17TH International *C. elegans* Meeting

University of California, Los Angeles • June 24–28, 2009

Program and Abstracts

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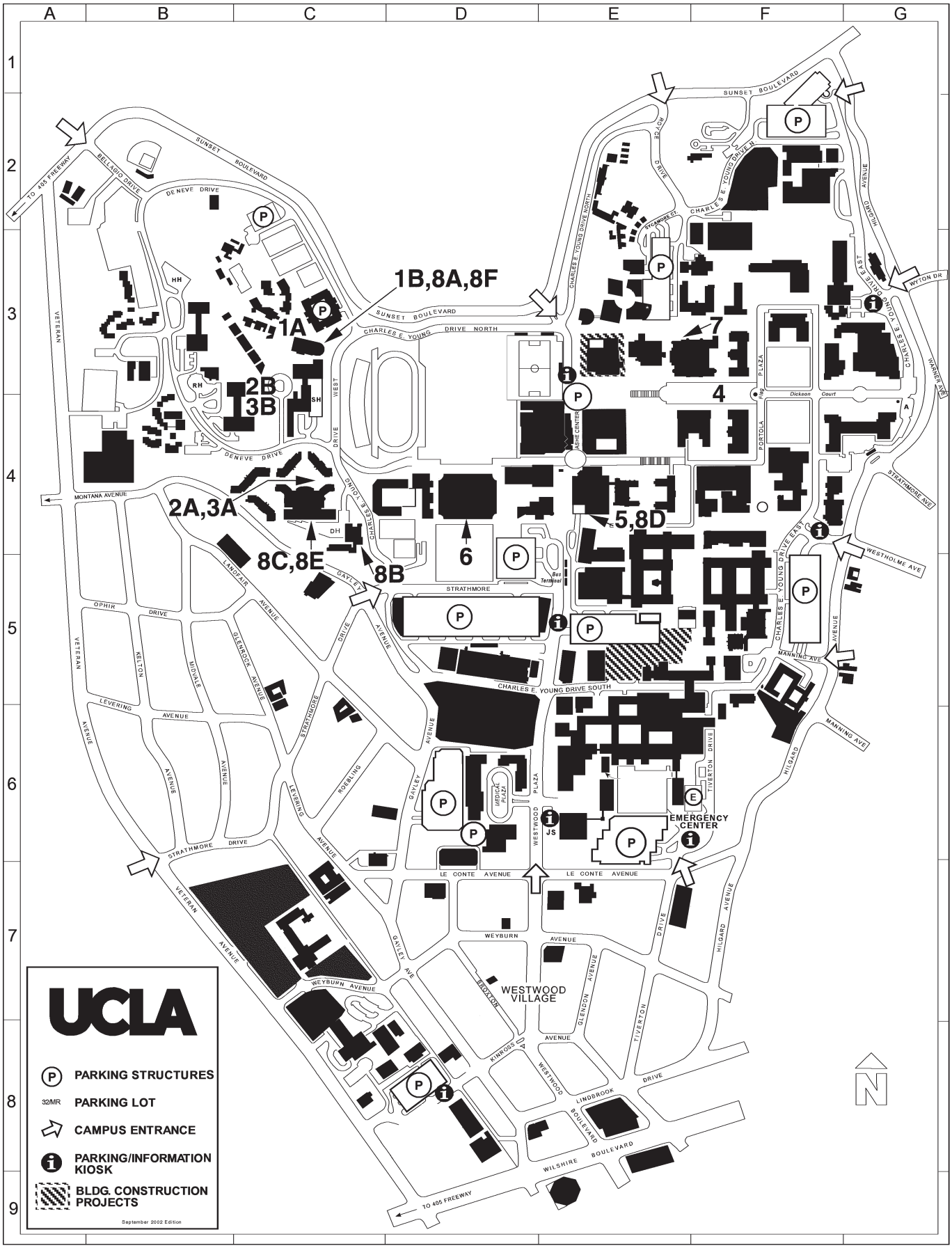
SCHEDULE OF ALL EVENTS

17th International *C. elegans* Meeting

• **WEDNESDAY, JUNE 24**		
1:00 pm - 9:00 pm	**Registration**	Sunset Village Plaza
7:00 pm - 8:00 pm	**Keynote Speaker** Barbara Meyer, University of California, Berkeley	Royce Hall
8:15 pm - 11:00 pm	**Plenary Session 1: Genomics, Developmental Timing and Evolution** *Chairs:* David Raizen and Judith Yanowitz	Royce Hall
11:00 pm - 12:00 midnight	**Opening Mixer**	Royce Quad
• **THURSDAY, JUNE 25**		
8:00 am - 1:00 pm	**Registration**	Covel Commons
9:00 am - 12:00 noon	**Parallel Sessions I**	
	Neuronal Development *Chair:* Robyn Lints	Ackerman Grand Ballroom
	Evolution *Chair:* Christian Braendle	Sunset Village Study Lounge
	Germline *Chair:* Gillian Stanfield	Grand Horizon Ballroom
	Genomics *Chair:* Asher Cutter	De Neve Plaza Room
	Morphogenesis, Migration and the Cytoskeleton *Chair:* Zhirong Bao	De Neve Auditorium
	Aging *Chair:* Catherine Wolkow	Bradley International Ballroom
1:30 pm - 4:30 pm	**Plenary Session 2: Germline Development, Embryogenesis and Morphogenesis** *Chairs:* Emily Troemel and Jennifer Ross Wolff	Royce Hall
5:00 pm - 6:30 pm	**Concurrent Workshops**	
	Advanced Microscopy *Organizers:* Harald Hutter, Steve Grill and Benjamin Podbiliewicz	Bradley International Ballroom
	Evolution and Genomics *Organizers:* Ron Ellis, Ilya Ruvinsky and Erich Schwarz	De Neve Auditorium
	Microfluidic Approaches to the Analysis of Behavior, Physiology, and Development in *C. elegans* *Organizer:* Nikos Chronis	Grand Horizon Ballroom
	Approaches for Studying Metabolism in *C. elegans* *Organizer:* Amy Walker	Sunset Village Study Lounge
	Tools and Resources for High-Resolution Protein Localization Analysis *Organizer:* Mihail Sarov	De Neve Plaza Room
7:00 pm - 8:00 pm	**Genetics Society of America Faculty Mentoring Social**	Fowler Museum Courtyard

7:30 pm - 8:30 pm	**Teaching Workshop I: What is Life Like at a Predominantly Undergraduate (i.e. Teaching) Institution and How Can I Get a Job at One?** *Organizer:* Jennifer Miskowski	De Neve Auditorium
7:30 pm - 9:00 pm	**Focus Group on GENETICS Journal** *Organizer:* Tracey DePellegrin Connelly	Sunset Village Study Lounge
8:00 pm - 11:00 pm	**Poster Session 1/Exhibits/Art Show**	Pauley Pavillion
10:00 pm - 12:00 midnight	**Evening Social**	Pauley Pavillion
• **FRIDAY, JUNE 26**		
8:30 am - 12:30 pm	**Registration**	Covel Commons
9:00 am - 12:00 noon	**Parallel Sessions II**	
	Synaptic Function, Neural Circuits and Neural Methods *Chair:* Alexander Van Der Linden	Bradley International Ballroom
	Gene Expression *Chair:* Weiwei Zhong	Ackerman Grand Ballroom
	Cell Death and Neurodegeneration *Chair:* Itzhak Mano	Sunset Village Study Lounge
	Developmental Timing and Dauer Larvae *Chair:* Allison Abbott	De Neve Plaza Room
	Polarity and Trafficking *Chair:* Erin Cram	Grand Horizon Ballroom
12:15 pm - 1:15 pm	**Genetics Society of America Student Mentoring Luncheon**	De Neve Private Dining Room
1:30 pm - 4:30 pm	**Plenary Session 3: RNAi, Regeneration and Neurobiology** *Chairs:* Marc Hammarlund and Jill Bettinger	Royce Hall
5:00 pm - 6:30 pm	**Plenary Session 4** Special Presentation by Martin Chalfie	Royce Hall
7:30 pm - 8:30 pm	**Teaching Workshop II: Post-tenure Life at a Predominantly Undergraduate Institution: Sabbaticals, Administrative Responsibilities, Mentoring, and More** *Organizer:* Jennifer Miskowski	De Neve Auditorium
8:00 pm - 11:00 pm	**Poster Session 2/Exhibits/Art Show**	Pauley Pavillion
10:00 pm - 12:00 midnight	**Evening Social**	Pauley Pavillion
• **SATURDAY, JUNE 27**		
8:30 am - 12:30 pm	**Registration**	Covel Commons
9:00 am - 12:00 noon	**Parallel Sessions III**	
	Behavior *Chair:* Laura Bianchi	Ackerman Grand Ballroom
	Mechanisms and Function of RNA Interference and Small RNAs *Chair:* Jonathan Whetstine	De Neve Auditorium

• **SATURDAY, JUNE 27, continued**		
9:00 am - 12:00 noon	**Stress Responses** *Chair:* Pankaj Kapahi	Grand Horizon Ballroom
	Cell Cycle, Chromosome Dynamics and Nuclear Organization *Chair:* Alisa Piekny	Bradley International Ballroom
	Pathogenesis *Chair:* Scott Alper	Sunset Village Study Lounge
	Cell Fate Patterning *Chair:* Sophie Jarriault	De Neve Plaza Room
12:30 pm - 2:30 pm	**Teaching Workshop III: Biology Education: Focus on Successful Pedagogical Techniques for Large Lecture Classes** *Organizer:* Jennifer Miskowski	De Neve Auditorium
	Genetics Society of America Town Hall Meeting *Organizers:* David Kushner and Sherry Marts	Sunset Village Study Lounge
1:30 pm - 4:30 pm	**Poster Session 3/Exhibits/Art Show**	Pauley Pavillion
4:45 pm - 6:15 pm	**Concurrent Workshops**	
	WormBase: Linking Genomic Sequence to Biological Functions *Organizer:* Erich Schwarz	De Neve Auditorium
	Dynamic Imaging in *C. elegans* Neurons *Organizers:* Sandhya Koushika and Bill Schafer	Bradley International Ballroom
	The National Science Foundation: Funding Opportunities, Evaluation Criteria and Successful Strategies *Organizers:* Jo Anne Powell-Coffman and Aixa Alfonso	De Neve Plaza Room
	Whole Genome Sequencing for Mutant Identification *Organizer:* Oliver Hobert	Grand Horizon Ballroom
6:15 pm - 8:00 pm	**Closing Dinner**	Royce Quad
7:30 pm - 8:00 pm	**GSA Postdoc Mentoring Social**	Royce Hall
8:00 pm - 8:15 pm	**Genetics Society of America Poster Awards**	Royce Hall
8:15 pm - 8:30 pm	**Worm Art Show Awards**	Royce Hall
8:30 pm - 9:00 pm	**Worm Comedy Show**	Royce Hall
9:00 pm - 11:30 pm	**Closing Party**	Ackerman Grand Ballroom
• **SUNDAY, JUNE 28**		
9:00 am - 12:00 noon	**Plenary Session 5: Pathogenesis, Dauer, Aging and Death** *Chairs:* Malene Hansen and Kotaro Kimura	Royce Hall



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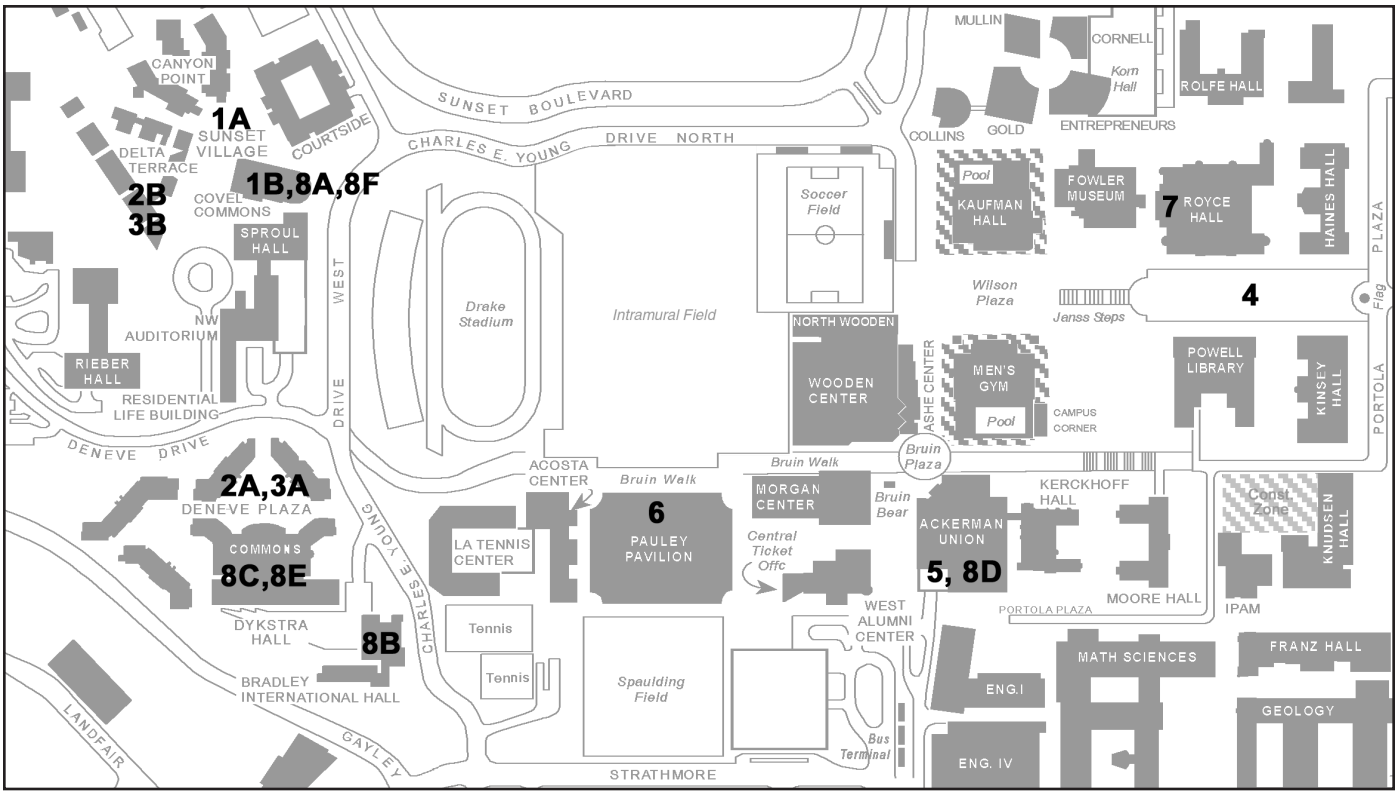
UNIVERSITY OF CALIFORNIA, LOS ANGELES, CAMPUS
Northwest Sector



