, anterior, and ventral blastomeres have migration defects during gastrulation. To better understand the role of PMR-1 in cell migration, we performed gene interaction experiments. We hypothesized that reducing activity of ITR-1/IP3R and UNC-68/RyR, channels that release calcium from secretory stores, would suppress phenotypes caused by reduced activity of PMR-1, which transports calcium back into secretory stores. Consistent with this hypothesis, we found that depletion of *unc-68* partially suppresses *pmr-1(lof)* phenotypes. In contrast, an *itr-1(lof)* allele enhances *pmr-1(lof)* lethality, while an *itr-1(gof)* allele suppresses, opposite our expectations. This analysis identifies a new role for UNC-68 and indicates that ITR-1 may have a non-redundant or opposing role in regulating calcium levels important for cell migration during *C. elegans* development.

1154A. LAWD-1, a potential scaffold protein with a WD40 domain involved in epithelial morphogenesis. **Mengmeng Qiao**[1], Jonathan Hodgkin[2], Patricia Kuwabara[1]. 1) School of Biochemistry, Univ of Bristol, Bristol, UK; 2) Biochemistry Dept., Univ of Oxford, Oxford, UK.

Epithelial morphogenesis is a dynamic process whereby the interaction of polarity and adhesion complexes combined with cell rearrangements establishes cellular architecture and contributes to the development of organs and body plan. Vab (Variable ABnormal) mutants, such as *vab-1* and *vab-2*[(1,2)], which encode an ephrin receptor and ligand, respectively, have provided important insights into *C. elegans* morphogenesis. The *lawd-1(cr7)* (lumen associated with WD40 domain) mutant (previously named *vab-17*) also displays a highly penetrant viable Vab phenotype; however, lethality occurs when *cr7* is placed in trans to a deficiency. Although *lawd-1(cr7)* mutants are viable, they also show synthetic lethality when combined with a mutation in the *sma-1* gene, which encodes the cytoskeletal b-H spectrin protein. Synthetic lethality was also detected in *lawd-1*; *vab-18* mutants; we have recently identified the gene responsible for the *vab-18* phenotype by whole genome sequencing. We have also characterised a *lawd-1* deletion mutant *tm4605* (kind gift of Shohei Mitani) that displays a Vab phenotype. Germline rescue has established that the *lawd-1* gene encodes a protein with multiple isoforms. The largest isoforms carry an N-terminal WD40 domain that we hypothesize functions as a scaffold for protein-protein interactions based, in part, on the identification of multiple lethal synthetic interactions involving *lawd-1*. We have integrated *lawd-1* fosmids recombineered with mCherry or GFP to examine the intracellular localization of LAWD-1. We find that LAWD-1 is expressed throughout development in tissues such as the pharynx, intestine, vulva and spermatheca. Co-localization studies using the *hmp-1* and *dlg-1* epithelial markers reveal that LAWD-1 is apically enriched in epithelial cells. Using Western blot analysis, we have been able to detect multiple GFP-tagged LAWD-1 isoforms. We are presently attempting to identify LAWD-1 interacting proteins using a proteomic approach. **Refs.** [1]Chin-Sang ID et al. (1999) Cell. 99: 781. [2]George SE et al. (1998) Cell. 92: 633.

1155B. Structure-function analysis of the cell-fusion protein EFF-1. **Hadas Raveh-Barak**[1], C. Valansi[1], O. Avinoam[1,2], T. Krey[3], J. Perez-Vargas[3], FA. Rey[3], B. Podbilewicz[1]. 1) Department of Biology, Technion, Haifa, Israel; 2) The European Molecular Biology Laboratory, Heidelberg, Germany; 3) Structural Virology Unit, Pasteur Institute, Paris, France.

Cell membrane fusion is an important process in the life course of many organisms. It is thought, and in some cases demonstrated, that specialized proteins mediate the cell fusion process. The membrane protein EFF-1 mediates most of the cell fusions in C. elegans, which are crucial to embryonic development. The crystal structure of the extracellular region of EFF-1 was recently solved; it shows a remarkable homology to the fold of class II viral membrane-fusion proteins. These viral proteins oligomerize into trimers, go through conformational changes upon acidification, exposing a fusion loop which is inserted into the target endosomal membrane. The trimers fold back, into a hairpin conformation, which clamps the two membranes and leads to their fusion. The viral fusion proteins act in a unidirectional way from the viral membrane. EFF-1, on the other hand, is required in both fusing membranes and functions in a bidirectional way. While EFF-1 structure and function are conserved from C. elegans to viruses, the mechanism of eukaryotic membrane fusion mediated by EFF-1 is not identical to that mediated by viral fusion proteins. However, it does share a few characteristics, such as oligomerization and a hemifusion intermediate. We aim to analyze EFF-1-mediated fusion mechanism and the contribution of different residues and domains to its function. We are currently constructing different EFF-1 mutants that harbor mutated residues or lack domains, and comparing their fusion activity to that of wild-type EFF-1. We use mammalian cells and virus-like particles (VLPs) as two heterologous systems, in which we express EFF-1 wild-type and mutants. These two systems are used for cell-cell and VLP-cell fusion assays. We found that one of the mutations that reduces EFF-1 trimerization reduces also EFF-1 fusion activity when expressed in mammalian cells. Also, we showed that addition of monomeric soluble extracellular region to cells expressing EFF-1 inhibit cell fusion, while soluble trimers stimulate it. These results suggest that EFF-1 trimerization is essential for cell fusion.

1156C. Formins Play a Role in the C. elegans Embryonic Elongation. **Osama M. Refai**[1], Christopher A. Vanneste[1], David Pruyne[2], Paul E. Mains[1]. 1) Dept Biochemistry and Molecular Biology, Univ of Calgary, Calgary, Alberta, Canada; 2) Department of Cell and Developmental Biology, State Univ of New York Upstate Medical Univ, Syracuse, NY 13210, USA.

During the C. elegans embryonic development, the embryo undergoes dramatic changes to elongate from a spheroid into a long, thin worm. The epidermis of the embryo provides the driving force for elongation. The epidermal cytoskeleton is a highly organized structure of actin microfilaments, microtubules and intermediate filaments. The C. elegans Rho-binding kinase (LET-502) and myosin phosphatase (MEL-11) are essential regulators for the actomyosin-mediated embryonic elongation and act in parallel with FEM-2/PP2c phosphatase and PAK-1/p21 activated kinase (GALLY et al. 2009; PIEKNY et al. 2000). Actin nucleation is the rate limiting step of actin filament polymerization. We previously identified fhod-1 (formin homology domain), which is highly similar to the human actin nucleators FHOD1 and FHOD3. Our genetic analysis revealed that fhod-1 acts in the mel-11/let-502 pathway, in parallel to fem-2/pak-1. fhod-1 mutants exhibit less than 25% elongation defects, suggesting redundancy with other formins (or other genes) for actin nucleation. Potential candidates include six other C. elegans formins: daam-1, inft-1, inft-2, frl-1, fozi-1 and cyk-1. Only two of these genes, inft-1and cyk-1, appear to have a role in elongation based on let-502 RNAi in mutant backgrounds. Muscle is required for elongation past the 2 fold stage, and perhaps is required redundantly with let-502/mel-11 at earlier stages. However, we found that the ilk-1 Pat (paralyzed at two fold) mutant did not suppress mel-11.

1157A. Towards the Complete Embryonic Cell Lineage. **Anthony Santella**[1], Zhuo Du[1], Zidong Yu[1], Yicong Wu[2], Hari Shroff[2], Zhirong Bao[1]. 1) Developmental Biology, Sloan Kettering Institute, NY, NY; 2) National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD.

The invariant lineage has been a cornerstone of C. elegans biology. John Sulston's initial lineage 30 years ago used multiple embryos to assemble the invariant pattern. Complete continuous lineaging has never been performed for a single animal. While image analysis software has facilitated lineage creation during early embryogenesis, embryo movement at later stages has hampered the analysis of later development. We have made progress towards lineage tracing during this developmental period through a combination of innovative computational and imaging methods. Our efforts on image analysis are focused on the challenge of reliably following small and crowded nuclei over long periods of time in under sampled images. Our nuclear detection method uses per slice segmentation and a learned shape model to robustly detect and segment nuclei in crowded configurations. The detection results are merged into a cell lineage using multiple linking steps, which select from a set of possible causes and actions, including cell movements, divisions, or detection errors. This is based on probabilistic models of nuclear appearance and local spatial configuration. These computational improvements are bolstered by a qualitative improvement in image quality through the dual-view inverted Selective Plane Illumination Microscope (diSPIM), which captures isotropically sampled volumes at speeds that largely eliminate motion artifacts even through the final stage of embryogenesis. We anticipate that our combined effort will allow not just lineage tracing, but that the detailed dynamics of development (including cell positions and expression patterns) can be followed at high spatiotemporal resolution to build a quantitative model of development. A long term application is the production of WormGUIDES, a 4D atlas tracking both cell positions and neuronal outgrowth.

1158B. A Complex Issue: Understanding vMSP Receptor Heteromeric Complexing Behavior. **Jessica L. Schultz**[1], Sung Min Han[1,2], Se-Jin Lee[1], Michael Miller[1]. 1) CDIB, Univ of Alabama at Birmingham, Birmingham, AL; 2) CNNR program, Dept. of Genetics, Yale Univ, New Haven, CT.

Amyotrophic Lateral Sclerosis (ALS) is a lethal neurodegenerative disease with an unknown pathogenesis and limited therapeutic treatments. A major limitation hindering the development of therapeutic treatments is a lack of understanding of the disease's molecular pathways. In humans, a P56S point mutation in the VAPB/ALS8 MSP domain is associated with ALS and late-onset spinal muscular atrophy (SMA) (Funke et al., 2010; Millecamps et al., 2010; Nishimura et al., 2004). The N-terminal MSP domain is cleaved from the C-terminus of the VAPB protein, and is secreted in a cell-type specific manner (Tsuda et al., 2008). However, the P56S mutation inhibits secretion of the MSP domain. Genetic and biochemical evidence support the hypothesis that the MSP domain interacts with the VAB-1 Eph receptor, ROBO/SAX-3 receptor, and CLR-1 Lar-like protein tyrosine phosphatase receptor, which are collectively called growth cone guidance receptors (Miller et al, 2001; Miller et al., 2003; Tsuda et al., 2008; Han et al., 2012). In *C. elegans*, secreted vMSP acts on CLR-1 and ROBO/SAX-3 receptors expressed in striated muscle, promoting Arp2/3-dependent actin remodeling. This remodeling is critical for proper placement of mitochondria to actin-rich myofilament I-bands (Han et al., 2012). We hypothesize that the vMSP receptors form heteromeric complexes to promote signaling critical for actin remodeling and correct mitochondria placement. To begin testing this hypothesis, I am expressing combinations of VAB-1, SAX-3, and CLR-1 in cultured cells and investigating putative complex formation via co-immunoprecipitation. My preliminary data suggest that SAX-3 complexes with both VAB-1 and CLR-1. Data will also be presented on the role of *C. elegans* VAPB/VPR-1 in regulating mitochondria in motor neurons. The results could provide insight into growth cone guidance receptor interactions and pathways involved in ALS.

1159C. DBL-1 TGF-b localization and the physiological basis of body size regulation in *C. elegans*. **Robbie D. Schultz**[1], E. Ann Ellis[2], Tina L. Gumienny[1]. 1) Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX; 2) Microscopy & Imaging Center, Texas A&M Univ, College Station, TX.

Transforming Growth Factor-b (TGF-b) is a family of secreted cell signaling ligands. DBL-1, a *C. elegans* TGF-b superfamily member, is secreted from nervous tissue, but must be trafficked to the epidermis where it binds to receptors. Alteration of the DBL-1 pathway generates distinct, dose-dependent phenotypes, where animals with increased or decreased signaling are long or small respectively. However, how body size is altered in *dbl-1* pathway variants is still poorly understood. The goals of this project are to determine subcellular localization of secreted DBL-1, analyze its regulation between secreting and receiving cells, and elucidate the molecular and physiological underpinnings of body length regulation by DBL-1 pathway signaling.

We found that that secreted GFP-tagged DBL-1 localizes in a discrete punctate pattern along the dorsal and ventral nerve cords. Using a whole-mount microwave-based immunofluorescence method we developed, we found that mouse BMP4, a DBL-1 homolog, rescues body size defects in *C. elegans* caused by loss of DBL-1 pathway signaling, but also co-localizes with GFP-tagged DBL-1. Immunocytochemical studies also show that these punctae co-localize with cell-cell contact sites and caveolar bodies in the ventral nerve cord. These studies are the first to decipher the subcellular localization of DBL-1, expanding the knowledge of TGF-b trafficking in *C. elegans*. Microarray analyses have revealed DBL-1 signaling regulates transcription of cuticular components, which may together affect body size. Our genetic and pharmacological studies reveal that DBL-1 signaling affects cuticular permeability, altering sensitivity to soluble anesthetics based on the dose of DBL-1. Using transmission electron microscopy, we found that ultrastructural composition of the cuticle is affected in a dose-dependent manner in *dbl-1* variants.

These results show that DBL-1-mediated body size differences directly correlate with changes in cuticular organization. These studies expand our understanding of the molecular mechanisms involved in regulation of body size by the DBL-1 pathway.

1160A. An In Vivo Analysis of Critical Functional Domains of a-catenin in *C. elegans*. **Xiangqiang Shao**[1], Jeffrey Simske[3], Anjon Audhya[2], Jeff Hardin[1]. 1) Program in Genetics, Department of Zoology, Univ of Wisconsin-Madison; 2) Department of Biomolecular Chemistry, Univ of Wisconsin-Madison; 3) Rammelkamp Center for Research, Cleveland, Ohio.

Stable intercellular adhesions are critical for normal embryonic development in metazoans, during which tissue integrity is maintained as cells undergo morphogenetic movements. The adherens junction, a key cell-cell adhesion structure, contains a highly conserved cadherin-catenin complex (CCC). a-catenin is an important regulator that connects the CCC to F-actin at adherens junctions. *C. elegans* provides a good model to study the function of a-catenin in vivo, since *hmp-1* is the sole a-catenin homolog. Given the potential role of a-catenin as a physical linker between CCC and the actin cytoskeleton, we hypothesized that recruiting a-catenin to junctions irrespective of its normal binding partners is sufficient for its function. To test this, we fused full length HMP-1 with the junctional transmembrane protein VAB-9 (VAB-9::HMP-1::GFP). This fusion protein localizes correctly to junctions in

embryos, and is able to rescue the hmp-1 strong loss-of-function mutant allele *zu242*. HMP-1 is normally recruited to junctions via HMP-2/b-catenin. The presumed HMP-2/b-catenin binding site of HMP-1 is located in the N-terminus of HMP-1. To test if this binding site has additional functional roles, we fused a truncated form of HMP-1 lacking the HMP-2/b-catenin binding site with VAB-9 (VAB-9::HMP-1(315-927)::GFP). This fusion protein also localizes correctly to junctions; however, it only partially rescues *zu242* embryos. Our results suggest that *hmp-1* is a stable physical linker between the cadherin-catenin complex and F-actin, while its N-terminal interaction with *hmp-2* may indeed be required for robust linkage. The N terminus of HMP-1 may do so by recruiting additional binding partners that strengthen the connection to F-actin at the junction. In order to find potential binding partners for *hmp-1*, we performed co-immunoprecipitation followed by mass spectrometry and are currently testing the candidates to determine functional roles for these proteins.

1161B. LET-653, a secreted ZP-domain and mucin-related protein, functions in the excretory duct/pore, and not the excretory canal cell. Corey Poggioli, Kevin Bickard, **Meera V. Sundaram**. Dept of Genetics, Univ of Pennsylvania Perelman School of Medicine, Philadelphia, PA.

Polarized epithelia secrete a specialized extracellular matrix (ECM) that strongly influences their cell morphology and tissue integrity. For example, the basal ECM includes collagens and laminins that interact with cell surface receptors such as integrins to influence cytoskeletal organization and junction strength. The apical ECM frequently contains glycoproteins such as mucins or zona pellucida (ZP)-domain proteins, as well as (in ecdysozoa) cuticle components such as collagen or chitin. There is increasing evidence that the apical ECM also can modulate cytoskeletal organization and junction strength, although in general this role is not well understood.

Mutants lacking the C. elegans ZP-domain and mucin-like protein LET-653 arrest as “rod-like” L1 larvae with severe defects in morphology of the excretory canal cell (Buechner et al, 1999; Jones and Baillie, 1995), leading to a hypothesis that LET-653 might be part of the canal cell apical ECM. Here we show instead that *let-653* functions in two other tubular components of the excretory system, the duct and pore cells, which connect the excretory canal cell to the outside environment to allow for fluid waste excretion. *let-653* reporters are expressed in the excretory duct and other cuticle-lined epithelia, and *let-653* transgene expression in the duct or pore, but not in the canal cell or body muscle, is sufficient to rescue *let-653* lethality. The most prominent early phenotype of *let-653* mutants is a swelling of the duct lumen. We propose that LET-653 is a component of the duct/pore apical ECM, and that canal cell defects in *let-653* mutants are a secondary consequence of luminal defects in the duct/pore.

1162C. SPV-1, a RhoGAP and F-BAR domain protein, regulates spermatheca contractility. **Pei Yi Tan**[1], Ronen Zaidel-Bar[1,2]. 1) Mechanobiology Institute, Singapore; 2) Department of Bioengineering, National Univ of Singapore, Singapore.

C. elegans ovulation involves the passage of an oocyte through an epithelial “pouch” termed the spermatheca, where the oocyte is fertilized and subsequently propelled into the uterus. Actomyosin contractility is known to provide the force to squeeze the spermatheca, but how it is regulated in a cyclical manner is not known. We identified SPV-1, an F-BAR and RhoGAP protein, as a negative regulator of spermatheca contractility. Loss of SPV-1 resulted in over-constriction of the spermatheca, leading to premature embryo exit. Conversely, overexpression of SPV-1 transgene extended oocyte retention in the spermatheca. Expression of deletion constructs in the mutant background point to an essential role for the RhoGAP domain in regulation of contractility and for the F-BAR domain in localization of SPV-1. Full length SPV-1::GFP fusion protein revealed exclusive spermatheca expression, with transient localization to the apical cell membrane. We observed detachment of SPV-1::GFP from the membrane upon spermatheca cell stretching by an incoming oocyte, and speculated this to be due to the membrane curvature-sensing ability of its F-BAR domain. We tested this idea by expressing the F-BAR domain of SPV-1 fused to GFP in HeLa cells. In adherent cells the F-BAR domain was cytoplasmic. Upon trypsinization, when cells round up and form membrane folds, we observed translocation of the F-BAR domain to the plasma membrane. Taken together, our findings support a model in which SPV-1 localization to the apical membrane of the spermatheca is mediated by its F-BAR domain in a curvature-dependent manner. When localized to the membrane it inhibits contractility by inactivating RHO-1 through its RhoGAP domain. Oocyte entry stretches the spermatheca, straightens out the curvature and thus leads to detachment of SPV-1. We hypothesize that cytoplasmic SPV-1 looses its RhoGAP activity, possibly due to autoinhibition, permitting the level of active RHO-1 to rise beyond the threshold needed to induce contraction. In summary, we have identified an important regulator of spermatheca function and suggest a mechanism for feedback between membrane tension and actomyosin contractility that may be operating in other contractile systems.

1163A. Regulation of the cadherin-catenin complex by the ULP-2 SUMO protease. **Assaf Tsur**, Ulrike Bening-Abu-Shach, Orit Adir, Limor Broday. Cellular and developmental biology, Tel-Aviv Univ, Sackler faculty of medicine, Tel-aviv, Israel.

In multicellular organisms, adherens junctions (AJs) provide robust cell-cell adhesion, maintaining tissue strength and architecture. During morphogenesis AJs must also retain high plasticity to facilitate remodeling of cell-cell contacts. We revealed that ULP-2, a predicted SUMO protease, has an important role at AJs during epidermal morphogenesis. Depletion of *ulp-2* by RNAi causes embryonic lethality; 35% of the affected embryos arrest at late gastrulation and the rest are arrested at ventral enclosure failing to cover the embryo with epidermis (28%), or at early stages of elongation (37%). To determine whether the epidermal phenotypes are independent of the late gastrulation failure, we induced expression of ULP-2^{C743S}, an enzymatic inactive form, under the regulation of the *hsp-16* promoter at the end of gastrulation and the onset of epidermal morphogenesis. This resulted in a strikingly uniform hammerhead phenotype. Interestingly, this phenotype was partially rescued by over expression of HMR-1/cadherin::GFP. When knocked down by RNAi using feeding, reduced levels of *hmr-1* causes low embryonic lethality (~10%). This phenotype is dramatically increased (~50%) in the background of *ulp-2(gk916250)* hypomorph. Confocal analysis of *ulp-2(RNAi)* treated embryos using fluorescent reporters of the cadherin-catenin complex (CCC) showed abnormal clearance of HMR-1::GFP and HMP-1/a-catenin::GFP from AJs of seam cells at early elongation. This may indicate that the stability of the CCC is compromised causing the complex to disassemble under the mechanical forces generated during elongation. To determine if the actin cytoskeleton is impaired by the loss of the CCC components, we performed phalloidin staining and live imaging of VAB-10ABD::mCherry. The typically observed circumferential pattern of actin in the dorsal and ventral epidermal cells during elongation is lost in *ulp-2(RNAi)* embryos and the filaments are severely disorganized. Taken together, these data suggest that the SUMO pathway control the flexibility of the CCC, an essential property during epidermal

morphogenesis.

1164B. UNC-54 and Y54E5B.2 work in concert to inhibit ectopic membrane extensions away from the nerve cord in *C. elegans* body wall muscle. **Ryan Viveiros**[1], Ralf Schnabel[2], Robert Barstead[3], Donald Moerman[1]. 1) Dept Zoology, Univ British Columbia, Vancouver, BC, Canada; 2) Univ Carolo-Wilhelmina of Braunschweig, Institute of Genetics, Braunschweig, Germany; 3) Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, U.S.A.

Adult *C. elegans* have 95 body wall muscle cells arranged in 4 quadrants, each consisting of two longitudinal rows of cells running along the length of the animal. The two ventral quadrants flank the ventral nerve and the two dorsal quadrants flank the dorsal nerve cord. These muscle cells, both those proximal and those distal to their adjacent nerve cord, extend specialized membrane extensions called muscle arms to establish contact with the nerve cord. These are the only membrane projections extended by adult body wall muscle cells.

In a screen for mutants with muscle defects, we have identified a temperature sensitive strain, GE6583, which exhibits ectopic membrane extensions away from the nerve cords at the non-permissive temperature. These projections can be quite extensive. We have observed processes originating from a ventral or dorsal cell extending across the animal to make contact with a cell in a quadrant on the opposite side of the animal. Mapping and sequencing studies of the strain reveal two tightly linked candidate genes, *unc-54*(t3197), the *C. elegans* myosin heavy chain B homologue, and Y54E5B.2(t3198), a uncharacterized WD motif containing protein. Analysis of the *unc-54*(e190) null allele revealed that loss of unc-54 alone is sufficient to induce these ectopic membrane extensions, though not at the level of penetrance observed in GE6583. Fosmid rescue experiments using constructs containing either wildtype Y45E5B.2 or *unc-54* were able to partially rescue the mutant phenotype, suggesting that the two genes are required to prevent ectopic muscle membrane extensions. We are currently in the process of characterizing Y54E5B.2 and determining why the loss of the *unc-54* myosin results in these membrane extensions.

1165C. Mechanical forces in *C. elegans* embryo elongation. **Thanh TK Vuong**, Michel Labouesse. Development & Stem Cell, IGBMC, 67404 Ilkirch, France.

Embryo development requires precise coordination of mechanical forces and their failure can lead to diseases. During the morphogenesis of *C. elegans* embryo, the cooperation of epidermal acto-myosin network and muscle contractions is essential. The acto-myosin activity in the epidermis, which has been shown to be more important in lateral than in dorsal-ventral cells, squeeze the embryo and make it elongate [1]. Muscle contractions become active around 1.7-1.8 fold stage. They have been showed to induce a mechano-transduction pathway [2], which is important for elongation. However, it is unclear how the contractions along the anterior-posterior axis help to increase the length of the embryo. Our project aims to elucidate the mechanical role of muscle contractions and its coordination with acto-myosin forces. The experiments are designed following a working model where muscle contractions induce a change in the elasticity of the embryo. We are using a laser nano-dissection technique to investigate cortical tension and elasticity of epidermal cells before and after the onset of muscle contractions. In parallel, we are evaluating the relative changes of acto-myosin forces with a FRET sensor [3] inserted in HMP-1 - a component of the adherens junctions. I will present our observations and preliminary results of the epidermal cortex nano-dissection experiments and measures of acto-myosin forces exerted on adherens junctions. References 1.Gally C, Wissler F, Zahreddine H, Quintin S, Landmann F, Labouesse M. Myosin II regulation during C. elegans embryonic elongation: LET-502/ROCK, MRCK-1 and PAK-1, three kinases with different roles. Development. 2009 Sep;136(18):3109-19. Epub 2009 Aug 12. 2.Zhang H, Landmann F, Zahreddine H, Rodriguez D, Koch M, Labouesse M. A tension-induced mechanotransduction pathway promotes epithelial morphogenesis. Nature. 2011 Mar 3;471(7336):99-103. 3.Grashoff C, Hoffman B, Brenner M, Zhou R, Parsons M, Yang M, McLean M, Sligar S, Chen C, Ha T, Schwartz M. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature. 2010 July 8; 466(7303): 263-266.

1166A. NOCA-1 isoforms regulate non-centrosomal microtubule array formation in different C. elegans tissues. **Shaohe Wang**[1,2], Arshad Desai[1], Karen Oegema[1]. 1) Ludwig Institute for Cancer Research, UCSD; 2) Biomedical Sciences Program, UCSD.

In contrast to the radial microtubule arrays organized by centrosomes in dividing cells, many differentiated cells assemble non-centrosomal microtubule arrays adapted for specific cellular functions. In a genome-wide RNAi screen, we identified NOCA-1, a novel protein essential for organizing non-centrosomal microtubule arrays in C.elegans germline and epidermis. NOCA-1 has six described isoforms and we identified a seventh that is necessary and sufficient for its germline function. A combination of live imaging and immunofluorescence revealed that NOCA-1 is found in multiple tissues, including the germline, epidermis, intestine, muscles and neurons. In the germline, NOCA-1 inhibition results in a catastrophic phenotype similar to inhibition of the microtubule nucleating protein g-tubulin. NOCA-1 and g-tubulin both target to the plasma membrane in the germline, but independently of each other. Due to its essential germline function, embryos homozygous for a noca-1 deletion grow up to become sterile adults. We also find that deletion of noca-1 is synthetically larval lethal with a deletion of the gene encoding the C. elegans homolog of the microtubule minus end-binding protein Patronin (PTRN-1). We used MosDEL to generate a null allele of ptrn-1 that deletes the majority of its coding sequence including the start codon, and found the deletion worms are superficially wildtype. Compared to wildtype control or to worms harboring the single deletions, noca-1D;ptrn-1D worms grow much slower post-embryonically and most die during the first three days after L1. The synthetic interaction of NOCA-1 and PTRN-1 likely occurs in the epidermis of larval and adult worms, since the synthetic lethal phenotype can be rescued by a NOCA-1 isoform that is mainly expressed in the epidermis. Our results suggest that different isoforms of NOCA-1 function together with microtubule minus-end associated proteins to generate non-centrosomal microtubule arrays that are essential to support the architecture of the syncytial germline and to maintain the barrier function of the epidermis.

1167B. Searching for regulators of LIT-1 localization in the amphid sensory compartment. **Wendy M Wang**, Shai Shaham. The Rockefeller Univ, New York, NY 10065.

Sensory structures are often arranged with neuronal endings enclosed by neighboring glial cells that form a specialized compartment important for neuronal function. For instance, Müller cells are glia in the vertebrate retina that surround photoreceptor cells; these funnel light to the photoreceptors and can regenerate certain retinal cell types when injured. To understand how such neuronal-glial compartments form, we are using the C. elegans amphid, the primary chemosensory organ of this animal, as a model system for compartment morphogenesis. The amphid consists of sensory neurons, a

glial sheath cell, and a glial socket cell. These glial cells contribute discrete parts of the amphid channel, a tubular structure through which the sensory neurons extend ciliated dendrites to reach the external environment. Previous work from our lab has shown that the DAF-6 and LIT-1 proteins oppose each other in controlling morphogenesis of the amphid sheath channel. DAF-6 is a Patched-related protein that lines the lumen of the amphid sheath channel, and daf-6 mutants have a bloated channel that blocks access of sensory dendrites to the environment. daf-6 genetically interacts with the Dispatched gene, che-14, to regulate amphid lumen morphogenesis. LIT-1 is a Nemo-like kinase identified in a daf-6 suppressor screen. lit-1 mutants have an excessively narrow channel, and daf-6; lit-1 double mutants have grossly normal glial channels. Like DAF-6, LIT-1 localizes along the length of the channel compartment. Importantly, in sensory neuron cilia mutants, both DAF-6 and LIT-1 are mislocalized, concentrating in the anterior end of the channel. This suggests that neuronal cues may control DAF-6 and LIT-1 localization and sensory compartment morphogenesis. To identify such neuronal cues, we are using a GFP::LIT-1 line to screen for mutants in which LIT-1 is mislocalized within the amphid channel. Thus far, we have identified 8 independent mutants. In all these mutants, the amphid sheath channel is bloated, with LIT-1 concentrated more at the anterior end of the channel. We are screening for additional mutants, and plan to map and clone the relevant genes, to identify potential signals involved in neuronal-glial communication.

1168C. LIN-29/EGR1, a zinc-finger transcription factor, controls the depth of anchor cell invasion in C. elegans. **Zheng Wang**, Shelly McClatchey, Lara Linden, Qiuyi Chi, David Sherwood. Duke Univ, Durham, NC.

Cell invasion through basement membrane (BM) occurs in various developmental and physiological contexts. It is a precisely controlled process. Deregulation of cell invasion causes a variety of diseases, most notably, cancer metastasis. Owing to the complexity of the microenvironment, cell invasion has been challenging to experimentally examine in vivo and thus the underlying mechanisms are poorly understood. Taking advantages of visual tractability and convenient genetic manipulability, we use anchor cell (AC) invasion in C. elegans as an in vivo invasion model. The AC is a specialized gonadal cell that invades through the juxtaposed gonadal and ventral epidermal BMs and contacts the central 1°-fated vulval precursor cells. AC invasion is followed by uterine-vulval attachment. The underlying vulval cells invaginate dorsally to form the vulva. The anchor cell sits at the apex of the invaginated vulva, mediating the attachment between the vulva and the uterus. To identify genes that regulate anchor cell invasion, we performed a forward mutagenesis screen for mutants with defect in invasion. From the screen, we isolated a mutant with a novel overinvasion phenotype. These mutant ACs continue to invade further ventrally to the apex of the vulva during uterine-vulval attachment. Cloning of this gene revealed that it encodes LIN-29, a conserved zinc-finger transcription factor related to the EGR family. The expression pattern and site-of-action analysis indicated that LIN-29 acts within the AC to control the depth of invasion. This study uncovers a previously unknown role for LIN-29 in invasion depth control and reveals that the invading cell has the intrinsic transcriptional program to regulate the degree of invasion.

1169A. Anillin non-autonomously regulates epidermal morphogenesis during *C. elegans* embryogenesis. **Wernike D.**, Fotopoulos N., Piekny A. Concordia Univ, Department of Biology, 7141 Sherbrooke St. West, Montreal, QC, H4B 1R6, Canada.

Ventral enclosure (VE) is a key part of epidermal morphogenesis, where the ventral surface of the *C. elegans* embryo is enclosed in a layer of epithelial cells. VE requires the coordinated migration of ventral epidermal cells and their adhesion at the ventral midline. Known regulators of VE include RhoGTPases, nucleators of F-actin and the catenin/cadherin complex. There likely are additional proteins that regulate F-actin for cell shape change, migration and/or adhesion that have not yet been identified. One candidate is anillin, ANI-1, a multi-domain scaffolding protein that coordinates actomyosin contractility in the early embryo. We show that *ani-1* is required for epidermal morphogenesis. In *ani-1* RNAi embryos expressing AJM-1:GFP (adherens junctions marker), ventral epidermal cells fail to meet and adhere at the ventral midline. Although they migrate at a rate comparable to control embryos, they often are not properly aligned with their contralateral neighbors. Interestingly, ANI-1 does not localize to adherens junctions or epidermal F-actin, but is present in HAM-1-expressing neuroblasts. Neuroblasts lie underneath the epidermis and are hypothesized to serve as a substrate for ventral epidermal cell migration, likely by providing chemical cues for their guidance. ANI-1 localizes to the cleavage furrows of dividing neuroblasts, which fail to divide upon ANI-1 depletion, suggesting that ANI-1 regulates neuroblast cytokinesis. In support of ANI-1's non-autonomous regulation of VE, *ani-1* RNAi enhances VE phenotypes caused by hypomorphic alleles of the catenin/cadherin complex. Also, ANI-1 and alpha-catenin co-suppress one another when one is over-expressed and the other is mutated or depleted by RNAi. Therefore, strengthening the junctions between cells (e.g. by alpha-catenin over-expression) could make the substrate partially redundant and likewise, strengthening the cytoskeleton of the substrate (e.g. by ANI-1 over-expression) could make junctions partially redundant. These data support the model that mechanotransduction between multiple tissues in the developing embryo is essential for epidermal morphogenesis.

1170B. FAX-1 and PROMININ function in migration and morphogenesis. **Bruce Wightman**, Emily Bayer. Dept Biol, Muhlenberg College, Allentown, PA.

The FAX-1 nuclear receptor of *C. elegans* functions in neuron identity and is orthologous to human PNR nuclear receptor (NR2E3). Mutations in *PNR* lead to defects in photoreceptor development and retinal degeneration. Mutations in the human *Prominin-1* gene lead to photoreceptor dysmorphogenesis and retinal degeneration (Stargardt Disease). The *Prominin-1* gene encodes a five-pass transmembrane protein that is localized to membrane protrusions. Prominin-1 protein is the CD133 antigen, an important surface marker for stem cells. Co-expression and similarity of function between *PNR* and *Prominin-1*, suggested there might be a relationship between *fax-1* and the *C. elegans prmn-1* gene. Unlike flies and vertebrates, *C. elegans* has only one Prominin ortholog. FAX-1 protein is expressed in both DTCs during their migratory period from L2 to late L4, and in a subset of dorsal vulval cells during L4 morphogenesis. We found that mutations in *fax-1* cause defects in the navigation of the DTCs, including premature turns, delayed turns, and navigational errors. Mutations in *vab-3*, which cause continued migration of the DTCs into adulthood, also cause continued expression of *fax-1::gfp* in the DTCs into adulthood, indicating that *fax-1* expression is a property of a migratory DTC and suggesting that *fax-1* expression may be downstream of *vab-3* activity. *fax-1(RNAi)* knockdown does not enhance the DTC migration defects of *unc-5* and *unc-6* mutations, suggesting that *fax-1* might function in a linear pathway with the netrin system. Mutations in *fax-1* do not cause a strong vulva phenotype, indicating that *fax-1* function in the vulva may be subtle. Mutations in *prmn-1* also cause DTC migration phenotypes, which are not substantially increased in *fax-1; prmn-1(RNAi)* doubles, suggesting a linear pathway. However, we have not been able to detect expression of *prmn-1::gfp* in the DTCs, so the *prmn-1* DTC migration phenotype may be non-autonomous. In the process of this analysis, we discovered that PRMN-1 protein accumulates on the lumen-facing plasma membrane of L4 vulval cells

undergoing morphogenesis. It is retained in the adult external-facing “lips” of the vulva. Thus we are evaluating possible vulva morphogenesis functions for *prmn-1*.