Legend

1. Registration
   - A. Sunset Village Plaza
   - B. Covel Commons
2. Residence Halls
   - A. De Neve Plaza
   - B. Sunset Village
3. Cafeterias
   - A. De Neve Plaza
   - B. Sunset Village Dining Hall
4. Saturday Night Barbecue: Royce Quad
5. Closing Party: Ackerman Grand Ballroom
6. Poster Sessions/Exhibits/Art Show: Pauley Pavilion
7. Plenary Sessions, Exhibits, Barbara Meyer and Martin Chalfie Talks: Royce Hall
8. Parallel Sessions
   - A. Grand Horizon Ballroom
   - B. Bradley International Ballroom
   - C. De Neve Auditorium
   - D. Ackerman Grand Ballroom
   - E. De Neve Plaza Room
   - F. Sunset Village Study Lounge

- **Registration and Information Desk**
  The Conference registration desk will be open according to the following schedule:

Wednesday, June 24	1:00 pm – 9:00 pm	Sunset Village Plaza
Thursday, June 25	8:00 am – 1:00 pm	Covel Commons
Friday, June 26	8:30 am – 12:30 pm	Covel Commons
Saturday, June 27	8:30 am – 12:30 pm	Covel Commons

- **Instructions for Speakers**
  Please arrive 60 minutes before the beginning of your session with your CD, zip disk or memory stick to load your presentation on the MAC meeting computer. If you plan to show Quicktime movies, please DO NOT attach them to your presentation. Include them as separate files on the disk and be prepared to place them back into your presentation after loading on the conference computer. All plenary speakers should enter through the door marked "Artist's Entrance" at the back of Royce Hall.

- **Poster Sessions**
  All posters will be displayed in Pauley Pavilion for the entire conference. Presenters may mount abstracts beginning at 8:00 am on Thursday, June 25. All abstracts will be up for the entire conference. Authors are expected to present at their posters for only one time slot, according to the following schedule:

Thursday, June 25	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 26	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 27	1:30 pm – 3:00 pm	Presenters of all even "C" posters
	3:00 pm – 4:30 pm	Presenters of all odd "C" posters

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

Thursday, June 25	8:00 am – 11:00 pm
Friday, June 26	8:00 am – 11:00 pm
Saturday, June 27	8:00 am – 4:30 pm

  All presenters should remove their abstracts by 4:30 pm on Saturday, June 27. 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. The posterboards will be removed starting at 4:30 on Saturday, June 27.

- **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 graduate students 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 held in Pauley Pavilion throughout the poster viewing time. The prizes will be awarded on Saturday, June 27, at 8:15 in Royce Hall, prior to the Worm Comedy Show.

- **Teaching Poster Session**
  Posters that describe the use of *C. elegans* in teaching will be grouped together and their authors will present on Thursday, June 25, from 9:30 pm until 11:00 pm.

- **Social Events**
  Opening Mixer: Meet friends and colleagues at the Opening Mixer on Wednesday, June 24, in Royce Quad immediately following Plenary Session 1. Complimentary beer, wine and sodas will be available.

  Evening Socials: There will be evening socials on Thursday and Friday outside Pauley Pavilion from 10:00 pm until 12:00 midnight. Complimentary beer, wine and sodas will be available.

Genetics Society of America Faculty Mentoring Social: 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. The Social will take place in the courtyard of the Fowler Museum on Thursday, June 25, from 7:00 pm until 8:00 pm.

Genetics Society of America Student Mentoring Luncheon: The Genetics Society of America, the organizing committee, and other faculty have organized a lunch on Friday, June 26, from 12:15 pm until 1:15 pm for faculty members and senior graduate students. Faculty members will be available to informally discuss procuring postdoc positions, including such topics as how to choose a postdoctoral mentor and laboratory, how to most effectively apply for a postdoctoral position, and what to expect in the interview. The lunch will be held in the Private Dining Room (PDR) in the De Neve Cafeteria. No need to register. After getting your lunch, you can proceed to the private dining room to get a seat.

Genetics Society of America Postdoc Mentoring Social: This event is organized by *C. elegans* faculty for postdocs. The social will take place in the reception area of Royce Hall on Saturday, June 27, from 7:30 pm until 8:00 pm.

Closing Evening Events: A barbecue is planned for Saturday, June 27, 6:15 pm until 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 Comedy Show, 8:30 pm until 9:00 pm. The closing party will be held on Saturday, June 27, 9:00 pm until 11:30 pm in the Ackerman Ballroom. Beer, wine and soft drinks will be available.

- **Meal Times and Locations**

	De Neve Plaza Commons	Sunset Village 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

- **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-mail. The hours are:

Monday–Thursday, June 22 –25	7:30 am – 8:00 pm
Friday, June 26	7:30 am – 6:00 pm
Saturday, June 27	9:00 am – 6:00 pm
Sunday, July 28	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.

- **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.

- **Smoking**
  Smoking is allowed only in designated outdoor areas.

As exhibitors at the 17th International *C. elegans* Meeting, the following companies have contributed to the support of this meeting. Registrants are encouraged to visit the exhibits during the poster sessions in Pauley Pavilion at the times listed below. Please be certain to stop by.

- **Cold Spring Harbor Laboratory Press**
  500 Sunnyside Boulevard
  Woodbury, NY 11797
  Tel: (516) 422-4100 Fax: (516) 422-4097
  E-mail: cshpress@cshl.edu
  URL: www.cshlpress.com

  Featured at our booth are these *C. elegans* classics: *C. elegans* Atlas (Hall and Altun); *C. elegans* II (Riddle, Blumenthal, Meyer, and Priess); and The Nematode *Caenorhabditis elegans* (Wood), along with other recently published books from CSHL Press. All books will be discounted 10%.

- **Leica Microsystems**
  2345 Waukegan Road
  Bannockburn, IL 60015
  Tel: (800) 248-0123 Fax: (847) 405-0164
  E-mail: info@leica-microsystems.com
  URL: www.leica-microsystems.com

  Leica Microsystems, a world leader in micro imaging, will display the Leica M205 FA automated fluorescence stereomicroscope with FusionOptics™ technology, which allows crisp, clear imaging across the entire magnification range. We will also show the Leica MZFLIII Fluo Combi stereo and compound micro imaging workstation. The Leica S8 APO, the world's only 10x to 80x apochromatic zoom stereomicroscope and the Leica DMI6000 B inverted research microscope with Intelligent Automation will be demonstrated. Fluorescence applications in gene expression, transgenics, and developmental biology will benefit from Leica Microsystems imaging systems.

- **Union Biometrica, Inc.**
  84 October Hill Road
  Holliston, MA 01746
  Tel: (508) 893-3115 Fax: (508) 893-8044
  E-mail: tream@unionbio.com
  URL: www.unionbio.com

  Union Biometrica produces large-bore flow cytometers which automate the analysis, sorting and dispensing of objects too big for common cytometers, using parameters including size, and fluorescent labels. These objects include small model organism, large cells and cell clusters, combinatorial chemistry beads—virtually any object between 20–1500μm. We deliver high throughput with increased accuracy and reproducibility.

The exhibitors will be available at their displays during the following times:

Thursday, June 25	8:00 pm – 11:00 pm	Pauley Pavilion
Friday, June 26	8:00 pm – 11:00 pm	Pauley Pavilion
Saturday, June 27	1:30 pm – 4:30 pm	Pauley Pavilion

• **Thursday, June 25 5:00 PM–6:30 PM**

Advanced Microscopy

Room: Bradley International Ballroom

Organizers: **Harald Hutter, Steve Grill and Benjamin Podbiliewicz**

Microscopy techniques have always been central to the *C. elegans* field. Nomarski optics, Differential Interference Contrast (DIC), was and still is essential for lineaging and in combination with serial section electron microscopy solved the complete anatomy of the worm including its mind. The introduction of fluorescent proteins for in vivo studies has revolutionized many areas of microscopy and has led to tremendous advances in microscopic techniques, many of which have been developed and/or adopted by the *C. elegans* research community. This workshop will explore technical aspects of microscopic setups and techniques for:

Laser nanosurgery, Dr. Ben-Yakar
Automated lineaging, Waterston lab
Correlative light and electron microscopy, Dr. Kolotuev
Microscopy Techniques to Study
Cell division (TBA)
Morphogenesis, Dr. Simske

• **Thursday, June 25 5:00 PM–6:30 PM**

Evolution and Genomics

Room: De Neve Auditorium

Organizers: **Ron Ellis, Ilya Ruvinsky and Erich Schwarz**

Ten years ago, Mark Blaxter had to remind biologists that *Caenorhabditis* elegans was not "the worm," but only one nematode species out of many. Although extensive phylogenies already existed for the Drosophila genus, the number of well-studied Caenorhabditis species was paltry. The lack of a rich phylogeny and of associated ecological data hampered evolutionary studies. Since then, the number of known close relatives of *C. elegans* has grown, serious genetic and physiological studies of other Caenorhabditis species have begun, and comparative genomics has changed from an esoteric hope to a routine procedure including distantly related nematodes.

This workshop will provide in-depth discussion of the latest technical work in both classical and genomic evolution of nematodes, with emphasis on the newly sequenced gonochoristic species *C. remanei*, *C. brenneri*, and . It will highlight methods, resources and future directions.

Some Things You Always Wanted to Know About Nematode Ecology and Evolution*
(*But Were Afraid to Ask)

The Five Genomes
- Identification of orthologous genes
  WormBase-Compara, Michael Han
  OrthoCluster and genBlastG, Jeffrey Chu
- Evolution of cis-regulatory sequences, Sujai Kumar
- Problem of heterozygosity, Eric Haag
- *Discussion*

Phylogenetic Diversity and Ecology
- Phylogeny and distribution of Caenorhabditis and relatives, Karin Kiontke
- Nematode habitats and food, Marie-Anne Félix
- Nematode-host associations, Ralf Sommer
- Population genomics approaches, Matt Rockman
- Developing additional genetic systems, Ron Ellis
- *Discussion*

• **Thursday, June 25 5:00 PM–6:30 PM**

Microfluidic Approaches to the Analysis of Behavior, Physiology, and Development in *C. elegans*

Room: Grand Horizon Ballroom

Organizer: **Nikos Chronis**

Microfluidics (the science of fluid systems with physical features on the scale of tens to hundreds of microns) has begun to revolutionize the biological sciences by providing microscopic, high-throughput tools for manipulation of biological entities from DNA molecules to micro-organisms. In *C. elegans* research, microfluidics has been used to build devices for worm restraint, optofluidic imaging, automated sorting and screening, neuronal ablations, localized chemical stimulation, and long-term culture. This workshop has two main objectives. First, it will serve as a tutorial on the materials, equipment, and methods for building and using microfluidic devices. Second, it will illustrate the remarkable utility of microfluidic devices by presenting a broad range of real-world examples. By bringing together experts from the engineering and biology disciplines, we aim to foster collaboration and interaction between them with the purpose of identifying potential novel applications of microfluidics to *C. elegans* biology.

Speakers:

Lizzie Hulme, Microfluidic devices for long-term examination of *C. elegans*

Nikos Chronis, Microfluidics for calcium imaging assays

Shawn Lockery, Materials, machines, and methods for microfluidics

Hang Lu, Microfluidics for high-throughput, high-resolution quantitative imaging and screening

Dirk Albrecht, *C. elegans* behavior in microfluidic environments

• **Thursday, June 25 5:00 PM–6:30 PM**

Approaches for Studying Metabolism in *C. elegans*

Room: Sunset Village Study Lounge

Organizer: **Amy Walker**

Many groups have discovered the utility of metabolism studies in *C. elegans*, which offers a whole organism approach for investigating how genetics, diet and environment influence metabolism. In recent years, studies on *C. elegans* metabolic pathways have identified links to other biological processes such as aging and behavior. In addition, several labs have adapted diverse techniques to measuring and quantitating metabolites. In this workshop, we will discuss a variety of technologies available for lipid or carbohydrate metabolic studies in *C. elegans*. In addition, we will cover the various strengths and weakness of these techniques. Finally, we would like to further encourage interactions and collaboration between the labs in this area to strengthen this facile area of *C. elegans* research.

Speakers:

Amy Walker (MGH Cancer Center), Introduction to metabolic pathways studied in *C. elegans*

Bart Braekman (Biology Department, Ghent University), Assessment of energy metabolism in *C. elegans*

Jennifer Watts (School of Molecular Biosciences, Washington State University)

Marc Van Gilst (Division of Basic Sciences, Fred Hutchinson Cancer), Isotope enrichment and molecular tracing

• **Thursday, June 25 5:00 PM–6:30 PM**

Tools and Resources for High-Resolution Protein Localization Analysis

Room: De Neve Plaza Room

Organizer: **Mihail Sarov**

Protein localization is directly linked to molecular function but observing proteins in vivo is technically challenging. Several recent developments have significantly improved our ability to address protein localization. Transgenic technology has now made it straightforward to express fluorescently-tagged proteins under native regulatory control. Improved automated imaging methods can translate the observed patterns into structured, high information content data. Extending this approach to a genome scale would allow systems biology approach to protein function in vivo and would greatly improve our understanding of wide range of biological problems. The purpose of this workshop is to present some of these innovative technologies and to discuss how they can be translated to a genome scale in a way that is open and accessible to the community.