1171C. DYF-7 prevents rupture of a sensory epithelium made of neurons and glia. **Claire R. Williams**[1,2], Maxwell G. Heiman[1,2]. 1) Harvard Medical School, Boston, MA; 2) Boston Children's Hospital, Boston, MA.

Cells come in a variety of shapes, all finely tuned to perform specific functions. To understand how these essential shapes arise, we have turned to neurons with highly stereotyped morphologies, those of the amphid sense organ in *Caenorhabditis elegans*. The amphid contains twelve neurons that each extend a single, unbranched dendrite to the nose tip, and two glia which ensheathe the distal endings of these dendrites; together, these cells, which make apical junctions with one another, form a sensory epithelium. We previously showed that amphid dendrites grow by a process termed retrograde extension, in which the dendrite tip remains stationary at the nose as the neuron cell body migrates away, stretching out the dendrite behind it. This process is dependent on DYF-7, a zona pellucida (ZP) domain protein, as without it the dendrite tip breaks away from the nose, resulting in a short dendrite phenotype. We tagged DYF-7 with superfolderGFP and found that its extracellular ZP domain is secreted and forms a cap structure surrounding the dendrite tip. When expressed ectopically in other regions of the embryo, DYF-7 re-localizes to dendrite tips, but also marks the luminal surfaces of the digestive tract, a classic epithelium. Immunostaining these embryos with antibodies against AJM-1 showed that DYF-7 localizes in the apical extracellular matrix adjacent to epithelial apical junctions, including junctions between amphid dendrites and their ensheathing glia. Precociously expressed DYF-7 does not become localized until after apical junctions have been established. In contrast to our previous model of DYF-7 acting as an anchor that holds sensory dendrites at the nose, we now propose that DYF-7 forms filaments across the apical surface of a sensory epithelium composed of neurons and glia, and prevents rupture of this epithelium in response to mechanical forces that result from cell migration. Further, since the primary domain of DYF-7 is a ZP domain, and ZP domains are found at the surfaces of nearly all epithelia in nearly all animals, we speculate that an ancestral function of ZP domain proteins may be to reinforce epithelia by forming a similar meshwork of filaments over their apical surfaces.

1172A. Role of integrin in neuronal cell migration. **Jing Wu**, Richa Manglorkar, Myeongwoo Lee. Biology, Baylor Univ , Waco, TX.

Cell migration, based on the interaction between cells and the extracellular matrix (ECM), is a process during which certain molecular signals from external environment, such as matrix proteins and guidance cues, are needed for the migrating cell to recognize its moving direction and correct path. Specifically we are interested in neuronal migration, of which the disorder results in defects of neurogenesis. Integrins are trans-membrane receptors that mediate the attachment of cells to ECM, which contribute in guiding migrating cells. In this study, we first took a molecular approach to investigate the function of integrins in neuronal migration using transgenic *C. elegans* mutants with a *pat-3* integrin defect. The bpat-3 (sp) and bpat-3 (TTAA) lines showed path-finding and organizational defects in the posterior ganglion. Next we employed RNAi technique to remove certain path-finding molecules from ECM in order to determine what other extracellular cues were influencing neuronal migration. The UNC-6/netrin gene was knocked down which caused severe misplacement of many neurons, especially in the posterior area of the worms. The UNC-129/TGF-b RNAi also exhibited neuron-migration defects. Lastly, UNC-52/perlecan RNAi showed defects in anterior nerve ring structure in bpat-3 (TTAA) and posterior ganglion in bpat-3 (sp). Taken together, it is demonstrated that b integrin, netrin, TGF-b, and perlecan play important roles in the organization of neurons in the nematode. This research is ongoing and will continue with the knocking down of more genes through RNAi to identify more factors that are involved in neuronal migration.

1173B. The Ezrin/Radixin/Moesin protein ERM-1 controls actin-mediated cell shape changes during vulval invagination. **Qiutan Yang**, Juan Restrepo, Alex Hajnal. Institute of Molecular Life Sciences, Zurich, Switzerland.

During vulval development, a linear array of epithelial cells is converted into a three-dimensional tubular organ. Contraction of the actomyosin network generates forces that induce the cell shape changes during vulval invagination and the subsequent morphogenesis. Vulval invagination begins in L3 larvae at the Pn.pxx stage. At this stage, the gonadal anchor cell (AC) located dorsal of the vulval cells establishes a contact with the two central vulval cells (vulF). Then, the two vulF cells begin to move dorsally and initiate the formation of the vulval lumen. We have found that the AC induces the progressive shortening of the lateral plasma membranes between the two vulF cells along the dorsal-ventral axis, causing the vulF cells to reorganize their apical junctions and detach from the cuticle to form the tip of the vulval lumen. In the absence of the AC or in fos-1(ar105) mutants, in which the basal laminae between the AC and vulval cells are not removed and hence no direct AC-vulF connection is formed, the lateral vulF membranes do not shorten and an abnormally shaped vulval lumen is formed. In a screen for genes controlling vulval morphogenesis, we have discovered that erm-1 is required for proper vulval lumen formation. erm-1 encodes the only C. elegans member of the Ezrin/Radixin/Moesin (ERM) protein family, which functions as a linker between plasma membrane proteins and the actin cytoskeleton (Ivetic and Ridley, 2004).erm-1 mutants exhibit defects in intestinal apical lumen morphogenesis (Van Fürden et al., 2004; Göbel et al., 2004). Loss of erm-1 function in the vulva results in an abnormal, bulged lumen. Interestingly, erm-1(lf) also prevents the shortening of the lateral vulF membranes, similar to the absence of the AC. An ERM-1::mCherry reporter is expressed in the AC and vulval cells, where it partially co-localizes with cortical actin microfilaments. Moreover, basolateral F-actin staining is reduced while apical F-actin is increased in erm-1(lf) mutants. Taken together, we propose that ERM-1 controls the subcellular distribution of the cortical actomyosin network to maintain a balance between the apical and basolateral contractile forces that shape the vulval cells during invagination .

1174C. Study of Aurora-B Kinase Regulators in *C. elegans* Meiosis. **Elisabeth Altendorfer**[1,2], Saravanapriah Nadarajan[2], Monica Colaiacovo[2]. 1) Max F. Perutz Laboratories, Univ of Vienna, Vienna, Austria; 2) Dept. of Genetics, Harvard Medical School, Boston, MA, USA.

Meiosis is the cell division program creating haploid germ cells from diploid precursor cells. This is achieved by following a single round of DNA replication with two consecutive rounds of cell divisions (meiosis I and II). A crucial meiotic event is the stepwise loss of a meiosis-specific component of sister chromatid cohesion (SCC) REC-8, resulting in the separation of homologs at meiosis I, and of sister chromatids at meiosis II. Errors in this process lead to chromosome missegregation. Thus, understanding the molecular mechanisms regulating the stepwise loss of SCC is highly important. At meiosis I, proteolysis of REC-8 located between homologs (mid-bivalent) is mediated by Separase. However, cohesion between sister chromatids is protected until meiosis II by Shugoshin and PP2A in mammals, and LAB-1, HTP-1 and PP1 in worms. In *C. elegans*, Aurora B kinase (AIR-2) is observed forming two closely

juxtaposed rings restricted to the mid-bivalent during late meiotic prophase. AIR-2 has been proposed to phosphorylate REC-8 in this region thereby enhancing its cleavability by Separase. However, how the localization of AIR-2 is regulated throughout species is not fully understood. We applied a functional genomics approach to find further regulators that promote the restricted localization of AIR-2 to the mid-bivalent. Specifically, we used an AIR-2::GFP transgenic line in which we depleted 213 germline-enriched genes by RNAi. We identified several candidates resulting in impaired AIR-2 localization in late meiotic prophase I: Class I) 22 candidates exhibited premature loss of SCC as suggested by the presence of twelve uncoupled, instead of six tightly juxtaposed, AIR-2::GFP rings; Class II) 5 candidates resulted in a failure of AIR-2 to load onto the chromatin; and Class III) 14 candidates (including three from Class I, possibly due to partial RNAi depletion) led to unrestricted loading of AIR-2 throughout the bivalent. Analysis of several putative candidates is ongoing. New insights into the molecular mechanisms restricting AIR-2 localization on the bivalents will be presented.

1175A. MAIN CHROMOSOME ABERRATIONS FOUND AMONG 4617 PEDIATRIC PATIENTS AT A TIRHD LEVEL CHILDREN MEXICAN HOSPITAL. **Juan M. Aparicio**[1,4], Maria de L Hurtado H[2], Margarita Barrientos P[3], Hortencia Chavez O[4], Sergio Chatelain M[5]. 1) Dept Gen; 2) Cytogenetics; 3) Endocrinology, Hosp para el Nino Poblano, Puebla, Puebla; 4) Estomatology, Benemerita Universidad de Puebla; 5) Biotechnology, Universidad Autonoma Metropolitana, Mexico.

Chromosome mutations are considered changes in the chromosome number or structure. They are due to gametogenesis inborn error (meiosis) or during the zygote first cellular divisions. All these alterations might be observed during metaphase from the cellular cycle, where DNA loses are seen (clastogenic processes) due to DNA repair processes deficiency o total absence, among others. 4617 chromosomal studies were performed at Hospital Para El Niño Poblano (Pediatric Hospital) in Mexico. During 19 years period of time (from 1992 to 2011) were 34.6% (1596 patients) showed different chromosomal alterations. Among the studies population, male and female pediatric patients with different genetic diseases were chosen. These chromosome changes are classified as numeric or structural alterations, respectively. Another group of genetic alterations are known as mutations and can be inherited among generations. A wide variety of pediatric patients with genetic diseases due to chromosome aberrations are described in this study analyzing their clinical characteristics, medical or surgical treatments and their medical evolution according to their genetic change. An early patient diagnosis is important for a better quality of life.

1176B. Spindle assembly checkpoint proteins regulate and monitor meiotic synapsis in *C. elegans*. **T. Bohr**, P. Lamelza, N. Bhalla. Molecular, Cellular and Developmental Biology, Univ of California Santa Cruz, Santa Cruz, CA.

In order to achieve proper meiotic chromosome segregation homologous chromosomes must pair and synapse in prophase I to facilitate crossover recombination. Improper chromosome segregation can lead to aneuploidy, which is associated with miscarriages, birth defects and tumorigenesis. Cell cycle checkpoints ensure accurate chromosome segregation by monitoring key events during cell division to ensure genomic integrity. In *C. elegans* the synapsis checkpoint monitors synapsis of homologous chromosomes and triggers cell death in the event of asynapsis to reduce the probability that aberrant cell divisions will produce aneuploid gametes. The synapsis checkpoint requires *cis*-acting sites near the end of each chromosome, termed pairing centers (PCs), for activation. PCs promote pairing and synapsis by establishing transient connections with the cytoplasmic microtubule network via attachment to the nuclear envelope, but how they activate the synapsis checkpoint is currently unknown.

In an RNAi screen to identify new synapsis checkpoint components, we isolated shugoshin (*sgo-1*). In organisms other than *C. elegans*, shugoshin has also been shown to be involved in the spindle assembly checkpoint (SAC). The SAC monitors microtubule attachment at kinetochores during the metaphase to anaphase transition. Like the synapsis checkpoint, the SAC uses *cis*-acting sites, centromeres, as platforms for activation and microtubule attachment. The similarities between the SAC and the synapsis checkpoint led us to speculate that other proteins required for SAC activation may also be required for the synapsis checkpoint. We have found that SAC proteins, MDF-1, MDF-2, BUB-3 and SGO-1, are required for the synapsis checkpoint. Furthermore, SAC components, MDF-1, MDF-2 and ZWL-1 localize to meiotic nuclei during the time when the synapsis checkpoint is active. We also demonstrate that synapsis occurs faster in SAC mutant backgrounds, suggesting that SAC proteins are required to both monitor and regulate synapsis. These findings are entirely unexpected and suggest exciting possibilities about the role that these proteins play in regulating chromosome dynamics during cell division.

1177C. Characterization and comparative profiling of the mitotic spindle proteome reveals a glycosylation factor, OSTD-1 as being necessary for cell division and ER morphology. **Mary Kate Bonner**, Ahna Skop. Dept Genetics, Univ Wisconsin, Madison, Madison, WI.

Cell division is important for many cellular processes including cell growth, reproduction, wound healing and stem cell renewal. Failures in cell division can often lead to tumors and birth defects. To identify factors necessary for this process, we implemented a comparative profiling strategy of the mitotic spindle proteome and corresponding *C. elegans* homologs. Of our published candidate mammalian proteins, we determined that 72% had homologs in *C. elegans*, 18% of which were associated with human disease. Of the *C. elegans* candidates (n=143), we determined that 34 genes functioned in embryonic development and 56% of these were predicted to be membrane trafficking proteins. A secondary, visual screen to detect distinct defects in cell division revealed 21 genes that were necessary for cytokinesis. One of these candidates, OSTD-1, a protein involved in glycosylation, was further characterized. Depletion of OSTD-1 resulted in cell cycle delays, spindle orientation defects, aberrant karyomere fusion and cytokinesis failures. In particular, 65% of all *ostd-1* RNAi-treated embryos failed to correctly position cleavage furrows. OSTD-1 also functions in maintaining ER organization during mitosis. We propose that OSTD-1 may play a necessary role in ER organization during mitosis leading to proper spindle dynamics and placement of the cleavage furrow.

1178A. SMCL-1 interacts with condensin proteins and modulates their function in chromosome dynamics. **Lucy Fang-I Chao**[1], Meha Singh[2], John Yates III[2], Kirsten Hagstrom[1]. 1) Dept of Molecular Medicine, Univ of Massachusetts Medical School, Worcester, MA; 2) Department of Chemical Physiology,The Scripps Research Institute, La Jolla, CA.

Proper chromosome organization is important for cell function. A crucial player in packaging DNA into chromosomes is condensin. In *C. elegans*, two condensin protein complexes promote proper chromosome segregation, and a third regulates X chromosome gene expression. At the heart of each five-subunit condensin complex is a heterodimer of proteins from the SMC (structural maintenance of chromosomes) family of chromosomal ATPases.

Eukaryotes have different SMC heterodimer pairs at the core of several complexes involved in chromosome dynamics. Here, we identify a new and unusual SMC protein, SMCL-1 (SMC Like-1), that interacts specifically with condensin SMC subunits and may negatively regulate condensin function. SMCL-1 is atypical in several ways. First, while most SMC proteins bind one other SMC protein, SMCL-1 may interact simultaneously with both members of an SMC heterodimer. Second, although other condensin SMC proteins bind stiochiometrically to all five complex subunits, SMCL-1 preferentially interacts with the SMC subunits. Third, although SMCL-1 associates with condensin SMC proteins, it does not localize to mitotic or X chromosomes like other condensin subunits. Fourth, while mutations of condensin subunits cause lethality, SMCL-1 null mutants are viable. Interestingly, an SMCL-1 null mutant in combination with a condensin hypomorphic mutant partially rescues the lethality of the condensin mutant. This genetic suppression implies that SMCL-1 may negatively regulate condensin function in chromosome segregation and/or X gene regulation. Also, while SMCL-1 shares homology with SMC proteins, it lacks certain domains and has a non-canonical ATPase motif. Together, our results lead us to hypothesize that SMCL-1 negatively regulates the function of one or more condensin complexes by sequestering and preventing activity of their core SMC subunits.

1179B. Suppressor mutations for the CAND-1 regulator of cullin-RING ubiquitin ligases. **Snehal N Chaudhari**, Edward T Kipreos. Cellular Biology, Univ of Georgia, Athens, GA.

The regulated degradation of proteins in the nucleus and cytosol is mediated in large part by the ubiquitin/26S proteasome pathway. Cullin-RING ubiquitin ligases (CRLs) are the largest class of ubiquitin ligases in eukaryotes. CRLs regulate diverse cellular processes including the cell cycle, transcription, signal transduction, and developmental programming. Perturbations of CRL activity have been linked to a broad array of cellular and developmental defects including cancer. CAND1 (cullin-associated nedd8-dissociated 1) is a conserved protein that binds all cullins in animals and is required for the activity of a subset of CRLs in vivo. Depletion of CAND1 results in alterations of the abundance of specific CRL complexes. It has been proposed that CAND1 acts as an exchange factor that regulates the CRL-adaptor repertoire. Altered expression of CAND1 has been correlated with the development of prostate cancer and lung tumors. Another CRL regulatory pathway involves the COP9 signalosome (CSN), an evolutionarily conserved multiprotein complex that sequesters CRL complexes, and is also required for CRL activity in vivo. The CSN complex along with CAND1 presumably maintains CRLs in a dynamic equilibrium between active and inactive states that is required for full CRL activity in vivo. How CSN and CAND1 activities are regulated in vivo is not well understood. To uncover CAND1 regulatory pathways and to further delineate the molecular roles of CAND-1 in C. elegans, our lab has screened for cand-1 suppressor mutations using random mutagenesis. The suppressor mutations can rescue the cand-1 mutant phenotype, and make the cand-1 mutant resistant to CSN RNAi, which normally acts as an enhancer of the cand-1 mutant. Using SNP mapping, we have identified a mutation on Chromosome I that is linked to the suppression. We are currently using molecular and genetic techniques to identify which of three genes near the mutation contribute to the cand-1 suppression.

1180C. Investigating loss-of-function suppressors of C. elegans centrosomal defective mutants. **Chien-Hui Chuang**, Sean O'Rourke, Bruce Bowerman. Univ of Oregon, Eugene, OR.

Centrosomes act as microtubule organizing centers, and are critical for cell division and cell polarity. Identifying the regulators and components of centrosomes is an important step in understanding cell division. To this end, our lab has completed genome-wide modifier screens to identify genes that, when reduced in function using feeding RNAi, can suppress lethality in three conditional centrosome-defective mutants: *spd-2(or183ts)*, *spd-5(or213ts)* and *zyg-1(or278ts)*. The *spd-2* gene encodes a coiled-coil protein essential for centrosome duplicaion and maturation; *zyg-1* encodes a kinase required for daughter centriole formation; and *spd-5* encodes a coiled-coil protein required for centrosome maturation. We have isolated 9 non-essential genes and one essential gene that specifically suppress the embryonic-lethality associated with one or more of the three mutants. We are currently investigating these 10 genes for their roles in regulating microtubule dynamics and mitotic spindle assembly and positioning. One gene, H37N21.1/*hpo-11*, strongly and specifically suppresses the lethality of *spd-5* after RNAi knockdown. When we use RNAi to knock down H37N21.1/*hpo-11* in wild-type embryos, we observe a substantial delay in rotation of the first mitotic spindle and a highly penetrant loss of spindle rocking during anaphase at the one-cell stage. These results suggest there may be abnormalities in microtubule dynamics in embryos lacking H37N21.1. Interestingly, H37N21.1 is conserved, with a mammalian ortholog called MADM (Mlf-1 adaptor molecule). MADM encodes a kinase-like protein localizes to centrosomes, and physically associates with the Mlf1 oncoprotein, which is involved in acute myeloid leukemia. However, the role of MADM at centrosome and in leukemia is little understood. We are investigating the cellular localization and function of H37N21.1, and of the other suppressors, to understand how they influence centrosome fuction and suppress lethality.

1181A. CHL-1 is required for DNA replicative integrity in *Caenorhabditis elegans*. **George Chung**, Ann M Rose. Department of Medical Genetics, Univ of British Columbia, Vancouver, Canada.

Previously, we have described *chl-1* in *C. elegans*, an ortholog of the human *DDX11/ChlR1* gene associated with Warsaw breakage syndrome. Our published results have shown that CHL-1 is indispensable for cell proliferation and for genome integrity. Using the continuously-replicating, mitotic nuclei of the *C. elegans* germline, we have begun to assay the recruitment of repair factors to the DNA under various non-damaging and damaging conditions. Even in the absence of DNA damaging agents, the *chl-1* mutant DNA is marked by an abnormally high number of RAD-51 foci, indicating that the CHL-1 protein is required for the normal, breakage-free completion of mitotic replication.

1182B. PP1 phosphatases, GSP-3 and GSP-4, are required for chromosome segregation in sperm meiosis. **Thais G. Cintra**, Joseph Beyene, Jui-Ching Wu, Diana Chu. Deparment of Biology, 1600 Holloway Avenue, San Francisco State Univ, San Francisco, CA.

Proper meiotic chromosome segregation is crucial to maintaining the integrity of chromosomes and avoiding the inheritance of genetic disorders. Although oocyte and sperm both undergo meiosis during their development, each exhibit differences in molecular machinery and progression of meiotic divisions. In mouse and worms, key players in regulating sperm meiosis are sperm-specific PP1 phosphatases. For example, deletion of the genes encoding the *C. elegans* PP1 proteins, GSP-3 and GSP-4 (GSP-3/4), causes chromosome segregation defects during sperm meiosis. However, how GSP-3/4 regulates meiotic divisions is unclear. We have determined that GSP-3/4 localize in a manner similar to kinetochore proteins, which connect microtubules and

chromosomes during meiotic divisions. Thus, **we hypothesize that GSP-3/4 regulate chromosome-microtubule interactions and dynamics during spermatogenesis**. Using live-imaging of strains expressing fluorescently-labeled histone and tubulin, we first defined the coordinated changes in chromosome and microtubule dynamics required for wild-type sperm meiotic divisions and then found specific features that are altered in *gsp-3/4* mutants. These include defects in X-chromosome resolution and sister chromatid reorientation between meiosis I and II divisions, and sister chromatid segregation during meiosis II that result in multinucleate or anucleate sperm. We previously found the outer kinetochore CEN-P proteins, HCP-1 and HCP-2, are differentially localized in sperm and oocyte meiosis, suggesting HCP-2 may be important in mediating specific aspects of sperm meiotic divisions. Indeed, using immunolocalization we find that HCP-2 is mislocalized during the transition from meiosis I and II in *gsp-3/4* mutants. Preliminary results also suggest other kinetochore components may be mislocalized. GSP-3/4 do not regulate the removal of the cohesin proteins REC-8 and COH-3/4 nor AIR-2, a key player in cohesin removal, as they are not mislocalized in *gsp-3/4* mutants. Thus, GSP-3/4 specifically regulate changes in kinetochore localization required for the reorientation of sister chromatids during meiotic divisions necessary for successful sperm formation.

1183C. Oocyte meiotic spindle assembly. **Amy Connolly**[1], Valerie Osterberg[1], Sara Christensen[1], Chenggang Lu[2], Kathy Chicas-Cruz[1], Shawn Lockery[1], Paul Mains[2], Bruce Bowerman[1]. 1) Univ of Oregon; 2) Univ of Calgary.

We are investigating the genetic requirements for oocyte meiotic spindle assembly. In an ongoing screen for temperature-sensitive, embryonic-lethal mutants, we have identified alleles of three essential genes known to be required for meiotic spindle assembly. These genes encode the scaffolding protein ASPM-1 with two IQ motifs and one CH domain, the kinesin 12 family member KLP-18, and the microtubule severing complex katanin subunit MEI-1. We more recently identified two alleles of the kinesin 13 family member *klp-7*, a gene not previously known to be required for oocyte meiotic spindle assembly. We compared the *aspm-1, klp-18*, and *mei-1* mutant phenotypes using spinning disk confocal microscopy to image both chromosomes and microtubules during Meiosis I in live oocytes. While previous studies of these genes have focused largely on meiotic spindle positioning, we focused instead on earlier steps in bipolar spindle assembly. *aspm-1(-)* spindles are bipolar, but initially large and loosely organized before compacting into a shape similar to wild-type. In contrast, bipolar spindle assembly fails in *mei-1(-)* and *klp-18(-)* oocytes. In *mei-1(-)* oocytes, chromosomes are dispersed at ovulation and remain dispersed until polar body extrusion. In *klp-18(-)* oocytes, the initially dispersed chromosomes aggregate into a tight cluster by the time of polar body extrusion. We therefore hypothesized that the *klp-18(-)* spindles are monopolar and *mei-1(-)* spindles are apolar. In support of this conclusion ASPM-1 localizes to a single focus in *klp-18(-)* mutants, but is absent in *mei-1(-)* mutants. We have found that *klp-18(-), mei-1(-)* double mutants resemble *mei-1(-)* single mutants, indicating that *mei-1* is required for the formation of a single pole in *klp-18(-)* mutants and therefore likely to drive pole formation in wild type. Using *klp-18(-)* monopolar spindles as a model for identifying factors required for spindle pole focusing, we are currently investigating the requirements for two distinct *mei-1*- mediated activities: katanin function and recruitment of ASPM-1 to meiotic spindle poles. Our results suggest that the katanin activity and ASPM-1 may work together to assemble and focus oocyte meiotic spindle poles.

1184A. Dissection of the temporal requirements for cell division proteins. **Tim Davies**[1], Shawn Jordan[1], Vandana Chand[1], Kimberley Laband[3], Mimi Shirasu-Hiza[2], Julien Dumont[3], Julie Canman[1]. 1) Dept of Pathology and Cell Biology, Columbia Univ, New York, NY; 2) Department of Genetics and Development, Columbia Univ Medical School, New York, NY; 3) Institut Jacques Monod, CNRS, UMR 7592, Université Paris Diderot, Paris, France.

We have developed novel technology for rapid temperature shift experiments using a custom-built machine we call “The Therminator”. We use the Therminator to dissect the precise temporal requirement for proteins in cytokinesis, or the physical division of one cell into two. Cytokinesis requires a stereotypical and highly synchronized series of events within only 5-10 minutes in the first mitotic division of the *C. elegans* embryo. Because many of the proteins required for division also play crucial roles during oogenesis and development, they are difficult to study by traditional genetic approaches. Combining the Therminator with fast-acting (£20s), temperature-sensitive (*ts*) cytokinesis-defective mutants allows us to bypass developmental requirements and identify the precise temporal window during which specific proteins contribute to cytokinesis. We set out to examine the temporal requirements of five key proteins or protein complexes known to play a role in cell division including the myosin-II motor (NMY-2), a formin family actin filament nucleator (CYK-1), a regulator of Rho GTPase signaling (CYK-4), a midzone microtubule organizer (ZEN-4), and the master regulatory kinase Aurora-B (AIR-2). All of these proteins are assumed to function throughout cytokinesis; however, only myosin-II (Liu *et al*., Dev. Biol. 2010) has been functionally studied at high temporal resolution. Unexpectedly, we find that each protein exhibits a unique temporal profile of functional requirement. These results challenge several current models of the cellular mechanisms underlying cytokinesis. Furthermore, these data illustrate the power of fast-acting *ts* mutants to dissect the temporal requirement for protein function during a complex cellular event such as cytokinesis.

1185B. Developmental regulation of telomere anchoring in *C. elegans*. **Helder C. Ferreira**, Benjamin Towbin, Thibaud Jegou, Susan M. Gasser. Friedrich Miescher Institute, Basel, Switzerland.

Telomeres are specialized protein-DNA structures that protect chromosome ends. In lower eukaryotes, such as budding yeast, telomeres show a distinct nuclear organization, being bound to the nuclear periphery. By imaging telomere position in the metazoan *C. elegans*, we find that, here too, telomeres are preferentially located near the nuclear envelope. Peripheral telomere localization increases during embryogenesis and persists in later larval stages. We show that in early embryogenesis telomere position is independent of the Ku complex and of H3 K9 methylation but instead is regulated by the PIAS-like SUMO E3 ligase *gei-17* and the nuclear envelope protein SUN-1. Moreover, we find that the shelterin component POT-1 but not POT-2 is required to maintain telomeres at the nuclear periphery. Interestingly, POT-1 inhibits recombination mediated telomere maintenance. To our knowledge, this is the first description of telomere position in *C. elegans*.

1186C. Characterizing the role of ATX-2, the C. elegans ortholog of Ataxin-2, in cell division. **Megan Gnazzo**, Ahna Skop. Department of Genetics, Univ of Wisconsin Madison, Madison, WI.

Mutations in Ataxin-2 give rise to the devastating neurodegenerative disease spinocerebellar ataxia type 2 (SCA2). In SCA2 an expansion of CAG repeats encoding polyglutamine in Ataxin-2 is observed. Many individuals with amyotrophic lateral sclerosis (ALS) also have expanded polyglutamine repeats in Ataxin-2. The reasons why mutations in Ataxin-2 lead to neurodegeneration are unknown, and the cellular functions of Ataxin-2 remain unclear. Our lab

identified the ataxin-2 gene from isolated mammalian midbodies and the corresponding C. elegans ortholog, ATX-2, displayed defects in cytokinesis (Skop et al, 2004). To determine why ATX-2 leads to cytokinesis defects, we are characterizing its role in the early C. elegans embryo. Bioinformatic analysis revealed that ATX-2 is very highly conserved. ATX-2 contains several RNA binding motifs suggesting a role for ATX-2 in the control of mRNA translation. Local control of mRNA translation has been proposed as a mechanism for regulating synapse plasticity. We hypothesize that ATX-2 may play a role in mediating the local translation of RNAs found in the midbody during cytokinesis. We have identified four ATX-2 isoforms and would like to know how these isoforms function throughout embryonic development. We are currently constructing GFP constructs to two of the identified isoforms (full-length and C-terminal) to determine the localization of these constructs in the early embryo. Live imaging analysis has revealed defects in both meiotic and mitotic cytokinesis. Here, the second polar body often fails to be extruded and during the first division late failures in cytokinesis are observed. We will present our current analysis of ATX-2 function in cytokinesis.

1187A. Transcriptional Regulation of Centrosome Duplication in *C. elegans*. **Jacqueline Goeres**, Kevin O'Connell. Biochemistry and Genetics, NIDDK, NIH, Bethesda, MD.

The formation of a bipolar mitotic spindle organized by centrosomes at each pole is a key determinant for the accurate segregation of chromosomes during cell division. In order to ensure spindle bipolarity, centrosomes must be duplicated once and only once per cell cycle. Precise regulation of centrosome duplication is therefore essential for proper cell growth and division. Several lines of evidence have indicated that signaling through Ras and the MAP kinase pathway causes centrosome amplification. However, the molecular mechanism underlying these events has not been elucidated. Genetic analysis in *C.elegans* has identified a basic framework of five conserved factors that are required for centrosome duplication, which include the master regulator kinase ZYG-1 (Plk4 in humans). Mutation or depletion of these factors leads to a failure in centriole duplication and results in the formation of monopolar spindles in the *C. elegans* embryo. The *zyg-1(it25)* mutant fails to duplicate its centrioles, resulting in embryonic lethality. We found that mutation of the transcription factor *dpl-1* restores centrosome duplication in the *zyg-1(it25)* mutant and suppresses its embryonic lethality, indicating a genetic interaction between the transcriptional regulator and the centrosome duplication pathway. DPL-1 is the homolog of human DP, a binding partner of the E2F transcriptional regulator that is responsible for the G1-to-S phase transition in vertebrates. Mutation of the E2F homolog, *efl-1*, also suppresses *zyg-1(it25)* lethality, implicating that the DPL-1/EFL-1 complex negatively regulates centrosome duplication. Current studies are focused on determining whether centrosome duplication factors are direct targets of the DPL-1/EFL-1 transcriptional regulator complex. Notably, DPL-1 has been shown to be negatively regulated by Ras signaling. We found that overactivation of the Ras signaling pathway through the expression of a gain-of-function allele of Ras also suppresses *zyg-1(it25)* lethality. This work elucidates a transcriptional network that is regulated by Ras signaling and controls expression of the centrosome duplication factors, providing a mechanistic link between Ras and centrosome amplification.

1188B. Functional dissection of MEL-28, a chromatin-binding protein with essential roles in nuclear envelope function and chromosome segregation. **Georgina Gómez-Saldivar**[1,4], Anita G. Fernandez[2,3,4], Allison Lai[2], Carly Bock[2], Cristina González-Aguilera[1], Fabio Piano[3], Peter Askjaer[1]. 1) CABD, CSIC-Univ. Pablo de Olavide, Seville, Spain; 2) Fairfield Univ Biology Department, Fairfield CT, 06824, USA; 3) NYU Center for Genomics and Systems Biology, New York, NY 10006, USA; 4) These authors contributed equally.

MEL-28/ELYS is a large AT-Hook protein required for the proper structure and function of the nuclear envelope during interphase in nematodes and vertebrates. In addition, MEL-28 has critical roles in chromosome congression and segregation during mitosis. MEL-28 localizes to nuclear pores and chromatin during interphase and shuttles to the kinetochore during cell division. Other than the AT-Hook domains, which suggest that MEL-28 binds DNA directly, primary structure analysis has not revealed functional domains. Biochemical studies have demonstrated that MEL-28 interacts with the NUP107 subcomplex at nuclear pores, but its targeting mechanism to kinetochores is unknown. We are interested in understanding 1) the function and mode of MEL-28 chromatin binding and 2) which regions of the MEL-28 protein are required for its different functions. To this end we have used DamID to define the chromatin regions with which MEL-28 associates. Interestingly, MEL-28 is enriched in active chromatin, suggesting that it may be involved in regulation of gene expression. In addition we are using a structure/function approach to determine which domains of the MEL-28 protein are required for MEL-28 localization and function. We have generated truncated versions of MEL-28 that lack different domains and fused these to GFP to track their localization in live embryos. Biological function of these fusions is evaluated by crossing them into *mel-28* mutants and scoring for rescue of embryonic lethality. In doing this we have defined separate domains that are implicated in localization of MEL-28 to the nuclear envelope, chromatin and kinetochores. Surprisingly, we have found that removal of the AT Hooks does not affect MEL-28 localization although it does disrupt MEL-28 function. These studies illuminate the functioning of an essential and conserved protein.

1189C. ZTF-15 is required for the meiotic synapsis checkpoint in *C. elegans*. **Tom Hwang**, Matt Ragle, Needhi Bhalla. UC Santa Cruz, Santa Cruz, CA.