Speakers:

Mihail Sarov, Transgenic platform for in vivo localization of fluorescently tagged proteins expressed under native regulatory control

Daniel Blick, Automated, continuous analysis of embryonic gene expression with cellular resolution in *C. elegans*

Fuhui Long, Automatic Recognition of Cells (ARC) for 3D Images of *C. elegans*.

Jorge Ripoll, Optical Projection Tomography for 3D visualization of fluorescent protein localization and anatomy in *C. elegans*.

Stephan Preibisch, Selective Plane Illumination Microscopy for isotropic high resolution 3D imaging in *C. elegans*

• **Thursday, June 25 7:30 PM–8:30 PM**

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

Room: De Neve Auditorium

Organizer: **Jennifer Miskowski**

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.

• **Thursday, June 25 7:30 PM–9:00 PM**

Focus Group on GENETICS Journal

Room: Sunset Village Study Lounge

Organizer: **Tracey DePellegrin Connell**y

• **Friday, June 26 7:30 PM–8:30 PM**

Teaching Workshop II: Post-tenure Life at a Predominantly Undergraduate Institution: Sabbaticals, Administrative Responsibilities, Mentoring, and More

Room: De Neve Auditorium

Organizer: **Jennifer Miskowski**

This workshop should be of primary interest to faculty members who are mid-career faculty members at predominately undergraduate institutions (PUIs), we well as those considering a career at a PUI or in positions at other types of institutions. The discussion will focus on issues confronting faculty members who have survived the tenure process such as when to take a sabbatical and how to make the most of the experience, how to handle administrative roles in an effective and efficient manner, how to best mentor junior faculty, and how to maintain a thriving research program with all these new responsibilities.

• **Saturday, June 27 12:30 PM–2:30 PM**

Teaching Workshop III: Biology Education: Focus on Successful Pedagogical Techniques for Large Lecture Classes

Room: De Neve Auditorium

Organizer: **Jennifer Miskowski**

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 provide information on successful pedagogical techniques they have employed to enhancing the learning experience, with a focus on large lecture courses which can pose unique challenges. Topics might include the use of classroom response systems (i.e. clickers), small group work interspersed with traditional lecture, case studies, etc.

• **Saturday, June 27 4:45 PM–6:15 PM**

WormBase: Linking Genomic Sequence to Biological Functions

Room: De Neve Auditorium

Organizer: **Erich Schwarz**

WormBase has long been a standard tool for designing and interpreting experiments involving *C. elegans*' genes and genomic sequences. At this workshop, you can learn about both classic features and new functions of WormBase from its team of developers, get on-the-spot answers to your technical questions, and propose new WormBase features. We will first describe how to take full advantage of the latest developments in the Genome Browser and the overall user interface. Several short talks will then explain WormBase's tools for studying physiology, functional genomics, and models of human disease.

Speakers:

Introduction, Erich Schwarz

Latest advances in GBrowse and the WormBase GUI, Todd Harris

Phenotypes, Jolene Fernandes

Anatomy, Raymond Lee

Orthologs and disease, Norie de la Cruz

The Journal GENETICS and WormBase, Tracey DePellegrin Connelly

Nbrowse 2009, Kris Gunsalus

Closing Remarks, Erich Schwarz

• **Saturday, June 27 4:45 PM–6:15 PM**

Dynamic Imaging in *C. elegans* Neurons

Room: Bradley International Ballroom

Organizers: **Sandhya Koushika and Bill Schafer**

Imaging neurons in *C. elegans* to understand behavior and neuronal cell biology has been making very rapid progress. This workshop seeks to review recent progress in neuronal imaging to make these methods more widely accessible. The workshop will broadly cover imaging of dendritic growth, organelle transport, calcium responses, and new PDMS devices/microscopy setups to acquire data.

Dynamic imaging of the PVD neurons reveals new roles for the EFF-1 fusogen in arborizatio. Meital Oren-Suissa, Gidi Shemer, Millet Treinin, and Benjamin Podbilewicz Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel

Imaging organelle transport in *C. elegans* mechanoreceptor neurons. Shikha Ahlawat, Sudip Mondal, Guruprasad Reddy Sure, Bikash Chowdhary, Sandhya P. Koushika Neurobiology, NCBS-TIFR, Bangalore, India

Neuronal calcium imaging from live and dissected worms. James Cregg, Ithai Rabinowitch, Yoshinori Tanizawa, Victoria Butler, William Schafer, MRC-LMB, Cambridge, UK

Imaging calcium responses in *C. elegans* sensory and interneurons. Sreekanth Chalasani, Nikos Chronis, Manuel Zimmer and Cornelia I. Bargmann HHMI/Rockefeller University, New York, NY

Subcellular calcium imaging during laser axotomy and neural regeneration in C. elegans. Christopher Gabel, Aravinthan Samuel Department of Physics, Harvard University, Cambridge, MA

Optical systems for manipulating sensation and memory. Atsushi Kuhara, Ikue Mori, Department of Molecular Biology, Nagoya University, Japan

• **Saturday, June 27 4:45 PM–6:15 PM**

The National Science Foundation: Funding Opportunities, Evaluation Criteria and Successful Strategies

Room: De Neve Plaza Room

Organizers: **Jo Anne Powell-Coffman and Aixa Alfonso**

The National Science Foundation provides funding for basic research, scientific infrastructure, and programs to train and retain a world-class and broadly inclusive workforce. All NSF grants are evaluated for both intellectual merit and on the broader impacts of the proposed activities. The purpose of this workshop is to describe some of the funding opportunities available and to discuss strategies for success. The panel of speakers will answer questions about their experiences with NSF-funded programs, NSF's view on "Broader Impact" and on the grant evaluation process.

Speakers and panelists include:

Aixa Alfonso, NSF program director

Jo Anne Powell-Coffman, ADVANCE Institutional Transformation Project

Patrick Phillips, Programs for graduate training, including IGERT and DIG opportunities

Maureen Peters, Research at undergraduate institutions

Piali Sengupta, Individual research grants

Robyn Lints, Individual research grants

• **Saturday, June 27 4:45 PM–6:15 PM**

Whole Genome Sequencing for Mutant Identification

Room: Grand Horizon Ballroom

Organizer: **Oliver Hobert**

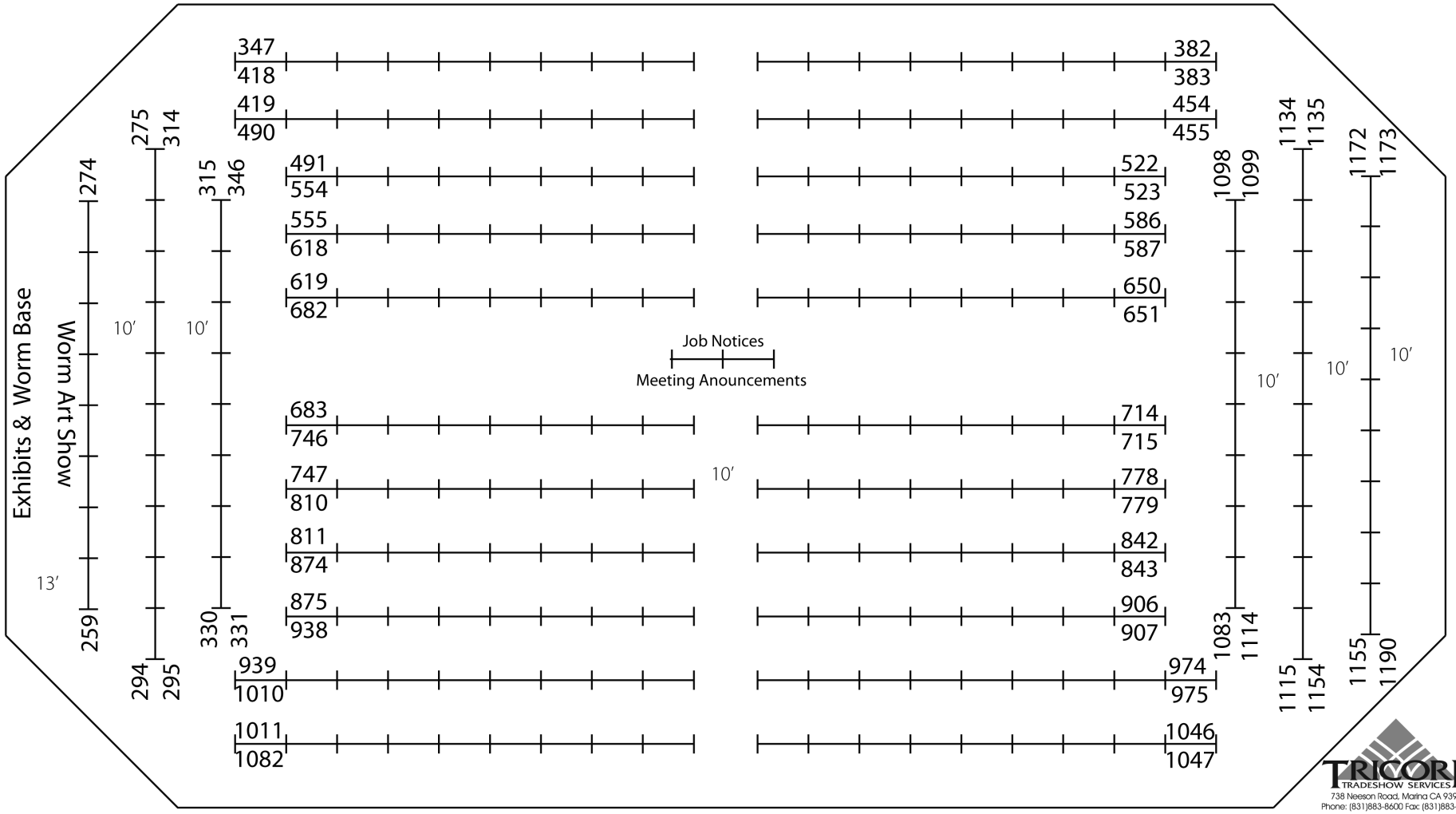
This workshop presents background and practise of using whole-genome Deep Sequencing technology as a means to identify molecular identity of mutant animals. Members of the Hobert lab (Maria Doitsidou and Henry Bigelow) will give a practical overview (with some theory) of the Illumina Genome Analyzer II technology, present a comparison of alternative protocols that can be used to identify sequence variants (e.g., paired end vs. single run), provide information on costs and give a detailed account of the labs experience using this technology to clone a multitude of mutants retrieved from various genetic screens in the lab. Furthermore, all computational steps needed to convert raw signal intensity to a list of gene-annotated variants and indels will be introduced. A Web interface, MAQWeb for running MAQ (Mapping and Assemply with Quality) software and annotating the resulting sequence variants and indels by mutation type (missense, premature stop, splice site, read-through, etc.) will be presented. The final output is easily converted to a single Excel spreadsheet to be sorted and filtered as needed. These contents can also be found on a poster by Bigelow, Doitsidou and Hobert. Representatives from other laboratories will also give short presentations of their experience using whole genome sequencing (Don Moerman, Shai Shaham, Andrew Singson, Sternberg lab). The overall goal of this experience- and "know-how"-sharing is to help propagate the use of this effective approach as a standard operating procedure for genetic analysis in worms.

17th International C. *elegans* Meeting

June 24 - 28, 2009

Poster Sessions/Exhibits/Art Show

UCLA Pauley Pavillion



259 - 433 Physiology
434 - 635 Neurobiology
636 - 829 Development and Evolution
830 - 986 Gene Regulation and Genomics
987 - 1153 Cell Biology
1154 - 1162 Academic Teaching
1163 - 1190 Late Abstracts

Wednesday, June 24 8:15 PM–11:00 PM
Royce Hall

Plenary Session 1: Genomics, Developmental Timing and Evolution

Chairs: David Raizen and Judith Yanowitz

1- 8:15
GENETICS, the peer-edited journal of the Genetics Society of America, reports on updates and special projects, including article links to WormBase. **Tracey DePellegrin Connelly**, Tim Schedl.

2- 8:21
Balancing selection maintains paternal-effect-by-zygotic incompatibility among *C. elegans* wild isolates. **Hannah Seidel**, Matthew Rockman, Michael Ailion, Leonid Kruglyak.

3- 8:33
DNA-mediated transformation in *Pristionchus pacificus* reveals novel functions of Wnt signaling in vulva induction. **Xiaoyue Wang**, Ralf J. Sommer.

4- 8:57
RNA Polymerase II Accumulates on Promoters of Growth and Development Genes During L1 Arrest. **L. Ryan Baugh**, John DeModena, Paul Sternberg.

5- 9:09
Driving with a clutch: cadherin acts with Rac signaling to regulate cell movements during gastrulation. **Gidi Shemer**, Minna Roh, Joe McClellan, Bob Goldstein.

6- 9:21
Comprehensive identification of endogenous Argonaute-bound miRNAs and mRNA target sequences at nucleotide level resolution in *C. elegans.*. **Dimitrios Zisoulis**, Michael Lovci, Tiffany Liang, Melissa Wilbert, Gene Yeo, Amy Pasquinelli.

9:33 - **Break**

7- 10:03
Environmental programming of gene expression in *C. elegans*. **Sarah E. Hall**, Matthew H. Beverly, Carsten Russ, Chad Nusbaum, Piali Sengupta.

8- 10:15
Cell cycle re-entry in terminally differentiated body wall muscle cells. **Jerome Peter Korzelius**, Vincent Portegijs, Marian Groot Koerkamp, Frank Holstege, Inge The, Sander Van den Heuvel.

9- 10:27
Electron tomography: a new tool for exploring *C. elegans* cellular anatomy. **David H. Hall**, Kristin A. Politi, K. D. Derr, William J. Rice, Chris Crocker, Kevin Fisher, Leslie Gunther, Ken C. Q. Nguyen.

10- 10:30
Correlative microscopy in *C. elegans*. **Irina Kolotuev**, Yannick Schwab, Michel Labouesse.