In order to achieve proper meiotic chromosome segregation, homologous chromosomes must pair and synapse in prophase I to facilitate crossover recombination. Defects in meiotic prophase events can result in programmed cell death (apoptosis) or cell cycle arrest, indicating that these events are monitored by checkpoint mechanisms to avoid the production of aneuploid gametes. In *C. elegans*, unsynapsed chromosomes activate germline apoptosis independently of a DNA damage checkpoint that monitors recombination. While independent of one another, both of these checkpoints require the pro-apoptotic factor, *egl-1*. In response to asynapsis or DNA damage, *egl-1* is transcriptionally upregulated to promote apoptosis so that defective meiotic nuclei are removed and do not go on to form aneuploid gametes. When the DNA damage checkpoint is activated, the *C. elegans* ortholog of tumor suppressor p53, *cep-1*, is responsible for promoting transcription of *egl-1*. However, *cep-1* is not required for the synapsis checkpoint. We are interested in how *egl-1* transcription is upregulated when asynapsis occurs.

In an RNAi screen to identify new checkpoint components, we discovered *ztf-15* is required for the synapsis checkpoint. ZTF-15 contains 5 C2H2 zinc finger domains, a motif that often mediates binding to specific DNA sequences. We speculate that ZTF-15 is the synapsis checkpoint counterpart of *cep-1* and that it acts as a transcription factor to promote *egl-1* transcription in the event of asynapsis. To test this hypothesis, we plan to use MOSSci to fluorescently tag ZTF-15 as well as create an antibody that recognizes ZTF-15 to determine where it localizes in the *C. elegans* germline. If its localization is consistent with a role in apoptosis, we can further test *ztf-15*'s relationship with *egl 1* through use of qPCR and gel mobility shift assays. Through these

experiments, we hope to elucidate how meiotic events are monitored by cell cycle checkpoints to protect against chromosome missegregation and aneuploidy, which can contribute to infertility, birth defects, and cancer predisposition.

1190A. Intertwined Functions of Separase and Caspase in Chromosome Separation and Programmed Cell Death. **Pan-Young Jeong**[1], Ashish Kumar[1], Pradeep Joshi[1,2], Joel H. Rothman[1,3]. 1) 1Department of Molecular, Cellular, and Developmental Biology, and Neuroscience Research Institute, Univ of California, Santa Barbara, Santa Barbara, CA; 2) Dept. of Zoology, Univ of British Columbia, Vancouver, BC Canada; 3) School of Biological Sciences, Univ of Auckland, Auckland 1010 New Zealand.

Timely sister chromatid separation, promoted by separase, is essential for faithful chromosome transmission during mitosis and meiosis. We report that the C. elegans pro-apoptotic caspase CED-3, which was previously known exclusively for its role in regulating apoptosis, cleaves cohesin SCC-1 specifically and functions with separase SEP-1 to allow mitotic and meiotic chromosome separation. CED-3 function alone is necessary for the normal rate of embryonic development and germline meiotic chromosome disjunction independent of its role in apoptosis. Loss of the endogenous soma-specific (CSP-3) and germline-specific (CSP-2) caspase inhibitors results in CED-3-dependent suppression of embryonic lethality and meiotic chromosome non-disjunction, respectively, in animals compromised for separase function. Further, removal of SEP-1 blocks developmental apoptosis and suppresses a mutant defective for the apoptotic suppressor *ced-9/Bcl-2*. Thus, caspases and separases orchestrate both apoptosis and chromosome segregation, supporting the view that co-option of components in chromosome segregation may have led to the innovation of programmed cell suicide early in metazoan evolution.

1191B. Systematic characterization and positional cloning of temperature-sensitive, embryonic-lethal *C. elegans* mutants. **Reza Keikhaee**, Chien-Hui Chiang, Amy Connolly, Josh Lowry, John Yochem, Bruce Bowerman. Institute of Molecular Biology, Univ of Oregon, Eugene, OR, USA.

While most genetic screens for embryonic-lethal *C. elegans* mutants have focused on a few narrow sub-classes of mutant phenotypes, we now seek to isolate and systematically categorize by mutant phenotype 2000 new temperature-sensitive, embryonic lethal *C. elegans* mutants. For every new mutant we find, we initially use Differential Interference Contrast (DIC) microscopy to examine the early embryonic cell divisions in live mutant embryos. We broadly classify all new mutants in four categories: (i) meiotic and mitotic cell division defective, (ii) eggshell defective, (iii) delayed P1 division, and (iv) wild-type early embryogenesis but penetrant embryonic lethality. To date 626 mutants from the 890 new mutants that we have found are categorized in above categories as follows: ~28% exhibit cell division defects, ~16% are eggshell defective, ~9% exhibit delays in P1 cell division, and ~44% appear to have wild type early cell divisions. We also are categorizing each mutant using two additional criteria. First, our mutants are isolated by scoring embryonic lethality after shifting L4 worms to the restrictive temperature. We therefore also have shifted mutant worms to the restrictive temperature as arrested L1s, and then scored for larval lethality and sterility. Finally, to further classify embryos with normal eggshells and early cell divisions, we examine terminal mutant phenotypes to classify their development with respect to proper morphogenesis. Thus far we have found several mutants with abnormal phenotypes. For example, ~40% of *or867*ts and *or1687*ts resemble *vab* (variable abnormal morphology) mutants with mis-shapen but elongated two- to three-fold embryos developing but failing to hatch. We will use next generation DNA sequencing and single nucleotide polymorphism (SNP)-based mapping strategy to rapidly map and identify affected loci in mutants with penetrant and consistent phenotypes. Once we have identified causal mutations, we will further explore the requirements for genes that are conserved in humans but have not been extensively characterized in *C. elegans*.

1192C. Condensin depletion licenses an alternate meiotic DSB repair pathway. **Teresa W. Lee**, Barbara J. Meyer. HHMI & UC Berkeley.

During meiosis, crossovers are essential to orient and segregate homologous chromosomes. Disruption of condensin I or condensin II, SMC-family complexes that structure chromosomes for cell division, perturbs the number and distribution of meiotic crossovers (COs). In *C. elegans*, wild type animals have just one CO per chromosome; in animals depleted of condensin, some chromosomes have two or three. This increase in COs is strongly correlated with an extension of the chromosome axis and an increase and shifted distribution of CO precursors (double-strand breaks, DSBs). In many organisms, COs are resolved from DSBs by one of two pathways: the ZMM family of proteins or the Mus81-Mms4 endonuclease. The ZMM pathway is thought to be the dominant method of generating COs in *C. elegans*, but it is not known whether the extra COs in condensin-depleted animals are resolved by this pathway. In animals treated with gamma-irradiation, CO number increases but the numbers of a cytological marker for COs, COSA-1 foci, do not differ from non-irradiated controls (Yokoo et al 2012). Animals depleted of subunits in condensin I (*dpy-26/+*), condensin II (*kle-2/+*), or both, have more COs than wild-type animals, but retain wild-type levels of COSA-1 foci. Therefore, condensin mutants have COs that are marked by COSA-1 and not marked by COSA-1.

To determine whether the unmarked COs are resolved by the MUS-81 pathway, we examined the suppression of COs by *mus-81* in condensin mutants. In *mus-81; dpy-26/+* and *mus-81; kle-2/+* animals, we observed no suppression by *mus-81*. However, in *mus-81; kle-2/+; dpy-26/+* animals, we did observe some suppression. In *kle-2/+; dpy-26/+* animals, 20% of X chromosomes had double COs and 2% had triple COs, while in *mus-81; kle-2/+; dpy-26/+* animals, 6% of X chromosomes had double COs and none had triple COs (p value=.00001), similar to levels observed in *dpy-26/+*. Condensin depletion may induce repair by MUS-81 through changes in chromosome structure, formation of excess DSBs, or a reduction in condensin complexes. To better understand the conditions required to invoke this alternate CO pathway, we are examining whether DSBs induced by gamma irradiation use MUS-81 for their resolution as COs, and whether the requirement for MUS-81 is caused by excess DSBs or exogenous damage from irradiation.

1193A. Suppression of Cell Cycle Defects through Knockdown of Tumor Suppressor Genes. **Y. Liu**, D. Tobin, M. Saito. Geisel School of Medicine, Dartmouth College, Hanover, NH.

Defects in cell cycle control can be damaging or even fatal to an organism. Cell cycle control processes have many fundamental similarities between *C. elegans* and humans. Since the loss of cell cycle regulation is a hallmark of cancer, understanding the function of genes that control the cell cycle in *C. elegans* will expedite the development of cancer detection and treatment methods for human patients. Our laboratory's goal is to characterize the normal function of putative tumor suppressor genes, previously identified in a genome wide screen for animals defective in maintaining cell cycle quiescence (see abstract by Tobin et al). *C. elegans* Cyclin D(*cyd-1*) mutants animals arrest in larval development with fewer intestinal cells than wild-type animals. RNAi was used to knockdown specific genes, to test whether or not cell cycle defects of the *cyd-1(lf)* animals could be suppressed. Suppression of *cyd-1(lf)* would indicate that the tumor suppressor gene functions downstream of or in parallel to *cyd-1*. Several RNAi clones were found to suppress intestinal cell

division defects, such as *ubc-25, C56G2.1, B0393.6* and *cdc-14*. Suppression of *cyd-1* by B0393.6 was novel and interesting given the particular genetic interactions of B0393.1. *cdc-14, ubc-25*, and *lin-35* all suppress cell division by inhibiting CDK-2/CYE-1 activity through distinct mechanisms. This was determined using genetic enhancement tests, for example *cdc-14(lf)* with *ubc-25(lf)* is additive. Interestingly, B0393.6 knockdown combined with mutation of any of these three pathways causes an additive effect on intestinal cell number, indicating that B0393.6 is part of a previously uncharacterized G1/S inhibitory pathway. Further studies will be conducted to uncover the mechanism by which B0393.6 promotes cell cycle quiescence.

1194B. Cell cycle uncoupling and centriole elimination in the endoreduplicating intestinal cells of *C. elegans*. **Yu Lu**, Richard Roy. Dept Biol, McGill Univ, Montreal, PQ, Canada.

The centrosome cycle is most often coupled with the mitotic cell cycle through the shared activity of various cell cycle regulators, thus ensuring that the centriole is duplicated only once per cell cycle. However this coupling can be altered in specific developmental contexts. In highly differentiated tracheal cells hundreds of centrioles are generated *de novo* in the absence of DNA proliferation to form the cilia typical of these cells, whereas in *Drosophila* follicle cells the centrosomes are eliminated prior to the onset of the successive rounds of DNA replication that normally occur in this tissue. How such cell cycle uncoupling of centrosome cycle occurs in these unique developmental contexts still remains misunderstood. In *C. elegans*, the larval intestinal cells undergo one nuclear division followed by four endocycles, characterized by successive rounds of S-phases without intervening mitoses. Under normal conditions S-phase CDK activities also promote centriole duplication therefore we questioned what would happen to the centriole in these situations of successive rounds of DNA replication. We therefore monitored the levels of SPD-2, a protein critical for centriole duplication in *C. elegans*, together with other centriolar markers and found that centrosomes lose their pericentriolar material following the nuclear division that occurs during the L1 stage and is thereafter never re-gained. Centriole duplication is subsequently uncoupled from the S phase of the following endocycle prior to their elimination during the L2 stage. Furthermore, we show that SPD-2 plays a central role in the numeral regulation of centrioles as a potential target of CDK activities, while the phosphorylation on SPD-2 by Polo-like kinase, transcriptional regulation of genes that affect centriole biogenesis, concomitant with the timely function of the ubiquitin/proteasome degradation pathway, contribute to final stability/elimination of the centrioles during the L2 stage.

1195C. A forward genetic screen for suppressors of an allele of microtubule-bundling factor, *spd-1*, crucial for central spindle formation during cytokinesis. E. Pablo-Hernando[2], B. Esmaeili[1], **M. Mishima[1]**. 1) Warwick Medical School, Univ of Warwick, Coventry, United Kingdom; 2) Gurdon Institute, Univ of Cambridge, Cambridge, United Kingdom.

Cytokinesis is the final step of cell division that divides the whole cytoplasm between segregating sister chromatids. Failure of cytokinesis can cause aneuploidy, which is frequently associated with cancer. A key player in cytokinesis is the central spindle, a microtubule bundle structure formed between segregating chromosomes. It plays a central role in the coordination of cytokinesis with chromosome segregation by localizing the molecular signals for cleavage furrow induction. The central spindle is compacted by the fully ingressed furrow and matures into a structure called the midbody. This structure provides a platform for vesicle transport and membrane fusion systems that are essential for abscission, the final separation of the two daughter cells. Two essential microtubule-bundling proteins required for the formation of the central spindle are PRC1 (SPD-1 in *C. elegans*) and centralspindlin (a stable complex of ZEN-4 kinesin and CYK-4 GTPase-activating protein). We previously reported that the direct interaction between SPD-1 and CYK-4 plays an important role in stable formation of the central spindle under mechanical tension. To further our understanding of their cooperative action, we performed a screen for genetic suppressors of a temperature sensitive allele of *spd-1, oj5*, which disrupts the SPD-1 and CYK-4 interaction and thus causes the central spindle defect. We have screened 1.6x10^5 genomes mutagenized by EMS and isolated 8 independent extragenic suppressors. Interestingly, a novel allele of *zen-4* was identified by a combination of single nucleotide polymorphism and whole genome sequencing. At the meeting, we will provide an update on characterisation of this mutation found in the ZEN-4 motor domain and other *spd-1(oj5)* suppressors isolated in our screen.

1196A. Methods to Study Toxic Transgenes: Analysis of Protease-Dead Separase in Membrane Trafficking. **Diana Mitchell**, Lindsey Uehlein, Joshua Bembenek. Univ of Tennessee, Knoxville, TN.

The *C. elegans* embryo provides an ideal system to study the processes of egg activation and cell division. We have demonstrated a novel role for separase, a conserved protease that facilitates chromosome segregation, in the regulation of membrane trafficking during cortical granule exocytosis and cytokinesis. We are interested in determining the role of separase's protease function in the regulation of membrane trafficking. To address this, we generated protease-dead separase mutant tagged with GFP (GFP::SEP-1-PD). We find that GFP::SEP-1-PD accumulates more strongly than wt GFP::SEP-1 at the furrow and midbody during anaphase and cytokinesis, suggesting it could be substrate trapping and have a role in membrane trafficking. The expression of GFP::SEP-1-PD causes embryonic lethality, suggesting that it is a dominant-negative transgene that interferes with endogenous separase function. To enable further study of GFP::SEP-1-PD, we developed methods that allow propagation of worm lines containing toxic transgenes. We are using these methods to enable the study of the protease function of separase in membrane trafficking in the *C. elegans* embryo. The first method involves feeding worms *gfp*(RNAi) bacteria to silence the transgene. Transgene expression takes on average 5 generations after removal from *gfp*(RNAi). Transgene re-expression is also temperature sensitive, allowing further control over its re-expression. We are using this method to obtain large numbers of embryos expressing GFP::SEP-1-PD for biochemical and proteomics analysis, which may allow us to identify novel separase substrates. The second method takes advantage of the *pie-1* promotor, which drives transgene expression, and is not expressed in the male germline. Male worms containing GFP::SEP-1-PD can used to propagate the transgene and circumvent the generation delay in the *gfp*(RNAi) feeding method. We are employing this method to examine genetic interactions of PD-separase with genes in the membrane trafficking pathway known to be involved in cortical granule exocytosis and/or cytokinesis. The general application of these methods facilitates the study of dominant-negative/toxic transgenes using standard techniques in the *C. elegans* model system.

1197B. The nuclear envelope protein LEM-2 is critical for nuclear positioning and centrosome attachment. **Adela Morales Martinez**, Agnieszka Dobrzynska, Cristina Ayuso, Peter Askjaer. CABD, CSIC-Univ. Pablo de Olavide, Sevilla, Spain.

LEM domain proteins (for LBR, Emerin and MAN1) are typically localized in the inner nuclear membrane where they serve as bridging molecules between

the nuclear membranes, the nuclear lamina and chromatin proteins, such as BAF. In addition, LEM domain proteins regulate gene expression through specific interaction with transcription factors and chromatin modifying enzymes at the nuclear envelope. Mutations in LEM domain proteins or in interacting nuclear envelope proteins cause a wide range of severe human diseases, collectively known as laminopathies. Most laminopathies are still poorly understood and no efficient treatment has been identified. To explore the mechanisms behind LEM domain protein activities we are characterizing the C. elegans *lem-2* gene, which encodes a protein homologous to vertebrate inner nuclear membrane proteins LEM2 and MAN1. Previous experiments have reported that LEM-2 shares redundant roles with emerin/EMR-1. We find that LEM-2 is expressed in all cell types but is enriched in the germ line and intestine relative to EMR-1. Moreover, LEM-2 is recruited significantly earlier than EMR-1 during nuclear envelope reassembly in telophase, suggesting that LEM-2 carries specific functions. Indeed, live imaging reveals that nuclear morphology is irregular in embryos lacking expression of LEM-2 and EMR-1 protein turnover is accelerated in *lem-2* adults. The nuclear envelope is responsible for nucleus-centrosome attachment and alterations in the nuclear envelope can cause abnormal nuclear and centrosome positioning. In C. elegans nuclear positioning is mainly studied in mid-stage embryos and early larvae but we observe that mutation of *lem-2* causes defects already in the second embryonic division. During AB division nuclei separate slower than in wild type embryos and nucleating centrosomes are observed at abnormal distance from nuclei. Thus, although expressed in the inner nuclear membrane our data suggest that LEM-2 is involved in the anchoring of centrosomes to the outer nuclear membrane. To identify potential interaction partners in this process, we are performing a genome-wide RNAi screen for enhancers of *lem-2* phenotypes.

1198C. Mechanistic Insights Into The Recruitment of the Spindle Checkpoint Protein MDF-1 To Unattached Kinetochores. **Mark Moyle**, Karen Oegema, Arshad Desai. Ludwig Institute for Cancer Research, La Jolla, CA.

Correct alignment and segregation of chromosomes during cell division is essential for proper development and growth. When chromosomes are improperly attached an intracellular signaling cascade, termed the spindle checkpoint, generates a “wait anaphase” signal which delays cell cycle progression until all chromosomes are properly aligned. This signal originates from unattached kinetochores, the macromolecular spindle microtubule-binding machines built on the centromeric regions of chromosomes. Central to the “wait anaphase” signal is the protein MDF-1 (Mad1 in human), but it is still unclear how MDF-1 is recruited to and activated at unattached kinetochores. To address this question, we performed a yeast two-hybrid screen with MDF-1 and a library of kinetochore constituents. We identified an interaction between MDF-1 and BUB-1, a conserved kinase implicated in spindle checkpoint signaling. We then performed a mutagenic yeast two-hybrid screen, which identified alleles of MDF-1 that no longer interact with BUB-1. Prior work had shown that BUB-1 is required for the kinetochore localization of MDF-1. Employing the MosSCI system, we generated MDF-1 mutants that do not interact with BUB-1 and found that the mutant forms of MDF-1 are compromised in their localization to unattached kinetochores. Consistent with this, the MDF-1 mutants that do not interact with BUB-1 were defective in generating a spindle checkpoint signal. As BUB-1 is a kinase, we generated kinase-dead mutants of BUB-1 and found that these mutants no longer interact with MDF-1 in the two-hybrid assay, suggesting that the kinase activity of BUB-1 is required for this interaction. Currently, we are investigating BUB-1 kinase-dead mutants in vivo. Finally, we performed a compensatory mutagenic yeast two-hybrid screen and found a mutation in BUB-1 that restores interaction with a MDF-1 mutant, strongly suggesting that MDF-1 and BUB-1 directly interact. Overall, our findings elucidate the recruitment of MDF-1 to unattached kinetochores via the kinase BUB-1, and show that the specific interaction underlying this recruitment is required for generation of the “wait anaphase” checkpoint signal.

1199A. Regulation of meiotic recombination by the MAP kinase cascade. **Christian R. Nelson**, Tom Hwang, Needhi Bhalla. MCD Biology, UC Santa Cruz, Santa Cruz, CA.

Proper chromosome segregation during meiosis requires the formation of double-strand breaks (DSBs) and the repair of a subset of DSBs into inter-homolog crossovers (COs). In *C. elegans*, crossover control ensures that a CO forms between each homolog pair but that the overall number of COs is limited. This regulation is thought to be accomplished through licensing and designation steps, whereby only a subset of DSB repair intermediates are allowed to mature into inter-homolog COs. In addition to these regulatory mechanisms, the MAP kinase cascade contributes to meiotic recombination by promoting a switch in DNA repair from use of homologous chromosomes to a homolog-independent repair pathway. The interplay between these events, which occur contemporaneously in the worm germline, is not well understood.

The conserved RING-domain protein ZHP-3 was previously shown to be required for CO formation. ZHP-3 initially localizes along the synaptonemal complex in early pachytene, but re-localizes to form bright foci marking CO sites by late pachytene. ZHP-3 contains a putative MAP kinase docking site (RSK domain) as well as six putative MAP kinase phosphorylation sites. We have shown that ZHP-3 is phosphorylated by murine MAP kinase *in vitro* and mutation of the consensus sites to alanine abolishes phosphorylation. Furthermore, mutation of the MAP kinase docking site in ZHP-3 (*zhp-3-dock*) reduces embryonic viability to ~10% *in vivo*. Importantly, this mutant still localizes to the synaptonemal complex correctly in early pachytene, but shows a reduced number of foci in late pachytene as well as an increase in the number of achiasmate chromosomes at diakinesis. Together, these results suggest that regulation of ZHP-3 by MAP kinase is an important step in promoting CO formation. The defects seen in *zhp-3-dock* may reflect a failure in crossover designation, mis-regulation of the switch in DSB repair modes or both. Future experiments will distinguish between these models and will determine the effects of ZHP-3 phosphomutants/mimetics on crossover formation.

1200B. Centriole Copy Number Control Is Mediated by Protein Phosphatase 1-Beta in *C. elegans*. **Nina Peel**[1], Jyoti Iyer[2], Michael Dougherty[2], Kevin O'Connell[2]. 1) Department of Biology, The College of New Jersey, Ewing, NJ 08628; 2) Laboratory of Biochemistry & Genetics, NIDDK, NIH, Bethesda, MD 20892.

Centrioles are small cylindrical structures that play essential roles during cell division and development. In mitotic cells, centrioles direct formation of centrosomes, microtubule-organizing centers that promote bipolar spindle formation. In post-mitotic cells, centrioles serve as basal bodies to nucleate the formation of cilia and flagella. Under control of the kinase ZYG-1, centrioles duplicate precisely once per cell cycle with one daughter centriole assembled next to each pre-existing mother centriole. The fidelity of this process is essential for the proper segregation of chromosomes, as too few or too many centrioles lead to the formation of mono- or multipolar spindles. While defects in centriole number control have been linked to cancer and other diseases, the mechanisms that constrain centriole assembly to one daughter per mother are poorly understood. We have found that loss-of-function mutations that

affect either of two highly conserved PP1 regulators (referred to here as I-2 and SDS-22) can suppress the centriole duplication defect incurred by partial loss of ZYG-1 activity. Likewise, reduced expression of GSP-1, the worm PP1-beta catalytic subunit also suppresses a *zyg-1* mutant. Proteomic analysis indicates that both I-2 and SDS-22 bind GSP-1, but function in distinct PP1 complexes. Significantly, we find that loss of PP1-beta activity leads to the over-expression of ZYG-1, suggesting a likely mechanism of suppression. Most interestingly, in a *zyg-1(+)* background, strong inhibition of I-2 or SDS-22 activity leads to centriole over-duplication whereby mother centrioles produce more than a single daughter. Our results show that by limiting ZYG-1 expression, PP1-beta acts as a key determinant of centriole number.

1201C. The NR4A Orphan Nuclear Receptor NHR-6 Plays an Important Role in Cell Cycle Progression and Cell Differentiation during Spermatheca Development. **Brandon Praslicka**, Chris R. Gissendanner. College of Pharmacy, Univ of Louisiana-Monroe, Monroe, LA.

NR4A nuclear receptors are a conserved, functionally diverse group that regulates many cellular processes including proliferation, differentiation, migration, apoptosis and DNA repair. The C. elegans NR4A ortholog, NHR-6, is expressed in all spermathecal cells from mid-L3 until terminal differentiation is reached in the early adult. nhr-6(lg6001) mutants display severely disorganized cellular structure in the spermatheca and spermatheca-uterine valve as well as a ~50% decrease in spermatheca cell number indicating a dualistic function for NHR-6 in cell proliferation and differentiation. Preliminary data indicate that the cell proliferation defect in nhr-6 mutants is due to an inability to complete the normal number of spermatheca cell divisions. A cell lineaging analysis was performed to determine which cells in nhr-6 mutants fail to divide. In nhr-6 mutants, cell divisions are blocked in the lineages that give rise to the distal spermatheca. We then utilized the S-phase marker, rnr::GFP, to assess cell cycle progression in these animals. rnr::GFP; nhr-6(-) animals failed to show expression in the distal cell lineage at the same time as wild type animals indicating a delay or block in S-phase entry. Furthermore, loss of the negative G1/S transition regulator fzr-1 was able to strongly suppress the cell number phenotype in fzr-1;nhr-6 double mutants, indicating that NHR-6 promotes G1/S entry. The spermathecal cells still failed to differentiate properly in fzr-1;nhr-6 mutants leading to the formation of a nonfunctional organ, further indicating a dualistic function for NHR-6. To determine the target genes regulated by NHR-6, we identified NHR-6 binding sites using ChIP-seq. The analysis was performed at the mid to late L3 and the early to mid L4 stages. We identified ~2,500 binding sites, including sites specific to the L3 and L4 stages. We find binding sites associated with genes with a wide variety of functions including signaling proteins and other transcription factors. These data will provide a unique in vivo framework for dissecting NR4A NR activity during organogenesis.

1202A. Evolutionary comparisons reveal a positional switch for spindle pole oscillation, and divergent regulation of GPR in Cænorhabditis embryos. **Soizic Riche**[1], Melissa Zouak[1], Françoise Argoul[2], Alain Arnéodo[2], Jacques Pécréaux[3], Marie Delattre[1]. 1) Laboratory of Molecular Biology of the Cell, 46 allée d'Italie, ENSL, 69007 Lyon, France; 2) Physics Laboratory , 46 allée d'Italie, ENSL, 69007 Lyon, France; 3) Institute of Genetics and Developmental biology of Rennes, CNRS UMR 6061, Université Rennes 1, 2 avenue du Prof. L. Bernard, F-35043 Rennes, France.

Mitotic spindle positioning is essential for oriented cell division. During the first embryonic division in *C. elegans*, the mitotic spindle is pulled towards the posterior pole of the cell and undergoes vigorous transverse oscillations. We identified variations in spindle trajectories by analyzing the outwardly similar one-cell stage embryo of its close relative *C. briggsae*. Compared to *C. elegans*, *C. briggsae* embryos exhibit an anterior shifting of nuclei in prophase and reduced anaphase spindle oscillations. By combining physical perturbations and mutant analysis in both species, we show that differences can be explained by inter-species changes in the regulation of the cortical Ga/GPR/LIN-5 complex. However, we uncover that in both species 1) a conserved positional switch controls the onset of spindle oscillations, 2) GPR posterior localization may set this positional switch, and 3) the maximum amplitude of spindle oscillations is determined by the time spent in the oscillating phase. By investigating microevolution of a subcellular process, we therefore identify new mechanisms that are instrumental to decipher spindle positioning (1). Interestingly, GPR is poorly conserved at the amino acid level between these species. We are currently analyzing this unexpected divergence. To this end, we are performing protein replacement between species, as well as analysis of protein chimeras. By correlating sequence divergence, GPR localisation and phenotypes of nuclei and spindle positioning we hope to identify new regulatory modules of GPR, an essential but poorly caracterized player in the control of spindle positioning. (1) Riche, S. & al. Evolutionary comparisons reveal a positional switch for spindle pole oscillation in Cænorhabditis embryos. Journal of Cell Biology, 2013 In press.

1203B. Chromosome bi-orientation in the first spermatocyte meiotic division prevents abnormal spindle organization in the second division. **Mara Schvarzstein**, Anne Villeneuve. Developmental Biology, Stanford Univ School of Medicine, Stanford, CA.

Chromosomes are known to play a prominent role in organizing spindles in the context of cell divisions where centrosomes are absent. Here we show that chromosome structure is also important for normal spindle organization during spermatocyte meiosis, where centrosomes are present. Specifically, we show that chromosome structure during meiosis I influences spindle organization during meiosis II. By imaging spermatocytes in meiotic mutants lacking connections between homologous chromosomes at meiosis I, we found that a subset of these mutants exhibited a high frequency of multipolar or linked spindles in meiosis II. Presence or absence of aberrant spindles correlated with the ability of chromosomes to bi-orient at meiosis I. Specifically, aberrant structure of meiosis II spindles was observed in mutants in which sister chromatid pairs mono-orient and retain cohesion during the first meiotic division (e.g. spo-11). In contrast, aberrant meiosis II spindle structures were absent in mutants in which sister chromatids bi-orient and segregate at the first division (e.g. rec-8) (although lack of sister chromatid connections during meiosis II in such mutants result in abnormal chromosome segregation). Multipolar and linked meiosis II spindles were not observed in triploid worms with a mixture of bi-orienting and mono-orienting chromosomes at meiosis I. These and other data imply that the presence of chromosomes that are structurally proficient for bi-orientation at meiosis I ensures the formation of bipolar spindles at meiosis II. Moreover, one or two bi-oriented chromosomes in meiosis I are sufficient to prevent the multipolar and linked spindle phenotype in meiosis II. Further, live imaging of dividing spermatocytes suggests that meiosis I chromosome structure may influence meiosis II spindle organization by enabling sufficient separation of chromosomes at anaphase I to ensure that meiosis II spindles will form in well-separated spatial domains.

1204C. Are there changes in nucleo-cytoplasmic volume ratio during early embryonic development of *Caenorhabditis elegans*? **Jitka Simandlová**, Christian Lanctôt. Institute of Cellular Biology and Pathology, First Faculty of Medicine, Charles Univ in Prague.

After fertilization, the *C. elegans* embryo goes through a series of cleavages to give rise to particular cell lineages and to form in turn the whole organism.

During the early development of this nematode, the embryo does not grow despite the fact that the genome undergoes duplication before each cell division. In other words, the nuclear volume decreases during this process, which means that there must be sort of rearrangement in the structure and organization of the chromatin in the nucleus. Using *C. elegans* as a model organism has several advantages in the study of nuclear architecture. The most significant of which is that it develops according to an invariant cell lineage, allowing us to compare nuclear architecture in cells that are rigorously equivalent in terms of history, developmental potential and gene expression profile. In our experiments we performed the direct measurement of nuclear and cytoplasmic volumes and compared the nucleo-cytoplasmic ratio between the individual cells within one embryo during early development. The measurements were performed on 3D images of embryos of the OD95 strain using high-speed, high-resolution confocal spinning disk microscopy. These embryos express mCherry-histone and GFP targeted to the cytoplasmic membrane.

1205A. RNA-binding Protein ATX-2 Interacts with SZY-20 to Regulate Centrosome Assembly in C. elegans Embryos. Michael Bobian, Madeline Topitzes, John Ross, Jake Crumb, Abigail Meisel, Dongyan Zhang, **Mi Hye Song**. Biological Sciences, Michigan Technological Univ, Houghton, MI 49931.

Centrosomes are critical sites for orchestrating microtubule dynamics, and undergo dynamic changes in size during the cell cycle. As cells progress to mitosis, centrosomes recruit more pericentriolar materials (PCM), and nucleate more microtubules to establish bipolar mitotic spindles. Thus, the amounts of PCM contribute to the centrosome size, which positively correlates with the levels of microtubule-nucleation. *szy-20* mutants possess enlarged centrosomes, leading to abnormal microtubule processes and embryonic lethality. SZY-20 acts as a negative regulator of centrosome size by limiting the amounts of centrosome-associated components. The *szy-20* gene encodes a novel centrosome-associated RNA-binding protein that negatively regulates centrosome assembly. SZY-20 contains novel RNA-binding domains; mutating these domains perturbs RNA-binding by SZY-20 in vitro and its capacity to regulate centrosome size and microtubule-dependent processes in vivo. It has been demonstrated that a number of RNAs and RNA-binding proteins associate with centrosomes and microtubules, and that they function to assemble mitotic spindles. To further understand the roles of SZY-20 and its RNA-binding in centrosome assembly and size, we utilized proteomics to identify proteins complexed with SZY-20, and identified a handful of proteins that are reproducibly pulled-down with SZY-20, including known RNA-binding proteins. ATX-2 and PAB-1, other known RNA-binding proteins are identified in SZY-20 immuno-complex. Knocking down either *atx-2* or *pab-1* by RNAi resulted in abnormal cell divisions and centrosome behavior. Genetic analyses suggest that both *atx-2* and *pab-1* function in centrosome assembly, which is closely associated with *szy-20* or *zyg-1*. Using the range of genetic, cell biological, biochemical and optical approaches, we are characterizing ATX-2 and PAB-1 in centrosome assembly to understand how this RNA-binding protein complex function together with SZY-20 to achieve proper centrosome assembly and size.

1206B. Characterization of *lin-5* mRNA localization in the early embryo. **Zoltán Spiró**, Pierre Gönczy. EPFL, Lausanne, Switzerland.

Asymmetric division is essential for generating cell diversity during development. The *C. elegans* zygote is an attractive model system to investigate the mechanisms governing asymmetric cell division. The asymmetric distribution of force generator complexes acting on astral microtubules at the cell cortex drives the unequal cleavage of the one-cell stage embryo. These complexes are composed of the Ga subunits GPA-16 and GOA-1, the GoLoco motif proteins GPR-1 and GPR-2, the coil-coiled protein, LIN-5, as well as the dynein complex. According to the current model, the mechanisms driving asymmetric division of the one-cell stage embryo occur solely at the protein level. We found that *lin-5* mRNA is enriched around centrosomes in early embryos of *C. elegans* and *C. briggsae*. This enrichment is slightly asymmetric during anaphase, with more *lin-5* transcript on the anterior side, an asymmetry that is regulated by anterior-posterior polarity cues. Moreover, we found that centrosomes are necessary and sufficient for *lin-5* mRNA enrichment, and that microtubules are needed as well. Intriguingly, we observed that *lin-5* mRNA is mislocalized in *lin-5* mutant embryos, raising the possibility that LIN-5 protein is needed for the localization of its own mRNA. Unexpectedly in addition, we found that the enrichment of lin-5 mRNA around centrosomes is independent of its 3'UTR, in contrast to the situation for many localized mRNAs. We aim at identifying the cis-acting elements on the *lin-5* mRNA that are necessary for centrosome enrichment, which should put us in a position to mislocalize *lin-5* mRNA and thus address the functional relevance of transcript localization.

1207C. Identification and characterization of *mel-43*, a gene required for the meiosis-to-mitosis transition in *C. elegans*. Maryam Ataeian, **J. Tegha-Dunghu**, Martin Srayko. Biological Sciences, Univ of Alberta, Edmonton, Alberta, Canada.