11- 10:36
The posterior connectome of the *C. elegans* male. **Scott W. Emmons**, Yi Wang, Travis Jarrell, Meng Xu, David H. Hall, Donna G. Albertson, Nicole Thomson.

12- 10:42
C. elegans Gene Knockout Consortium Report: Progress, New Technologies and Funding. **Mark Edgley**, Don Moerman, Stephane Flibotte, Jeff Holmes, John Rummage, Bob Barstead.

13- 10:48
WormBase or Worm**S**Base? **Anthony S. Rogers**, The WormBase Consortium.

14- 10:54
Caenorhabditis Genetics Center. **Ann E. Rougvie**, Theresa Stiernagle.

Thursday, June 25 9:00 AM–12:00 noon
Ackerman Grand Ballroom

Neuronal Development

Chair: Robyn Lints

15- 9:00
zyg-8 promotes microtubule stability in touch neurons for proper mechanotransduction. **Jean-Michel Bellanger**, Anne Debant.

16- 9:12
Regulatory logic of dopaminergic neuron differentiation. **Nuria Flames**, Oliver Hobert.

17- 9:24
Antagonistic Wnt pathways define motor neuron-specific inputs. **Judsen Schneider**, Rachel L. Skelton, Steve Von Stetina, David Miller.

18- 9:36
The transcription factor *hbl-1* controls the timing of DD neuron synaptic remodeling. **Katherine L. Thompson-Peer**, Jihong Bai, Joshua Kaplan.

19- 9:48
An ER-resident membrane protein complex regulates nAChR subunit composition at the synapse. R. Almedom, **J. Liewald**, G. Hernando, D. Rayes, J. Pan, H. Hutter, C. Bouzat, A. Gottschalk.

20- 10:00
USP-46 is a Deubiquitinating Enzyme that Regulates the Synaptic Abundance of the Glutamate Receptor GLR-1. **Jennifer R. Kowalski**, Peter Juo.

10:12 - **Break**

21- 10:42
Molecular mechanisms activated by the UNC-40/DCC receptor to direct synaptic target selection. **Daniel A. Colon-Ramos**.

22- 10:54
zig-5 and *zig-8* regulate sax-7/L1 CAM in maintaining nervous system architecture. **Claire Bénard**, Oliver Hobert.

23- 11:06
A G-Protein coupled receptor (GPCR) mediates UNC-6/Netrin signaling in axon guidance. **Jasmine T. Plummer**, Joseph Culotti.

24- 11:18
Watching Neurons Grow: The Rac GTPases, UNC-34 and the Arp2/3 complex are Important for Growth Cone Structure and Dynamics. **Adam Norris**, Erik Lundquist.

25- 11:30
C. elegans glia require the thermotaxis gene *ttx-1* for temperature and dauer dependent functions. **Carl Procko**, Yun Lu, Shai Shaham.

26- 11:42
The Na^+/Cl^--dependent betaine transporter *snf-3* or the phospholipase Cβ *egl-8* blocks neurodegeneration. **Aude S. Ada-Nguema**, Randi L. Rawson, Guoliang Jiang, You-Jun Fei, Vadivel Ganapathy, Erik Jorgensen.

Thursday, June 25 9:00 AM–12:00 noon
Sunset Village Study Lounge

Evolution

Chair: Christian Braendle

27- 9:00
New Caenorhabditis species: phylogeny and evolution. **Karin C. Kiontke**, Marie-Anne Félix, David H. A. Fitch.

28- 9:12
The secret life of *E. coli* OP50. Joe Hedden, Nick Loman, Claudia Boehnisch, Mark Pallen, **Robin May**.

29- 9:24
Eating organic: effect of wild microbes on *C. elegans'* metabolism. **Buck S. Samuel**, Christian Braendle, Marie-Anne Felix, Gary Ruvkun.

30- 9:36
Quantitative genetic analyses of *C. elegans* intraspecies variation in responses to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. **Erik C. Andersen**, Zachary Okhah, Kirthi Reddy, Dennis H. Kim, Leonid Kruglyak.

31- 9:48
Life is not fair: larger chromosomes are transmitted to males in *C. elegans*. **John Wang**, Pei-Jiun Chen, George Wang, Winship Herr, Laurent Keller.

32- 10:00
The genomes of gonochoristic versus hermaphroditic Caenorhabditis species. **Erich M. Schwarz**, *Caenorhabditis* Genome Analysis Consortium.

10:12 - **Break**

33- 10:42
Evolutionary Comparisons of Nematode Dosage Compensation and Sex Determination. **Te-Wen Lo**, Qinwen Liu, Eric Haag, Barbara Meyer.

34- 10:54
The role of *gld-1* in *C. briggsae* germline sex determination. **Alana V. Doty**, Eric Haag.

35- 11:06
The Tip60/NuA4 HAT complex promotes spermatogenesis in *C. briggsae*. **Yiqing Guo**, Ronald E. Ellis.

36- 11:18
Inactivation of TRA-2 and SWM-1 Creates *C. remanei* Hermaphrodites. **Christopher C. Baldi**, Ronald E. Ellis.

37- 11:30
Germline RNAi insensitivity exhibits genetic complexity and heterogeneity in wild *C. elegans*. **D. A. Pollard**, M. J. Kramer, M. V. Rockman.

38- 11:42
Evolutionary Conservation of Cell Fusion in Nematodes. **Ori Avinoam**, Benjamin Podbilewicz.

Thursday, June 25 9:00 AM–12:00 noon
Grand Horizon Ballroom

Germline

Chair: Gillian Stanfield

39- 9:00
cye-1 and *cdk-2* may link the proliferation versus meiotic entry decision with the mitotic cell cycle. **Paul M. Fox**, Valarie Vought, Min-Ho Lee, Eleanor Maine, Tim Schedl.

40- 9:12
GLD-1-mediated repression of cyclin E/Cdk2 maintains totipotency during meiosis. Bjoern Biedermann, Mathias Senften, **Jane Wright**, Irene Kalchhauser, Gautham Sarathy, Min-Ho Lee, Rafal Ciosk.

41- 9:24
The Puf RNA-binding Proteins FBF-1 and FBF-2 Inhibit the Expression of Structural Components of Meiotic Chromosomes in Germline Stem Cells. **Chris Merritt**, Geraldine Seydoux.

42- 9:36
Germline Chromatin Structure is Regulated by XND-1. Cynthia Wagner, **Judith Yanowitz**.

43- 9:48
LAB-1 cooperates with cohesin to ensure accurate homolog segregation during meiosis I. **Yonatan B. Tzur**, Carlos E. de Carvalho, Ivo van Bostelen, Monica Colaiácovo.

44- 10:00
A High-Content Screen to Functionally Classify Proteins Required for Cell Viability and Division. **Rebecca A. Green**, Anjon Audhya, Karen Oegema, Arshad Desai.

10:12 - **Break**

45- 10:42
P granule-nuclear pore complexes are principal sites of mRNA export in *C. elegans* germ cells. **Ujwal Sheth**, Jason Pitt, James R. Priess.

46- 10:54
Nup98/NPP-10: a Link between Nuclear Pores and Germ Granules in *C. elegans* and Mouse. **Ekaterina Voronina**, Geraldine Seydoux.

47- 11:06
Self-aggregation of PGL proteins plays a crucial role in P granule assembly. Momoyo Hanazawa, Masafumi Yonetani, **Asako Sugimoto**.

48- 11:18
An EGG complex that is required for the oocyte-to-embryo transition. **Jean M. Parry**, Nathalie V. Velarde, Matthew H. Zegarek, Marina Druzhinina, Lindsay K. Kelly, Ariel J. Lefkovith, Julie Hang, Jon Ohm, Barth D. Grant, Fabio Piano, Andrew Singson.

49- 11:30
Males *try-5* to signal sperm activation. **Joseph Smith**, Gillian Stanfield.

50- 11:42
DAF-16 regulates prostaglandin signaling and sperm guidance. **Wes Edmonds**, Jeevan Prasain, Dixon Dorand, Michael Miller.

Thursday, June 25 9:00 AM–12:00 noon
De Neve Plaza Room

Genomics

Chair: Asher Cutter

51- 9:00
Transcriptome profiling of 7 *Pristionchus* species: Horizontal gene transfer, novel gene families and gene turnover. **Christoph Dieterich**, Werner Mayer, Lisa Schuster, Matthias Herrmann, Ralf Sommer.

52- 9:12
Of operons and outrons: a genomic and experimental analysis. **Tom Blumenthal**, Jason Morton, Mary Ann Allen, Peg MacMorris, LaDeana Hillier, Bob Waterston.

53- 9:24
Germline expression influences operon organization in the C. elegans genome. **Asher D. Cutter**, Valerie Reinke.

54- 9:36
Quantitative screen for alternative splicing regulation factors in vivo. **Karine G. M. Rebora**, Ilyass Zniber, Genta Ohno, Hidehito Kuroyanagi, Laura Fontrodona, Julian Ceron, Simo Schwartz, Jr., Denis Dupuy.

55- 9:48
Genomic Strategies to Map the *C. elegans* Transcriptome. **William Clay Spencer**, Joseph Watson, Rebecca McWhirter, Kathie Watkins, Ashish Agarwal, Mark Gerstein, Shenglong Wang, Nurith Kern, David M. Miller III.

56- 10:00
Comparative fnctional analysis of the *Caenorhabditis elegans* and *Drosophila melanogaster* proteomes. Sabine Schrimpf, Manuel Weiss, Lukas Reiter, Christian H. Ahrens, Marko Jovanovic, Johan Malstroem, Erich Brunner, Sonali Mohanti, Martin J. Lercher, Peter E. Hunziker, Ruedi Aebersold, Christian von Mering, **Michael O. Hengartner**.

10:12 - **Break**

57- 10:42
The landscape of *C. elegans* 3'UTRs. **Marco Mangone**, Arun Prasad Manoharan, Han Ting, Oliver Attie, Emily Mis, Philip MacMenamin, Charles Zegar, Vishal Khivansara, Kevin Chen, Wei Chen, Nikolaus Rajewsky, Kourosh Salehi-Ashtiani, Marc Vidal, Yuji Kohara, Jean Thierry-Mieg, Danielle Thierry-Mieg, Fabio Piano, John Kim, Kristin Gunsalus.

58- 10:54
A Genetic Interaction Map of Early Embryogenesis in *C. elegans*. **Patricia Giselle Cipriani**, Huey-Ling Kao, Amelia White, Kristin Gunsalus, Fabio Piano.

59- 11:06
An intricate network of nuclear hormone receptors regulates energy balance in the nematode *C. elegans*. **H. Efsun Arda**, Stefan Taubert, Colin Conine, Marc Van Gilst, Reynaldo Sequerra, Lynn Doucette-Stamm, Keith Yamamoto, Albertha J. M. Walhout.

60- 11:18
Temperature and light-entrained circadian rhythms in *C. elegans*. **Alexander M. van der Linden**, Sebastian Kadener, Michael Rosbash, Piali Sengupta.

61- 11:30
Searching for signaling balance through the identification of genetic interactors of the Rab guanine-nucleotide dissociation Inhibitor, *gdi-1* in *C. elegans*. **Anna Y.-W. Lee**, Richard Perreault, Sharon Harel, Elodie Boulier, Matthew Suderman, Michael Hallett, Sarah Jenna.

62- 11:42
Rapid high resolution SNP-CGH mapping in *C. elegans*. **Stephane Flibotte**, Mark Edgley, Jon Taylor, Iasha Chaudhry, Robert Waterston, Donald Moerman.

Thursday, June 25 9:00 AM–12:00 noon
De Neve Auditorium

Morphogenesis, Migration and the Cytoskeleton

Chair: Zhirong Bao

63- 9:00
A New Role for the Anchor Cell in Vulval Development: Initiating Dorsal Lumen Formation. **Kathleen Estes**, Wendy Hanna-Rose.

64- 9:12
LET-4 is a transmembrane protein required for *C. elegans* excretory system development. **Vincent P. Mancuso**, Meera Sundaram.

65- 9:24
daf-6/Patched, *che-14*/Dispatched and Wnt Components Interact To Direct Amphid Lumen Morphogenesis. **G. Oikonomou**, E. Perens, Y. Lu, S. Shaham.

66- 9:36
Up- and down-regulation of TOR-Raptor and TOR-Rictor complexes by semaphorin in *C. elegans*. **Akira Nukazuka**, Shusaku Tamaki, Hajime Fujisawa, Yoichi Oda, Shin Takagi.

67- 9:48
Identification of an essential component of the subapical intermediate filament anchorage machinery in *C. elegans*. **O. Bossinger**, K. Hüsken, T. Wiesenfahrt, H. Gerhardus, F. Geisler, D. Ueberbach, R. Leube.

68- 10:00
The Conserved Glc7/PP1 Phosphatases GSP-3 and GSP-4 Regulate Sperm Morphogenesis. **Aiza Go**, Jui-ching Wu, Mark Samson, Hongkang Zhou, Susan Mirsoian, Diana Chu.

10:12 - **Break**

69- 10:42
The role of Glycogen Debranching Enzyme during *C. elegans* development and aging. **Jeffrey S. Simske**.

70- 10:54
Investigating the role of PAR-3 in *C. elegans* embryonic epithelial cells. **Annita Achilleos**, Jeremy Nance.

71- 11:06
Circumferential actin and elongation. **Christopher Allen Vanneste**, Paul E. Mains.

72- 11:18
The ROCK homolog LET-502 is temporally and spatially regulated by LIN-12 Notch and LIN-1 ETS during vulval morphogenesis. **Sarfarazhussain Farooqui**, Ivo Rimann, Alex Hajnal.

73- 11:30
Screening for essential muscle genes using high throughput RNAi identifies a copine involved in early sarcomere assembly. **Adam D. Warner**, Teresa Rogalski, Aruna Somasiri, Don Moerman.

74- 11:42
Molecular interactions reveal multiple roles for UNC-53 in cell migration, intracellular trafficking, and innate immunity. Kristopher Schmidt, **Eve Stringham**.

Thursday, June 25 9:00 AM–12:00 noon
Bradley International Ballroom

Aging

Chair: Catherine Wolkow

75- 9:00
The anticonvulsant ethosuximide disrupts sensory function to extend *C. elegans* life span. James J. Collins, Kimberley Evason, **Christopher L. Pickett**, Daniel L. Schneider, Kerry Kornfeld.

76- 9:12
Relating cellular and organismal aging phenotypes in *C. elegans*. **Javier Apfeld**, Walter Fontana.

77- 9:24
Strongyloides ratti: An unusual nematode with extraordinary plasticity in aging. **David Gems**, Fiona Thompson, Michael Gardner, Mark Viney.

78- 9:36
Genetic interactions between *apl-1*, a gene encoding an amyloid precursor-related protein, and *daf-16*, a regulator of lifespan. **Collin Ewald**, Chris Li.

79- 9:48
Attenuated immune response during aging in *C. elegans*. **Matthew J. Youngman**, Odile Kamanzi, Annie R. Inman, Dennis H. Kim.

80- 10:00
An age-dependent switch in the functional balance of mitogen-activated protein kinase pathways affects infection susceptibility and lifespan. Kwame Twumasi-Boateng, Man-Wah Tan, **Michael Shapira**.

10:12 - **Break**

81- 10:42
TGF-β Sma/Mab Signaling is a Novel Regulator of Reproductive Aging and the Maintenance of Germline and Oocyte Quality in *C. elegans*. **Shijing Luo**, Gunnar A. Kleemann, Wendy Shaw, Jasmine Ashraf, Coleen T. Murphy.

82- 10:54
HCF-1 modulates lifespan and stress responses in *C. elegans* by regulating the transcriptional activities of DAF-16 and SIR-2.1. **Gizem Rizki**, Ji Li, Max Jan, Coleen T. Murphy, Siu Sylvia Lee.

83- 11:06
HSF-1 is an Essential Downstream Mediator of Resveratrol-induced, SIR-2.1-dependent Longevity in C. elegans. **Marton L. Toth**, Balazs Dancso, Peter Csermely, Csaba Soti.

84- 11:18
A soma-to-germline transformation in long-lived *C. elegans* mutants. **Sean P. Curran**, Xiaoyun Wu, Christian G. Riedel, Gary Ruvkun.

85- 11:30
Mutations in *egl-1* and *daf-2* improve oocyte quality in aging females. **Qing Wei**, Sara Audux, Ronald E. Ellis.

86- 11:42
A sperm signal downstream of DAF-16/FOXO induces vitellogenin gene expression in C elegans. **Ana S. DePina**, Wendy B. Iser, Catherine A. Wolkow.

Thursday, June 25 1:30 PM–4:30 PM
Royce Hall

Plenary Session 2: Germline Development, Embryogenesis and Morphogenesis

Chairs: Emily Troemel and Jennifer Ross Wolff

87- 1:30
Starvation-induced adult reproductive diapause protects germline stem cells and extends reproductive longevity in *C. elegans*. **Giana Angelo**, Marc Van Gilst.

88- 1:42
Motor-Driven Motion Associated with Meiotic Chromosome Pairing. **David Wynne**, Pete Carlton, Abby Dernburg.

89- 1:54
Toward a Unified-Field Theory of the Adult Hermaphrodite Gonad. **J. Amaranath Govindan**, Saravanapriah Nadarajan, Seongseop Kim, Todd Starich, David Greenstein.

90- 2:06
VAP MSP domains are secreted metabolic factors that bind to Eph and Roundabout receptors. **Sung Min Han**, Youfeng Yang, Hiroshi Tsuda, Chao Tong, Claire Haueter, Hugo J. Bellen, Michael Miller.

91- 2:18
MPK-1 ERK regulates NOS-3, FEM-3/E3 ubiquitin ligase complex and TRA-1 cascade during oogenesis to execute cell membrane organization and morphogenesis. **Swathi Arur**, Mitsue Omachi, Amanda Hay, Tim Schedl.

92- 2:30
EFA-6 induces microtubule catastrophe in early *C. elegans* embryos. **Sean O'Rourke**, Sara Christensen, Ronald Kwong, Bruce Bowerman.

2:42 - **Break**

93- 3:12
Establishment of PAR polarity in *C. elegans* through a reaction-diffusion mechanism. **Nathan W. Goehring**, Debanjan Chowdury, Philipp Khuc Trong, Ernesto Nicola, Stephan W. Grill, Anthony Hyman.

94- 3:24
PAR-2 is a microtubule-binding protein that links microtubules and cortical polarity in *C. elegans* zygotes. **Fumio Motegi**, Seth A. Zonies, Geraldine Seydoux.

95- 3:36
PPH-6 Regulates AIR-1 Distribution to Modulate Cortical Pulling Forces during Spindle Positioning in One-cell Stage Embryos. **Katayoun Afshar**, Pierre Gönczy.

96- 3:48
A tug-of-war model for the centrosome centering in *C. elegans* early embryo. **K. Kimura**, A. Kimura.

97- 4:00
A "Notch + Forkhead" code in the *C. elegans* pharynx. **Jeffrey P. Rasmussen**, Kathryn English, James R. Priess.

98- 4:12
A Tension-dependent Pathway Promoting Fibrous Organelle Maturation During Embryonic Morphogenesis. **Huimin Zhang**, Hala Zahreddine, Marie Diogon, Frédéric Landmann, Yasuko Nagamatsu, Michel Labouesse.

99- 4:24
DRAG-1 is a cell type specific modulator of the Sma/Mab TGF-beta pathway in *C. elegans*. **C. Tian**, D. Sen, H. Shi, M. Foehr, Y. Plavskin, A. Lindy, A. Al-Barwani, J. Liu.

Friday, June 26 9:00 AM–12:00 noon
Bradley International Ballroom

Synaptic Function, Neural Circuits and Neural Methods

Chair: Alexander Van Der Linden

100- 9:00
A single immunoglobulin domain protein required for the localization of acetylcholine receptors at the *C. elegans* neuromuscular junction. **Georgia Rapti**, Janet Richmond, Jean-Louis Bessereau.

101- 9:12
Synaptic vesicle acidification is a checkpoint for vesicle fusion. **Glen G. Ernstrom**, Mark T. Palfreyman, Shigeki Watanabe, Erik Jorgensen.

102- 9:24
Dysferlin/*fer-1* promotes cholinergic synaptic transmission in *C. elegans* and mice. **P. Krajacic**, M. Mosqueira, J. Hermanowski, O. Lozynska, J. Snitzman, X. Shen, P. Arratia, T. S. Khurana, T. Lamitina.

103- 9:36
BKIP-1 is a Novel Regulatory Protein Critical to SLO-1 Function in vivo. **Bojun Chen**, Qian Ge, Haiying Zhan, Zhao-Wen Wang.

104- 9:48
A Genetic Suppressor Screen with Whole Genome Sequencing identifies novel effectors of RHO-1 in the nervous system. **Andrew Porter**, Rachel McMullan, Stephen Nurrish.

105- 10:00
Neuroligin deficient mutants of *C. elegans* are hypersensitive to oxidative stress and some heavy metals. **Jim Rand**, Jerrod Hunter, Greg Mullen, Jessica Heatherly, John McManus, Angie Duke.

10:12 - **Break**

106- 10:42
Glial DEG/ENaC channel ACD-1 functions in odor sensation in *C. elegans*. Ying Wang, **Laura Bianchi**.

107- 10:54
AWC sensory neurons release two neurotransmitters to regulate exploratory behavior upon removal from food. **Sreekanth H. Chalasani**, Dirk Albrecht, Saul Kato, Cornelia I. Bargmann.

108- 11:06
Dopaminergic neuronal cell-fate mutants retrieved through automated screening. **Maria Doitsidou**, Nuria Flames, Oliver Hobert.

109- 11:18
Development of a tetracycline controllable expression system for *C. elegans*. **U. Schaeffer**, A. Feldmann, R. Baumeister.

110- 11:30
Microfluidic in vivo screening for compounds affecting neural regeneration in *C. elegans*. **Chrysanthi Samara**, Christopher Rohde, Cody Gilleland, Stephanie Norton, Stephen Haggarty, Mehmet Yanik.

111- 11:42
A microfluidic chip enabling high-throughput in vivo femtosecond laser nanoaxotomy for nerve regeneration studies. Frederic Bourgeois, Samuel Guo, Navid Ghorashian, Trushal Chokshi, Massimo Hilliard, Nikos Chronis, **Adela Ben-Yakar**.

Friday, June 26 9:00 AM–12:00 noon
Ackerman Grand Ballroom

Gene Expression

Chair: Weiwei Zhong

112- 9:00
Trimethylation of lysine 36 of histone H3 is a chromatin mark for expressed exons in C. elegans, humans, and mice. **Paulina M. Kolasinska-Zwierz**, Thomas Down, Isabel Latorre, Tao Liu, Shirley Liu, Julie Ahringer.

113- 9:12
Novel proteins bound to SL1 and Sm Y RNAs: possible roles in recycling Sm proteins. Peg MacMorris, Tassa Saldi, **Tom Blumenthal**.

114- 9:24
Spatiotemporal analysis of promoter functions implicates spindle assembly checkpoint genes in polyploidization in *C. elegans*. **Maja Tarailo-Graovac**, Jun Wang, Jeffrey Shih-Chieh Chu, Domena Tu, David L. Baillie, Nansheng Chen.

115- 9:36
DNA adenine methyltransferase identification (DamID) analysis implies that DAF-16 is a master regulator. **Eugene F. Schuster**, Joshua McElwee, Jennifer Tullet, Ryan Doonan, Filip Matthijssens, Jacques R. Vanfleteren, David Gems.

116- 9:48
elt-2 and *sbp-1* are the only two transcription factors required in the intestine for viability after endoderm specification. **John M. Kalb**, Renee A. Larson, Jessica Nowak, James D. McGhee.

117- 10:00
Transcriptional regulation by the conserved regulatory complex mediator in *C. elegans*. **Stefan Taubert**, Jess Porter Abate, T. Keith Blackwell.

10:12 - **Break**

118- 10:42
Targeting the Condensin-like Dosage Compensation Complex to X. **Rebecca Pferdehirt**, Barbara Meyer.

119- 10:54
Automatic Gene Expression Profiling at Single Cell Resolution in both *C. elegans* and *C. briggsae*. **Zhongying Zhao**, John Murray, Zhirong Bao, Thomas Boyle, Max E. Boeck, Dan Blick, Matthew Sandel, Elicia Preston, Dionne Vafeados, Stephane Flibotte, Don Moerman, Robert Waterston.

120- 11:06
A new model of *C. elegans* embryogenesis with cell contacts and spatio-temporal gene expressions generated with VW-Base and Endrov. Jurgen Hench, **Johan Henriksson**, Martin Lüppert, Akram Abou-Zied, Konstantin Cesnulevicius, Krai Meemon, David L. Baillie, Thomas R. Burglin.

121- 11:18
Combination of *cis*-regulatory modules for temporal regulation of gene expression in *C. elegans*: Towards promoter design. **Akihiro Mori**, Y. Kohara.

122- 11:30
The *C. elegans* TransgeneOme: a fosmid transgene resource for genome scale protein function analysis. **Mihail Sarov**, John Murray, Mei Zhong, Valerie Reinke, Stuart K. Kim, Robert Waterston, Michael Snyder, Anthony Hyman.

123- 11:42
Using Mos1 elements to modify the genome. **Christian Frøkjær-Jensen**, M. Wayne Davis, Christopher E. Hopkins, Blake Newman, Rachel Lofgren, Morten Grunnet, Søren-Peter Olesen, Erik Jorgensen.

Friday, June 26 9:00 AM–12:00 noon
Sunset Village Study Lounge

Cell Death and Neurodegeneration

Chair: Itzhak Mano

124- 9:00
A polyQ-repeat protein promotes the novel, morphologically conserved death of the linker cell. **Elyse S. Blum**, Shai Shaham.

125- 9:12
The life versus death decision of the sexually dimorphic CEM neurons: a story beyond *egl-1* BH3-only. **Ralda Nehme**, Phillip Grote, Tatiana Tomasi, Stefanie Loeser, Heinke Holzkamp, Barbara Conradt.

126- 9:24
Bilaterally asymmetric deployment of alternative apoptotic pathways is determined by a novel left-right symmetry breaking mechanism. **Shin Sik Choi**, Joel H. Rothman, Tim A. Bloss.

127- 9:36
Induction of Germ Cell Apoptosis by Multiple Isoforms of IFG-1 are CED-4/Apaf-1 Dependent in *C. elegans*. **Vince Contreras**, Melissa Henderson, Enhui Hao, Brett Keiper.

128- 9:48
C. elegans Rab GTPase Activating Protein, TBC-2, Promotes Cell Corpse Degradation by Regulating the Small GTPase RAB-5. Weida Li, **Wei Zou**, Dongfeng Zhao, Jiacong Yan, Zuoyan Zhu, Jing Lu, Xiaochen Wang.

129- 10:00
The role of F58G11.6 in apoptotic corpses degradation in *C. elegans*. Cristina Nieto, Johann Almendinger, Stephan Gysi, Andres Kaech, Ralf Schnabel, Michael O. Hengartner, Sergio Moreno, **Juan Cabello**.

10:12 - **Break**

130- 10:42
How do necrotic cells expose “eat me” signals to attract engulfing cells? **Victor Venegas**, Zheng Zhou.

131- 10:54
The RhoGAP SRGP-1 regulates cell killing and clearance through CED-10 in *C. elegans*. **Lukas J. Neukomm**, Andreas P. Frei, Juan Cabello, Xhong Ma, Lisa B. Haney, Marko Jovanovic, Bernd Wollscheid, Kodimangalam S. Ravichandran, Sergio Moreno, Michael O. Hengartner.

132- 11:06
A mechanism for exchange of RAB-5 for RAB-7 on the surface of the phagosome. **Jason M. Kinchen**, Kodi S. Ravichandran.