The segregation of chromatin during meiosis and mitosis in the one-cell *C. elegans* embryo occurs via two distinct microtubule-based spindle structures. Meiotic and mitotic spindles in these embryos differ in both their morphology and mechanism of chromatin segregation. Meiotic spindles form in the absence of centrosomes, with microtubules initially nucleating around chromatin and then organising into a bipolar array. In contrast, during mitosis the centrosomes nucleate and organise the microtubules into a bipolar spindle. In most animals, female meiotic divisions occur sometime after fertilization, with the sperm-derived centrosomes present in the cytoplasm but remaining quiescent until meiosis is complete.

To understand how the meiosis-to-mitosis transition is regulated, we are studying *mel-43*. *mel-43(sb41)* was identified as a dominant maternal-effect mutation that is required for early embryonic viability[1]. In *mel-43(sb41)* embryos, anaphase II meiotic spindle morphology was abnormal, chromatin segregation usually failed, and polar body II extrusion did not occur. Because the earlier stages of meiosis II appeared normal, the *sb41* phenotypes are consistent with a failure to exit meiosis II. Genetic duplication analysis showed that *mel-43(sb41)* is a hypermorphic gain-of-function mutation. Genomic sequencing (BC Genome and D. Moerman, UBC) revealed a missense mutation in the coding region of *sb41* worms, however, *mel-43(RNAi)* embryos did not exhibit any obvious phenotype. *mel-43* encodes a novel protein, with two highly similar paralogs in *C. elegans*, suggesting that it could be a redundant gene. Consistent with this idea, *mel-43(RNAi)* rescued the *sb41* mutant phenotype. Furthermore, RNAi directed against all paralogs resulted in meiotic defects. Interestingly, these embryos displayed defects in polar body extrusion at the end of meiosis I before proceeding directly into mitosis, without forming a meiosis II spindle. Together, our data indicate that *mel-43* and its paralogs are required to specify the meiosis II program of the meiosis-to-mitosis transition in *C. elegans*. 1. Mitenko, N. L., *et al.,* 1997. *Genetics.* **147**, 1665-74.

1208A. Critical targets of CYD-1/CDK-4 in the control of cell cycle entry. **Inge The**[1], Suzan Ruijtenberg[1], Javier Muñoz[2], Martine Prinsen[1], Albert Heck[2], Sander van den Heuvel[1]. 1) Developmental Biology; 2) Biomolecular Mass Spectrometry and Proteomics Group, Utrecht Univ, The Netherlands.

Whether to continue or to arrest cell proliferation is a key developmental decision. Ultimately, this decision depends on the activity of cyclin-dependent kinases (CDKs) in the G1 phase of the cell cycle. G1 cyclin/CDKs counteract inhibition of cell-cycle entry by the retinoblastoma (Rb) tumor suppressor protein. It is currently unknown if additional critical regulators of G1 progression are regulated by CDK phosphorylation. We have previously shown that *C. elegans* has single *cyd-1* Cyclin D and *cdk-4* Cdk4/6 genes that are essential for G1 progression. Inactivation of *lin-35*, the sole *C. elegans* Rb family member, partly overcomes CYD-1/CDK-4 requirement. However, cell proliferation remains limited in double mutants of *lin-35* and *cyd-1*, or *lin-35* and *cdk-4*, indicating the presence of critical CYD-1/CDK-4 target(s) in addition to the LIN-35 protein. To identify such targets, we performed a genetic screen for mutations that restore cell proliferation in the absence of *cyd-1* and *lin-35* function. The screen identified a single mutant with normal development and cell proliferation patterns. We found that the responsible mutation results in incomplete loss-of-function of *fzr-1* Cdh1, a substrate specificity factor of the anaphase promoting complex (APC/C). The APC/C^{Cdh1} E3 ubiquitin ligase targets proteins for degradation and is critical for exit from mitosis and inhibition of cell-cycle entry in G1. Using *in vitro* kinase assays, we found that CYD-1/CDK-4 phosphorylates LIN-35 on specific sites that correspond to Rb-inactivating phosphorylation in mammalian cells. In addition, CYD-1/CDK-4 phosphorylated eight different serine residues in the N-terminal region of FZR-1 Cdh1. In other systems, phosphorylation of the Cdh1 N-terminus by mitotic CDKs prevents APC/C association and premature APC/C^{Cdh1} activation. We propose that similar phosphorylation by CYD-1/CDK-4 overcomes APC/C^{Cdh1} activity in G1, thereby promoting cell cycle entry. Our data support that the LIN-35 Rb and FZR-1 Cdh1 proteins are the only two critical phosphorylation targets of Cyclin D/CDK-4 in the control of cell-cycle entry.

1209B. The *C. elegans* UBE2Q2 homolog, UBC-25, Promotes Cell Cycle Quiescence by Inhibiting Cyclin E Expression. **David V Tobin**[1], Sarah H Roy[1], Nadin Memar[2], Barbara Conradt[2], R. Mako Saito[1]. 1) Geisel School of Medicine at Dartmouth, Hanover, NH; 2) Ludwig-Maximilians-Universität.

Cell cycle quiescence is important for coordinating differentiation with development. In *C. elegans*, vulva precursor cells (VPCs) remain quiescent for over two larval stages before re-entering the cell cycle to generate vulva and hypodermal tissues. A forward genetic screen identified several novel regulators of VPC quiescence, including the *cdc-14* phosphatase. *cdc-14* promotes VPC quiescence through stabilization of *cki-1*, contrary to its requirement for mitosis in yeast. We conducted an RNAi screen to identify additional genes necessary to maintain VPC quiescence. We focus on the screen positive *ubc-25*: it is highly conserved with human UBE2Q2 and *ubc-25(lf)* produces severe extra intestinal nuclear divisions. Additionally, studies of human UBE2Q2 have provided conflicting functions, which may be clarified from our studies in *C. elegans*. Lineage analysis of predicted null *ubc-25(ok1732)* embryos found that the extra nuclei originate from shorter cell cycles in the E lineage. Genetic analyses suggest that *ubc-25* acts in a linear pathway with SCF component *cul-1*, in parallel to *cki-1* and *lin-35* to restrict intestinal nuclear divisions. Consistent with the role of *cul-1* in limiting Cyclin-E expression, animals lacking *ubc-25* display 1.5 to 8.7 fold increased CYE-1 expression by western blot analysis. Interestingly, the phenotype of *ubc-25(lf)* is not as severe as *cul-1(lf)*. Other ubiquitin-conjugating enzymes compensate for *ubc-25(lf)* since inhibition of several *ubc* genes substantially enhanced intestinal nuclei number in *ubc-25(lf)* animals. Broad expression of a mCherry::UBC-25 fusion protein suggests that *ubc-25* activity may promote quiescence in diverse tissues. This hypothesis is supported by the dramatic enhancement of rare ectopic VPC divisions in *ubc-25(lf)* animals treated with *lin-35* RNAi. Our experiments support a model in which *ubc-25* activity broadly supports cell cycle quiescence though inhibition of CYE-1 expression, but through the action of parallel processes only appears rate limiting in the intestine.

1210C. A splice mutation in pat-3 b integrin reveals genetic interactions between the extracellular matrix and cki-1/$p27^{KIP1}$. **Eun-Jeong Yu**, Lena Al-Rashed, Myeongwoo Lee. Biology, Baylor Univeristy, Waco, TX.

The cell-extracellular matrix (ECM) interaction plays an essential role in maintaining tissue shapes and regulating cell behaviors such as cell adhesion, differentiation and proliferation. The cell-ECM interaction is particularly important for progression and arrest of cell cycle. However, the mechanism by which the ECM influences the cell cycle in vivo is poorly understood. Our previous study demonstrated that the b integrin PAT-3 controls the localization and expression of CKI-1, a C. elegans homologue of the cyclin dependent kinase inhibitor $p27^{KIP1}$. In transgenic nematodes expressing wild type *pat-3 (+)*, CKI-1::GFP localized to nucleoli in hypodermal cells, whereas in animals expressing mutant *pat-3 (sp)* defective in splicing intron 7 of the *pat-3* gene, CKI-1::GFP appeared clumped and scattered in the nucleus. In addition, the level of CKI-1::GFP protein was elevated in the animals expressing the splice mutant, *pat-3 (sp)*. RNAi experiments linked cell adhesion and SCF E3 ubiquitin ligase complex genes to the CKI-1 expression. *unc-52*/perlecan, *ina-1*/a integrin, *pat-4*/ILK, and *unc-97*/PINCH appeared to involve in the localization of CKI-1::GFP. The SCF E3 ubiquitin-ligase complex genes, *skpt-1*/SKP2, *cul-1*/CUL1 and *lin-23*/F-box, is also required for the proper localization and expression of CKI-1, suggesting that integrin signaling and SCF E3 ligase work together to regulate the cellular distribution of CKI-1. These data demonstrated that perturbed integrin signaling leads to the inhibition of SCF ligase activity, mislocalization and elevation of CKI-1/$p27^{KIP1}$ protein. We continue to study the localization of CKI-1; we are interested in finding additional elements contributing to CKI-1 localization in the cell. Series of RNAi analysis identified the suppressors of *pat-3 (sp)* mislocalization defects. RNAi of genes involved in ribosome biogenesis, rRNA processing, and ubiquitin-mediated protein degradation resulted in the suppression of CKI-1::GFP nuclear localization, suggesting that integrin signaling links to the function of many genes acting in nucleus, nucleolus, and protein degradation. Further analysis on the regulation of CKI-1::GFP localization is currently underway.

1211A. The lipid binding and GAP domains of CYK-4 are essential for cytokinesis. **Donglei Zhang**, Andy Loria, Michael Glotzer. Department of Molecular Genetics and Cell Biology, Univ of Chicago, Chicago, IL 60637, USA.

Cytokinesis is a fundamental biological processes in which the parent cell physically separates and sequesters the duplicated genetic materials into two daughter cells. RhoA is a central regulator of cytokinesis, whose activity is redundantly controlled by centralspindlin and NOP-1 [1, 2]. Centralspindlin is a heterotetramer of a kinesin protein ZEN-4 and a Rho family GAP CYK-4. In addition to organizing the central spindle, this complex also plays key roles during furrow initiation, ingression and completion. CYK-4 contains numerous functional domains, including a coiled-coil domain, a C1 domain, and a GAP domain [3].

In order to better investigate functions of these domains of CYK-4 on cytokinesis, we generated strains carrying RNAi-resistant alleles of CYK-4::GFP by MosSCI. The RNAi-resistant alleles of CYK-4 allow us to examine transgenic CYK-4 variants by time-lapse fluorescence microscopy while specifically depleting endogenous CYK-4 by RNAi. We found that wild type CYK-4::GFP was properly localized in *cyk-4(RNAi)* embryos and fully compensated for

depletion of endogenous CYK-4 in wild type and *nop-1* mutants. Although CYK-4(DC1)::GFP also localized to the central spindle, furrow regression occurred in *cyk-4(RNAi)* strains, and it failed to induce a furrow in *nop-1; cyk-4(RNAi)* strains. These results suggest that the C1 domain plays a role at an early stage of cytokinesis (i.e. RhoA activation). Consistent with this, we found that CYK-4::GFP was recruited to the equatorial membrane, where RhoA is activated, while CYK-4(DC1)::GFP was not. Finally, we found that a catalytically inactivated allele, CYK-4(R459A)::GFP, failed to support full furrow ingression in *cyk-4(RNAi)* embryos. The defect was observed as a late regression phenotype as the furrow ingressed to ~90% of embryo width. These results suggest that CYK-4 GAP activity is required at a late stage of cytokinesis.

1. Glotzer, M., Science (2005); 2. Tse, Y.C. et al., Mol Biol Cell (2012); 3. White, E.C. et al., Cytoskeleton (2012).

1212B. Efficient single-cell transgene induction in Caenorhabditis elegans using a pulsed infrared laser. **Matthew A Churgin**, Liping He, John I Murray, Christopher Fang-Yen. Univ of Pennsylvania, Philadelphia, PA.

The coupling of transgenes to heat shock promoters is a common method for regulating C. elegans gene expression. Gene induction can be performed in a temporally defined manner, through timing of heat shock, and in a tissue-specific manner, via targeted rescue in hsf-1 mutants. However, cell-specific targeting methods are limited by the availability of cell-specific promoters. Here we present a method for evoking gene expression in single cells by local activation of heat shock. Our technique builds on the IR-LEGO method by Kamei et al (Nat. Meth. 2009) in which a continuous-wave, focused infrared laser beam is used to locally induce a heat shock response. However, continuous irradiation generates significant heating away from the laser focus due to the diffusion of heat, which increases the total heated volume. We have developed an improved method using a pulsed infrared laser that minimizes off-target heating by allowing time for thermal energy to dissipate between laser pulses. The resultant total heated volume is ~32 times smaller than that of IR-LEGO. Using a Phsp-16.2::GFP transgene as a reporter of heat shock we have optimized parameters to achieve a single cell gene induction rate of over 75% in seam cells (n=30), almost doubling the previously reported efficiency. To test the spatial resolution of our method, we successfully used it to induce GFP expression in the nerve ring neuron ADL in L2 larvae, and we observed no off-target induction despite the presence of neighboring neurons within 5 microns. To explore applications in lineage tracing, we used our method to individually heat shock each cell of the four-cell embryo. Resulting GFP expression patterns at bean stage were consistent with the expected positions of the descendants of the heat-shocked cell. In addition to direct transgene expression through heat shock, we also used an Phsp-16.2::FLP construct in combination with an FRT-flanked expression cassette to induce permanent GFP expression in single cells. Our laser-induced heat shock method is modest in cost and similar in technical complexity to widely used laser ablation systems. We therefore expect that our technique will be a broadly useful tool for C. elegans research.

1213C. A Functional Genomic Screen for the Telomerase RNA in *C. elegans*. **Robert D. Cohen**, Christopher Smith, Diana Chu. San Francisco State Univ, San Francisco, CA.

Telomerase maintains telomere length in eukaryotes by synthesizing telomere repeat patterns on the chromosome ends. It is composed of a protein component (TERT) with reverse transcriptase activity, and an RNA component (TERC) that acts as a template for the reverse transcriptase domain. As dysfunction in telomere length maintenance has been implicated in cancer and aging, the study of telomerase has emerged as an attractive research area for therapeutic intervention. Although identification of TERT genes in newly sequenced genomes has been achieved by homologous search with known TERT genes, the same cannot be said for TERC genes, as primary sequence is not well conserved. TERC identification has relied on co-purification experiments; however, these methods are costly and labor intensive, and as a result, TERC discovery has been limited.

To identify candidate TERC genes in a set of genomes from closely related species, we have developed a novel bioinformatic strategy. *C. elegans* is an ideal organism for the technique, because its TERC gene is unknown, a rich body of bioinformatic resources are available, and RNAi assays are available to test candidate gene function by reverse genetics. Although primary sequence is not well conserved in TERCs, one feature is dependably reproduced - the 1.5x template repeat pattern (eg, 9 bases of the telomere repeat: TTAGGCTTA), which serves as a template for TERT. Searching the *C. elegans* genome for all instances of this sequence yields 20503 loci. We used the Mauve genome aligner to identify syntenic regions between the *Caenorhabditis* genomes. 1.5x template repeats conserved at the same syntenic locus in each species are high probability TERC gene candidates. As a positive control, this bioinformatic approach analyzed five vertebrate genomes to identify the human TERC gene, which has been previously published. We have verified expression and RNAi knockdown of two TERC gene candidates, and are currently developing a *C. elegans* telomere length assay to confirm a reduced telomere length phenotype. Identification of the *C. elegans* TERC would provide an important tool to elucidate telomerase function in *C. elegans*.

1214A. Exploring the role of mechanosensory extracellular matrix components in the structure and function of primary cilia. **Deanna Michele De Vore**[1], Karla Knobel[2], Maureen Barr[1]. 1) Genetics, Rutgers Univ, Piscataway, NJ; 2) Cellular and Molecular Neuroscience, Univ of Wisconsin-Madison, Madison, WI.

PKD2 encodes a transient receptor potential polycystin (TRPP) channel receptor protein found in non-motile, primary cilia of mammalian cells 1. In humans, PKD2 mutations result in Autosomal Dominant Polycystic Kidney Disease (ADPKD). In the nematode *Caenorhabditis elegans*, the polycystin-2 homolog, PKD-2, localizes to the primary cilia of male-specific sensory neurons (CEMs and RnBs in the head and tail respectively) where it is required for male mating behaviors. Given the ancient and evolutionarily conserved role for polycystin-2 in cilia, I am using *C. elegans* as a model to identify new genes required for ciliary receptor localization. Our laboratory performed a forward genetic screen for PKD-2::GFP ciliary localization (Cil) defective mutants (Bae et al 2008). The *cil-2(my2)* mutants exhibit excess unusual PKD-2 accumulation at the ciliary base, cilium proper, and around the distal dendrite. We performed three-factor and deficiency mapping, whole genome sequencing, and single gene rescue experiments and determined that the *cil-2(my2)* allele is a mutation in the gene, *mec-5*. MEC-5 is a unique collagen protein found in the extracellular matrix (ECM) surrounding touch receptor neurons (Du et al 1996). In touch receptor neurons (TRNs), MEC-1, MEC-5, and MEC-9 are found in the ECM, play a role in mechanosensation, and may localize degenerin/epithelial sodium channels (DEG/ENaCs) along the TRN processes (Du et al 1996, Emtage et al 2004). Our preliminary data indicates that *mec-1* and *mec-9* regulate PKD-2::GFP localization in male-specific sensory neurons. We are currently determining how these ECM proteins regulate localization and channel properties of the PKD-2 TRP polycystin channel. These studies will shed light on how sensory receptors like PKD-2 are targeted to cilia, and may advance the understanding and treatment of ADPKD. References: 1) Bae YK et al, Dev Dyn. 2008 Aug;237(8):2021-9. 2) Du H. et al, Neuron. 1996 Jan;16(1):183-94. 3) Emtage L et al, Neuron. 2004 Dec 2;44(5):795-807.

1215B. Study of arrhythmogenic mutations in the pharynx using electrophysiological and optogenetic approaches. **E. Fischer**[1], C. Schüler[1], S. Wabnig[1], K. Erbguth[1], P. Hegemann[2], L. L. Looger[3], A. Gottschalk[1]. 1) Buchmann Institute for Molecular Life Sciences, Goethe Univ, Frankfurt, Germany; 2) Experimental Biophysics, Humboldt-Univ, Berlin, Germany; 3) HHMI Janelia Farm, Ashburn, USA.

The pharynx muscular pump comprises 20 muscle cells which are gap junction coupled, and possess orthologues of the human voltage-dependent Ca^{2+} channel $Ca_v1.2$ (i.e. EGL-19), the SR-Ca^{2+} storage protein CASQ2 (i.e. CSQ-1) and the ryanodine receptor (RyR; UNC-68). The similar physiology, pumping frequencies, and shape of the action potential imply the pharynx as a potential model for the human heart, possibly allowing to study mechanisms of arrhythmia like catecholaminergic polymorphic ventricular tachycardia (CPVT) or Timothy Syndrome (LQT8). Pharyngeal pumping was recorded by electropharyngeograms (EPGs) in *unc-68* and *csq-1* deletions, or in *egl-19* point mutants, as well as in pharmacologically treated animals. By engineering animals carrying CPVT related mutations of the RyR (e.g. R4743C) or *csq-1* as well as using *egl-19* alleles resembling mutants implicated in LQT8, we analyze in detail whether arrhythmic pumping can be provoked. Even though the RyR is not required for basal pumping, activation of RyRs by caffeine increased the pumping frequency. The RyR point mutant R4743C could rescue *unc-68* (r1162) mutants, based on swimming assays, indicative for a functional rescue in body wall muscles. As spontaneous pumping is not regular enough to analyze arrhythmia faithfully, we use the light-activated cation channels Channelrhodopsin-2 (ChR2) or its color-shifted variant C1V1, expressed in pharyngeal muscle, to depolarize the plasma membrane, evoke muscle contraction, and to "pace" the pharynx at up to 4 Hz. For the UNC-68(R4743C) mutant, we observed an increase in irregular pump events, and we will perform similar analyses of *egl-19* and of engineered CPVT-related *csq-1* mutants. We will further analyze these mutants by Ca^{2+} imaging with the red-fluorescent Ca^{2+} indicator RCaMP, which, as we could show, can faithfully report Ca^{2+} dynamics in the pharynx.

1216C. MiniMos and Universal MosSCI sites - new methods for *C. elegans* transgenesis. **C Frokjaer-Jensen**[1], MW Davis[1], M Sarov[2], X Liu[3], K Rebora[4], J Taylor[5], S Flibotte[5], A Pozniakovski[2], SK Kim[6], D Dupuy[4], DG Moerman[5], EM Jorgensen[1]. 1) Biology, HHMI, U Utah, SLC, UT, 84112; 2) TransgeneOmics Facility, Max Planck I, Dresden, Germany; 3) School of Life Sciences, Tsing-Hua U, Beijing 100084, China; 4) INSERM U869, Institut ECB, Pessac, France; 5) Dep. Zoology, UBC, Vancouver, BC, Canada; 6) Depts. Dev. Bio. And Genetics, Stanford U Medical School, Stanford CA 94305.

We have developed two new methods for stable insertion of single-copy transgenes into the *C. elegans* genome. **First**, we find that a modified Mos1 element (MiniMos) can efficiently insert transgenes (60% efficiency). The transposon can carry 45kb fosmids with high fidelity; all analyzed fosmid insertions were full length by CGH. The MiniMos element can be used with genetic (*unc-119*, *unc-18*) and antibiotic selections (NeoR and PuroR) in N2, *C. briggsae* and natural *C. elegans* isolates. From insertions of P*pie-1*:GFP we observe germline expression in 47% of animals. The non-fluorescent lines appear to mainly be silenced by position effects (e.g. X chromosome inactivation). Although we cannot rule out RNA epigenetic (RNAe) silencing, several lines crossed into *mut-7* were not re-activated. **Second**, we have developed a set of universal MosSCI insertion sites. We found that MiniMos can carry a wild-type Mos1 element and have used this to insert the *ttTi5605* Mos1 element and flanking genomic sequence into dozens of sites in the genome. These create a set of universal MosSCI landing sites that are compatible with a single targeting vector (e.g. pCFJ150). The insertion sites are flanked by a co-insertion marker (NeoR or P*myo-2*:GFP) that can be followed in crosses. We have characterized a standard insertion site on each autosome that allows robust germline expression. We have used these tools to determine somatic and germline position effects. In general, we see that the center of autosomes permit robust expression whereas insertions into autosomal arms or Chr. X are frequently partially or fully silenced. To quantify silencing, we have generated a set of >150 bright fluorescent insertions at identified locations across the genome and quantified expression of a subset by automated imaging techniques (at cellular resolution in L1 or by flow-cytometry) .

1217A. A conditional knockout system based on the single/low-copy integration of transgenes in *C. elegans*. E. Kage-Nakadai, **R. Imae**, O. Funatsu, S. Hori, Y. Suehiro, S. Yoshina, S. Mitani. Dept Physiology, Tokyo Women's Med Univ Sch Med, Tokyo, Japan.

Single/low-copy transgene integration is essential for avoiding overexpression, ectopic expression and gene silencing in the germline. We developed a simple method using ultraviolet trimethylpsoralen (UV/TMP), to create single- or low-copy chromosomal integrated strains. We integrated low-copy transgenes from extrachromosomal arrays using positive selection based on temperature sensitivity with a *vps-45* rescue fragment and negative selection based on benzimidazole sensitivity with a *ben-1* rescue fragment. Quantitative PCR revealed that strains generated by this method contain single- and low-copy transgenes. Single copy integration of excisable transgenes enables the complete gene excision that is crucial for generating conditional knockout mutants. Many Let or Ste mutants isolated so far, remain to be stably analyzed. Thus, we applied the UV/TMP methods to a conditional knockout system using Cre/LoxP. To visualize Cre/LoxP-mediated excision, tester strains possessing single copy floxed NLS::GFP were generated and crossed into transgenic animals that express Cre recombinase under various tissue-specific or heat-shock promoters. As a result, Cre-dependent excision was observed in many promoters tested. We also developed transgenic strains to test Cre/LoxP-mediated gene expression. Currently, we are constructing a conditional knockout system using the combination of these methods and pre-existing mutant strains.

1218B. Developing a high-throughput approach for identifying genetic interactions in *C. elegans*. **Calvin A. Mok**[1], O.A. Thompson[1], M. Edgley[2], L. Gevirtzman[1], C. Huynh[1], D.G. Moerman[2], R.H. Waterston[1]. 1) Genome Sciences, Univ of Washington, Seattle, WA., US; 2) Dept. of Zoology, Univ of British Columbia, Vancouver, BC, CA.

The investigation of gene function through genetic interactions (GIs) may provide insights on many human disorders. Recently, the Million Mutation Project (MMP) generated a library of 2007 fully-sequenced strains carrying over 820,000 single nucleotide variants (SNVs). Each strain has an average of ~400 SNVs, creating a compact library well-suited for studying GIs. We hypothesize that mutations in some genes related to development or survival fail to display phenotypes due to buffering interactions with other genes, thus obscuring their function. We will investigate this phenomenon using MMP strains in conjunction with RNAi targets in a high-throughput approach to identify GIs. We are interested in identifying GIs related to fitness using a mixed population competitive growth assay. Using the MMP library, we will grow pools of strains together on the same media over several generations and then assess their proportional representation. Each strain carries specific SNVs that we can track using molecular inversion probes (MIPs). MIPs can accurately identify the proportion of each strain within any given pool. Combining this assay with RNAi, we aim to identify GIs related to population fitness through increases or decreases in overall strain proportions. This method allows a group of 100 strains to be assayed in tandem against 90 RNAi's in a single

experiment to interrogate more than 1 million bi-allelic pairings. To test this approach on a smaller scale we chose a pilot group of strains, focusing on transcript factor (TF) mutants. TFs play an important role throughout the life cycle as key regulators of gene expression. In *C. elegans* there are over 800 predicted TFs with data to identify spatiotemporal and binding patterns although little is known of their function. We have currently chosen 112 transcription factors across 212 MMP strains to investigate TF interactions during early development. The development of this high-throughput method will allow multiple research groups to coordinate the study of genetic interactions and gene function.

1219C. Systematic comparison of bacterial feeding strains for increased yield of C. elegans males by RNA interference induced non-disjunction. **Vaishnavi Nagarajan**[1], Nadeem Asad[1], Hayley Luna[2], Jordan Martinez[2], Zachary Moore[3], Lisa Timmons[1]. 1) Univ of Kansas, KS; 2) Lawrence High school, KS; 3) Free State High School, KS.

Since gender in C. elegans is based on the number of X chromosomes, the presence of XO male progeny derived from a self-fertilized XX hermaphrodite indicates the occurrence of meiotic non-disjunction. In addition to the 16 Him genes uncovered by such assays, Him phenotypes are observed in RNAi-based screens and in mutants from unrelated genetic screens. Since him genes are non-essential for viability, him mutations are introduced into strains to create double mutants so that males can be easily obtained. But, it can be challenging to introduce a him mutation into more complicated strains, for eg., double or triple mutants harboring transgenes. More recently, bacterial strains expressing dsRNA corresponding to him sequences have been made available, and this is a good strategy to produce males from more complicated strains. We have also used an RNAi feeding strategy ("male food") to generate large numbers of males from mut-7 and rde-2 homozygotes, males that were then used successfully in numerous genetic crosses. While mut-7 and rde-2 mutants have Him phenotypes, mutant males derived from defective mut-7 or rde-2 disjunction mechanisms are not fertile. By contrast, rde-2 or mut-7 males derived from nondisjunction induced by "male food" (or from genetic crosses to wild-type males) are fertile, as they do not inherit the mut-7 or rde-2 epigenetic defects. Thus, "male food" has proven to be a useful tool, allowing for genetic crosses to be made. We reasoned that we might be able to improve the "male food" methodology by screening for dsRNA-expressing bacteria that are more effective in eliciting X-chromosome non-disjunction. We compiled a list of genes associated with Him phenotypes based on data included in publications and online databases, and we tested bacterial clones from the Source BioScience library to identify the most effective "male food". This project provided us with a better tool for generating males from complicated strains, and as the experiments were relatively simple to perform, it also afforded us the ability to include high school students in our research program.

1220A. Characterization of the *hmgr-1* mutant, which lacks the *C. elegans* Homolog of HMG-CoA reductase. **Parmida Ranji**, Marc Pilon. Dept Chem Mol Bio, Univ of Gothenburg, S-405 30 Gothenburg, Sweden.

The main trunk of the mevalonate pathway is conserved throughout the animal kingdom and consists of the steps through which acetyl-CoA is gradually transformed into isopentenyl diphosphate (IPP), then into farnesyl diphosphate (FPP). FPP can be converted to different important biomolecules including isopentenyl adenosine (important for t-RNA modification), dolichol and dolichol phosphate (important for protein glycosylation), CoQ (a soluble antioxidant that is also part of the respiratory chain in mitochondria), geranylgeranyl diphosphate (GGPP) (lipid moiety that, like FPP, can be attached to proteins to promote membrane association) and cholesterol (precursor for bile acids and steroid hormones). Using *C. elegans* as a model organism enables us to study the non-cholesterol branches of this pathway since the cholesterol branch present in mammals is lacking in *C. elegans* whereas the other branches are retained.

We are characterizing the *hmgr-1(tm4368) mutant*, which bears a 620-bp deletion that spans the first three exons and is likely a null mutant. The *hmgr-1(tm4368)* mutants are not viable in the absence of mevalonate but can be fully rescued by including 20 mM mevalonate in the culture plates. We also designed a transcriptional reporter (*Phmgr-1::GFP*) and a translational reporter *(Phmgr-1::hmgr-1::GFP)* to examine the expression of this gene in *C. elegans*. Based on these reporters, we show that the *hmgr-1* gene is expressed in several tissues including the pharyngeal muscle cells, excretory canals, intestine, spermatheca and the vulva muscle. Incidentally, the translational reporter can rescue the *hmgr-1* mutant, indicating that it is fully functional. In the *hmgr-1(tm4368)* mutant, mevalonate withdrawal results in the activation of unfolded protein response (UPR^{er}) but not UPR^{mt}, as well as a loss of prenylation and muscle mitochondrial defect.

We conclude that *hmgr-1* is an essential gene in *C. elegans* that is expressed in several tissue types and that can be rescued with exogenously supplied mevalonate.

1221B. Expanding the repertoire of mutations amenable to identification by whole-genome sequencing. Sijung Yun, Michael Krause, **Harold E. Smith**. NIH/NIDDK, Bethesda, MD.

Whole-genome sequencing provides a rapid and powerful method for identifying mutations on a global scale. The technique has spurred a renewed enthusiasm for classical genetic screens. In a typical experiment, chemical mutagenesis is followed by screening for a phenotype of interest. The most commonly characterized category of mutation consists of monogenic, recessive traits (due to their genetic tractability) resulting from single-nucleotide polymorphisms (the predominant class of chemically-induced lesions). Therefore, most of the mapping strategies and bioinformatics tools for mutation identification by whole-genome sequencing are directed toward alleles that fulfill these criteria (i.e., single-gene, homozygous SNPs). However, these approaches are not entirely suitable for the characterization of a variety of more challenging mutations: insertions/deletions, dominant/semi-dominant alleles, and multigenic traits. Therefore, we have developed alternative strategies for the identification of each of these classes of mutations. We also report a method for high-resolution mapping in gene-rich regions, and a solution to the technical challenge of sequencing non-conditional lethal or sterile strains. These tools extend the applicability of whole-genome sequencing to a broader spectrum of mutations, including classes that are difficult to map by traditional means.

1222C. Acute, High-throughput RNAi in *C. elegans*. **Elizabeth J Thatcher**, Victor Ambros. Univ of Massachusetts Medical School, Worcester, MA.

C. elegans have previously demonstrated a robust ability to utilize exogenous dsRNA to silence endogenous genes and transgenes by RNA interference (RNAi). Typically, dsRNA is delivered for RNAi by microinjection, soaking in naked dsRNA, or feeding of bacteria expressing dsRNA. Microinjection requires

training and expensive equipment and is usually applied to relatively small numbers of worms. Soaking and feeding are more amenable to higher throughput, although soaking requires a larger quantity of dsRNA, and both feeding and soaking can involve a relatively long time course between application of the dsRNA and when knockdown is observed.

We have developed a method for the use of electroporation to efficiently deliver dsRNA to C. elegans larvae or adults for silencing of somatic or germline genes. Electroporation offers a number of significant advantages over previous methods for RNAi in C. elegans. Electroporation results in rapid delivery of dsRNA to populations of worms without expensive equipment or training. Electroporation requires a lower concentration of dsRNA than does soaking, and results in more rapid knockdown than does feeding or soaking. Electroporation is most effective at long-term knockdown when using long dsRNA rather than short (25 base pairs) dicer substrate RNA or siRNA (22 base pairs, with 2 nt 3' overhangs). Interestingly, short dsRNAs and siRNAs result in acute but transient knockdown (for example of a GFP transgene), whereas long dsRNA results in long-term (and heritable) knockdown. Long dsRNA can be easily *in vitro* transcribed from widely available RNAi plasmids. Electroporation can also be completed using dsRNA extracted from bacteria expressing RNAi plasmids. This allows for efficient screening of RNAi libraries using a 96-well format. Thus, electroporation can be easily employed for rapid and either transient or long-term knockdown of endogenous genes or transgenes on a high-throughput scale.

1223A. Genomic variation data in WormBase. **Mary-Ann Tuli**, Paul Davis, Michael Paulini, Gary Williams, Kevin Howe. EMBL-EBI, Hinxton, United Kingdom.

We continue to see growth in volume and diversity of nematode genomic variation data, in large part due to increasing research effort in whole-genome sequencing (WGS) of numerous *C.elegans* mutant and wild-isolate strains. WormBase have responded to the challenges presented by this growth by making changes to the way in which we curate, store and display variation data. One significant change has been to more-clearly distinguish between naturally-occurring polymorphisms and laboratory-induced mutations at the display level. These are now show in two separate tables on Gene Summary pages, with laboratory-induced alleles identified by the allele designation of the laboratory of origin, and naturally-occurring polymorphisms identified by WormBase variation accessions. We have also begun to consolidate redundant data from independent wild-isolate sequencing projects. Previously, if a specific molecular variation had been identified by multiple independent projects, and/or in multiple strains, a separate variation object would have been created for each. Now, a single reference variation is created which cross-references all studies that have characterised that variation and all strains that carry it. A new version of the WormBase website was launched in early 2012, and we continue to refine and improve the display of variation data. Coloured fields in the Strain widget on the Variation Summary Page clearly show which strains carry a variation and whether the strain is available from the CGC. The Gene Summary Page now allows customisation in the the way Variations are viewed; both variation tables can be sorted by various properties, including type of molecular change, effect on the protein, and the number of associated phenotypes. We have also increased the complement of variation tracks on the genome browser, clearly separating classical alleles from those generated by large-scale sequencing projects, and creating additional tracks for single-nucleotide variants that confer a putative change-of-function on a protein. We we continue to refine the presentation of this data, and welcome feedback from the C.elegans research community.