133- 11:18
The neural protein PKC-2 suppresses Duchenne muscular dystrophy. **A. Reedy**, C. Lecroisey, M. C. Mariol, H. Salter, L. Ségalat, K. Gieseler.

134- 11:30
Use of *C. elegans* to Identify and Characterize VPS41 as a New Therapeutic Target for Parkinson’s Disease. **A. Harrington**, Q. Ruan, S. Hamamichi, J. Schieltz, D. G. Standaert, K. A. Caldwell, G. A. Caldwell.

135- 11:42
The Coenzyme Q Synthesis Gene *coq-1* Protects *C. elegans* GABA Neurons from Calcium-Dependent Programmed Necrosis. **Mallory L. Hacker**, Laurie R. Earls, Joseph D. Watson, David M. Miller III.

Friday, June 26 9:00 AM–12:00 noon
De Neve Plaza Room

Developmental Timing and Dauer Larvae

Chair: Allison Abbott

136- 9:00
Heterochronic genes and lateral signaling during vulval precursor cell (VPC) fate specification in *C. elegans*. **Ji Li**, Iva Greenwald.

137- 9:12
lin-28 and lin-46 act at distinct molecular steps to control hbl-1 and stage-specific seam cell fates. **Eric G. Moss**, Bhaskar Vadla, Christian Heine, Kevin Kemper.

138- 9:24
The Conserved NAB Family Transcriptional Co-factor *mab-10* Acts with the Heterochronic Gene *lin-29* to Regulate Terminal Differentiation in Hypodermal Lineages. **David T. Harris**, Bob Horvitz.

139- 9:36
The 3-β-hydroxysteroid dehydrogenase (3βHSD) family member HSD-1 controls dauer arrest via regulation of nuclear DAF-16/FoxO. Xi Wang, Kirk Benjamin Burkhart, Chunfang Guo, Kathleen Dumas, Elizabeth Adams, Hena Alam, **Patrick J. Hu**.

140- 9:48
Genetic analysis of dauer formation in *Pristionchus pacificus* reveals conserved and diverse aspects of signaling mechanisms. **Akira Ogawa**, Ralf Sommer.

141- 10:00
Endocannabinoid signaling has a role in dauer formation. **Mark Lucanic**, Jason Held, Mark White, Ida Klang, Brad Gibson, Gordon J. Lithgow, Matthew Gill.

10:12 - **Break**

142- 10:42
Methylation of sterols by *strm-1* as a novel mechanism for regulation of dauer larva formation. **Eugeni V. Entchev**, J.-Thomas Hannich, Fanny Mende, Hristio Boytchev, René Martin, Gabriele Theumer, Isabelle Riezman, Howard Riezman, Hans-Joachim Knölker, Teymuras V. Kurzchalia.

143- 10:54
Modulation of Insulin Secretion by DAF-8/R-Smad and NHR-69/HNF4α. **Donha Park**, Donald L. Riddle.

144- 11:06
Specific insulin-like peptides translate distinct sensory information to regulate C. elegans development. **Astrid Cornils**, Mario Gloeck, Joy Alcedo.

145- 11:18
NHR-8, A Nuclear Receptor Influencing Reproduction, Lipid, and Cholesterol Homeostasis. **Daniel Magner**, Dongling Li, Joshua Wollam, Adam Antebi.

146- 11:30
The nuclear hormone receptor *nhr-25* is a heterochronic gene that has dual roles in both promoting and inhibiting *C. elegans* adult programs. **Ryusuke Niwa**, Kazumasa Hada, Hiroshi Hasegawa, Masako Asahina, Yasunori Kanaho, Frank Slack.

147- 11:42
Identification of New Components of Dauer Regulation using Full-Genome RNAi Screens. **Dhaval S. Patel**, Li Fang, Shyam Bhansali, Gary Ruvkun, Weiqing Li.

Friday, June 26 9:00 AM–12:00 noon
Grand Horizon Ballroom

Polarity and Trafficking

Chair: Erin Cram

148- 9:00
Flows and tension during cortical polarization of the *C. elegans* zygote. **M. Mayer**, M. Depken, J. S. Bois, F. Juelicher, S. W. Grill.

149- 9:12
PAR-4 regulates actomyosin contractility through ANI-2 during early C. elegans embryonic divisions. **Nicolas T. Chartier**, Paulina Salazar, Amy S. Maddox, Labbé Jean-Claude.

150- 9:24
Directional control of PAR-dependent polarization in *C. elegans*. **Yukinobu Arata**, Tetsuya Kobayashi, Hitoshi Sawa.

151- 9:36
SUMO is essential for IFB-1 function and assembly. **Rachel Kaminsky**, Carilee Denison, Andrew D. Chisholm, Steven P. Gygi, Limor Broday.

152- 9:48
The Myotubularin complex MTM-6/MTM-9 and the PX domain containing protein SNX-3 play a crucial role in MIG-14/Wls recycling in Wnt producing cells. **M. Harterink**, M. Silhankova, M. Betist, R. G. H. P. van Heesbeen, H. C. Korswagen.

153- 10:00
A ZYG-12-dynein interaction at the nuclear envelope defines microtubule architecture and germ line nuclear position in the *C. elegans* gonad. **Kang Zhou**, Melissa M. Rolls, David H. Hall, Christian J. Malone, Wendy Hanna-Rose.

10:12 - **Break**

154- 10:42
UNC-83 is a nuclear-specific cargo adaptor for kinesin-1 mediated and dynein regulated nuclear migration. **Heidi N. Fridolfsson**, Marina Meyerzon, Nina Ly, Daniel A. Starr.

155- 10:54
The Distinct Roles of Self-assembly and GTP Hydrolysis in Regulating Dynamin's Association with Target Membranes and the Removal of Apoptotic Cells in *C. elegans*. **Bin He**, Xiaomeng Yu, Moran Margolis, Xiaohong Leng, Yael Etzion, Dganit Danino, Zheng Zhou.

156- 11:06
A *C. elegans* model of the human disease orotic aciduria reveals enlarged lysosome-related organelles in embryos lacking *umps*-1 function. **Steven Levitte**, Becca Salesky, Maddie Cole, Micah Depper, Greg Hermann.

157- 11:18
AMPH-1/Amphiphysin/BIN1: A Novel Regulator of Endocytic Recycling. **S. Pant**, M. Sharma, K. Patel, S. Caplan, C. Carr, B. Grant.

158- 11:30
New roles for ciliary kinesins in male-specific CEM sensory neurons. **Natalia Morsci**, Maureen Barr.

159- 11:42
A *C. elegans* homologue of Joubert syndrome-associated Arl13B associates with the ciliary membrane and is required for proper targeting and motilities of ciliary transmembrane and IFT proteins. Sebiha Cevik, Oktay Kaplan, Katarzyna Kida, Tiina Toivenon, David Cottell, **Oliver Blacque**.

Friday, June 26 1:30 PM–4:30 PM
Royce Hall

Plenary Session 3: RNAi, Regeneration and Neurobiology

Chairs: Marc Hammarlund and Jill Bettinger

160- 1:30
Position and precision of the germline stem cell proliferation boundary are regulated by a miRNA regulatory network. **Pradeep M. Joshi**, Jess Porter Abate, Joel H. Rothman.

161- 1:42
A dsRNA-specific endocytosis pathway for environmental RNAi. **Deborah L. De Jong**, Craig P. Hunter.

162- 1:54
siRNAs regulate transcription elongation. **Shouhong Guang**, Aaron Bochner, Kirk Benjamin Burkhart, Derek M. Pavelec, Scott Kennedy.

163- 2:06
The Argonaute CSR-1 interacts with 22G-RNAs targeting germline-expressed genes to promote chromosome segregation in *C. elegans*. **J. M. Claycomb**, P. J. Batista, K. Pang, W. Gu, J. J. Vasale, D. A. Chaves, M. Shirayama, S. Mitani, D. Conte, C. C. Mello.

164- 2:18
The core apoptotic executioner proteins CED-3 and CED-4 promote neuronal regeneration in *C. elegans*. **Berangere Pinan-Lucarre**, Christopher Gabel, Elizabeth Hulme, Sergey Shevkoplyas, Daniel Slone, Jian Xue, Sarah Weisberg, George Whitesides, Aravinthan Samuel, Monica Driscoll.

165- 2:30
Dissecting Calcium/cAMP mediated axon regeneration pathways. **A. Ghosh Roy**, Z. Wu, A. Goncharov, Y. Jin, A. D. Chisholm.

166- 2:42
The DLK-1 kinase pathway promotes CEBP-1 mRNA stability and local translation in *C. elegans* synapse maintenance and axon regeneration. **D. Yan**, Z. Wu, A. D. Chisholm, Y. Jin.

2:54 - **Break**

167- 3:24
Single-Stranded DNA Binding Protein (SSDP) regulates *C. elegans* PLM synaptic branching and synaptogenesis. **Qun Zheng**, Michael Nonet.

168- 3:36
Different sensory dendrites use distinct machineries to attain their lengths. **Maxwell G. Heiman**, Shai Shaham.

169- 3:48
EFF-1 sculpts neuronal trees via membrane bending, branch retraction and self-cell fusion. **Meital Oren-Suissa**, Gidi Shemer, Millet Treinin, Benjamin Podbilewicz.

170- 4:00
Identification of a novel dense-core vesicle trafficking pathway. **Michael Ailion**, Andrea Pappas, Susan Dalton, Kim Schuske, Patrick Hullett, Erik Jorgensen.

171- 4:12
Analysis of Endophilin endocytic function suggests a model for coupling exo- and endocytosis. **Jihong Bai**, Jeremy Dittman, Edward Pym, Joshua Kaplan.

172- 4:24
Eyeless but not blind: Phototransduction in C. elegans. **Alex Ward**, Jie Liu, Lijun Kang, Jingwei Gao, Yong Yu, Nana Nishio, Hitoshi Inada, Di Ma, Brandon Decaluwe, Ikue Mori, Zhixiong Xie, X. Z. Shawn Xu.

173- 4:36
Olfactory adaptation is regulated by a peptide signal in *C. elegans*. **K. Yamada**, T. Hirotsu, M. Matsuki, H. Kunitomo, Y. Iino.

174- 4:48
Complex properties of C. elegans oxygen sensing neurons shape different behavioural responses to oxygen. **Patrick Laurent**, Karl Emanuel Busch, Robin Murphy, Mario de Bono.

Friday, June 26 5:00 PM–6:30 PM
Royce Hall

Plenary Session 4

Chairs: Monica Driscoll and Ding Xue

175- 5:00
Special Presentation. **Martin Chalfie**.

Saturday, June 27 9:00 AM–12:00 noon
Ackerman Grand Ballroom

Behavior

Chair: Laura Bianchi

176- 9:00
RGEF-1b, a Diacylglycerol (DAG)-regulated GTP Exchange Factor, Facilitates Chemotaxis by Activating LET-60 in AWC and AWA Neurons. **Lu Chen**, Charles Rubin.

177- 9:12
Modulation of chemosensation in ASH through extrasynaptic dopaminergic and neuropeptide signaling. **Marina Ezcurra**, Peter Swoboda, William Schafer.

178- 9:24
Exclusively optical interrogation and monitoring of neural circuits in *C. elegans*. **Zengcai Guo**, Anne C. Hart, Sharad Ramanathan.

179- 9:36
Neuropeptides are essential for the serotonergic stimulation of aversive responses mediated by the ASH sensory neurons. **Gareth P. Harris**, Rachel Wragg, Vera Hapiak, Amanda Korchnak, Philip Summers, Richard W. Komuniecki.

180- 9:48
Serotonin enhances feeding only in response to familiar food. **B. Song**, L. Avery.

181- 10:00
Fat storage and metabolic genes regulate quiescence as a result of satiety. **Young-jai You**, Leon Avery.

10:12 - **Break**

182- 10:42
Distributed neural sex differences modify *C. elegans* locomotory behaviors. William Mowrey, **Douglas Portman**.

183- 10:54
An integrated model of *C. elegans* locomotion: from swimming to crawling. **Stefano Berri**, Jordan H. Boyle, Manlio Tassieri, Ian A. Hope, Netta Cohen.

184- 11:06
The worms crawl in, the worms crawl out: An analysis of C. elegans locomotory gaits. **Christopher Fang-Yen**, Risa Kawai, Sway Chen, Matthieu Wyart, Quan Wen, Dmitri Chklovskii, Aravinthan Samuel.

185- 11:18
HPL-2 and MUT-7 in odor-adaptation of the AWC neuron. **Bi-Tzen Juang**, Noelle L'Etoile.

186- 11:30
The Role of the Calcium/Calmodulin-dependent Protein Kinase Cascade in Mechanosensory Habituation. **Tiffany A. Timbers**, Catharine H. Rankin.

187- 11:42
A Genetic and Cellular Circuit for *C. elegans* Long-term Associative Memory. **Amanda L. Kauffman**, Geneva M. Stein, Coleen T. Murphy.

Saturday, June 27 9:00 AM–12:00 noon
De Neve Auditorium

Mechanisms and Function of RNA Interference and Small RNAs

Chair: Jonathan Whetstine

188- 9:00
A cell-free *C. elegans* embryonic system reveals maternal translation control by the miR-35-42 microRNA family through deadenylation. Edlyn Wu, Mathieu Flamand, Caroline Thivierge, Marc Fabian, Géraldine Mathonnet, James Wohschlegel, Nahum Sonenberg, **Thomas F. Duchaine**.

189- 9:12
Multiple germline 22G-RNA pathways maintain genome integrity in *C. elegans*. **Weifeng Gu**, Masaki Shirayama, Darryl Conte, Jr., Jessica J. Vasale, Pedro Batista, Julie Claycomb, Daniel Chaves, Jennifer Keys, Craig Mello.

190- 9:24
New Roles for Mitotic Condensin Proteins in RNAi and Chromatin-Based Gene Silencing. **James F. Carey**, James Thompson, John R. Yates III, Kirsten A. Hagstrom.

191- 9:36
RDE-8 encodes a novel protein with a conserved domain required for RNAi. **Hsin-Yue Tsai**, Chun-Chieh G. Chen, James J. Moresco, Weifeng Gu, John R. Yates III, Craig Mello.

192- 9:48
26G RNAs regulate gene expression during spermatogenesis and larval development. **Ting Han**, Arun Prasad Manoharan, Colin Fitzpatrick, Diana Chu, John Kim.

193- 10:00
Small RNA Pathways Required to Produce Thermo-tolerant Sperm in C. elegans. **Colin Conine**, Pedro Batista, Hsin-Yue Tsai, Craig Mello.

10:12 - **Break**

194- 10:42
SID-1 expression in neurons enhances neuronal RNAi in *C. elegans*. **Andrea Calixto**, Chelur Dattananda, Topalidou Irini, Chalfie Martin.

195- 10:54
Distinct mechanisms of microRNA post-transcriptional regulation by LIN-28 and LIN-42. **Priscilla M. Van Wynsberghe**, Zoya Kai, Valentino M. Gantz, Amy Pasquinelli.

196- 11:06
A novel nuclear export route for miRNAs? **Ingo Buessing**, Monika Fasler, Helge Grosshans.

197- 11:18
Genes containing binding sites for multiple microRNAs. **Yoshiki Andachi**.

198- 11:30
Systematic analysis of dynamic miRNA-target interactions during C. elegans development. Liang Zhang, Molly C. Hammell, **Brian A. Kudlow**, Victor Ambros, Min Han.

199- 11:42
Roles of Rb and its related genes in regulating RNA interference. **Mingxue Cui**, Min Han.

Saturday, June 27 9:00 AM–12:00 noon
Grand Horizon Ballroom

Stress Responses

Chair: Pankaj Kapahi

200- 9:00
Rictor/TOR complex 2 regulates fat metabolism, feeding, growth, and lifespan in *C. elegans*. **Alexander Soukas**, Elizabeth Kane, Christopher Carr, Buck S. Samuel, Justine Melo, Gary Ruvkun.

201- 9:12
Transketolase is a new target of SBP-1 and is required for lipid homeostasis in *C. elegans*. **Veerle Rottiers**, Amy K. Walker, Jennifer Watts, Anne C. Hart, Anders M. Näär.

202- 9:24
Hormonal Control of the Dietary Restriction Response in C. elegans. **Bree Heestand**, Adam Antebi.

203- 9:36
Intraflagellar transport/Hedgehog-related signaling components couple sensory cilium morphology and serotonin biosynthesis in *C. elegans*. **Mustapha Moussaif**, Ji Ying Sze.

204- 9:48
Neuronal Regulation of L1 Survival Under Starvation Condition. **Brian Han Lee**, Kaveh Ashrafi.

205- 10:00
Identification of a novel conserved master stress response pathway. **Natalia V. Kirienko**, David S. Fay.

10:12 - **Break**

206- 10:42
Pore-forming toxins, stress and the hypoxia pathway in *C. elegans*. **Audrey Bellier**, Chang-Shi Chen, Raffi V. Aroian.

207- 10:54
Unfolded protein response (UPR) protects *C. elegans* from hypoxic injury. **Xianrong Mao**, C. Michael Crowder.

208- 11:06
Ascaroside Signalling and Lifespan in C. elegans. **Andreas H. Ludewig**, Rabia Malik, Chirag Pungaliya, Bennett Fox, Frank Schroeder.

209- 11:18
Live imaging and quantification of proteasome activity using C. elegans. **Geert Hamer**, Olli Matilainen, Carina Holmberg.

210- 11:30
p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress. **Natascia Ventura**, Shane Rea, Alfonso Schiavi, Alessandro Torgovnick, Roberto Testi, Thomas Johnson.

211- 11:42
eIF-4G post-transcriptionally remodels stress response and cellular homeostasis gene expression in *C. elegans.*. **Aric N. Rogers**, Di Chen, Gregg Czerwieniec, Gawain McColl, Alan Hubbard, Brad Gibson, Simon Melov, Gordon J. Lithgow, Pankaj Kapahi.

Saturday, June 27 9:00 AM–12:00 noon
Bradley International Ballroom

Cell Cycle, Chromosome Dynamics and Nuclear Organization

Chair: Alisa Piekny

212- 9:00
The axial element protein HTP-3 promotes cohesin loading and assembly of the meiotic chromosomal axis. **Aaron Severson**, Barbara Meyer.

213- 9:12
Dynamic features of chromosome organization during *C. elegans* meiosis revealed through chromosome painting in the context of intact 3-D nuclear architecture. **Kentaro Nabeshima**, Anne Villeneuve.

214- 9:24
Mechanism and regulation of meiotic recombination. **Simona Rosu**, Anne Villeneuve.

215- 9:36
Condensin I promotes accurate mitotic and meiotic chromosome segregation. **Karishma Collette**, Uchita Patel, Gyorgyi Csankovszki.

216- 9:48
Three-Dimensional Positioning of Dosage Compensation Complex Binding Sites. **Emily Crane**, Satoru Uzawa, David Mets, Deborah Thurtle, Barbara Meyer.

217- 10:00
Differentiation associated spatial separation of active and silent loci. **Benjamin D. Towbin**, Peter Meister, Brietta L. Pike, Susan M. Gasser.

10:12 - **Break**

218- 10:42
Lateral microtubule bundles promote chromosome alignment during acentrosomal oocyte meiosis. **Sarah Wignall**, Anne Villeneuve.

219- 10:54
Female meiotic spindle dynamics in *C. elegans*: Roles for ASPM-1 and BMK-1. **M. H. Price**, V. Davis Haug, D. W. Turnbull, M. L. Drummond, E. A. Johnson, S. R. Lockery, B. A. Bowerman.

220- 11:06
Action of CED-3 in meiotic and mitotic chromosome segregation. **Ashish Kumar**, Joel H. Rothman.

221- 11:18
RACK-1 directs dynactin-dependent RAB-11 endosomal recycling during mitosis. **Erkang Ai**, Daniel S. Poole, Ahna R. Skop.

222- 11:30
Anoxia-induced suspended animation requires a nucleoporin protein for prophase arrest in blastomeres and oocytes in *C. elegans*. **Brent A. Little**, Vinita A. Hajeri, Mary L. Ladage, Pamela A. Padilla.

223- 11:42
Global analysis of histone subtype composition in *C. elegans* sperm using MudPIT mass spectrometric analysis. **Tammy F. Wu**, Colin Fitzpatrick, Catherine Wong, Michael C. Yee, Kieran Hervold, Aiza Go, John R. Yates III, Diana Chu.

Saturday, June 27 9:00 AM–12:00 noon
Sunset Village Study Lounge

Pathogenesis

Chair: Scott Alper

224- 9:00
The PMK-1 p38 MAPK pathway regulates *C. elegans* innate immunity through phosphorylation of the bZIP transcription factor ATF-7. **Robert P. Shivers**, Daniel J. Pagano, Tristan Kooistra, Naoki Hisamoto, Kunihiro Matsumoto, Dennis H. Kim.

225- 9:12
Further dissection of the epidermal antifungal response. **Olivier Zugasti**, Jerome Belougne, Jonathan Ewbank.

226- 9:24
Tissue- and pathogen-specific innate immune signalling. Sophie Cypowyj, Jonathan Ewbank, **Nathalie Pujol**.

227- 9:36
HDA-4/MEF-2 Is a DKF-2A Target-Effector that Regulates Inducible Innate Immunity. **Y. Fu**, M. Ren, H. Feng, M. Zeng, C. S. Rubin.

228- 9:48
The small GTPase RHO-1 is required to alter cell morphology during the *C. elegans* innate immune response. **Rachel McMullan**, Jennifer Winter, Stephen Nurrish.

229- 10:00
Single gene immunological trade-offs in the nematode *C. elegans*. **Elizabeth Marsh**, Maiike van Den Berg, Robin May.

10:12 - **Break**

230- 10:42
The IRE-1 Branch of the Unfolded Protein Response Functions in C. elegans Pathogen Resistance During Larval Development. **Claire E. Richardson**, Tristan Kooistra, Dennis H. Kim.

231- 10:54
A multi-prong genome-wide approach to studying cellular defenses against the bacterial pore-forming toxin. **Cheng-Yuan Kao**, Ferdinand C. O. Los, Shinichiro Wachi, Youn Sagong, Christine Ha, Danielle L. Huffman, Larry J. Bischof, Raffi V. Aroian.

232- 11:06
Intestinal epithelial cell destruction during *S. aureus* pathogenesis in *C. elegans*. **Javier E. Irazoqui**, Frederick M. Ausubel.

233- 11:18
Cell vacuolation, a manifestation of *Vibrio cholerae* hemolysin in *C. elegans* intestine. **Hediye N. Cinar**, Mahendra Kothary, Atin Datta, Ben D. Tall, Robert Sprando, Kivanc Bilecen, Fitnat Yildiz, Barbara McCardell.

234- 11:30
Using quantitative proteomics to identify proteins involved in innate immunity of *C. elegans*. **Karina T. Simonsen**, Jakob Møller-Jensen, Anders Riis Kristensen, Jens S. Andersen, Donald L. Riddle, Birgitte H. Kallipolitis.

Saturday, June 27 9:00 AM–12:00 noon
De Neve Plaza Room

Cell Fate Patterning

Chair: Sophie Jarriault

235- 9:00
Wnts regulate asymmetric spindle to generate asymmetry of β-catenin localization. **Kenji Sugioka**, Hitoshi Sawa.

236- 9:12
Two ARF GTPase cycles and a Frizzled pathway collaborate to regulate the asymmetric cell division of the Q.p neuroblast. **Jerome Teuliere**, Aakanksha Singhvi, Shaun Cordes, Karla Talavera, Guangshuo Ou, Ron Vale, Gian Garriga.

237- 9:24
The Homeodomain protein CEH-51 and the T-box factor TBX-35 share function in specification of the MS blastomere. **Morris F. Maduro**, Gina Broitman-Maduro, Wendy Hung, Melissa Owraghi.

238- 9:36
Analysis of the early steps of trans-differentiation *in vivo* in *C. elegans*. **Konstantinos Kagias**, Nadine Fischer, Sophie Jarriault.

239- 9:48
Mechanisms that exclude somatic differentiation in the *C. elegans* germline. **Nate R. Dudley**, Joel H. Rothman.

240- 10:00
The BTB/Zinc finger protein EOR-1 and its obligate binding partner EOR-2 may be direct targets of MPK-1 ERK. **Kelly L. Howell**, Swathi Arur, Tim Schedl, Meera Sundaram.

10:12 - **Break**

241- 10:42
The LET-60/Ras effectors RGL-1 (RalGEF) and LIN-45 (Raf) play antagonizing roles during vulval fate patterning. **Tanya P. Zand**, Vanessa González-Pérez, Channing J. Der, David J. Reiner.

242- 10:54
A tetraspanin promotes Notch signaling in *C. elegans*. **Cory David Dunn**, Iva Greenwald.

243- 11:06
Computational Modeling of Vulval Development Reveals Cell-cycle Regulation of LIN-12 Notch Signaling. S. Nusser, I. Rimann, N. Piterman, A. Hajnal, **J. Fisher**.

244- 11:18
Evolution of developmental mechanisms in vulva formation in nematodes C. elegans and C. briggsae. **B. P. Gupta**, N. Bojanala, P. Cumbo, S. Marri, A. Seetharaman, B. Thillainathan.

245- 11:30
ngn-1 and *hlh-2* are required for a left-right asymmetric neurogenesis decision. **Shunji Nakano**, Ronald E. Ellis, Bob Horvitz.

246- 11:42
Computational analysis of quantitative information of cell division dynamics of early embryos. **K. Kyoda**, E. Adachi, K. Shimada, J. Kuramochi, S. Onami.

Sunday, June 28 9:00 AM–12:00 noon
Royce Hall

Plenary Session 5: Pathogenesis, Dauer, Aging and Death

Chairs: Malene Hansen and Kotaro Kimura

247- 9:00
The monounsaturated fat vaccenic acid negatively regulates innate immunity through its effect on insulin signaling. **Madhumitha Nandakumar**, Man-Wah Tan.

248- 9:12
Microsporidia are natural intracellular parasites of *C. elegans*. **Emily Ruth Troemel**, Marie-Anne Félix, Noah Whiteman, Antoine Barrière, Frederick M. Ausubel.

249- 9:24
Spatio-Temporal Regulation of the Dauer Decision. **Oren Schaedel**, Paul Sternberg.

250- 9:36
C. elegans chemical biology: lessons from small molecules. **Frank Schroeder**, Chirag Pungaliya, Jagan Srinivasan, Fatma Kaplan, Arthur Edison, Paul Sternberg.

251- 9:48
The C. elegans Gen-1 Holliday Junction Resolvase links DNA double strand break repair and DNA damage signalling. Aymeric Bailly, Anne-Cécile Déclais, Julie Hall, Alasdair Freeman, Arno Alpi, David Lilley, Shawn Ahmed, **Anton Gartner**.

252- 10:00
CPS-5, a substrate of the CED-3 cell death protease, promotes cell death in *C. elegans* upon cleavage by CED-3. **Akihisa Nakagawa**, Yong Shi, Ding Xue.

10:12 - **Break**

253- 10:42
Fat metabolism: a missing link between reproduction and longevity. **Meng Wang**, Eyleen J. O'Rourke, Gary Ruvkun.

254- 10:54
A Transcription Elongation Factor that Links Signals from the Reproductive System to Lifespan Extension. **A. Ghazi**, S. Henis-Korenblit, C. Kenyon.

255- 11:06
Systemic regulation of starvation response in *C. elegans*. **Chanhee Kang**, Leon Avery.

256- 11:18
Molecular aging driven by the ELT-3/ELT-5/ELT-6 GATA transcription circuit in *C. elegans*. **Yelena V. Budovskaya**, Stuart K. Kim.

257- 11:30
Serine/Threonine Phosphatases in the regulation of Insulin/IGF-1 signaling. **S. D. Narasimhan**, S. Padmanabhan, A. Mukhopadhyay, H. A. Tissenbaum.