1224B. A cGMP reporter for *C. elegans*. **Mary Bethke**[1], Chantal Brüggemann[1], O. Scott Hamilton[1], Damien O'Halloran[2], Bi-Tzen Juang[1], Klaus Kruttwig[1], Ben Barsi-Rhyne[3], Mihn Pham[3], Dominique Glauser[4,5], Miriam Goodman[4], Piali Sengupta[6], Miri Van Hoven[3], Noelle L'Etoile[1]. 1) Department of Cell and Tissue Biology, Univ of California, San Francisco, 521 Parnassus Ave., San Francisco, CA 94143-0640, USA; 2) Department of Biological Sciences, George Washington Univ, 2023 G St. NW Washington, DC 20052, USA; 3) Department of Biological Sciences, San Jose State Univ, 1 Washington Square, Duncan Hall, San Jose, CA 95192, USA; 4) Department of Molecular and Cellular Physiology, Stanford Univ School of Medicine, 279 Campus Drive, Stanford, CA 94305, USA; 5) Current Address: Department of Biology, Zoology, Chemin du Musée 10, CH-1700 Fribourg, Switzerland; 6) Department of Biology and National Center for Behavioral Genomics, Brandeis Univ, Waltham, Massachusetts 02454, USA.

Cyclic guanosine monophosphate (cGMP) is a pervasive and critical component of many pathways currently being examined by Caenorhabditis elegans researchers. A small number of cGMP reporters have been developed in other systems but, to date, there are no genetically encoded cGMP reporters available for use in C. elegans. Here we report the development of a genetically encoded single fluorescent protein cGMP biosensor called WincG that expresses stably in ASER and PHB and can be used to elucidate cellular dynamics across a broad range of biological functions. Changes in WincG fluorescence were detected in response to stimuli in living animals in the ASER amphid and PHB phasmid neurons. Expression of the WincG construct did not cause changes in worm behavior. We are currently designing experiments to determine which stimuli evoke optimal responses and further investigating the effect of other stimuli, changes in experimental conditions and expression in mutant backgrounds.

1225C. Endrov, a general imaging framework, to visualize C. elegans gene expression, 4D models, and lineage. Johan Henriksson[1], Jurgen Hench[2], Martin Luppert[1], Akram Abou-Zied[3], Lois Tang[1], Yong-Guang Tong[1], David Baillie[4], **Thomas R. Burglin**[1]. 1) Biosciences & Nutrition Karolinska Institutet Huddinge, Sweden; 2) Dept of Pathology Univ Hospital Basel Switzerland; 3) Dept of Zoology Suez Canal Univ Ismailia, Egypt; 4) Molecular Biology and Biochemistry Simon Fraser Univ Canada.

Events that specify cell fates during embryogenesis are highly dynamic. We have developed an image analysis and microscopy framework, Endrov (www.endrov.net), and used it to create multi-channel 4D recordings of live GFP expression throughout embryogenesis. The software can increase the dynamic range of recordings by changing the camera exposure time over time. We have applied this to homeodomain transcription factors and have so far recorded over 60 homeobox gene expression patterns using DIC and promoter::GFP. We have selected one of these, ceh-5 for in-depth analysis (see poster by Gangishetti et al.). Our data complements the EPIC dataset (Murray et al., Genome Res 2012), with 15 genes in common. We also observed differences, e.g., ceh-14 is detected earlier in our dataset. We have also shown that our recordings correlate with microarray data (Yanai and Hunter, Genome Res 2009). Genes expressed at similar location or time point can be found from clusterings. A website with all the data is currently being set up. Endrov is a general open source image analysis framework, but has many features for the worm community. It allows gene expressions to be extracted and shown on single-cell level, in 3D and on the lineage. In addition to the embryo model (Hench et al., Dev Biol 2009) the 3D EM model of the adult larvae (C. Grove, pers. com.) can be displayed and linked to the lineage. Endrov is also capable of importing the gene expression patterns from the EPIC dataset. Several genes can be overlapped and visualized. Coordinates made using SIMI Biocell can be imported. Lineaging can be done manually and with

automatic lineaging algorithms. Endrov can be used with almost any computerized DIC/fluorescent microscope, making it a versatile tool for many labs. Gene expression and morphology can be studied in wild-type or mutant embryos. Endrov allows quantitative comparison of many expression patterns simultaneously, in 3D and on the lineage.

1226A. A non-trapping method for live imaging of specific neuronal connections. **Muriel Desbois**, Hannes Buelow. Albert Einstein College of Medicine, Bronx, NY.

The nervous system is a complex network that senses and processes information and is essential for the survival of both vertebrates and invertebrates. Information within the network is transmitted through specialized cell-cell contacts, including synaptic connections and GAP junctions. Importantly, the network is not static and is believed to change during development, learning but also during pathological or normal age-related decline. Previous strategies to label specific synapses in living animals 'trap' the synapses by fixing the connections, thus precluding dynamic studies. To circumvent this problem, we are adapting a technique called BLINC (Biotin Labeling of INtercellular Contacts) for live imaging of specific synapses in *C. elegans*. BLINC is based on the biotinylation of an acceptor peptide by the *E. Coli* biotin ligase BirA, both are fused to two interacting proteins. In analogy to work in cell culture, we fused the BirA ligase to neurexin and expressed the fusion in a set of neurons. We then fused the acceptor peptide to neuroligin and expressed this construct in another group of neurons, known to form connections with the former set of neurons. During the formation of synapses/connections between the two groups of neurons, the neuroligin and the neurexin form a complex, leading the ligase to transfer a biotin to the acceptor peptide. This "biotin mark" is specifically detected by a monovalent streptavidin fused to a fluorescent protein which is transgenically secreted from the coelomocytes. In preliminary findings we observe specific staining in living animals consistent with known connections between both sets of neurons. We will report on our progress, but expect that this new technique will allow to visualize formation and dynamics of specific neuronal connections *in vivo* under different experimental conditions.

1227B. *In vivo* calcium imaging of motor circuit during spontaneous locomotion using improved G-CaMPs. **K. Gengyo-Ando[1]**, Y. Kagawa-Nagamura[1], M. Ohkura[1], X. Fei[2], M. Suzuki[3], K. Hashimoto[2], J. Nakai[1]. 1) Brain Science Institute Saitama Univ, Saitama, Japan; 2) Graduate School of Information Sciences, Tohoku Univ, Miyagi, Japan; 3) Microbeam Radiation Biology Gr., JAEA, Gunma, Japan.

Caenorhabditis elegans is a powerful model to investigate how the nervous system generates and regulates locomotion behavior. We previously showed that both body wall muscles and GABAergic DD/VD motoneurons in the ventral nerve cord exhibited increase in Ca^{2+} levels during backward locomotion in unrestraind worms expressing G-CaMP4. To further analyze functional dynamics of the neuromuscular circuit, we have developed a new imaging device in which an auto-tracking unit is integrated into a fast-scanning confocal laser microscope to enable prolonged recording of multicolor fluorescence and transmitted images from freely moving worms. We also applied our newly designed G-CaMPs (G-CaMP6 and G-CaMP7) that allow greater signals than previous versions, to reliably obtain high-resolution images on the single cell levels. Using our imaging system, we found that, in contrast with the DD/VD motoneurons, GABAergic RME motoneurons that innervate the head muscles were persistently inhibited during spontaneous backward movement. Further studies for GABAergic regulation in the locomotion behavior are in progress.

1228C. Mapping the entire connectome of C. elegans L1 larvae. **Valeriya Laskova(*)[1]**, Quan Wen(*)[2], Richard Shalek(*)[2], Daniel Berger[3], Asuka Guan[1,4], Bobby Kasthuri[2], Verena Kaynig-Fittkau[2], Hanspeter Pfister(*)[2], Jeff Lichtman(*)[2], Aravi Samuel(*)[2], Mei Zhen(*)[1,4]. 1) Samuel Lunenfeld Research Institute, Toronto, Canada; 2) Harvard Univ, Cambridge, USA; 3) Massachusetts Institute of Technology, Cambridge, USA; 4) Univ of Toronto, Toronto, Canada.

To investigate the poorly understood mechanisms of development and function of the nervous system, connections between neurons must be deciphered first. The adult nervous system of C. elegans was mapped to near completion by Dr. John White and his colleagues in 1970s. However, neuronal wiring in C. elegans larvae differs from that of an adult, since it undergoes multiple rounds of neuronal birth, apoptosis and rewiring during development. We utilize an Automatic Tape-Collecting Ultramicrotome (ATUM) to section an entire L1 stage animal into thousands cross-sections, followed by the automated imaging with a scanning electron microscope (SEM) to map an entire neuronal wiring diagram with synaptic resolution. The acquired wiring diagram will guide and be validated by calcium imaging to map the functional connection of the juvenile motor circuit.

1229A. Development of a comprehensive image analysis software package for the analysis of lifespan, locomotion, body length, and egg laying of *C. elegans*. **S.K. Jung**, B. Aleman-Meza, C.M. Riepe, W. Zhong. Biochemistry and Cell Biology, Rice Univ, Houston, TX.

It is time-consuming to manually quantify *C. elegans* phenotypes in lifespan, locomotion, body size, and egg laying We developed an automated image acquisition and analysis system to quantify multiple *C. elegans* phenotypes. The imaging system is composed of a microscope equipped with a digital camera, a motorized stage, and image analysis software. The image analysis software package contains Lifespan Assay, Locomotion Assay, WormLength Assay, and Egg Counter software. The Lifespan Assay software counts the number of moving worms using two time-lapse images. Inspired by the Parallel Worm Tracker developed by the Goodman lab, the Locomotion Assay software conducts fully automated video analysis and computes the velocity of moving worms. The WormLength Assay and Egg counter software have been developed for body size measurement and automated egg counting, respectively. This software suite can run on any operating system since they were written in Java. We evaluate the performance of the software by various benchmarks. We also demonstrate the application of the software in a pilot chemical screen.

1230B. WormView: a library of modular Matlab functions for static and dynamic image analysis. **Gunnar Kleemann[1,2]**, Lance Parsons[2], Coleen Murphy[1,2]. 1) Dept Molecular Biol, Princeton Univ, Princeton, NJ; 2) Lewis-Sigler Institute, Princeton Univ, Princeton, NJ.

We present *WormView*, a library of Matlab functions, which can be interchangeably combined to conduct automated image analysis and data visualization. *WormView* is designed to be flexible and easy enough to use that users with little familiarity with programming can construct new applications. The modular architecture of *WormView* allows a user to rearrange interchangeable parts into novel applications without requiring new code generation. At the same time, the open source Matlab code can be easily modified by more advanced users to generate new functions without affecting the other parts of the program. The components of the *WormView* library fall into four progressive functional categories (1) particle identification, (2)

particle verification (optional), (3) shape and time series analysis, and (4) data compilation and figure generation. In a typical analysis, single images or images in a series are passed into *GetWorm*. *GetWorm* collects worm data as binary thresholded images, as well as position, shape and size data. The *GetWorm* output is passed to through an optional quality assurance step involving manual perusal or automated verification by SVM (a support vector machine; machine learning). The processed dataset is then used collect data on the worm path (*WormTrip*), worm posture (*SpineWorm*), and movement patterns. Finally the processed files are passed into a final analysis for compilation of time course data, particle counts, shape and size analysis and figure generation. We will present sample configurations of the library, which can be used to measure aspects of worm shape, size, posture, position and color as they change over time.

1231C. Development and applications of TEM approaches adapted for *C. elegans* research. **Irina Kolotueva**. BIOSIT - UMS 3480, Université de Rennes 1 2 avenue du Pr Léon Bernard, 35043 Rennes, France.

TEM in *C. elegans* has endorsed as a powerful tool for cell and developmental biology questions. A large library of processing techniques together with the publically available image data collections provides a valuable background for further TEM studies. The majority of existing methods still remain time and expertise consuming. The aim of my project is to improve the existing techniques and adapt new techniques, successful in other systems, but underrepresented in *C. elegans* research. Resin embedded sample preparation (chemical or high pressure frozen) is commonly used to study *C. elegans* ultrastructure. Recently we developed a procedure aimed to facilitate correlative microscopy and efficient localization of the region of interest (Kolotuev et al, 2010, *Biol Cell*). Based on this procedure, I developed chemical and HPF protocols adding the upgrades in processing equipment. Modifications in embedding permit a higher resolution of the samples after the embedding and acquisition of high quality DIC images. This in turn allows the recognition of cells and organs in embedded samples in a way similar to live studies, enabling spatial correlation in minimal time. Additional modifications in embedding permit simultaneous sectioning of several worms together, up to twenty in one sectioning day. Large numbers of analyzed samples provide statistically significant numbers, tackling the known disadvantage of TEM analysis. Cryo-preparation by Tokuyasu is an efficient method for better immuno-EM labeling compared to resin embedded samples (Tokuyasu, 1986, *J Microsc*). Though widespread in other fields, Tokuyasu method did not gain popularity in *C. elegans* studies, mainly due to technical difficulties related to sample preparation and sectioning axis orientation. The primary goal was to reach high level of sample preservation and enable reproducible sectioning conditions. Adaptation of existing Tokuyasu protocols for *C. elegans* is possible and gives satisfying tissue morphology. Based on results with standard antibodies, Tokuyasu labeling is more efficient compared to samples embedded in resin. Currently I test the efficiency of specific antibodies in different conditions in collaboration with several *C. elegans* groups.

1232A. Probing intercellular lipoprotein transport in Caenorhabditis elegans by fluorescent nanodiamond imaging. **Yung Kuo**[1], Tsung-Yuan Hsu[2], Yi-Chun Wu[2], Huan-Cheng Chang[1]. 1) Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 10617, Taiwan; 2) Institute of Molecular and Cellular Biology, National Taiwan Univ, Taipei 10617,Taiwan.

Using fluorescence lifetime imaging microscopy (FLIM) and fluorescent nanodiamonds(FNDs) as biolabels, a background-free detection method has been developed to observe the intercellular transport of yolk lipoproteins in Caenorhabditis elegans over a time period of more than 50 min. The yolk lipoproteins in the nematode are similar to human serum low- density lipoproteins, serving as an intercellular transporter of cholesterol and triacylglycerol. FNDs were first coated with yolk lipoprotein complexes (YLCs) and then microinjected into the intestinal cells of the living organism. Real-time imaging with FLIM revealed the process of YLC-FND secretion from the intestine into the pseudocoelomic space, followed by endocytosis into oocytes and subsequent accumulation in developing multi-cellular embryos derived from these oocytes. Colocalization studies of *rme-2* adult hermaphrodites expressing green fluorescent protein (GFP)-tagged YLCs confirmed that the injected YLC-FNDs were taken up by oocytes through the RME-2 receptor-mediated endocytosis. Our results demonstrate for the first time that FNDs are useful as a lipoprotein carrier without significantly altering the functionality of the cargos for intercellular transport, cell-specific targeting, and long-term imaging applications *in vivo*.

1233B. 3-D Worm Tracker for *C. elegans*. **Namseop Kwon**[1], Ara B. Hwang[2], Seung-Jae Lee[1,2], Jung Ho Je[1,3]. 1) School of Interdisciplinary Bioscience and Bioengineering; 2) Department of Life sciences, and World Class Univ Information Technology Convergence Engineering; 3) Department of Materials Science and Engineering, Pohang Univ of Science and Technology, Pohang, Gyeongbuk, 790-784, South Korea.

Various tracking systems have been developed to quantify the behavior of *C. elegans*, a genetic model organism for studying animal behaviors. The tracking systems, however, provide two dimensional (2-D) imaging and thus have limitations in understanding the behaviors of worms freely moving in 3-D environments. Here, we introduce a 3-D worm tracker (3DWT) that is capable of analyzing trajectories and postures of worms crawling in 3-D environments. The prototype 3DWT was based on a stereoscopic imaging system that consisted of two cameras aligned at right angles, recording stereoscopic images of a single worm moving in a 3-D sample chamber. From these two 2-D images, 3-D information such as the trajectories or the postures of the worm in 3-D environments was reconstructed by stereomatching. We found that the trajectories and the postures in 3-D environments are not restricted in a single 2-D plane. This result confirms the idea that the movements of *C. elegans* in 3-D environments are more complex than those of worms moving on agar plates. For analyzing the kinematics of *C. elegans*, we introduced “bending vector” that is defined by the magnitude and direction of the bending at each body part of a worm. The bending vectors with various directions propagated from head to tail during the worms’ forward runs, suggesting that the bending vector can be a useful analytic parameter for understanding complex undulatory patterns of worms in 3-D environments. Currently, we are improving our 3DWT to automatically track a worm by recording the stereoscopic images on a single camera, instead of two cameras, using four mirrors. We are furthermore enhancing the throughput and accuracy of the reconstruction process by incorporating additional camera perpendicularly to the stereoscopic configuration. We believe that our 3DWT would provide new opportunities to analyze the behaviors of *C. elegans* in 3-D environments and would help understand its behaviors in nature.

1234C. WormSizer: Image-based analysis of nematode size and shape. **Brad T Moore**[1], James M Jordan[2], L Ryan Baugh[2,3]. 1) Computational Biology & Bioinformatics, Duke Univ, Durham, NC; 2) Department of Biology, Duke Univ, Durham NC; 3) Duke Center for Systems Biology, Duke Univerity, Durham, NC.

The fundamental phenotypes of growth rate, size and morphology are the result of complex interactions between genotype and environment. We developed a high-throughput software application, WormSizer, which computes size and shape of nematodes from brightfield images. Existing methods for estimating volume either coarsely model the nematode as a cylinder or assume the worm shape or opacity is invariant. Our estimate is more robust to changes in morphology or optical density as it only assumes radial symmetry. This open source software is written as a plugin for the well-known image-processing framework Fiji/ImageJ. WormSizer is designed to be used by biologists, and works with images from a standard dissection microscope. We evaluated the technical performance of this framework, and we used it to analyze growth and shape of several canonical *Caenorhabditis elegans* mutants in a developmental time series. We confirm quantitatively that a Dumpy (Dpy) mutant is short and fat and that a Long (Lon) mutant is long and thin. We show that *daf-2* insulin-like receptor mutants are larger than wild-type upon hatching but grow slow, and WormSizer can distinguish dauer larvae from normal larvae. We also show that a Small (Sma) mutant is actually smaller than wild-type at all stages of larval development. WormSizer works with Uncoordinated (Unc) and Roller (Rol) mutants as well, indicating that it can be used with mutants despite behavioral phenotypes. We used our complete data set to perform a power analysis, giving users a sense of how many images are needed to detect different effect sizes. Our analysis confirms and extends on existing phenotypic characterization of well-characterized mutants, demonstrating the utility and robustness of WormSizer.

1235A. Investigation of Simplified Dual-fluorophore Dissecting Stereomicroscopes. **Andy Papp**, Chris Aldrich. Tritech Research, Los Angeles, CA.

In previous studies (Papp et al., 2009, Papp et al. 2011) we demonstrated that high-power Light Emitting Diodes (LEDs) could be combined with good quality filters to visualize the expression of fluorescent transgenes and produce a low-cost fluorescent dissecting stereomicroscope system. Two of the cost saving methods were: (1) the use of oblique rather than epi- illumination and (2) putting emission filters into the eyepieces and/or camera port rather than using filter cubes with dichroic mirrors. While this system was shown to work well for multiple fluorophores (eGFP, dsRed, and mCherry), it took about 1 minute to change lamps and eyepieces, making it impractical to screen individual moving worms for two fluorophores expressed in the same location (we were able to view eGFP and dsRed simultaneously using a single excitation and emission filter set, but one is likely to obscure the other if they are co-localized).

In the present study, we investigate the use of multiple LEDs and filters in the same lamp assembly and filter sliders to reduce the switchover time between screening for two fluorophores. Preliminary data suggest that the switching can be done in under 3 seconds (a similar time scale to switching filter cubes in a conventional epifluorescence system) without a significant increase in cost or complexity as compared with the original low-cost system.

Papp, Andy et al. (2009) International Worm Meeting "Investigation of Low-cost GFP Microscopy."

Papp, Andy et al. (2011) International Worm Meeting "Investigation of Low-cost Fluorescence Microscopy.".

1236B. High-throughput approaches to motility analysis in *C. elegans* and parasitic nematodes. **Frederick A. Partridge**, David B. Sattelle. Faculty of Life Sciences, Univ Manchester, Manchester, United Kingdom.

Motility is a direct readout of the neuromuscular system, and indirectly of the health of an animal. It therefore presents a useful measurement for high-throughput phenotypic drug screens, whether using parasitic nematodes when searching for much-needed new anthelmintics, or *C. elegans* genetically-engineered to model aspects of human disease. We present algorithms for analysis of worms swimming in microtiter plates.

For worms like *C. elegans* that thrash reasonably rhythmically when swimming, our algorithm can automatically and sensitively determine the rate of thrashing with a throughput of approximately 5000 wells per hour. This analysis is non-invasive and can be performed repeated to determine the motility of worms over their lifespan.

We also present algorithms to determine the mobility of parasitic nematodes, such as *Haemonchus contortus* and *Trichuris muris,* which swim much less regularly than *C. elegans.* We have validated this assay using known anthelmintics, and are now able to screen libraries of compounds searching for lead molecules.

Finally we present our attempts to develop our algorithms further to allow estimation of the motility of multiple individuals within wells, without compromising the speed of our system. This will enable us to measure variation of motility within a population and heterogeneity in responses to applied compounds.

1237C. *C. elegans* imaging by combined, selective plane illumination microscopy and optical projection tomography in a microfluidic device. **M. Rieckher**[1,2], G. Zacharakis[2], J. Ripoll[3], N. Tavernarakis[1,4]. 1) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, 71110 Crete, Greece; 2) Institute of Electronic Structure and Laser, Foundation for Research and Technology-Hellas & Medical School, Univ of Crete, Heraklion, 70013 Crete, Greece; 3) Department of Bioengineering and Aerospace Engineering, Universidad Carlos III de Madrid, Spain; 4) Medical School, Univ of Crete, Heraklion 71003, Crete, Greece.

We present a custom-made combined, selective plane illumination microscopy (SPIM) and optical projection tomography (OPT) system for rapid three-dimensional imaging. OPT eliminates the need for serial optical sectioning of specimens, required with conventional confocal microscopy, and allows for robust, high resolution fluorescence, as well as, absorption imaging of whole specimens. The SPIM system is designed for high penetration depth, low photobleaching and high acquisition speeds when using fluorescently labeled specimens, enabling extended time-lapse *in vivo* experiments. The setup is designed to easily switch between SPIM and OPT and can be used to image fixed mammalian tissue, *Drosophila* embryos and live *C. elegans* animals. The system can be coupled to a variety of microfluidic devices to facilitate high-throughput imaging of *C. elegans* in longitudinal studies of the ageing process. Recorded data sets derived from OPT and SPIM, can be combined to merge absorption and fluorescence images. We apply novel reconstruction algorithms to improve the resolution of 3D images. Our setup offers significant advantages over currently available methods when imaging dynamic developmental processes and animal ageing *in vivo*; it permits monitoring of spatio-temporal gene expression and anatomical alterations with single-cell resolution and can be readily adapted to image a wide range of model organisms.

1238A. High-throughput behavioral imaging reveals the neurons responsible for mechanosensory memory in *C. elegans*. **Takuma Sugi**[1,2]. 1) Institute for integrated cell-material sciences, Kyoto Univ, Japan; 2) PRESTO, JST, Japan.

Behavioral genetic studies in *C. elegans* have provide a new paradigm by unveiling a role of genes and neurons in a neural circuit. The greatest advantage in *C. elegans* behavioral studies is that a variety of transgenic experiments, such as rescue experiments and RNAi-mediated gene disruption, can be done in a cell-specific manner. The throughput of these transgenic studies has been increased by developments of image processing software (Swierczek et al., Nat. Mathods., 2011), which accelerate analysis of acquired data. However, the throughput remains to be limited by behavioral assay itself, because discriminations of transgenic worms from nontransgenic worms are required for each strain before or during data acquisition, due to semi-stable inheritance of extrachromosomal transgenes. Here, I developed a multiplexed behavioral imaging system, which simultaneously quantifies behaviors of various different transgenic strains. In order to illuminate transgenic strains labeled by a fluorescent injection marker on the four assay plates placed side by side, I constructed the optical system by spatially multiplexing the beam from a high-power excitation laser and navigating them to all assay plates. Through the USB-based CCD cameras with the emission filters on each assay plate, I succeeded to record movements of fluorescence as behavior of transgenic strains in a wide-field of view (>30 worms), discriminating them from nontransgenic worms. I thus demonstrated high-throughput behavioral imaging by combining this system with the tapping system that delivers mechanical stimuli simultaneously to all assay plates in a temporally controlled manner and with machine-vision software. I tried to identify a site of action of CRH-1/CREB responsible for memory formation, on mechanosensory behavior. I created a variety of transgenic strains that each express the dominant negative form of CRH-1 under the various cell-specific promoters, and quantified their mechanosensory responses. The behavioral imaging indicated that CRH-1 acts in AVA and AVD neurons, suggesting that these neurons are responsible for mechanosensory memory. These results demonstrated that my system can be applied to high-throughput behavioral genetic studies.

1239B. Imaging lipid depositions with third harmonic generation microscopy. George Tserevelakis[1,2], Evgenia Megalou[3], George Filippidis[1], Barbara Petanidou[1,2], Costas Fotakis[1,2], **Nektarios Tavernarakis**[3,4]. 1) Institute of Electronic Structure and Laser, Foundation for Research and Technology, Heraklion 71110, Crete, Greece; 2) Physics Department, Univ of Crete, Heraklion 71003, Crete, Greece; 3) Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, Heraklion 71110, Crete, Greece; 4) Medical School, Univ of Crete, Heraklion 71003, Crete, Greece.

Lipids are the main components of cell membranes, function as signaling molecules, and are the main energy store of organisms. Excess energy is stored as fat in adipocytes leading to obesity. The energy control and metabolism pathways that control lipid metabolism are still unravelled. In this study, we developed a methodology based on Third Harmonic Generation (THG) imaging to visualize fat deposition in *C. elegans*. This approach alleviates the requirement for staining samples. We excluded the possibility that lipofuscin contributes to the THG signal and instead found that fat is the main contributor of high THG signal in the *C. elegans* intestine. To validate our approach, we showed that fluorescent, following lipid staining with Bodipy 500/510, Nile Red and Oil Red-O, and THG signals colocalized in wild type worms. To further support the efficacy of THG in detecting lipid droplets, we showed that worms deficient for FAT-7 and GLO-1 had fewer lipid droplets, while in DAF-2 had more lipid droplets compared to wild type animals. Finally, we showed that fat accumulated progressively until early adulthood, while it progressively decreased during the later stages of the worm lifespan. Our results demonstrate that THG can reliably and efficiently detect lipid droplets in live nematodes. We anticipate THG to be a versatile alternative to fluorescence and dye-based approaches and a widely used method to detect lipid droplets in various organisms.

1240C. High-speed, high-magnification tracking system for calcium imaging of neurite during free moving. **Y. Tsukada**[1], C. Min[2], X. Fei[2], K. Hashimoto[2], I. Mori[1]. 1) Grad Sch Sci, Nagoya Univ, Nagoya, Japan; 2) Grad Sch Info Sci, Tohoku Univ, Miyagi, Japan.

Genetically encoded calcium indicators enabled to monitor activity of neurons without invasive manipulation; the technique has been shedding light on the unknown relationship between specific neural activity and its function. Combination of calcium imaging and automated tracking system further enabled to monitor activity of neurons even in freely moving worms, thus making it possible to dissect behavioral regulation by neural circuits. However, there are still methodological problems to fully acquire neural activity of freely moving animals. First, tracking in high-magnification needs a very quick stage control to keep a target region in a microscopic field; second, focus change during the tracking is obstacle for acquiring enough quality of fluorescence images for estimation of calcium concentration change. These problems are often apparent when we focus on some of the interneurons, where most of the intensity changes occur in a small neurite region. To solve such problems, we have developed a high-speed tracking system that enables to track freely moving worms with high-magnification (50x) objective lens. Our system tracks an animal with high-speed (120fps) camera and continuous transparent light independent from fluorescence excitation light, thus photo-bleaching of fluorescence probes is reduced though keeping high-speed tracking. In addition, auto-focus system is implemented with three high-speed cameras with different focal depths that enable the system to perform high-speed autofocus by comparing simultaneously acquired images from the different depths. We are currently targeting AIY interneuron because the fluorescence intensity change of this neuron was prominent in the neurites during the calcium imaging of fixed worms. Using ratio-metric and high-sensitive calcium probe GEM-GECO, we are taking fluorescence images of AIY using thermotaxis behavior. The acquired fluorescence images showed enough resolution to capture the targeted neurite in freely moving animals. Our system provided the potential to monitor neural activity at thin region during free movement.

1241A. DiSPIM: time to leave your confocal behind. **Hari Shroff**[1], Yicong Wu[1], Peter Wawrzusin[1], Justin Senseney[2], Robert Fischer[3], Ryan Christensen[4], Anthony Santella[5], Andrew York[1], Peter Winter[1], Clare Waterman[2], Zhirong Bao[5], Daniel Colón-Ramos[4], Matthew McAuliffe[2]. 1) NIBIB, NIH, Bethesda, MD.NIBIB, NIH, Bethesda, MD; 2) Center for Information Technology, NIH, Bethesda, MD; 3) NHLBI, NIH, Bethesda, MD; 4) Dept. of Cell Biology, Yale Univ, New Haven, CT; 5) Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Optimal 3D time-lapse imaging requires microscopy that provides high resolution in all spatial dimensions, high speed, and minimal photobleaching and photodamage. By creating algorithms that register and appropriately combine two perpendicular specimen views, we developed a dual-view inverted selective plane illumination microscope (diSPIM) with 330 nm isotropic resolution and 200 Hz acquisition, enabling rapid 3D imaging at 2 volumes/second. Unlike spinning-disk confocal or Bessel beam illumination methods that dose the sample outside the focal plane, leading to significant photobleaching, we

maintain this spatiotemporal resolution over hundreds of volumes (hundreds of thousands of images) with negligible photobleaching. DiSPIM enables the study of biological systems that are intractable using other optical microscopes, including 4D microtubule tracking in live cells, improved nuclear imaging over 14 hours of nematode embryogenesis, and visualization of neural wiring during C. elegans brain development.

1242B. Lipid droplets distribution in different developmental stages of C. elegans by using Coherent Anti-stoke Raman Scattering (CARS) microscopy. **Yung-Hsiang Yi**[1,7], Cheng-Hao Chien[3,4,7], Wei-Wen Chen[3,5,6], Tian-Hsiang Ma[1], Kuan-yu Liu[2], Yu-sun Chang[1], Ta-Chau Chang[3,8], Szecheng J. Lo[1,2,8]. 1) Molecular Medicine Research Center, Chang Gung Univ, Kwei-Shan, Tao-Yuan,Taiwan, 333, R.O.C; 2) Department of Biomedical Sciences, College of Medicine, Chang Gung Univ, Kwei-Shan, Tao-Yuan 333,Taiwan, R.O.C; 3) Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106, Taiwan, R.O.C; 4) Institute of Biophotonics, National Yang-Ming Univ, Taipei 112, Taiwan; 5) Molecular Science and Technology Program, Taiwan International Graduate Program, Institute of Atomic and Molecular Sciences, Academia Sinica, Taipei 106, Taiwan; 6) Department of Chemistry, National Tsing Hua Univ, Hsinchu 300, Taiwan; 7) equal contribution; 8) corresponding author.

Lipid droplets (LDs) are intracellular organelles that are very important for energy storage in all kind of organisms. Here, we use a biophotonic tool named Coherent Anti-stoke Raman Scattering (CARS) microscopy which is a dye-free and non-invasive method to monitor LDs distribution in different developmental stages of living C. elegans. We found that the amount and size of LDs sequentially increased in intestine cells from L1 stage to adult worm. The lipid concentration of LDs in intestine cells and body wall sequentially increased from L1 to L4 stage. Interestingly, we found the lipid concentration of LDs in intestine wall significantly decreased in adult worms and this phenomenon not occurred in body LDs. Our data indicated that LDs in body or intestine have different fate during development and most lipid storage in intestine cells is depleted in adult C. elegans. It suggests that the energy (lipid) storage in larval L4 stage may play an important role to support gonad development and reproduction in C. elegans.

1243C. Towards correlated localization of synaptic proteins at light and electron microscopy using a new generation of quantum dots. **Hong Zhan**[1], Michel Nasilowski[2], Bénoît Dubertret[2], Christian Stigloher[3], Jean-Louis Bessereau[1]. 1) Institut de Biologie de l'École Normale Supérieure, Paris, France; 2) Laboratoire de Physique et d'Étude des Matériaux, ESPCI, Paris, France; 3) Theodor-Boveri-Institute, Univ of Wüzburg, Germany.

At the active zone (AZ) of chemical synapses depolarization of presynaptic terminals triggers the opening of Voltage-Dependent Calcium Channels (VDCCs). The rise of calcium concentration that can trigger the fusion of synaptic vesicles (SVs) is restricted to nanodomains within less than a 100 nm from the VDCCs. Therefore, both the number and the distribution of VDCCs are crucial parameters that control synaptic efficiency. Using electron tomography we previously demonstrated that SVs docked at the plasma membrane are retained in close contact with the dense projection of C. elegans neuromuscular junctions. However, the relative distribution of docked SVs and VDCCs at these synapses is still enigmatic. To localize VDCCs in 3D with a nanometer resolution we are implementing a novel approach combining in vivo labeling of VDCCs via genetically-encoded extracellular epitope tags and electron tomography. In a first step we genetically introduced a GFP tag at the extracellular N-terminus of UNC-36, the a-2-d subunit of VDCCs, without impairing channel function. We then use an improved generation of multishell quantum dot (QD) as both fluorescent and electron dense probe. QDs are coupled to anti-GFP antibodies and injected in the pseudoceolomic cavity of C. elegans to label the VDCCs present at NMJs. In order to have access to near-to-native ultrastructure, worms are instantly immobilized by high pressure freezing and subsequently freeze substituted. Worms are imaged with light microscopy just before freezing, which provides a means to later image region of interest by transmission electron microscopy and visualize QD distribution. Using electron tomography we should eventually get access to the tridimensional distribution of VDCCs at the active zone with a few nanometer resolution. Current progress will be presented at the meeting.

1244A. Robust Head Versus Tail Determination to Facilitate High Throughput Image Processing and Automation Techniques in Caenorhabditis elegans. **Mei Zhan**, Hang Lu. Georgia Institute of Technology, Atlanta, GA.

Many new technologies to expedite experimentation in the model organism Caenorhabditis elegans rely upon image processing techniques to extract relevant information and enable automated decision-making in the experimental platform. In both high resolution imaging studies and low magnification platforms to observe and stimulate freely moving worms, one of the fundamental necessities of many automation routines is the ability to quickly distinguish between the head and the tail of the worm. While many groups have individually devised methods to distinguish between the head and the tail of the worm, these techniques often involve the generation of strains with specific fluorescent head or tail markers, the use of fluorescent imaging and the use of specific heuristics or trained classifiers for determination. These methods have the disadvantage that they are often strain-, age- and application-specific and not easily generalizable across experimental platforms and genetic backgrounds. The use of fluorescent imaging in the determination also photobleaches the specimen unnecessarily and necessitates the opening/closing of shutters or changing the state of the excitation sources. Finally, while the use of trained classifiers may facilitate robust delineation between head and tail in applications of interest, these algorithms are often highly sensitive to slight deviations from the training conditions and must be re-trained to accommodate new conditions of interest. Therefore, there still exists a need for a robust, generalizable head-tail classifier that can be performed using only brightfield imaging of a specimen. Here, we propose and validate a computationally and conceptually simple and fast algorithm that is easily scaled for different magnifications and camera formats and is orientation, age and strain independent. Moreover, it is based upon identifying simple, strain and age consistent features in the pharynx of the animal and requires no training. We demonstrate the utility and generalizability of the algorithm by high fidelity head versus tail classification in both high resolution images of C. elegans at different magnifications and ages and low magnification videos of freely moving worms.

1245B. BLIND CEL-Seq: Employing multiplexed single-cell transcriptomics for high-resolution developmental time courses. **Leon Anavy**, Michal Levin, Sally Khair, Tamar Hashimshony, Itai Yanai. Department of Biology, Technion - Institute of Technology, Haifa, Israel.

Developmental transcriptomic analyses are typically carried out on a few samples, each comprising pools of morphologically- or temporally- staged embryos. Our CEL-Seq method enables affordable analysis of hundreds of samples, each comprised of a single embryo, or even a single cell. To demonstrate its usefulness, we generated an embryonic transcriptome timecourse at 10-minutes resolution for 80 individually collected C. elegans embryos, spanning the fertilized egg and the first larvae. To analyze such data we also developed a computational framework - Basic, Linear, INdexing,

Determination (BLIND) - which provides a summary-look, detects outlier samples, and corrects the ordering of the samples. BLIND uses Principal Components Analysis (PCA) and is based upon the observation that in a plane of the first two PCs, the position of the 80 C. elegans embryos traces the progression of development. The "order" of the embryos along development can thus be determined by identifying the shortest path among the samples. This notion is also borne out in six previously published embryonic timecourses of diverse species. BLIND allows for the random isolation of hundreds of samples followed by their relative developmental ordering in the analysis stage. We further found that transcriptomic entropy increases with developmental time allowing for the determination of the arrow of time in the timecourse ordering. BLIND CEL-Seq thus unlocks the power of high-resolution single embryo transcriptomic analyses for species with asynchronous development as well as synchronous ones.

1246C. Cell type-specific profiling of the transcriptome in *Caenorhabditis elegans*. **Meenakshi K. Doma**[1], Igor Antoshechkin[2], Paul W. Sternberg[1]. 1) Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, CA; 2) Millard and Muriel Jacobs Genetics and Genomics Laboratory, California Institute of Technology, Pasadena, CA.

Cell type-specific gene expression patterns underlie much of biology including development, physiology and behavior. However, current strategies for analysis of cell-specific gene expression in *C. elegans* do not allow monitoring of gene expression changes in a specific cell type within its native environment. Here, we present a new strategy for biosynthetic labeling of newly synthesized RNA from specific cells in *C. elegans* by spatially restricted expression of the uracil phosphoribosyltransferase (UPRT) from *Toxoplasma gondii*. We show that on providing the uracil analog, 4-thiouracil (4TU), only the cell types expressing *TgUPRT* will efficiently incorporate 4TU into newly transcribed RNA, thereby covalently labeling cell type-specific newly synthesized RNA, during Pol II transcription. We have purified and sequenced thiol-labeled RNA from a specific cell type. Our on-going efforts will be directed at purification and sequencing of thiol-labeled RNA from multiple cell types in specific biological settings. The most important advantage of this approach is that the production of the labeled-RNA occurs *in vivo*, thereby preserving the gene expression profile in a specific spatial and temporal context in *C.elegans*.

1247A. A high-throughput mechanism-based toxicity screen using *C. elegans*. **RB Goldsmith**[1], JR Pirone[2], WA Boyd[1], MV Smith[2], JH Freedman[1]. 1) DNTP/NIEHS/NIH, RTP, NC; 2) SRA International Inc., Durham, NC.

To quantitatively assess the effects of chemical exposures on transcription, an automated high-throughput *in vivo* toxicity assay was developed. This assays measures changes in the levels and cell-specificity of expression of individual genes in *C. elegans*. Transcriptional responses were measured in individual strains of transgenic *C. elegans* that express fluorescent proteins under the control of archetypical stress-inducible target genes: *ced-3, cyp-35A2, gcs-1, gst-38, gst-4, hsp-16.2, hsp-16.4, hsp-17, hsp-4, hsp-6, hsp-60, mtl-2, ugt-1* and *ugt-13*. As part of assay development, transgenic nematodes with large dynamic ranges (i.e., low constitutive levels of expression), sensitivity (high response at low concentrations) and specificity (concentration dependent response) were identified following exposure to archetypical toxicants: juglone, an oxidative stressor; *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, a DNA damaging agent; cadmium, a heavy metal; chlorpyrifos, an organophosphate neurotoxin; tunicamycin, an endoplasmic reticulum stressor; and heat shock, a protein denaturant. Concomitant with chemical exposure, florescence data were measured from images acquired using a high content imager and subsequently analyzed using CellProfiler's WormToolbox, high-throughput imaging analysis software designed for use with nematodes. For some of the target genes, high levels of constitutive expression limited their dynamic range and utility in this assay. As expected, not all of the chemicals affected all of the genes; however, the chemicals generally affected the stress-inducible genes that corresponded to their known mechanisms of action. For example, nematodes containing the *hsp-4* transgene, which responds to endoplasmic reticulum stress, showed increased fluorescence after tunicamycin treatment. In addition, strong correlations were observed between chemical concentration and fluorescent signal. The results obtained using the transgenic *C. elegans* were verified by measuring changes in the cognate steady-state mRNA levels by qRT-PCR. These results show that this assay can be used to assess the effect of toxicants on gene expression *in vivo*.

1248B. 3' End Profiling of Gametogenesis in C. elegans Using RNA-Seq. **Michelle Gutwein**[1], Desirea Mecenas[1], Rina Ahmed[1,2], Paul Scheid[1], Fabio Piano[1,3], Kris Gunsalus[1,3]. 1) NYU, New York, NY; 2) MDC, Berlin, DEU; 3) NYUAD, Abu Dhabi, UAE.

Post-transcriptional regulation of gene expression is largely mediated through sequence elements in the 3'UTR of protein-coding genes. One of our major interests is to understand post-transcriptional regulatory mechanisms during development. The C. elegans germline provides an excellent model to study post-transcriptional regulation because it is the primary determinant of gene expression in this organ (Merritt et al., Curr Biol 2008). We would like to characterize differences in 3'UTR isoform usage throughout gametogenesis by profiling 3'UTR ends in mitotic and meiotic regions of the gonad as well as in oocytes as a first step toward analyzing the contribution of putative regulatory elements in different regions of 3'UTRs.

Next-generation sequencing has been useful to provide a deep sampling of transcriptional landscapes. However, transcript 3' termini are under-represented using standard RNA-seq library preparation protocols, rendering targeted analysis challenging. A number of specialized protocols to characterize the exact position of 3'end cleavage and polyadenylation have been developed, but they generally require larger sample sizes than are practical to extract from very specific tissues or cells in model organisms like C. elegans.

Our aim is to develop new protocols that optimize 3'UTR endpoint analysis using small sample sizes for tissue-specific profiling with RNA-seq analysis. We have designed and tested a library preparation protocol for linear amplification and 3' end capture followed by deep sequencing on the Illumina HiSeq. We perform paired-end sequencing using a non-standard protocol designed specifically to avoid issues with sequencing the low-complexity polyA tail of transcripts. Here we present our progress in using this protocol with samples of RNA extracted from whole male and hermaphrodite N2 gonads, as well as dissected mitotic and meiotic regions and oocytes from hermaphrodite gonads in order to characterize 3'UTR diversity during germline development.

1249C. Ecotoxicity of anatase and rutile TiO2 nanoparticles on C. elegans in dark condition. **Chun-Chih HU**[1], Gong-Her Wu[2], Hsieh-Ting Wu[1], Oliver I. Wagner[2], Ta-Jen Yen[1]. 1) Department of Materials Science & Engineering, National Tsing-Hua Univ, Hsinchu, Taiwan; 2) Institute of Molecular & Cellular Biology, National Tsing-Hua Univ, Hsinchu, Taiwan.

Due to the widespread applications of titanium dioxide (TiO2) nanoparticles (NPs) in food, cosmetics and medical devices, it is receiving increasing

attention to evaluate its toxicities to the environment. Many toxicity studies using different organisms have concluded that the toxicity of anatase TiO2 NPs is based on its photocatalytic effect. However, the toxic effect of anatase TiO2 in the dark condition as not been evaluated. Recently, it has been shown that rutile TiO2 nanotubes are able to induce the differentiation of neuronal rat cells and provide decent biocompatibility. The most elusive question has been whether TiO2 NPs exert direct toxic effects to organisms. Here, we examine the ecotoxicity of both anatase and rutile TiO2 NPs on nematode C. elegans in dark condition. Our results indicate that both anatase and rutile TiO2 nanoparticles retard overall population, inhibit worm growth as well as body length of worms. We further evaluate the effect of anatase and rutile TiO2 NPs on worm locomotion and found that motility is highly affected by both TiO2 forms concluding that motor neurons may be effected. Indeed, we found that dendrite and axon growth in primary neuronal cultures is affected by TiO2 NPs. Moreover, micro-array analysis revealed an -6 fold downregulation of mtl-2, which encodes for a metallothionein protein that plays a role in regulating growth and fertility of worms consistent with our finding that TiO2 NPs reduce the worm population. It is also notable that MTL-2 plays a role in metal detoxification underlying the importance of this protein when TiO2 NPs appear to be increased in the environment.

1250A. Synchronization of *C. elegans* embryos. **Olga Minkina**, Megan Senchuk, Susan Mango. Molecular and Cellular Biology, Harvard Univ, Cambridge, MA.

The formation of a multicellular animal depends on exquisitely-timed processes such as fertilization, gastrulation, cell-fate specification and organogenesis. A molecular analysis of the mechanisms that drive developmental progression requires isolation of precisely staged embryos, which remains an ongoing technical challenge for the field of developmental biology. While the rapid fourteen-hour embryonic development of *C. elegans* makes it a well-suited organism for the study of embryogenesis, it also poses a challenge for the isolation of a large, synchronized population of embryos for molecular and biochemical analyses. We describe a new approach to obtain large numbers of well-synchronized *C. elegans* embryos, and we compare this method to three previously described techniques.

1251B. Ribosome profiling reveals features of C. elegans longevity. **Kristan K. Steffen**, Andrew Dillin. Howard Hughes Medical Institute and Department of Molecular and Cell Biology, Univ of California - Berkeley, Berkeley, CA.

Throughout evolution and in nearly all cell types, protein synthesis consumes more energy than any other biosynthetic process. Consequently, mRNA translation by the ribosome is highly regulated at multiple levels. While it is clear that altered translation by a wide variety of genetic or environmental interventions results in enhanced longevity in multiple model organisms, little is understood about how protein synthesis is regulated as organisms age. To gain insight into this process, we have adapted the ribosome profiling technique for C. elegans and are using it to identify regulatory features of mRNA translation at unprecedented resolution. Based on the deep sequencing of ribosome-protected mRNA fragments, ribosome profiling data from young and aged C. elegans has revealed novel features of protein synthesis, including translation of 5'UTR sequences, ribosome stalling, indications of translation occurring at regions annotated as noncoding, and translation of specific gene isoforms. Furthermore, ribosome profiling of long-lived C. elegans lacking a translation initiation factor has identified features of altered translation that provide insight into the mechanisms by which these animals live long. For multicellular organisms to survive during aging, individual tissues must coordinate their physiological activities to maintain the health of the whole animal. To better understand the contributions of individual cell and tissue types to the aging process, we have employed a ribosome tagging strategy to isolate tissue-specific mRNA messages by affinity purification. It is becoming increasingly clear that specific tissues within C. elegans can transmit longevity signals to modulate the aging process of the entire animal. Using our ribosome tagging strategy, coupled with ribosome profiling, we are working to identify key features of cell non-autonomous longevity responses.

1252C. Conserved ion and amino acid transporters identified as phosphorylcholine modified N-glycoproteins by metabolic labeling with propargylcholine in *Caenorhabditis elegans*. Casey Snodgrass, Amanda Burnham-Marusich, John Meteer, **Patricia M. Berninsone**. Biology Department, Univ Nevada, Reno, NV.

Phosphorylcholine (Pc) modification of proteins by pathogens has been implicated in mediating host-pathogen interactions. In parasitic nematodes, Pc modulates the host's immune response to favor nematode survival. *Caenorhabditis elegans* expresses Pc-modified N-linked glycans, offering an attractive non-parasitic model to study the biology of Pc-modification, but a lack of selective purification tools limits the number of known Pc targets. Here we show that Pc-modified N-glycoproteins can be identified in *C. elegans* embryonic cells by metabolic labeling with propargylcholine, an alkyne-modified choline analog. Cu(I)-catalyzed cycloaddition with biotin-azide enables streptavidin purification, high-throughput liquid chromatography and mass spectrometry identification of propargyl-labeled proteins. We report 21 novel Pc-modified N-glycoproteins and their sites of Pc-N-glycan attachment. Ion and amino acid transporters as well as synaptic proteins are among those identified as Pc-modified, suggesting a function for Pc beyond immunomodulation. This work provides a method to study Pc-modified proteins in *C. elegans* and related nematodes.

1253A. Analysis of protein-protein interaction by in vivo quantitative proteomics during C. elegans embryogenesis. **Jia-Xuan Chen**[1], Florian E. Paul[1], Miyeko Mana[2], Kris Gunsalus[2], Fabio Piano[2], Matthias Selbach[1]. 1) Max Delbrück Center for Molecular Medicine, 13125 Berlin, Germany; 2) New York Univ, New York, NY 10003, USA.

Protein-protein interactions (PPIs) are crucial for most biological processes. Many proteins exist as components of dynamic protein complexes which execute a plethora of cellular functions. Most studies on PPIs have so far used cell culture methods. Studying PPIs in an in vivo system provides the chance to reveal important information in a specific and functionally relevant biological context. To gain insights into the complex biological processes during early development of C. elegans, we developed an in vivo quantitative proteomics approach to study PPIs using C. elegans embryos. We combined label-free quantitative mass spectrometry with co-immunoprecipitation plus transgenic techniques to screen for the interaction partners of some key player proteins during early embryogenesis. In a proof-of-principle experiment using a P-granule component protein (CAR-1) as bait, we identified a number of novel putative interactions along with known interaction partners of CAR-1 and many other previously known P-granule components. A benchmark analysis confirmed the accuracy of our label-free quantification, although false positives remain a potential problem. Further bioinformatic analyses are consistent with previous findings that CAR-1 is involved in embryonic cell division, germ cell development and P-granule related processes and also suggest novel

roles of CAR-1 during embryogenesis. These results provide interesting candidates for follow-up experiments to explore new functional roles of CAR-1 during embryogenesis and suggest our approach can be adapted to studying interaction partners of a wider range of proteins in vivo.

1254B. Quantitative identifications of temperature-sensitive gene product by using proteomic analysis. **Narumi Enna**[1], Kanami Monobe[1], Yusuke Ishido[1], Yukako Tohsato[1], Toshiya Hayano[2], Masahiro Ito[1]. 1) Depart. of Bioinfo., College of Life Sci., Ritsumeikan Univ; 2) Depart. of Biomed. Sci., College of Life Sci., Ritsumeikan Univ.

Caenorhabditis elegans is a multicellular model with the optimum culture temperature of 20 °C. We focused on culture temperatures of *C. elegans*, because although a temperature of 25 °C is not lethal, it affects *C. elegans* phenotypes such as life cycle and number of eggs. Therefore, a culture environment with a temperature of 25 °C is extremely harsh for *C. elegans*.

We performed quantitative proteomic analysis at 3 developmental stages (early embryo, larval 1, and adult) of *C. elegans* cultured at 25 °C. For this purpose, we used the shotgun method with isobaric tags for relative and absolute quantitation (iTRAQ). iTRAQ analysis was performed 3 times at each of the 3 developmental stages. Proteins that were identified 2 or more times were subjected to further analysis to obtain highly reproducible data.

In this study, in total, 1,849 proteins were identified by iTRAQ analysis. However, of these, 1,228 proteins were identified 2 or more times. The expression levels of these 1,228 proteins were analyzed using the Tukey-Kramer method (multiple comparison test). The number of proteins that showed significant differences in the expression levels among the 3 developmental stages was 339: 128 in the embryo, 101 in the larval 1, 60 in the adult stage, and 50 in the multi stages. We focused on the 128 embryo-specific proteins, because the cell fate of *C. elegans* is determined at the early embryo stage. Surprisingly, approximately 30% (33 proteins) of these embryo-specific proteins were ribosomal proteins. According to the Wormbase database, the phenotype of 29 of these ribosomal proteins was embryonic lethal.

1255C. Comparative proteomics and transcriptomics in two *C. elegans* wild-type strains. **Polina Kamkina**[1,2], Michael Daube[1], Bernd Roschitzki[3], Jonas Grossmann[3], Rita Volkers[4], Basten Snoek[4], Jan Kammenga[4], Sabine Schrimpf[1], Michael Hengartner[1]. 1) Institute of Molecular Life Sciences,Univ of Zurich, Zurich, Switzerland; 2) Ph.D. Program in Molecular Life Sciences, Zurich, Switzerland; 3) Functional Genomics Center Zurich, Univ of Zurich and ETH Zurich, Zurich, Switzerland; 4) Laboratory of Nematology, Wageningen Univ, Wageningen, The Netherlands.

Natural variation of gene expression is a driving force for evolution. To investigate how naturally occurring alleles contribute to changes in gene expression, we compared mRNA and protein abundances in two divergent wild-type strains of *C. elegans*, N2 and CB4856. Proteins were isolated from staged L4 larvae and quantified by shotgun proteomics using SILAC labelling, whereas mRNAs were quantified using microarrays. We identified on average 3098 proteins in each SILAC experiment. Most of the proteins were highly abundant based on protein expression data in the PaxDb database. 2658 proteins were quantified in at least two biological replicates and used for further analysis. Surprisingly, only 23 proteins showed reproducible two-fold differential expression in at least two experiments (10 of them were detected in all three experiments); 7 proteins were down-regulated and 16 proteins were up-regulated in CB4856. According to the gene ontology enrichment analysis, 6 proteins play a role in developmental processes and reproduction, 8 proteins were assigned to metabolic processes, and 9 proteins have an unknown function. Interestingly, many more differences were seen at the transcriptome level than at the proteome level in N2 and CB4856. The data will be further used to generate gene interactive networks and to map the loci responsible for the observed changes in gene expression. This project is supported by the EU-FP7 HEALTH project PANACEA, contract nr. 222936.

1256A. Studying the effect of natural variation on protein abundance in C. elegans. **Kapil Dev Singh**[1], Polina Kamkina[1], Bernd Roschitzki[2], Mark Elvin[3], Miriam Rodriguez[4], Gino Poulin[3], L. Basten Snoek[4], Jan Kammenga[4], Sabine Schrimpf[1], Michael Hengartner[1]. 1) Institute of Molecular Life Sciences, Univ of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland; 2) Functional Genomics Center Zurich, Univ of Zurich and ETH Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland; 3) Faculty of Life Sciences, The Univ of Manchester, Oxford Road, Manchester, M13 9PL, UK; 4) Laboratory of Nematology, Wageningen Univ, 6708 BP Wageningen, The Netherlands.

Cancer, neurodegenerative diseases and autoimmune diseases are complex, polygenic pathologies caused by a combination of genetic and environmental factors, including lifestyle. Many of the signaling pathways (i.e. Apoptosis, Notch, MAPK and Wnt) involved in these diseases are conserved and also present in C. elegans. We are studying the influence of natural genetic variation on the abundance of proteins participating in these four signaling pathways in C. elegans. From the two genetically divergent wild-type strains Bristol N2 and Hawaii CB4856, 200 recombinant inbred lines were previously generated. Transcriptome analysis of these RILs showed significant heritable variation in gene expression at the mRNA levels, but very little is known about heritable variation at the protein level. To determine the effect of natural variation on protein abundance, we developed SRM assays for 156 proteins from the above mentioned signaling pathways. SRM measurements were acquired on a TSQ Vantage for two biological replicates of N2 and CB4856 and for one sample of four RILs (WN 31, WN 71, WN 105 and WN 186) at developmental stage L4. So far, by an automated data analysis using mProphet, 45 proteins (mostly represented by 1 or 2 peptides) could be quantified in all samples. Proteins with significant differences in abundance will be further confirmed in biological replicates of the RILs and QTL analysis will be done to determine the genetic locus (loci) responsible for the difference in protein abundance. Finally, these modifier loci will be crossed into sensitized genetic backgrounds to determine their potential to influence developmental signaling. This work is supported by the EU-FP7 HEALTH project PANACEA, contract nr. 222936.

1257B. in vivo isolation of telomeric proteins in the nematode Caenorhabditis elegans. **Sanghyun Sung**[1], Beomseok Seo[1], Junho Lee[1,2]. 1) Department of Biological Sciences and Research Center for Functional Cellulomics, Seoul National Univ, Seoul, South Korea; 2) World Class Univ Program Department of Biophysics and Chemical Biology, Seoul National Univ, Seoul, South Korea.

Telomeres are special nucleoprotein complexes that cap the ends of the eukaryotic linear chromosomes to maintain genomic integrity. Many interesting aspects of the telomeres of Caenorhabditis elegans have been studied; for example, the extension of organismic lifespan by longer telomeres and alternative lengthening of telomeres at the organismic level have been reported. It is conceivable that the protein components of the telomeres may have critical roles in these processes as regulators of length, stability, and yet unknown interesting mechanisms of telomeres. Because most telomeric proteins found in higher mammals (or even yeasts) do not have homologous partners in C. elegans, there is a strong need for biochemical approaches to identify

them in C. elegans. Previously, we used a nucleic acid affinity capture method to isolate specific proteins associated with telomeric DNA. Although this in vitro method was useful, it had limitations for describing all proteins that may act in the in vivo context. Therefore, in this study, we utilized the recently developed technique called proteomics of isolated chromatin segments(PICh). PICh uses unique nucleic acid to capture the endogenous segments of chromatin which are fixed before hybridization, so it can be a direct and quantitative tool. Currently, we are investigating the telomere-specific proteins of wild type C. elegans.

1258C. *C. elegans* metabolomics as a strategy in the fight against neurodegenerative diseases. **Liesbet Temmerman[1]**, Roel Van Assche[1], Bart P. Braeckman[2], Ute Roessner[3], Liliane Schoofs[1]. 1) Functional Genomics and Proteomics, Univ of Leuven, Belgium; 2) Laboratory for Aging Physiology and Molecular Evolution, Ghent Univ, Belgium; 3) Metabolomics Australia, Univ of Melbourne, Australia.

As average longevity increases in the human population, we are faced with an astonishing growing number of persons suffering from neurodegenerative diseases, in which the functionality or presence of certain neurons is impaired. Recognizing the enormous emotional and financial impact of these diseases on society, researchers are trying to gain insight into the (mal)functions of their pathology. This has in many cases led to the identification of genes that cause or forebode neurodegenerative diseases, resulting in improved diagnosis. In spite of these efforts, we only poorly understand the actual pathological mechanisms, painfully reflected in the lack of a cure or long-term effective treatment for most of these diseases.

The biochemical changes underlying early disease manifestation can best be studied using metabolomics, an approach that aims at identifying and quantifying all given metabolites in a biological sample. Because this method is extremely sensitive to environmental influences and therefore error-prone; highly controllable model systems are needed in order to exploit its full potential. We used a highly controllable *C. elegans* model for Alzheimer's disease in combination with mass spectrometry-based metabolomics (GC-MS & LC-MS) to explore its potential for neurodegenerative disease research. We established a method for sampling and fast generation of specific fingerprints, and interpreted our results in light of known metabolic changes in human disease manifestation. *C. elegans* can be a valuable model to significantly speed up fundamental research into the pathology of several debilitating diseases, currently afflicting millions worldwide.

1259A. Tissue-specific purification of protein complexes in *C. elegans*. **S. Waaijers[1]**, A.D. Zoumaro-Djayoon[2], S. Goerdayal[2], J. Muñoz[2], A.J. Heck[2], M. Boxem[1]. 1) Developmental Biology, Utrecht Univ, Utrecht, Netherlands; 2) Biomolecular Mass Spectrometry and Proteomics Group, Utrecht Univ, Utrecht, Netherlands.

Identifying the composition of protein complexes is essential to understand their biological function. Systematic efforts to purify protein complexes have mostly focused on single cell systems such as yeast and tissue culture cells. However, multicellular organisms consist of multiple cell types in which proteins may be part of distinct complexes. To obtain accurate information on the complexes formed *in vivo*, it is necessary to purify protein complexes from specific tissues.

We developed an affinity purification/mass spectrometry (AP/MS) based approach to enable tissue-specific protein purification. This allows us to identify and compare protein complexes from distinct cell types of *C. elegans*. We are currently applying this method to members of the PAR, Crumbs, and Scribble polarity groups. Several of these proteins are thought to act as scaffolds that recruit multiple protein partners, and many protein interactions likely remain to be discovered.

Our strategy is based on the *in vivo* biotinylation of a protein of interest tagged with GFP and an Avi tag, a small 15 aa peptide that can be recognized and biotinylated by the bacterial biotin ligase BirA. We use recombineering of fosmids for tagging to keep the gene of interest under control of its own regulatory sequences. The presence of GFP allows us to verify expression and localization of the fusion construct. When available, a mutant background is used to test for rescue by the fusion construct and to prevent incorporation of the endogenous wild type protein in protein complexes.

To obtain tissue-specific biotinylation, we cross animals expressing tagged proteins with strains expressing BirA from tissue-specific promoters. Protein complex members purified using streptavidin beads are then identified by mass spectrometry. Using this system, we were able to purify proteins when co-expressed with BirA, while no protein is purified if BirA is expressed in a neighboring tissue. We are currently processing worm strains with tagged DLG-1, LET-413, CDC-42, PAR-3, and LGL-1.

1260B. Analysis of immunogenic proteins in *C. elegans* (V). **A. Yamakawa[1]**, K. Susaki[1,2], Kei Onishi[1], H. Moriwaki[1], A. Aota[1], Y. Hashizume[1,3]. 1) Dept Material Sci, Wakayama National Col, Gobo, Japan; 2) Dept Environmental and Life Sci, Toyohashi Univ of Tech, Yoyohashi, Japan; 3) Tokyo Univ of Agri and Tech, Tokyo, Japan.

Many types of anti-*C. elegans* antibodies are contained in the serum from the mouse immunized with a whole body of *C. elegans*. Each type of them is able to bind to each of specific protein (immunogen) and is useful to find out it from the *C. elegans* proteins. In addition, the population of each type of them in serum may be closely related to the amount of each type antibody producing cells in the spleen from immunized mouse.

To pick up the highly immunogenic proteins from *C. elegans* proteins, we have made an effort to get a number of monoclonal antibodies (MAbs) against *C. elegans* proteins and investigated about a population of antibody types in these MAbs as follows.

At first, to get a number of MAbs, we immunized Balb/c mice with crude extracts from heterogenous population of *C. elegans*, and prepared the antibody producing cells after the fusion of spleen cells from these mice and P3U1 myeloma cells. Up to the present, 45 kinds of anti-*C. elegans* MAbs have been selected. In these, type A antibody (11 kinds of MAbs) binds to a single 180 kDa-protein, type B antibody (4 kinds of MAbs) binds to a single 130kDa-protein, type C antibody (2 kinds of MAbs) binds to a single 90kDa-protein, type D antibody (2 kinds of MAbs) binds to a single 55 kDa-protein, type E antibody (2 kinds of MAbs) binds to a single 35kDa-protein, type F antibody (2 kinds of MAbs) binds to a single 30kDa-protein, type G antibody (2 kinds of MAbs) binds to a single 25kDa-protein, type III antibody (3 kinds of MAbs) bind to multiple proteins, and others (10 kinds of MAbs) bind to each of single protein respectively.

From these results, type A antibody binding protein (180kDa-protein) was estimated as an especially high immunogenic one in *C. elegans* proteins.

1261C. Cell type-specific proteomic profiling in *Caenorhabditis elegans*. **Kai P. Yuet**[1], Meenakshi K. Doma[2], John T. Ngo[1], Paul W. Sternberg[2], David A. Tirrell[1]. 1) Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California, United States of America; 2) Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, California, United States of America.

How can we study a cell's proteome without the complications that arise from the isolation of proteins derived from all of the other cells in a complex eukaryote like *C. elegans*? We need systematic methods to enrich, identify and quantify by mass spectrometry low abundance proteins expressed in specific cells or tissues from whole-worm lysates. Here, we have engineered a family of mutant *C. elegans* aminoacyl-tRNA synthetases to make them capable of tagging proteins with reactive non-canonical amino acids. We provide the non-canonical amino acids by metabolic labeling of *E. coli*, which are then fed to worms. We achieved spatiotemporal selectivity by controlling expression of a mutant synthetase using cell-selective (body wall muscles, intestinal epithelial cells, pharyngeal and neuronal cells) or state-selective (heat shock) promoters in several transgenic lines. These tagged proteins are distinguished from other proteins through conjugation of the non-canonical amino acid side chain to probes that permit detection, isolation and visualization of the labeled proteins. This conjugation reaction is bio-orthogonal; i.e., the reaction is rapid and selective between labeled proteins and probes but remain inert to the other components found in worms. Proteins synthesized in cells that do not express the synthetase are neither labeled nor detected. This approach enables unbiased discovery of proteins uniquely expressed in a subset of cells.

1262A. Easy access to modENCODE data. **sergio contrino**[1], Marc Perry[2], Fengyuan Hu[1], Ellen Kephart[3], Paul Lloyd[3], Rachel Lyne[1], Peter Ruzanov[2], Richard Smith[1], E.O. Stinson[3], Quang Trinh[2], Nicole Washington[3], Zheng Zha[2], Daniela Butano[1], Adrian Carr[1], Kim Rutherford[1], Seth Carbon[3], Sheldon McKay[2], Suzanna Lewis[3], Gos Micklem[1], Lincoln Stein[2]. 1) Department of Genetics, Univ of Cambridge, Cambridge, UK; 2) Ontario Institute for Cancer Research, Toronto, ON, Canada; 3) Lawrence Berkeley National Laboratory; Genomics Division, Berkeley, CA, USA.

In an effort to comprehensively characterise the functional elements within the genomes of the important model organisms D. melanogaster and C. elegans, the NHGRI model organism Encyclopedia of DNA Elements (modENCODE) consortium has generated an enormous library of genomic data along with detailed, structured information on all aspects of the experiments. The modENCODE Data Coordination Center (DCC) is responsible for managing the integration and distribution of modENCODE raw and interpreted datasets, both within the project and to external resources such as FlyBase, WormBase and NCBI/GEO. It has provided the community with four main and interconnected avenues to access this vast amount of data, all available through http://www.modencode.org. Gbrowse: genome browsers giving a graphical view of all of the datasets created by modENCODE, plus a number of reference annotation tracks such as genes from WormBase and FlyBase. modMine: a data warehouse based on the InterMine system. modMine integrates modENCODE data with information from FlyBase, WormBase and other sources, and provides easy navigation through modENCODE experiments to view and export data. Data can be accessed from various different perspectives including: gene network graphs, gene expression heat maps and keyword search. Dataset Search: a faceted browser, which facilitates rapid discovery of datasets of interest via filtering on various criteria, e.g. organism, technique, target, etc. Then you can download the dataset, browse the relevant GBrowse tracks, view submission in modMine. modENCODE Wiki: the protocol and reagent wiki pages of released submissions are available to the community. The data and associated software components are already partially hosted on Amazon compute cloud, where all the public resources described here will be available by the end of the project.

1263B. Textpresso for *C. elegans* and Nematode. **James Done**[1], Yuling Li[1], Hans-Michael Müller[1], Paul Sternberg[1,2]. 1) Division of Biology, California Institute of Technology, Pasadena, CA; 2) Howard Hughes Medical Institute, Pasadena, CA.

Textpresso is a text-mining system for scientific literature whose capabilities go far beyond those of a simple keyword search engine. Its two major elements are a collection of the full text of scientific articles split into individual sentences and the implementation of categories of terms for which a database of articles and individual sentences can be searched. The categories are classes of biological concepts (*e.g.*, gene, allele, cell, etc.) and classes that relate two objects (*e.g.*, association, regulation, etc.) or describe an object. The corpus of articles and abstracts is marked up to identify terms of these categories. A search engine enables the user to search for one or a combination of these tags and/or keywords within a sentence or document. Due to the fact that ontology allows word meaning to be queried, it is possible to formulate semantic queries. Full text access increases recall of biological data types from 45% to 95%, and using category searches can yield a 3-fold increase in search efficiency. Textpresso for *C. elegans* (http://www.textpresso.org/celegans) with its access to over 20,000 full text research articles is used routinely by *C. elegans* researchers to find relevant information, and by WormBase (http://www.wormbase.org) to curate semi-automatically information such as gene-gene interaction, sub-cellular localization, disease-gene connections and much more. There is a new site, Textpresso for Nematode (http://www.textpresso.org/nematode/) containing more than 14,000 non-*C.elegans* nematode papers. Textpresso is extended for use by biologists to other model organisms and diseases. They can be accessed from http://www.textpresso.org/.

1264C. CisOrtho V 2.0: a comparative genomic approach to genome wide identification of transcription factor target genes. **Lori Glenwinkel**[1], Oliver Hobert[1,2]. 1) Dept Biology, Columbia Univ, New York, NY; 2) Dept Biochemistry and Molecular Biophysics, Columbia Univ, New York, NY.

CisOrtho V 2.0 is able to predict novel regulatory target genes using an experimentally derived PSSM and species conservation among five nematode genomes. CisOrtho ranks transcription factor (TF) binding sites (BS) by the PSSM log-odds score, BS enrichment, and inter-species preservation of BSs in orthologous regions. I have validated CisOrtho's predictive power using a bioinformatics approach and in vivo validation of novel regulatory targets. Three sets of regulatory target genes for the cholinergic, AIY, and ASE neuron terminal selector TFs (UNC-3,TTX-3/CEH-10,CHE-1) were compared to a control set of 1000 random genes or 93 collagen genes that we expect to exert their expression independently of these three TFs. Each criterion that CisOrtho uses to rank potential TF target genes was calculated for each orthologous region between 5 nematode species in upstream, intronic, exonic, and downstream regions. Known TF target genes ranked significantly better for all criteria in upstream promoter regions while intronic, exonic, downstream, and total gene ranking criteria were not always significantly different. For in vivo validation of CisOrtho, six highly ranked potential UNC-3 target genes were chosen. Four of these genes are completely uncharacterized while two have published expression patterns in the ventral nerve cord where UNC-3 exerts its regulation as a terminal selector of cholinergic motor neurons. GFP promoter fusions were made for four of the genes that are previously uncharacterized in the literature. Each reporter was injected into wild type *C. elegans* then crossed into an UNC-3 mutant, *unc-3(e151)*. The four uncharacterized genes showed

ventral nerve cord neuron expression and all 6 genes had significant loss of ventral nerve cord neuron expression in the UNC-3 mutant suggesting UNC-3 dependence. CisOrtho predicted target genes are also enriched in neurogenesis pathway genes for all three terminal selector genes providing ample candidates for further in vivo experimentation. These lines of evidence suggest that CisOrtho is able to successfully predict novel targets of TFs.

1265A. GExplore updated: more genome-scale data mining for worm researchers. Jinkyo Suh, **Harald Hutter**. Biol Sci, Simon Fraser Univ, Burnaby, BC, Canada.

Previously we implemented a simple web-based database interface for genome-scale data mining at the protein (rather than the DNA) level for experimental planning - hosted at: http://genome.sfu.ca/gexplore/. The interface allows individual or combinatorial searches for proteins with certain domains, expression in particular tissues or specific phenotypes. Genes in a given interval can be retrieved for vetting candidate genes in positional mapping experiments. The database, which contains also GO terms and homology information, has been updated regularly using Wormbase data. Recently new datasets including stage- and sex-specific RNAseq expression data (see Gerstein et al., Science 330:1775-87) have been processed and added. We are currently preparing a major update of GExplore to include several novel features and datasets. We included protein domain data using Pfam and SMART annotations for all core species in Wormbase (C. elegans, C. briggsae, C. brenneri, C. japonica, C. remanei and P. pacificus). We extended the manually curated list of protein domains that can be searched by simple abbreviations, e.g. 'IG' to retrieve all proteins containing Pfam or SMART domains referring to ImmunoGlobulin domains, to over 450 domains. Proteins containing a particular Pfam or SMART domain can be identified using Pfam or SMART identifiers. In addition to a graphic display of the domain organization of proteins, the protein sequences are available for display and download. A new interface allows users to observe where mutations are located within a protein of interest. The output can be limited to mutations affecting certain protein domains. Our database contains mutations accessible through Wormmart and will be updated as more recent Wormbase data will become available for general data mining. The database also includes mutations isolated by the Million Mutation Project, which have been released recently by the Moerman and Waterston labs (Thompson et al. submitted). Independently of GExplore we maintain a web-based interface to search for mutations from the Million Mutation Project (hosted at http://genome.sfu.ca/mmp/). We will present the current progress in updating the GExplore website and underlying database.

1266B. Gene Function (and Gene Dysfunction) Data in WormBase: Where and How to Find It. **Ranjana Kishore**, Kimberly Van Auken, Raymond Lee, Gary Schindelman, Karen Yook, WormBase Consortium. Dept of Biology, California Institute of Technology, 1200 E California Blvd, Pasadena, CA 91125.

WormBase curates biological data from the published literature towards presenting a complete picture about the function of a gene. The different categories of data are displayed in different widgets on WormBase gene pages and include: **1. Concise Descriptions.** These free-text summaries reside in the Overview widget and are meant to give the user a quick introduction to a gene. They describe a gene's molecular identity and orthology, processes and pathways, genetic and physical interactions, and expression at the tissue and sub-cellular levels. **2. Gene Ontology.** Gene Ontology (www.geneontology.org) curation provides computable annotations for a gene's Biological Process, Molecular Function and Cellular Component, using a controlled vocabulary and evidence codes, which are shared across multiple model organism databases. **3. Phenotypes.** Phenotypes are curated in great detail for mutations, RNAi knockdown experiments, anatomy function, overexpression studies and interactions, using a Worm Phenotype Ontology, developed by WormBase. Information about strains, conditions, and chemicals used in the experiment are also included. **4. Worm Models of Human Disease.** WormBase has begun to highlight data about how worm genes and biology inform the study of human disease. We now include free-text 'disease relevance' descriptions, and tag genes as 'potential' or 'experimental' models, based on orthology to a human disease gene, and/or experimental data, respectively. The details of these data and the different ways in which they can be accessed and mined will be discussed.

1267C. WDDD: Worm Developmental Dynamics Database. **Koji Kyoda**[1], Hatsumi Okada[1], Tomoko Sugimoto[1], Kenichi Henmi[1], Shihoko Yashiro[1], Shuichi Onami[1,2]. 1) Laboratory for Developmental Dynamics, RIKEN Quantitative Biology Center, Kobe, Japan; 2) National Bioscience Database Center, Japan Science and Technology Agency, Tokyo, Japan.

A collection of quantitative information about morphological dynamics under a wide variety of gene perturbations would provide the broader community of biologists with a novel resource for understanding molecular mechanism of development. Here we created a publicly available database, the Worm Developmental Dynamics Database (http://so.qbic.riken.jp/wddd/), which includes a collection of quantitative information about cell division dynamics in early *C. elegans* embryos with single genes silenced by RNAi [1]. Each set of information contains the three-dimensional coordinate values of the outlines of nuclear regions and the dynamics of the outlines over time during the first three rounds of cell division, which was obtained by combining four-dimensional (4D) DIC microscopy and computer image processing. The database provides free access to 50 sets of quantitative data for wild-type embryos and 136 sets of quantitative data for RNAi-treated embryos corresponding to 72 of the 97 essential embryonic genes on chromosome III. The database also provides sets of 4D DIC microscopy images on which the quantitative data was based. Using the collection, we developed several computational methods to analyze the cell division dynamics in early *C. elegans* embryo such as RNAi phenotype analysis. The results demonstrate that the database provides computational biologists with a novel opportunity for obtaining new insights into the mechanism of development. The quantitative information and 4D DIC microscopy images can be synchronously viewed through a web browser without a special software program, allowing easy access by experimental biologists. The database will be expanded to include data for all chromosomes and five sets of data for each gene.
[1] Kyoda, K. et al. (2013) WDDD: Worm Developmental Dynamics Database. *Nucleic Acids Res.* 41, D732-D737.

1268A. Simulation of embryonic development in *C. elegans* using agent-based modeling. William Decker[2], Josephine Cromartie[1], Daniel Brandon[1], Matthew O. Ward[2], **Elizabeth F. Ryder**[1]. 1) Dept Biol & Biotechnology, Worcester Polytechnic Inst, Worcester, MA; 2) Dept Computer Science, Worcester Polytechnic Inst, Worcester, MA.

Developmental biology is a very visual science; investigators typically generate and communicate hypotheses about how systems are working by drawing pictures of their latest model. Although we can draw complex diagrams of our favorite molecular pathways and cellular interactions, it is impossible for us to draw or imagine how all of these interactions occur during development in a real organism, where many processes are happening simultaneously. Thus,

there is a great need for computational simulations that provide a systems level understanding and visual representation of development. We have begun to design a simulation and visual representation of the development of *C. elegans* using autonomous agent modeling. In this approach, every cell in the animal is an agent; once it is born, the agent follows a set of rules that can be as simple or complex as desired. An agent might respond to a signal by an action, such as dividing, migrating, expressing a gene, or dying. A signal might be a molecular cue in the cell's environment, contact with another cell, or simply a set developmental time point. Our current simulation generates an embryo of about 520 cells, starting from the fertilized egg. It is completely deterministic; all the signals are simply developmental time points at which cells divide or move to determined locations. We use metaballs and marching cubes to generate a fairly realistic visual representation of cell surfaces. The embryo can be rotated, and viewed in cross-section, and cells of particular types or lineages can be colored similarly, or made invisible. Our goal is to create a more stochastic simulation, which would incorporate much more complex signaling among cells. Such a simulation would allow us to model hypotheses as rules governing the behavior of cells of interest. The intent is to use the simulation to test hypotheses and make predictions that can be experimentally verified. We hope to create a simulation that multiple investigators can use and contribute to, and we welcome input from the community regarding useful features to implement.

1269B. A method of estimating environmental friction based on a body dynamics model of *Caenorhabditis elegans*. **Zu Soh**[1], Kazuya Masaoka[2], Michiyo Suzuki[3], Yuya Hattori[4], Toshio Tsuji[2]. 1) Dept. Biomed. Sci., Hiroshima Univ., Hiroshima, Japan; 2) Dept. Sys. Cybern., Hiroshima Univ., Hiroshima, Japan; 3) Microbeam Radiat. Biol. Gr., JAEA, Gunma, Japan; 4) Radiat. Effect Analysis Gr., JAEA, Ibaraki, Japan.

Information on the forces generated by the *C. elegans* is important in exploring the mechanism of its locomotion. This study was performed to investigate a method for estimating environmental friction based on the use of a dynamics model and image analysis. The model is described using Newton-Euler equations of *N* rigid links serially coupled by rotational joints. The friction forces acting between the environment and the body are modeled using dynamic and viscous friction. Although the motion equation can be solved for friction coefficients as a function of the worm's motion, this straightforward solution is an ill-posed problem because the friction model contains four unknown coefficients, while a worm crawling on an x-y plane has only two degrees of freedom. To solve this problem, the proposed method involves simultaneous analysis of two different worm motions in the same environment for which identical friction coefficients are assumed. In addition, as the images used in analysis to determine a worm's motion inevitably contain noise, a coefficient-optimization approach was adopted to minimize the error between trajectories of an actual worm and the corresponding dynamics model in terms of the four friction coefficients.

To verify the proposed method, undulational motion of the dynamics model was artificially generated and simulated with randomly preset friction coefficients. With this configuration, the method was applied to estimate the preset coefficients. The results indicated that the percentage error between the preset and estimated coefficients was within 4%. The results of the method's subsequent application to actual worms showed that the dynamics model could trace their trajectories within a percentage error of 2% of the body length, and that the torque generated from the model was in a reasonable range as measured in previous studies (Ghanbari *et al.* 2008; Johari *et al*,, 2013) in a microstructured environment.

1270C. Cell-level modeling and simulation of the pharyngeal pumping in *Caenorhabditis elegans*. Yuya Hattori[1], **Michiyo Suzuki[2]**, Toshio Tsuji[3], Yasuhiko Kobayashi[2]. 1) Radiation Effect Analysis Gr., JAEA, Ibaraki, Japan; 2) Microbeam Radiation Biology Gr., JAEA, Gunma, Japan; 3) Dept. of System Cybernetics, Hiroshima Univ., Hiroshima, Japan.

The pharyngeal pumping in *Caenorhabditis elegans* is generated and controlled by the pharyngeal neurons and muscular cells. In this study, we proposed a simulation-based approach to estimate the mechanisms of oscillation generation in pharynx at cell level. To conduct the simulations, we previously developed a pharyngeal muscle model including 20 muscular cell models and 9 marginal cell models (Hattori, Y., *et al*, Artif. Life Robotics, 17, 2012). Output in each cell model was the membrane potential based on FitzHugh-Nagumo equations. These cell models were connected by gap junctions based on the actual connection structure of pharyngeal muscle in *C. elegans*. The gap junctions transmitted the outputs between cell models. The electropharyngeogram (EPG), which displays the summed activity of the electrophysiological responses of pharyngeal muscle cells and neurons, was used to measure the biological signals from pharyngeal pumping in *C. elegans*. In our simulation, we obtained the EPG using the outputs of individual cell models. To generate the EPG-like signals in our model, we tuned the parameters included in the various cell models. Consequently, we successfully generated an EPG similar to that observed in wild-type *C. elegans*. Subsequently, we virtually ablated certain gap junctions in our model so as to correspond to the defects in the *eat-5* mutant. As a result, our model could simulate the turbulent oscillation of the pharyngeal pumping in the *eat-5* mutant. In addition, we also simulated the turbulent oscillation in the *inx-6* mutant using the same technique of virtual ablation. Finally, we selected the parameter sets that could show all of the three types of oscillations of pharyngeal pumping (wild-type *C. elegans*, the *eat-5* mutant, and the *inx-6* mutant). Using the selected parameter sets that showed the characteristics of cells in *C. elegans*, the positions of the self-exciting oscillation-cells were suggested. Through a series of simulations, we confirmed the usefulness of the simulation-based approach for examining mechanisms of oscillation generation in the pharynx.

1271A. Visualizing Interactions and Pathways in WormBase. **Karen J. Yook**, Christian A. Grove, The WormBase Consortium. Division of Biology, California Institute of Technology, Pasadena, CA.

The term 'interaction' in biology can mean one of many different types of relationships (physical, genetic, cellular, chemical) within and between any class of biological entity: gene, RNA, protein, cell, small molecule from the environment, etc. Molecular genetics has successfully sought to identify these entities and show how they interact with others. WormBase is adopting and adapting ways in which these relationships can be displayed in a useful biological context. Many interaction visualization tools, such as Cytoscape, are adept at showing one-to-one relationships and are good at incorporating increasing numbers of entities and their relationships. The WormBase website includes a dynamic Cytoscape window in the interaction widget of gene pages, so users can explore curated relationships captured from the literature and large-scale datasets. User friendly tools now allow these displays to be specified, to show only certain relationships - physical, genetic, regulatory, etc., which cuts down enormously on the 'Hairball Effect' where entities and relationships are indistinguishable. Cytoscape views however, are only one-dimensional in that they are centered on the single entity, and therefore do not present these interactions into the larger biological context. To better visualize these intersecting details, we are using WikiPathways to diagram

genetic and physical relationships within cellular, anatomic, life stage, and environmental contexts. WikiPathways is a powerful community-driven pathways database with online and desktop editing tools. We invite the community to take part in creating pathways in WikiPathways, which will be incorporated into widgets on WormBase Process Pages. These Process Pages integrate genetic with anatomical, developmental, and temporal information to focus on the larger biological picture of the nematode rather than on the discrete biological entity, such as the gene. At this poster, we will walk people through our interaction pages and show how to customize your views of Cytoscape interactions. We will introduce WikiPathways, and demonstrate how to use the tools to build your own pathways, and how to submit them to WormBase.

1272B. Multi-well arrays for massively parallel cultivation and imaging of *C. elegans*. Matt Churgin, Chih-Chieh (Jay) Yu, **Chris Fang-Yen**. Dept of Bioengineering, Univ of Pennsylvania, Philadelphia, PA.

Many studies of *C. elegans* require longitudinal monitoring of individual animals, for example to track changes in behavior during development or aging. Current techniques for imaging uniquely identified worms are restricted to imaging single animals or several animals of a single developmental stage. To address these limitations, we have developed the WorMotel, a multi-well imaging platform for parallel monitoring of hundreds of larval or adult worms, each confined to an individual well. The devices are constructed using polydimethylsiloxane (PDMS) cast from a photopolymer 3D-printed mold. Each WorMotel contains an array of 240 concave, 3-mm-diameter wells optimized for worm cultivation and imaging. Each well is filled with 15 microliters of NGM agar and seeded with OP50 or DA837 bacteria. The next day, a single worm is added to each well, manually or using a COPAS worm sorter. The concave geometry of each well minimizes interference from optical scattering at the edge of each well and prevents worms from burrowing under the agar surface. A narrow liquid-filled moat surrounding each well prevents animals from escaping from the wells. After loading the devices, we use an automated imaging system to quantify size, movement, and (if needed) fluorescence of each worm over a period of hours to weeks. We are applying our method to measure locomotory quiescence during development and to track behavioral changes during aging. To facilitate high-throughput analysis, the devices are integrated with a standard 384-well microplate format and are compatible with both manual and automated liquid, plate, and worm handling tools. Our method will make it possible to conduct large-scale genetic screens for subtle mutations affecting development, behavior, and aging.

1273C. An Automated Microfluidic Multiplexer for Fast Delivery of C. elegans Populations from Multiwells. **Navid Ghorashian**[1], Sertan Gökçe[2], Sam Guo[3], William Everett[3], Adela Ben-Yakar[1,2,3]. 1) Biomedical Engineering, Univ of Texas, Austin, TX 78712; 2) Electrical and Computer Engineering, Univ of Texas, Austin, TX 78712; 3) Mechanical Engineering, Univ of Texas, Austin, TX 78712.

Automated microfluidic platforms are enabling high-resolution and high-content bioassays on small animal models. An upstream device that automatically delivers different animal populations to these bioassay platforms could enhance high-throughput biological studies. Current population delivery strategies rely on suction from conventional well plates through tubing exposed to air, which causes certain drawbacks: 1) bubble and debris introduction to the sample, which interferes with analysis in downstream systems, 2) experimental throughput reduction due to added cleaning steps, and 3) the requirement for complex mechanical manipulations of well plate position. To address these concerns, we developed a microfluidic platform that can deliver multiple distinct animal populations from on-chip wells through a multiplexed valve system and used it to deliver C. elegans worms. This Population Delivery Chip can operate autonomously as part of a relatively simple experimental setup that does not require any of the major mechanical moving parts typical of plate-handling systems to address a given well. The autonomous device setup could serially deliver 16 distinct worm populations from on-chip wells out of a single outlet without introducing any bubbles or debris, damaging the animals, or population cross-contamination. The device achieved delivery of more than 90 % of the population preloaded into a given well in 4.7 seconds; an order of magnitude faster than current worm delivery methods. This platform could potentially handle similarly sized model organisms, such as zebrafish and drosophila larvae or cellular micro-colonies. This proof-of-principle implementation with 16 wells can be easily expanded in future devices with many more built-in wells to process additional populations.

1274A. Microfluidic devices for longitudinal imaging of gently immobilized worms and live imaging of early embryos during acute drug treatment. Edgar Gutierrez[1], Rebecca Green[3], Sandra Encalada[2], Karen Oegema[3], **Alex Groisman**[1]. 1) Physics, Univ of California, San Diego, La Jolla, CA; 2) Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA; 3) Ludwig Institute for Cancer Research and Cellular and Molecular Medicine, UCSD, La Jolla, CA.

A major challenge for studying dynamic developmental processes in C. elegans is long-term high-resolution imaging. We have used a micro-machined elastomer chip that is pushed against a cover glass by the application of vacuum to trap worms between the chip and the cover glass. The pushing force is widely adjustable by varying the level of vacuum, enabling a user-defined degree of immobilization for a variety of worm sizes. Loading the device is simple and minimizes the loss of worms. Worms can be easily recovered from the device by separating the elastomer chip from the substrate. A high level of vacuum is applied to completely immobilize worms during short intervals of high-resolution imaging; the vacuum is reduced between the imaging to allow worms to feed and develop. Upon reduction of the vacuum, the worms recover their normal behavior. We applied the device to image the vesicular transport in neurons of adult worms and the developmental time course of the gonad region, using DIC and confocal microscopy. We have performed longitudinal imaging over a 48 hour time interval, with the immobilization remaining fully functional and with worms developing from larvae to young adults. Small molecule inhibitors are a valuable tool for the analysis of fundamental cellular functions and an entry point for the development of therapeutic agents. We identified a gene whose inhibition renders the C. elegans eggshell permeable and built a microdevice for in situ worm dissection and high resolution imaging of embryos. The microdevice is made of hard plastics and has a rectangular well with an array of microwells on the bottom. After the dissection of worms, the fragile embryos are gently swept towards the microwell array, where they are protected from flow by the microwell walls. Permeable embryos are acutely exposed to drugs after the existing medium is aspirated and a medium with the drug is dispensed into the well with a pipette.

1275B. Electrical recordings of naturalistic feeding behavior in a microfluidic environment. **Abraham W. Katzen**, Shawn R. Lockery. Univ of Oregon, Department of Biology & Institute of Neuroscience. Eugene, OR.

Food choice - the decision of what to eat - is critical to survival and reproduction. To advance the study of food choice in *C. elegans*, we have devised a

microfluidic device for measuring feeding behavior in single worms automatically and accurately in response to a variety of food types under naturalistic conditions. Feeding behavior in *C. elegans* is quantified by observing the pumping rate of the pharynx. There are currently two main methods for measuring pumping rate: manual observation of slow-motion videos of worms crawling in food, and electrical recordings, known as electropharyngeograms (EPGs). Neither method is practical for experiments requiring naturalistic feeding behavior with precise presentation of food stimuli. Manual observation is labor intensive, limited to short observation periods (~20 sec), and provides little spatiotemporal control of food presentation. EPG recordings, in which a submerged worm is sucked into a hollow recording electrode, necessarily isolate the pharynx from the environment, interfering with delivery of food to the worm. To overcome these limitations we have integrated EPG recording electrodes into an existing microfluidic device [1]. The worm is restrained with the longitudinal axis of its body aligned between two laminar fluid streams, while the head is free to move and explore either stream. The device allows the user to control the delivery, concentration, and type of food that is available to the worm in either fluid stream. Worms exhibit naturalistic feeding behavior in the microfluidic device, and stable recordings lasting 15 minutes are routine. Using this method, we are exploring the feeding response of *C. elegans* to familiar or novel foods of differing qualities. Additionally, we are determining the effect of food concentration on feeding latency and rate. We anticipate that this method will provide novel insights into the internal and external cues which contribute to adaptive feeding decisions. References: [1] McCormick et al. (2011) PLoS ONE 6(1):e25710.

1276C. Durable interrogation of response and adaptation in Caenorhabditis elegans. **Ronen B Kopito**, Erel Levine. Physics and fas center for systems biology, Harvard Univ, Cambridge, MA.

Animal response to change in environmental cues is a complex dynamical process that occurs at diverse molecular and cellular levels. To gain a quantitative understanding of such processes, it is desirable to measure many individuals for a long time periods, subjected to a repeatable and well-defined environment. We developed a microfluidic system, where worms are individually confined in a unique chamber optimized to the worm anatomy. Egg laying, pumping rate and other vital signs are all kept normal for more than 24 hours. Survival rate of rescued worms spent 24 hours in the device was found to be similar to worms on plate. We then explored dynamics of molecular response and gene expression to various stressful environmental conditions such as nutrient deprivation and high temperature. In particular we tested nuclear localization of DAF 16 and SKN-1, and expression profile of a heat shock protein. We characterized the spatio-temporal dynamics of the onset of response, identified a transition from local to systemic response. We found that stress response to extreme condition is not adaptive, and that response duration correlates with the duration of the stress.

1277A. Design of the microchip device to dissect the neural circuit based on thermotactic behavior in *C. elegans*. **Y. Nishida**[1], M. Nakajima[2], J. Jaehoon[2], M. Takeuchi[2], K. Kobayashi[1], T. Fukuda[3,4,5], I. Mori[1]. 1) Div. of Biol. Sci., Nagoya Univ, Nagoya, Japan; 2) Dept. of Micro-nano Syst. Eng., Nagoya Univ., Nagoya, Japan; 3) Faculty of Sci. and Eng., Meijo Univ., Nagoya, Japan; 4) Inst. for Adv. Res, Nagoya Univ., Nagoya, Japan; 5) Intelligent Robotics Inst., Sch. of Mechatronical Eng., Beijing Inst. of Tech., Beijing, China.

Animals cope with environmental stimuli by altering behaviors. Previous studies have characterized the thermotactic neural circuit, in which temperature is sensed and processed through the coordination of thermosensory neurons AFD and AWC, and interneurons AIY, AIZ and RIA in C. elegans (Mori et. al., 2007). This simple circuit is profitable to dissect neural activities of the respective neurons in response to temperature stimuli. However, it is unknown how the activities mediate the behavior to cope with environmental temperature. The recent study shows that RIA interneurons, which receive sensory inputs, encode head movement (Hendricks et. al., 2012). This finding provides a clue to tie the neural activities to not only the thermosensory inputs but also the behavioral outputs.In this study, we aim to study how the thermotactic neural circuit processes the temperature and mediates the behavior by monitoring the neural activities of the respective neurons for thermotactic behavior.

We are now designing the microchip device to control both temperature stimuli and head movements. First, we are optimizing the shape of microfluidic channel for stable fixation of the animals' body to ensure free head movements. We are planning to fabricate a device for handling head movements by manipulation of a micro-probe attached to head region of the animals. We are also fabricating temperature control system to present the animals with accurate temperature stimuli in the microchip device. Our study surely supports understanding how temperature is sensed and processed through the neural circuit to mediate thermotactic behavior.

1278B. Automated Suppressor Screen of Motor Degeneration Mutants Enabled by Microfluidics & Image Analysis. Ivan Cáceres[1], **Daniel Porto**[1], Ivan Gallotta[2], Josue Rodríguez-Cordero[3], Elia Di Schiavi[2], Hang Lu[1,4]. 1) Interdisciplinary Bioengineering Graduate Program, Georgia Institute of Technology, Atlanta, GA; 2) Institute of Genetics and Biophysics (ABT), CNR, Naples, Italy; 3) Electrical & Computer Engineering, Univ of Puerto Rico at Mayaguez, Mayaguez, Puerto Rico; 4) School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA.

Motor neuron degeneration is widely studied in C. *elegans*, yet the genetic factors affecting its mechanism are largely uncharacterized. Current phenotypic screens aim to find genes responsible for degeneration, but the requirement of manual operations lead to time-consuming experiments. Additionally, current methods require visual inspection of phenotypes, introducing human error and bias. Here we present an automated system for a suppressor screen of C. *elegans* motor degeneration mutants. To address the low-throughput issue of current screens, we used microfluidics to minimize the manual operations required. Our system also removes human bias by using a series of computer vision algorithms. By sequentially applying segmentation and morphological operations, fluorescent microscopy images are processed to determine the number of motor neurons present in the worm. The resulting number is then used for classification of phenotypes. The various components of the system are integrated by using automated software, enabling a high-throughput screen of roughly 300 animals per hour. We applied our system for a suppressor screen using a strain that expresses GFP in GABA neurons and in which *smn-1* is knocked-down only in GABAergic motor neurons. The silencing of *smn-1* causes the death of motor neurons, leaving only 7 or 8 out of the 19 GABAergic D-type motor neurons fluorescently visible. We performed an EMS suppressor screen of over five thousand animals and found over 10 double mutants alleles. These mutants show a reversal of the neurodegenerative phenotype, with at least 14 of the 19 total GABAergic motor neurons fluorescently visible. Our system is thus able to quickly and accurately screen for degenerative phenotypes, providing a method to highly increase the productivity of the study of genetic factors affecting motor neuron degeneration.

1279C. New high throughput analysis and redistribution technology for *C.elegans* to and from multiwell plates. **Rock Pulak**, Weon Bae, Bruce Holcombe, Mariya Lomakina, Mikalai Malinouski, Tom Mullins, Julia Thompson. Union Biometricia, Inc, Holliston, MA.

We have recently developed new technology for moving worm samples from wells of multiwell plates to our BioSorter® large particle flow cytometer. We call this autosampler the LP Sampler. The (Large Particle) LP Sampler is an automated sample introduction system designed specifically for gentle handling of *C. elegans* animals, and other sample types. The purpose of our experiments is to determine the overall performance of the LP Sampler. We show that worms deposited in wells of 96-well plates can be aspirated and delivered intact to the BioSorter instrument for analysis and redistribution. Sampling can be done from wells of 96-well plates and the analyzed worms can be redispensed into various multiwell plate formats, including 384-well plates. Worms from one well were distributed to many wells. Time per well is around 32-36 seconds, with a recovery that averages 89-95% over 96-wells. A wash step between wells was used to reduce carryover. Plates were inspected by microscopy and it was determined that carryover is less than 1%. Software was developed with the use of scripts we could modify. We established scripts for controlling the LP Sampler with the BioSorter resulting in the independent mapping of the plate of origin (which wells to sample from) and the output plate (which wells analyzed worms should be dispensed to). This type of flexible control allows the simple solution of moving the worms from one well of the origin plate to the corresponding well of a target plate after analysis, or the more difficult task of moving worms from one well to many wells of the target plate. We present our initial testing of the LP Sampler with proof of principle experiments using PD4793 [myo-2::GFP, pes-10::gfp, F22B7.9::gfp] and N2. The experiments we present simulate the types of experiments researchers working with RNAi libraries might require, as well as researchers screening deletion libraries and clonally distributing single worms for further growth and analysis.

1280A. Phenotypic profiling of synaptic sites for subtle mutant identification in automated genetic screens. **Adriana San Miguel**[1], Matthew Crane[1], Peri Kurshan[2], Kang Shen[2], Hang Lu[1]. 1) Georgia Institute of Technology, Atlanta, GA; 2) Howard Hughes Medical Institute, Stanford Univ, CA.

Many neurological diseases, caused by defects in neurotransmission, are suspected to show no obvious morphological alterations in neuronal patterning. Uncovering genes responsible for these disorders by identifying mutants with subtle changes in morphology of micron-sized synapses is a very challenging task that necessitates high-resolution and quantitative phenotyping, making genetic screening virtually impossible. In this work we exploit an integrated approach to perform automated genetic screens in *C. elegans* that enables the isolation of mutants with extremely subtle phenotypic differences in synaptic morphology. We take advantage of microfluidic devices for easy worm handling, paired with automated control of flow, image analysis, and decision-making. Computer vision algorithms allow the unsupervised detection of fluorescently labeled micron-sized synaptic puncta in an unbiased and quantitative manner. Phenotypic profiling of synaptic sites is performed by quantification of a large number of descriptors relevant for synapse morphology, such as synapse size and intensity. In order to take into account the heterogeneity present from isogenic populations, statistical analyses are performed based on phenotypic profiling of whole populations. Logistic regression models allow discerning between populations of true mutants and wild type animals, and suggest the most relevant features that differentiate them. The phenotypic differences of the mutants found in this work range from smaller, dimmer synaptic puncta, altered number, density and interpunctal distance, all extremely difficult to discern by eye. Performing full profile analysis of the found mutants, we are able to suggest putative genetic pathways in which the newly found alleles belong by analyzing their relationships with a representative collection of mutants of known pathways. Overall, the approach shown here provides a practical, feasible technique to perform extraordinarily difficult genetic screens in an unsupervised and unbiased manner and enables the isolation of mutants with extremely subtle phenotypic differences.

1281B. Pilot screening of phototoxicity of dyes by means of an automated motility bioassay using Caenorhabditis elegans. Javier I. Bianchi[1], Juan C. Stockert[2], Lucila Buzzi[1], Alfonso Blázquez-Castro[2], **Sergio H. Simonetta**[1]. 1) Fundacion Instituto Leloir,Buenos Aires, Argentina; 2) Universidad Autonoma de Madrid, Madrid, España.

Phototoxicity consists in the capability of certain innocuous molecules to become toxic when subjected to strong illumination. This has been particularly useful to develop new cancer therapies where patients are treated with the innocuous version of the drug, and once absorbed, the tumor area is focally illuminated in order to activate the phototoxic molecules and destroy cancer cells. In order to discover new compounds or characterize already existing molecules, it would be advantageous to count with simple and cheap biological tests which allow finding, in a short time, the best candidates. In this work, we present a pilot screening of 37 dyes to test for phototoxic effects in *C.elegans*. Nematodes were cultured in liquid medium and treated with different concentration of chemical dyes, and subsequently exposed to a pulse of light. We quantified behavioural effects of compound treatment through quantitation of global locomotor activity using an infrared tracking system (wmicrotracker). Of the tested compounds, 18 molecules were classified as positive in *C.elegans*, being Primuline and Phloxine B the most representatives. In order to characterize endogenous effect and mechanism of action of these chemicals, we retested the compounds washing them out of the medium before the pulse and also registered the staining of the nematodes. Intriguingly, 100% of the positive tested drugs were capable of permeating the animals and produce their phototoxic effects after the washout. We also tested through reporter strains the stress response being triggered by the compounds, endoplasmic reticulum stress response (hsp4::gfp strain), was activated in 22% of phototoxic compounds, and mitochondrial oxidative stress response (hsp6::gfp strain), was positive in 16% of phototoxic compounds. These results indicate a physiological effect in protein folding mechanism and oxidative stress similar as reported in cell culture lines. Our work shows for the first time the utility of *C.elegans* for running a phototoxic molecule screening and its potential application in the drug discovery pipeline.

1282C. A virtual reality running machine for worms-a highly integrated microscope system for olfactory behavior. **Yuki Tanimoto**[1], Kosuke Fujita[1], Yuya Kawazoe[1], Yosuke Miyanishi[1], Shuhei Yamazaki[1], Xianfeng Fei[2], Karl Emanuel Busch[3], Keiko Gengyo-Ando[4], Junichi Nakai[4], Koichi Hashimoto[2], Kotaro Kimura[1]. 1) Osaka Univ; 2) Tohoku Univ., Japan; 3) MRC, UK; 4) Saitama Univ., Japan.

A major function of the nervous system is to transform sensory information into an appropriate behavioral response. The neural mechanisms that mediate sensorimotor transformation are commonly studied by quantifying the behavioral and neural responses to a controlled sensory stimulus. Presenting a controlled chemical stimulus to freely behaving animals under a high-power microscope, however, is challenging. Here, we present a novel integrated microscope system that stimulates a freely moving worm with a virtual odor gradient, tracks its behavioral response, and optically monitors or

manipulates neural activity in the worm during this olfactory behavior. In this system, an unrestricted worm is maintained in the center of a bright field by an auto-tracking motorized stage that is regulated by a pattern-matching algorithm at 200 Hz [1]. In addition, the worm is stimulated continuously by an odor flowing from a tube, the concentration of which can be temporally controlled. The odor concentration used in this system is based on the concentration used in the traditional plate assay paradigm (Yamazoe et al., CeNeuro 2012), and can be monitored with a semiconductor sensor connected to the end of the tube when necessary. Using this system, we investigated the neural basis of behavioral responses to a repulsive odor 2-nonanone in worms. We monitored and modulated sensory neuron activity in behaving worms by using calcium imaging and optogenetics, respectively, and found that the avoidance behavior to 2-nonanone is achieved by two counteracting sensory pathways that respond to changes in temporal odor concentration as small as ~10 nM/s (Yamazoe et al., this meeting). Our integrated microscope system, therefore, will allow us to achieve a new level of understanding for sensorimotor transformation during chemosensory behaviors. [1] Maru et al., IEEE/SICE Int. Symp. Sys. Integr. Proc., 2011.

1283A. Chemical screens for factors affecting neuronal signaling using a semi-automated microfluidic electrotaxis platform. **J. Tong**[1], S. Salam[1], P. Rezai[2,4], R.K. Mishra[3], P.R. Selvaganapathy[2], B.P. Gupta[1]. 1) Biology, McMaster, Hamilton, ON, CA; 2) Mech Eng, McMaster, Hamilton, ON, CA; 3) PNB, McMaster, Hamilton, ON, CA; 4) Laboratory for Foodborne Zoonoses, PHAC, Guelph, ON, CA.

To accelerate the discovery of neuromuscular disease-related processes we have developed a novel microfluidic screening system that controls and quantifies Caenorhabditis elegans' locomotion inside a microchannel using Direct Current (DC) electric fields, which can robustly induce on-demand electrotaxis towards the cathode. We have previously established that defects in dopaminergic (DA) and other sensory neurons alter electrotactic swimming; therefore, abnormalities in neuronal signaling can be determined using locomotion as a read-out. In one initiative, we employ microfluidic electrotaxis to evaluate the neurotoxicity of heavy metals Ag, Cu, and Hg. Exposure-induced locomotion defects such as reduced speed are quantified using a computer tracking system. Microfluidic analysis is complemented with measurements of growth rate, brood size, lifespan, and specific neuronal toxicity. We have found that metal-exposed nematodes exhibit reduced electrotaxis speed as well as retarded growth, decreased fecundity, shortened lifespan, and neuronal damage in a dose-dependent manner. A second project seeks to identify candidate therapeutics for movement-related disorders, specifically Parkinson's disease (PD), using transgenic C. elegans that express human alpha-synuclein (aSyn), the main component of the intracellular inclusions that characterize PD. To confirm PD emulation, strains are subjected to well-established assays for nematode DA signalling, in addition to our microfluidic electrotaxis assay. Several stable lines expressing aSyn have been obtained and confirmed for accelerated DA neurodegeneration and defective behavioural responses. In summary, we present a novel methodology combining C. elegans with microfluidics to facilitate toxicological studies and to accelerate the discovery of new therapeutic candidates for PD. Our results suggest that microfluidic electrotaxis provides a rapid yet sensitive means to identify agents affecting metazoan neuronal health.

1284B. New Computational Techniques for Automated Mutant Sorting, and an Application to the Identification of Synaptic Mutants in *C. elegans*. **Charles L Zhao**[1], Ria Lim[2], Mei Zhen[2,3], Hang Lu[4]. 1) Department of Biomedical Engineering, Georgia Institute of Technology, Atlanta, GA; 2) Samuel Lunenfeld Research Institute, Mount Sinai Hospital; 3) Department of Molecular Genetics, Department of Physiology, Univ of Toronto, Toronto, ON, Canada; 4) School of Chemical & Biomolecular Engineering Georgia Institute of Technology, Atlanta, GA.

The advent of microfluidics has enabled the development of high-throughput mutant sorting, expediting a previously tedious, manual procedure, and enabling the sorting of tremendous numbers of worms. However, to be effective, automated sorting relies on two layers of computational decision making: feature extraction from detected objects, and mutant identification. In the context of synaptogenesis, we examine new and existing general techniques which improve decision accuracy. With the role of malformed synapses in disease, synaptogenesis has attracted much study, but the need for fluorescent markers and the subtlety of mutant phenotypes have made screening challenging. Pixel-based classification identifies putative varicosities, but misclassifies fat droplets and other similar-looking auto-fluorescent objects. Human observers perform better, relying on the arrangement of varicosities along axons. Using image sets taken from synaptically-labeled strains of C. elegans, we show that density-based clustering can enhance the accuracy of varicosity identification and, hence, feature extraction. When structure is obvious to a human, density-based clustering can refine object classification. This can enable more effective image processing for a variety of applications. We further demonstrate improvements in mutant detection. Multiple thresholding performs well, but may neglect subtle multi-feature mutants. We demonstrate that, with reasonable assumptions, an Independent Component Analysis (ICA) can greatly simplify Parzen outlier detection. The method reliably extracts difficult mutants, achieving higher true positives with similar false positives, while requiring smaller sample sizes than necessary for the full Parzen method. To gain further insight, we compare the performance of our method with SVDD, one-class SVMs, and the full Parzen method.

1285C. Acute inhibition of synaptic transmission using Mini-Singlet Oxygen-Generator (miniSOG)-mediated protein ablation. **Keming Zhou**[1], Yishi Jin[1,2]. 1) Dept. Biology, Univ of California San Diego,La Jolla, CA; 2) Howard Hughes Medical Institute.

Synaptic transmission occurs in milliseconds and involves hundreds of proteins that reside in microdomains of the synapse. Genetic mutations that eliminate or reduce protein function have been the traditional tools in investigating the roles of synaptic proteins in *C. elegans*. However, chronic compensation and homeostatic responses may arise from loss of specific proteins. It is thus of high interest to develop strategies to inducibly inhibit the function of synaptic proteins. MiniSOG (mini singlet oxygen generator) is a newly engineered green fluorescent flavoprotein[1]. Upon blue-light illumination, miniSOG efficiently generates singlet oxygen, a very short-lived reactive oxygen species. We have previously shown that miniSOG targeted to mitochondria can inducibly kill cells[2]. Here, using miniSOG-tagging of synaptic proteins, we have developed an optogenetic strategy for acute inhibition of synaptic transmission. The presynaptic protein UNC-13 is essential for synaptic transmission. The long UNC-13 isoform (UNC-13L) is localized to the presynaptic active zone via its N-terminal region. UNC-13L lacking the N-terminal region is diffuse in axons. Transgenic animals expressing miniSOG-tagged UNC-13L exhibit no discernable defects when grown in the dark. Upon brief exposure to blue light the animals rapidly display behavioral impairment, resembling *unc-13* loss of function, and recover after returning to normal culture condition. By electrophysiological recordings, we show that acute inactivation of miniSOG tagged full-length UNC-13L or UNC-13L lacking the N-terminal region preferentially inhibits the fast phase and slow phase of evoked release at the neuromuscular junctions, respectively. We also identify a specific role for the N-terminal region of UNC-13L in tonic release. These observations

demonstrate temporal and spatial specificity of miniSOG-mediated protein ablation. We envision that miniSOG-mediated photo-ablation will be a useful tool to transiently inhibit protein function in broad cellular contexts. 1. Shu, X. et al. PLoS Biol, 2011. 9(4): p. e1001041. 2. Qi, Y. B. et al. PNAS, 2012. 109: p7499.

1286A. Leveraging C. elegans cue-dependent behaviour to understand the host/parasite interaction for plant parasitic nematodes. **Anna Crisford**[1], Jessica Marvin[2], James Kearn[1], Vincent O'Connor[1], Peter E Urwin[2], Catherine Lilley[2], Lindy Holden-Dye[1]. 1) Centre for Biological Sciences, Univ of Southampton, Southampton, SO17 1BJ, UK; 2) Centre for Plant Sciences, Institute of Integrative and Comparative Biology, Univ of Leeds, Leeds LS2 9JT.

Plant parasitic nematodes (PPNs) are a global problem inflicting crop damage costing circa $125b per annum. The chemicals used to control these pests have an unacceptable environmental impact and are being withdrawn from use. This presents a challenge as without adequate crop protection the PPNs present a real threat to food security. The rationale for the study is that neurobiological underpinnings of C. elegans environmentally driven cue-dependent behaviour may be conserved in the PPNs and provide a route to new molecular targets for pest control. We have identified orthologues of C. elegans genes encoding key components of neurotransmitter signalling pathways in the potato cyst nematode Globodera pallida. We have characterised a G. pallida orthologue of C. elegans tph-1, which encodes the synthetic enzyme for 5-HT. C. elegans mutant tph-1(mg280) is defective in feeding and this was completely rescued by expression of the G. pallida orthologue from a pan neuronal promoter. Therefore this gene is most likely required for 5-HT synthesis in PPNs. In PPNs the host plant stimulates the activity of the stylet, a feeding tube that protrudes from the mouth of the worm and which is intimately involved in invasion of the roots (1). We show electrophysiological recordings from G. pallida provide a readout of stylet thrusts and we are currently investigating host plant cues in this paradigm. This will dovetail with RNAi experiments for tph-1 to delineate the role of 5-HT in host cue-dependent behaviour. Funded by the Biotechnology and Biological Sciences Research Council UK Grant no BB/K012495/1. We gratefully acknowledge CGC for provision of tph-1(mg280). 1.Holden-Dye, L., and Walker, R. (2011) Neurobiology of plant parasitic nematodes. Invertebrate Neuroscience, 1-11.

1287B. Structure of a plant peptide hormone and a root-knot nematode-encoded mimic. **Peter DiGennaro**[1], Benjamin Bobay[2], Elizabeth Scholl[1], Nijat Imin[3], Michael Djordjevic[3], David Mck. Bird[1,4]. 1) Department of Plant Pathology, NC State Univ, Raleigh NC; 2) Department of Molecular and Structural Biochemistry, NC State Univ, Raleigh NC; 3) Plant Science Division, Research School of Biology, College of Medicine, Biology and Environment, The Australian National Univ, Canberra ACT 0200, Australia; 4) Bioinformatics Research Center, NC State Univ, Raleigh NC.

Root-knot nematodes (RKN; *Meloidogyne* spp.) are a pervasive pest of vascular plants and cause substantial crop loss worldwide. The molecular basis underpinning the intimate plant-nematode symbiosis remains arcane. As an obligate parasite that must execute complex behavior to reproduce, mutations that are trivial in *C. elegans* are lethal in RKN. Further, transformation remains elusive, and RNAi unpredictable. Yet although the genetic tool-kit is limited, emergent technologies have greatly aided the exploration the host-parasite interaction, and permit traditional genetic manipulation. Using a targeted computational approach, we identified multiple families of plant peptide hormone mimics encoded within the RKN genome. One family, C-terminal Encoded Peptide (CEP) has been implicated in the suppression of lateral roots and formation of galls, a hallmark of RKN pathology. RKN uniquely encode and express mimics of CEP during parasitism and RKN encoded CEP phenocopy endogenous hormones in bioassays. To better understand their role in the parasitic interaction, we used NMR to compare tertiary structures of plant and nematode encoded CEP hormones. In-solution models reveal family-specific structural properties that have been implicated in receptor binding. Molecular dynamic simulations demonstrated relative conformational plasticity of host CEP. This is consistent with promiscuous interaction with multiple receptors to regulate diverse pathways throughout the plant. In contrast, the comparatively rigid structure of RKN-encoded mimics likely reflects their function in a singular niche. These analyses implicate RKN-encoded plant peptide hormones as core communicative signals in the plant-nematode interaction and constitute a new methodological paradigm in studying effectors at the host-parasite interface.

1288C. Treat worm infections with crystal protein expressing in probiotic like bacteria. **Yan Hu**, Melanie Miller, Alan Derman, Brian Ellis, Daniel Huerta, Joseph Pogliano, Raffi Aroian. Div Biological Sci, Univ California, San Diego, San Diego, CA. U.S.A.

Soil-transmitted helminths (namely hookworms, whipworms, and *Ascaris* large roundworms) are intestinal nematodes, which cause diseases of poverty that infect upwards of two billion people worldwide. These parasites are a major threat to health and development of hundreds of millions of children and pregnant women. Enormous hurdles must be overcome in order to develop and deliver urgently needed new therapies (anthelmintics) to replace old ones that perform sub-optimally and are losing efficacy. Any new therapy must be extremely cheap, be able to be produced in tremendous quantities to treat hundreds of millions of people, have a stable shelf life, and be capable of storage and delivery under adverse environmental conditions. Our research has uncovered a radical and unique new approach that solves each of these challenges: expression of vertebrate-safe, anthelmintic (anti-nematode) proteins in “probiotic-like” food-grade bacteria. Such bacteria can be produced cheaply, in great quantity, stored stably, and delivered under adverse conditions. Here we will discuss our work to develop such engineered bacterial therapy using the anthelmintic crystal protein Cry5B normally made by *Bacillus thuringiensis* (Bt). We will present data on how Cry5B can be expressed in a non-Bt bacterium related to food-grade bacteria, and the strong efficacy of such a bacterium in clearing hookworm infections in rodents. We will also update progress on engineering several food-grade bacteria to express Cry5B as a critical step towards implementation of this novel anthelmintic approach.

1289A. Development of quantitative methods for assessing the effects of anthelmintics on parasitic nematodes (soil transmitted helminths). **Melanie M. Miller**[1], Linda Z. Shi[2], Yan Hu[1], Arash Safavi[1], Sandy Chang[1], Michael Berns[2], Raffi V. Aroian[1]. 1) Division of Biology, Univ of California San Diego, La Jolla, CA; 2) Institute of Engineering in Medicine, UCSD 92093.

Soil transmitted helminthes (STHs) affect the poorest people with estimates of upwards of 2 billion people infected. There are currently only two drug classes (anthelmintics) used to treat STHs, both of which were developed originally for veterinary usage. Recent clincal data shows some increasing difficulties with the field efficacy of these drugs. A challenge in the development of new anthelmintic treatments is the ability to accurately quantitate the effects of drugs on parasitic nematodes. Methods for quantitating *C. elegans* motility, and development have been developed and explored, but not so much for larger, parasitic nematodes. Currently state of the art methods include touching worms and using a motility scale to classify live or dead, feeding

inhibition assays that employ the use of dyes such as Alamar Blue and MTT, cell viability assays including total ATP measurements, Live/Dead cell viability assays, egg hatching assays and isothermal calorimetry measurements using the xCELLigence systems to name a few. All of the described and published methods have their drawbacks. The touching assays have a level of subjectivity with them and take time. The feeding assays and viability assays give unreliable results that don't provide a dose curve. The XCELLigence system has shown to be promising when measuring the health of adult stage worms, but not larval stage worms . Egg hatching assays can only identify drugs that are able to penetrate the eggshell. Ultimately, we are looking for assays that are highly reproducible, provide reliable dose-response curves, allow reasonable throughput, and are accessible cost-wise. Our approach is primarily focused on two areas right now, quantifying the size of larvae in a larval development assay and using WormAssay developed at UCSF to quantify motility of both larvae and adult stage parasites. I will also present a number of other approaches we have investigated and put aside.

1290B. another can of worms - more genomes & sequences at WormBase. **Michael Paulini**, Paul Davis, Mary Ann Tuli, Gareth Williams, Kevin Howe. European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom.

Since 2011, the number of nematode species represented in WormBase increased by half from 12 to 19. These species can be divided into close relatives of *Caenorhabditis elegans* and parasitic nematodes. In addition the in-depth curation of a limited set of core species has been extended, with the support of the wider research community, to include *Brugia malayi*, a nematode of clade III and one of the causative agents of lymphatic filariasis in human. Support for multiple alternative reference genomes for a single species (like *Ascaris suum* somatic/germline assemblies) has been added. To support the extension of WormBase to non-*Caenorhabditis* species and provide a standardised nomenclature across nematodes, we have also extended our gene-naming service to include *B.malayi* and helped to provide a first pass gene nomenclature based on a combination of publications and predictions through orthologs. Another development focus was the mapping procedure for RNAi and expression probes, which now allows for more accurate mappings in a species-agnostic way. Also the use of RNASeq by WormBase increased and mappings of RNASeq libraries deposited in the Short Read Archive are not only provided as BAM files, but are also post processed and used in the curation process providing information on TSL sites, splicing, expression asymmetry, polycistronic transcripts and tissue and life-stage specific expression levels (available through SPELL). This is supplemented by the integration sequence features from modENCODE data, like transcription factor, RNA polymerase II and histone binding sites as well as genelets and transcripts.

1291C. *C. briggsae* genomic fosmid library. Mathew Tinney[1], Elisabeth Loester[1], Susanne Ernst[2], Siegfried Schloissnig[3], Andreas Dahl[4], **Mihail Sarov[1]**. 1) TransgeneOmics, MPI-CBG, Dresden, Germany; 2) Hyman lab, MPI-CBG, Dresden, Germany; 3) Computational Biology, HITS, Heidelberg, Germany; 4) Deep Sequencing, CRTD, TU Dresden, Germany.

We have previously developed techniques for protein function exploration *in vivo*, using BAC and fosmid genomic DNA clones as transgenes and have applied them on a genome wide scale in *C. elegans.* Extending this work to other nematode species would provide a platform for addressing a wide range of evo-devo problems. As a first step towards this goal we generated a fosmid gDNA library for *C. briggsae*. We have developed a new deep sequencing based approach for clone mapping that significantly reduces the cost and effort required to map a large number of clones compared to the traditional BAC/fosmid end sequencing. The method can be easily extended to more nematode species. While for *C. briggsae* the genome sequence is available, we are currently working on an approach that combines *de novo* assembly and clone mapping and can be used for unsequenced genomes. The physical scaffolding provided by the clones and the reduced complexity compared to direct gDNA sequencing greatly simplifies the assembly process. We have started to use the resource to compare orthologous groups of gene expression regulatory proteins (transcription factors and RNA binding proteins) in *C. elegans* and *C. briggsae*.

1292A. Genome and transcriptome of the zoonotic hookworm *Ancylostoma ceylanicum*. **Erich M. Schwarz[1,2,4]**, Yan Hu[3], Igor Antoshechkin[1], Paul W. Sternberg[1,2], Raffi V. Aroian[3]. 1) Division of Biology, California Institute of Technology, Pasadena, CA, 91125, U.S.A; 2) HHMI, California Institute of Technology, Pasadena, CA, 91125, U.S.A; 3) Section of Cell and Developmental Biology, UC San Diego, La Jolla, CA, 92093, U.S.A; 4) Department of Molecular Biology and Genetics, Cornell Univ., Ithaca, NY, 14853, U.S.A.

The hookworms *Necator americanus* and *Ancylostoma duodenale* infect up to 740 million human beings, stunting and impoverishing them. Existing drugs only partially cure hookworm infections, and failures of these drugs have been reported. Unlike *N. americanus* and *A. duodenale*, their zoonotic close relative *Ancylostoma ceylanicum* robustly infects both humans and other mammals, can be maintained in the laboratory, and thus provides an important model for hookworm disease. To identify new drug and vaccine targets, we assembled an *A. ceylanicum* genome sequence of 313 Mb which is ~95% complete. We also obtained RNA-seq data for an infectious progression from third-stage larvae to fully mature, fertile adults, along with responses to both the drug albendazole and the pore-forming toxin Cry5B. We detected expression in 23,855 genes encoding proteins of 100 or more residues. Genes upregulated during early infection only partially overlap with those induced by hookworm culture medium, a common model for infection; they include an Srx-class 7TM receptor gene expressed only during early infection, and ASPRs, encoding a strongylid-specific family of *Ancylostoma* Secreted Proteins (ASPs). Genes downregulated during early infection encode or modulate ligand-gated ion channels, G-protein coupled receptors, and potassium channels; similar downregulation occurs between the L3 and L4 larval stages of *C. elegans*. During later infection, three mannose and asialoglycoprotein receptor xenologs, acquired horizontally from deuterostomes, are strongly upregulated. A small number of genes are significantly affected by albendazole or Cry5B toxin, but transcriptional effects of Cry5B are greatly enhanced by inhibiting p38 MAP kinase. The *A. ceylanicum* genome should enable efficient tests of drug and vaccine candidates, along with RNAi and controlled immunomodulation.

1293B. C. elegans as an expression system for drug targets from parasitic nematodes. **Megan. A Sloan[1,2]**, Barbara. J Reaves[1], Adrian. J Wolstenholme[1]. 1) Deptarement of Infectious Diseases, Univ of Georgia, Athens, GA; 2) Department of Biology and Biochemistry, Univ of Bath, Bath, United Kindgom.

Many important neglected tropical diseases are caused by parasitic nematodes. Current control programs rely on very few effective anthelmintic drugs and resistance to these may develop. There is therefore a pressing need to find new drugs, which requires a better understanding of parasite genetics and physiology. However studies of parasitic nematodes are complicated and expensive, even when technically feasible, due to the need for passage though infected animals. The development of a system for expressing important parasite genes in C. elegans could make the study of parasitic nematodes much

easier and cheaper and would allow us to utilize the powerful genetic resources already available. We have shown that we can express ivermectin target genes, such as avr-14, from the parasite Haemonchus contortus in drug-resistant strains and cause them to revert to drug susceptibility, and also rescue behavioral phenotypes. We have also used this system to demonstrate that novel parasite genes can also act as drug targets. We are now extending these studies to genes encoding subunits of the neuromuscular junction dwelling levamisole-sensitive nicotinic acetylcholine receptor (nAChR) which are well conserved in parasitic nematodes - unc-29 and unc-38. We have cloned unc-29 and unc-38 orthologs from the clade V parasite, H. contortus, and the clade III species, Ascaris suum, downstream of the C. elegans promoters. These constructs are being expressed in unc-29 (ok2450) and unc-38(ok2896) mutants co-transformed with a pmyo-2::GFP marker plasmid to aid detection of transformed worms. The transgenic strains are assessed for levamisole sensitivity and uncoordinated phenotypes.

1294C. Worms in dirt: nematode diversity in restored tallgrass prairie. **Breanna Tetreault**, Andrea K. Kalis, Jennifer Ross Wolff. Carleton College, Northfield, MN.

Soil organisms play an integral role in ecosystems, influencing and being influenced by ecosystem processes and plant community dynamics. Nematodes are the most numerous soil mesofauna and are widely used as indicators of change in soil fauna communities. We aim to understand how the diversity of nematodes varies among a chronosequence of restored tallgrass prairie in the Cowling Arboretum of Carleton College, Northfield, MN. Continuing research of this restored prairie has focused on the soil nutrient content and above ground plant growth during the successional development of these prairies over time. Including information about soil invertebrate fauna will complement and enrich these studies.

In a pilot experiment, soil samples were taken from four fields restored to tallgrass prairie in 1996, 1998, 2000, and 2008. Replicate soil cores (10 cm deep x 2.5 cm in diameter) were taken in each restoration area in Fall 2012. Soil samples were homogenized and nematodes extracted using a Ludox floatation method. Initial phenotypic observations reveal a varied community of nematodes present in the Cowling Arboretum. From each sample of extracted nematodes, 96 individuals were lysed. We are in the process of PCR amplifying and sequencing an approximately 1 kb fragment of the small subunit ribosomal RNA gene in these individuals. To assess nematode diversity, each nematode sequence will be assigned to a molecular operational taxonomic unit (MOTU).

Future studies will increase the sample size in the different restorations and include sampling in exclosures that allow differing animal herbivory. Soil invertebrate fauna has been shown to enhance grassland succession and diversity. Thus, understanding the soil nematode content in the Cowling Arboretum may aid in restoration and conservation of tallgrass prairie.

1295A. Spectrum: Building Pathways to Biomedical Research Careers for Girls and Women of Color. **Diana S. Chu**, Audrey G. Parangan-Smith, Kimberly D. Tanner. Dept Biol, San Francisco State Univ, San Francisco, CA.

Women of color are largely absent from the biomedical research community. Despite some progress made in encouraging girls to pursue career paths in science, few materials exist that specifically attract girls of color to these careers. To address this need, the Science Education Partnership and Assessment Laboratory (SEPAL) at San Francisco State Univ (SFSU) developed the Spectrum program. This program partners SFSU biomedical scientists who are women of color (including Biology faculty, undergraduate students, Masters students, and alumni in doctoral and biotechnology positions) with middle and high school girls and teachers. These teams collaborate to: 1) co-sponsor after-school science clubs targeting girls of color in high needs public schools, 2) develop a mentoring community of women of color trainees in biomedical research, 3) create video biographies highlighting the research programs and experiences of women of color biologists, and 4) partner with the local and national Expanding Your Horizons organizations to disseminate Spectrum activities. During its initial four years, Spectrum engaged 456 middle and high school girls (45% Latina, 13% African American, 22% Asian, 11% Unknown, 7% White) across nine clubs providing ~20 hours of academic enrichment for each girl, including two field trips to the laboratories of SFSU women of color biologists. Evaluation data shows increases in the percentage of participating girls who agree with the following statements: 1) I have heard a woman scientist talk about why she likes science (pre: 72%, post: 96%), and 2) I have met a woman scientist who is like me (pre: 36%, post: 65%);. Spectrum has also developed two video resources highlighting women of color from SFSU. 1) *Women of Color Doing Biomedical Science: Inspiring Stories from Women of Color Biomedical Researchers* and 2) *From Us to Us: Advice on Careers in Biomedical Sciences for Girls & Women of Color*. These are available on the SEPAL website and have been distributed through partnerships with national organizations supporting girls in science. Spectrum is supported by the National Institutes of Health through award #R25RR024307.

1296B. *C. elegans* modules for multiple laboratory classes. **Janet S. Duerr**. Biological Sciences, Ohio Univ, Athens, OH.

At large public universities, it is often difficult to provide students with the optimum breadth and depth of laboratory experiences. To cope with the realities of team teaching, I have developed *C. elegans* based modules for three different lab courses: Introductory Biology (taught to 500 freshmen per semester), Genetics Lab (~50 upperclassmen per semester), and Cell Biology Lab (16 upperclassmen per semester). These modules provide a way to use *C. elegans* to introduce students to multiple subjects and techniques at different levels of complexity. **Introductory Biology:** The most basic module takes two meetings in the middle of the first term of a joint lecture-lab course; students have studied basic genetics and are studying cells. In week 1, students receive plates of N2, *dpy-5*, and *unc-52*. The students observe and record body bends per minute using a dissecting scope and learn about mutant phenotypes. In week 2, they analyze their data using Excel and use a shared fluorescent microscope to observe and photograph GFP+ worms. **Genetics Lab:** The module in this team-taught lab (which also uses *Drosophila*, bacteria, and yeast) introduces students to RNAi and gives them more experience with fluorescent microscopy. In week 1, students receive a regular or RNAi-hypersensitive GFP+ strain and plates with OP50 or *dpy-5* RNAi bacteria. In week 2, the students use upright (fluorescent) microscopes to observe the morphology of their treated worms vs. *dpy-5* mutants. The students take photographs and quantify worm length using ImageJ and Excel. **Cell Biology Lab:** "My" lab makes extensive uses of transgenic nematodes. Each student receives a different GFP-translational fusion strain near the beginning of the course. As an introduction to bioinformatics, the students use WormBase to gather information on their strains and GFP labeled proteins. The students use their strains to run protein gels and Westerns. They use fluorescent teaching scopes with digital cameras to observe and photograph their strains. They perform indirect immunofluorescence with an anti-GFP antibody, a second antibody, and phalloidin. The students are given supervised time at the confocal microscope to take images of their strain. The final class

presentations allow the students to discuss the cell biology of 'their' GFP-proteins with each other.

1297C. A semester-long investigative lab provides an authentic research experience in the cell biology of *C. elegans* embryos. **Sara K. Olson**, David Morgens. Pomona College, Claremont, CA.

Undergraduate biology education should provide students with authentic research experiences that allow them to think and act as scientists. The recent push to replace "cookbook" labs with inquiry-based investigative projects provides students with a more authentic research experience. At Pomona College, upper and lower division courses contain investigative laboratory components that typically last 1-4 weeks. In the fall semester of 2012, we piloted an investigative lab in an Advanced Cell Biology course that lasted an entire semester and was based on the research interests of the Olson Lab - *C. elegans* eggshell formation. Each lab group was assigned a different protein that was identified in a previous RNAi screen for genes involved in eggshell formation. None of the proteins were previously characterized, though worm strains expressing mCherry fusions were created prior to the start of the semester. Students spent the first 8 weeks conducting cell biological experiments on their assigned protein, learning methods such as worm picking, co-IP, mass spectrometry, Western blotting, RNAi, IF, and live cell fluorescence microscopy. Students then wrote a paper in the style of a journal article that highlighted their main findings, suggested a model for how their protein was involved in eggshell formation, and proposed future experiments that took into account current literature and classmates' data. Students then spent the next 5-6 weeks conducting experiments to address their hypotheses and proposed future directions. The students showed characteristics typical of dedicated scientists, such as taking ownership of "their proteins", returning voluntarily at night and on weekends to complete experiments, and being critical/skeptical of experiments conducted by other groups that did not meet perceived standards of experimental rigor. The investigative lab was a win-win for everyone. Students developed practical skills and learned what being a scientist is really like, while our research lab benefitted through generation of preliminary data for new projects and the recruitment of 5 new students to the lab (out of 20 enrolled in the course).

1298A. A continuous, discovery-based C. elegans laboratory for an intermediate-level undergraduate molecular and cellular biology course. **Yan Qi**[1,2], Jill Penn[2], Rachelle Gaudet[2]. 1) Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Molecular and Cellular Biology, Harvard Univ, Cambridge, MA.

C. elegans has been a widely-used model organism in instructional laboratories at the undergraduate level. Many genetics, molecular and cellular biology techniques in C. elegans research, such as RNAi, epi-fluorescence microscopy and PCR, can be readily mastered by undergraduate students and enable students to carry out meaningful research projects during their learning process. We designed a continuous, multi-module, discovery-based C. elegans laboratory to accompany an intermediate-level undergraduate course in molecular and cellular biology. Students first perform a GFP reporter-based RNAi screen to identify gene inactivations that modulate insulin signaling in worms. Each pair of students study a unique set of gene inactivations, and screen results are shared and analyzed across the entire class. Students then characterize their identified hits using both molecular and cellular biology techniques, including PCR amplification and sequencing of the RNAi clones, qPCR experiments to measure the expression level of the endogenous reporter gene, nuclear translocation assay of the transcription factor in the pathway, and a stress survival assay. We test-ran the RNAi screen and transcription factor nuclear translocation modules in the introductory cellular biology course enrolled by 100 students in seven laboratory sections led by instructors with varying expertise. This instructional laboratory aims to help students gain increased confidence in conceptual knowledge and practical skills by engaging students in real, cutting-edge research.

1299B. FIRE lab (Full Immersion Research Experience): Student-directed projects on ALA-dependent sleep in *C. elegans*. **Cheryl Van Buskirk**, Dany Roman, BIOL447/L students. Biology, California State Univ Northridge, Northridge, CA.

CSUN biology majors are eager for opportunities to work closely with faculty members and gain research experience. However, there are often not enough spaces in faculty labs to meet this need. The redesigned BIOL447/L, 'FIRE lab' helps to bridge that gap, allowing up to 18 students to participate in original, student-directed research. During the first half of the course, students are introduced to *C. elegans* and to a series of research articles that familiarize them with a specialized topic, in this case EGF-induced (ALA neuron dependent) sleep behavior[1]. During the second phase of the course, students design a series of experiments to address a unique question, and have the option of working individually or in groups. Prior to starting their project, each student applies for 'funding' by writing a grant proposal that undergoes rounds of instructor feedback and peer review. In the final week of the course, students present their results in a mock conference.

The chosen projects fell into two main groups: 1. Half of the class chose to perform a group RNAi screen of predicted protease genes, in order to identify the putative sheddase responsible for release of soluble EGF ligand from its transmembrane precursor. This involved identification and prioritization of candidate genes, and design of an RNAi strategy that was consistent with the twice-per-week class schedule.

2. The remaining students worked as individuals, investigating potential roles of ALA neuron-dependent sleep. Students chose stressors that are known to inhibit feeding and locomotion, such as bacterial toxins and tissue injury, and determined whether the quiescent state was ALA-dependent. This involved literature searches, establishment of quiescence assays, and comparison of WT and ALA-defective (ceh-17 mutant) animals. This poster serves as an opportunity for a FIRE lab student and the instructor to share the experimental results and learning outcomes of this course. 1. Van Buskirk, C. & Sternberg, P. W. Epidermal growth factor signaling induces behavioral quiescence in Caenorhabditis elegans. Nat Neuro. 10, 1300-1307 (2007).

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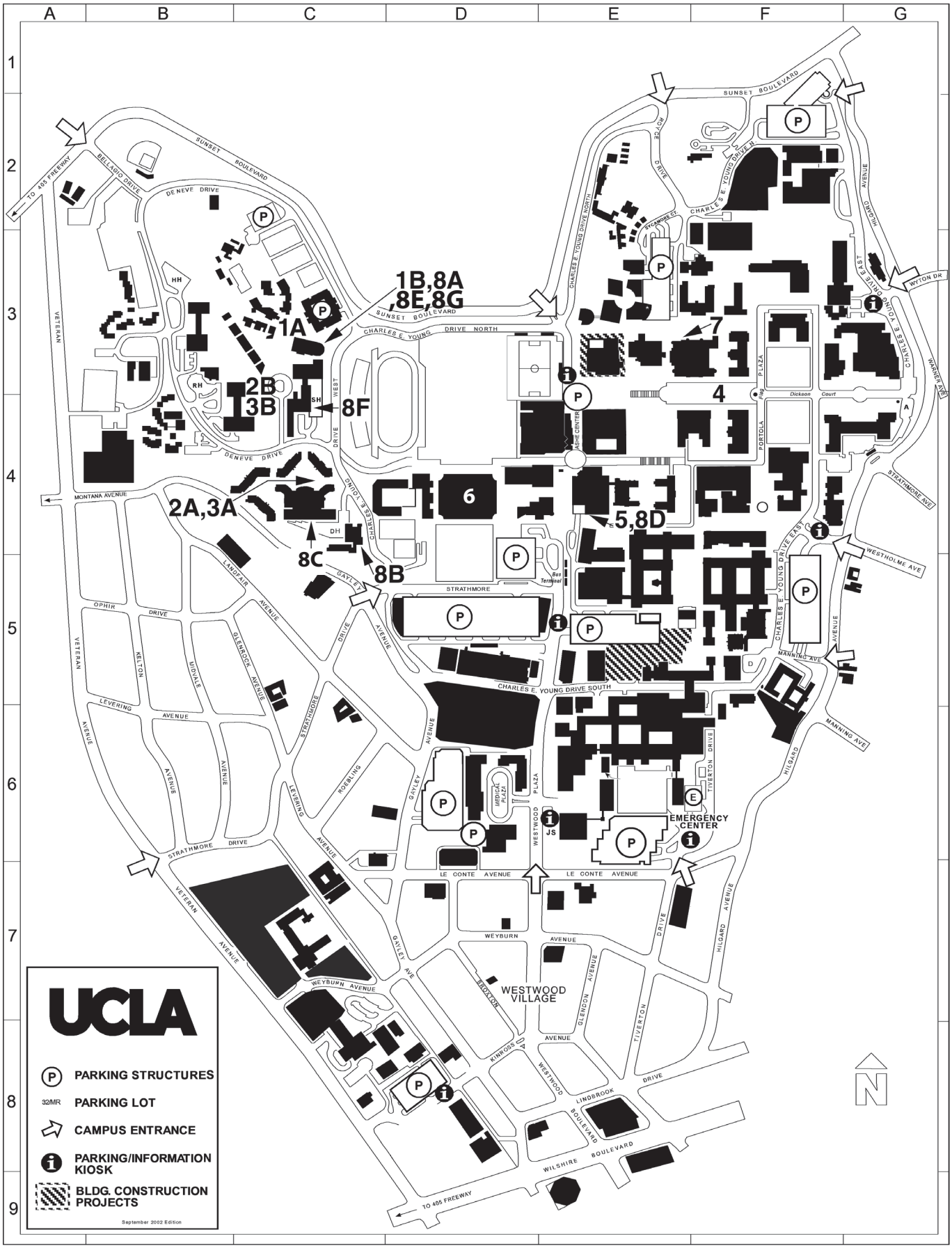

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