258- 11:42
RNAi screening reveals SKN-1 involvement in lifespan extension deriving from inhibition of translation. Jinling Wang, Stacey Robida, **T. Keith Blackwell**.

Physiology: Aging and stress

259A
A genetic screen for regulatory partners and effectors of DAF-16. **Daniel Ackerman**, David Weinkove, David Gems.

260B
C. elegans him-6 protein exhibits RecQ helicase activity. Hana Jung, Jina Lee, Moonjung Hyun, **Byungchan Ahn**.

261C
Osmotic stress extends C. elegans lifespan. Mark Corkins, **Edward N. Anderson**, Gerard Somers, Anne C. Hart.

262A
Prohibitin couples diapause signaling to mitochondrial metabolism during ageing in *C. elegans*. **Marta Artal-Sanz**, Nektarios Tavernarakis.

263B
Molecular mechanisms leading to extreme longevity in C. elegans. **S. Ayyadevara**, P. Bharill, R. Shmookler Reis.

264C
Transcriptional- and kinase-attenuation mechanisms associated with extreme longevity. **P. Bharill**, S. Ayyadevara, R. Shmookler Reis.

265A
The microRNA *mir-71* Is Involved in the Regulation of Longevity and Stress Responses in *C. elegans* . **Konstantinos Boulias**, Bob Horvitz.

266B
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GENETICS, the peer-edited journal of the Genetics Society of America, reports on updates and special projects, including article links to WormBase. **Tracey DePellegrin Connelly**[1], Tim Schedl[2]. 1) Genetics Society of America, Bethesda MD; 2) Washington University, St. Louis MO.

GENETICS, the peer-edited journal of the Genetics Society of America has partnered with WormBase (Arun Rangarajan, Hans-Michael Muller and Paul Sternberg) to produce interactive journal articles (in full text/HTML, XML and PDF outputs). A reader who clicks on a gene or protein name, allele, transgene (or potentially any object found in the database) is taken directly to the corresponding page in WormBase. This innovative project integrates two major modes of communication used in the biological sciences: journal articles and databases. The project offers several benefits to readers, including fast access to relevant information associated with a genetic object in the text. This information can be general, providing an overview (e.g. gene summary), or highly specific, providing an important experimental detail (e.g. the molecular lesion of an allele). Also, the project promotes standardization of individual object nomenclature (e.g. gene names) and simplifies connections when there is a nomenclature change. Finally, the objects remain connected but evolve with advancesin knowledge. The benefits to WormBase include increased use of and interest in the database, more efficient and extensive corrections of information in the database by the community, facile incorporation of new information, reverse integration of the database with the primary data in the literature, all with minimal ongoing cost. We will show examples of the article links to WormBase, and discuss a number of other initiatives being undertaken by the journal GENETICS.

2

Balancing selection maintains paternal-effect-by-zygotic incompatibility among *C. elegans* wild isolates. **Hannah Seidel**[1], Matthew Rockman[2], Michael Ailion[3], Leonid Kruglyak[1]. 1) Dept of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) Dept of Biology, New York University, New York, NY; 3) Dept of Biology, University of Utah, Salt Lake City, Utah.

C. elegans strains Bristol (N2) and Hawaii (CB4856) exhibit a paternal-effect-by-zygotic incompatibility involving two genes–*peel-1* and *zeel-1*. *peel-1* and *zeel-1* interact like a toxin and an antidote: *peel-1* is a paternal-effect gene that induces developmental arrest in embryos that do not inherit the zygotically expressed gene *zeel-1*. *peel-1* and *zeel-1* are located adjacent to one another in the Bristol genome, and Hawaii carries a deletion spanning both genes.

We have cloned *peel-1* and *zeel-1* by transgenic complementation. *peel-1* encodes a predicted transmembrane protein with no homology to any gene in any species. *peel-1::gfp* transcriptional fusions are expressed in spermatocytes, and immunostaining localizes PEEL-1 to the cell membrane of mature sperm. Embryos affected by the paternal-effect lethality arrest paralyzed at the two-fold stage, suggesting that PEEL-1 causes a defect in muscle development or function.

zeel-1 encodes a protein homologous to the substrate recognition subunit of an E3 ubiquitin ligase. Unlike most members of its family, ZEEL-1 contains a predicted transmembrane domain, and this transmembrane domain is required for the ability of *zeel-1* to rescue the paternal-effect lethality. *zeel-1::gfp* translational fusions are expressed ubiquitously in the embryo, beginning approximately 4 hours post-fertilization and ending before hatching. The observation that *zeel-1* expression does not begin until mid-embryogenesis raises the possibility that sperm-supplied PEEL-1 persists for several hours after fertilization.

The *zeel-1/peel-1* incompatibility is not specific to a cross between Bristol and Hawaii. We have genotyped a large panel of wild strains at the *zeel-1/peel-1* locus and found that approximately two thirds of strains carry *zeel-1* and *peel-1* (like Bristol), whereas the remaining strains carry a deletion of the genes (like Hawaii). The Bristol- and Hawaii-like haplotypes exhibit elevated sequence divergence, and the allele frequency spectrum at the locus is indicative of balancing selection. We propose that balancing selection acting on *zeel-1*, *peel-1*, or a tightly linked locus preserves both haplotypes in the population despite the lethality caused by their interaction.

3

DNA-mediated transformation in *Pristionchus pacificus* reveals novel functions of Wnt signaling in vulva induction. **Xiaoyue Wang**, Ralf J. Sommer. Evolutionary Biol, Max-Planck Inst, Tübingen, Germany.

Cell fate specification and cell-cell signaling have been well studied during the development of *C. elegans* and provide a paradigm for evolutionary developmental biology. To identify the molecular basis of evolutionary alterations in development, we have developed *Pristionchus pacificus* as a satellite organism, in which i) forward and reverse genetic studies can be performed, ii) a 10 X coverage of the genome is available and iii) DNA-mediated transformation allows experimental manipulation (Schlager et al., 2009). One developmental process that has been studied intensively is vulva formation and functional differences were identified in the molecular mechanisms of vulva induction between *C. elegans* and *P. pacificus*. While in *C. elegans* EGF is the key initiation signal of vulva development, forward and reverse genetic experiments revealed that Wnt signaling is crucial for vulva induction in *P. pacificus*. A *Ppa-bar-1*/β-catenin mutant is completely vulvaless. Reverse-genetic analysis indicated that multiple Wnt ligands and Fz-like receptors play a redundant role in vulva induction, including *Ppa-egl-20*/Wnt. Two striking features are a negative role of *Ppa-lin-17*/Frizzled, mutations in which result in a multivulva phenotype, and the function of *Ppa-egl-20*, that when mutated can suppress the *Ppa-lin-17* phenotype. Such genetic interactions suggest a role of this Frizzled receptor in ligand sequestration. Interestingly, *Ppa-egl-20* is only expressed in the rectal region, similar to *C. elegans*, representing the first inductive ligand for vulva induction that is expressed outside the gonad. To further elucidate the molecular mechanisms of *P. pacificus* vulva induction, we are combining forward genetics and transformation technology. First, another multivulva mutant, formerly known as *limu-1*, has been cloned and displays a novel aspect of Wnt signaling function. Second, a transgenic approach using heat-shock induced *Ppa-bar-1* and *Ppa-egl-20* reveals the temporal aspects of Wnt signaling. These studies show that at least three distinct Wnt signaling mechanisms are at work, which control i) vulva induction, ii) the polarity of the secondary cell fates and iii) the fate of the epidermal cell P8.p. In particular *Ppa-lin-17* has distinct functions in all three decisions, most of which are unknown from *Cel-lin-17*. This study provides the first molecular insight into vulva induction by a signaling center outside of the gonad and thereby extends our understanding of signaling mechanisms in nematodes.
References: Schlager, B. et al. (2009) Molecular cloning of a dominant Roller mutant and establishment of DNA-mediated transformation in the nematode *Pristionchus pacificus*. *Genesis* in Press.

4

RNA Polymerase II Accumulates on Promoters of Growth and Development Genes During L1 Arrest. **L Ryan Baugh**[1], John DeModena[2], Paul Sternberg[2]. 1) Dept Biol, Duke University, Durham, NC; 2) Dept Biol, California Institute of Technology, Pasadena, CA.

Animals cope with fluctuating nutrient availability by altering resource allocation in order to maintain energy homeostasis. For *C. elegans* life in the wild is characterized by feast or famine, so that organismal fitness presumably depends on developmental responses to nutrient availability. When *C. elegans* larvae hatch in the absence of food they reversibly arrest development and increase resistance to environmental stress (L1 arrest). Using L1 arrest and recovery as a model, we aim to characterize signaling pathways and gene regulatory mechanisms that maintain energy homeostasis during development. To study nutritional control of larval development, we characterized growth, mRNA expression, and RNA Polymerase II (Pol II) binding genome-wide during L1 arrest and recovery. Growth and gene expression analysis reveal that fed larvae are relatively slow to respond to starvation, while arrested larvae respond rapidly to feeding as if poised for recovery. Chromatin immunoprecipitation of RNA Pol II followed by deep sequencing (ChIP-Seq) shows that while Pol II continues transcribing starvation genes, it accumulates on the promoters of growth and development genes during L1 arrest, as if 'paused' or otherwise inhibited post-recruitment. Consistent with Pol II promoter accumulation poising arrested larvae for recovery, accumulation decreases in response to feeding, while elongation and mRNA levels increase. Therefore, accumulation of Pol II at promoters can anticipate nutrient-dependent gene expression during development. This work demonstrates the profound influence of energy homeostasis on gene regulation during development, and it suggests that nutritional control of Pol II promoter accumulation may be a general feature of gene regulation involving energy homeostasis.

5

Driving with a clutch: cadherin acts with Rac signaling to regulate cell movements during gastrulation. **Gidi Shemer**, Minna Roh, Joe McClellan, Bob Goldstein. Dep. Biology, University of North Carolina at Chapel Hill, NC.

We have studied early gastrulation in *C. elegans* as a model to understand how adhesion proteins interact with intracellular processes to regulate cell movements. *C. elegans* gastrulation starts at the 26-cell stage, with the internalization of the two endodermal precursors (E cells), accompanied by movements of the neighboring cells to cover the exposed ventral space. This process requires that the E cells adopt a proper cell fate and develop apico-basal polarity. Apical accumulation of activated non-muscle myosin in the E cells leads to apical constriction, resulting in their inward movements. We have taken a reverse genetic approach by knocking down candidate adhesion proteins, as well as members of signaling pathways that may interact with the adhesion machinery and the cytoskeleton. We have found that knockdown of HMR-1/cadherin together with the Rac activator CED-5 leads to failure of the E cells to internalize. Moreover, knockdown of other components of the cadherin-catenin complex, HMP-1 and HMP-2, in a *ced-5(-)* background resulted in similar gastrulation defects. We also found that CED-12 and CED-2, proteins that act with CED-5 as part of a RacGEF complex later in development, also function in gastrulation. Finally, we found that CED-10/Rac shows similar gastrulation defects. Thus, cadherin-catenin proteins act together with Rac signaling to allow gastrulation movements. Cell fate, polarity, and apical accumulation of myosin in the E cells are not compromised in *hmr-1;ced-5* mutant embryos. To better address the dynamics of apical constriction, we imaged wild type and mutant embryos for movement of myosin foci with respect to the zones where E cells contact their neighboring cells. Surprisingly, we have revealed that in wild type embryos, myosin undergoes centripetal movements along the apical cortex even before apical surfaces begin to shrink. This first phase of myosin movement is then followed by a second phase, where the contact zones between cells begin to narrow in concert with the myosin movements until apical constriction is completed and cells internalize. In contrast to wild type embryos, *hmr-1;ced-5* mutants showed defects in membrane dynamics. While centripetal movements of myosin took place normally, these movements were not followed by narrowing of the contact zones in the second phase, resulting in failure of the E cells to internalize. Our results suggest that gastrulation in *C. elegans* involves a clutch-like mechanism, whereby myosin motors move for some time without causing apical constriction. Only after this period do the cadherin-catenin complex and Rac signaling act together to serve as force carriers of cortical contraction to internalize the gastrulating cells.

6

Comprehensive identification of endogenous Argonaute-bound miRNAs and mRNA target sequences at nucleotide level resolution in *C. elegans*.. **Dimitrios Zisoulis**[1], Michael Lovci[2], Tiffany Liang[2], Melissa Wilbert[2], Gene Yeo[2], Amy Pasquinelli[1]. 1) Dept of Biology, University of California, San Diego; 2) Dept of Cellular and Molecular Medicine & Stem Cell Program, University of California, San Diego.

MicroRNAs (miRNAs) represent a family of genomically-encoded small RNAs that post-transcriptionally regulate gene expression. miRNAs guide Argonaute-containing protein complexes to the 3' UTR of target mRNAs resulting in degradation and/or translational repression. However, elucidation of the miRNA target recognition mechanism is challenging because miRNAs base-pair imperfectly with their target mRNAs. Furthermore, the limited availability of experimental data describing miRNA/mRNA interactions complicates the computational prediction of miRNA targets yielding results that may not be physiologically relevant.

To gain further insight into sites of functional miRNA:mRNA interactions we developed an unbiased, comprehensive strategy aimed at isolating endogenous mRNA target sequences bound by the ALG-1 Argonaute protein in *C. elegans*. To achieve this we combined the CLIP (Cross-linking and Immunoprecipitation) technology, employing a custom anti-ALG-1 antibody, with massive parallel sequencing. Our methodology is sensitive enough to detect previously established miRNA targets with a low false positive rate. We have identified approximately 13,237 unique Argonaute-bound sites in endogenous mRNAs, corresponding to 6,643 genes, about a third of the *C. elegans* genome. We intend to make the map of ALG-1-bound sequences available as a UCSC Genome Browser track, providing a valuable resource for miRNA-regulated genes to the *C. elegans* research community.

Computational analysis of the parallel sequencing data shows that: i) Argonaute-bound sites are significantly enriched at the 3' UTR and coding exons of mRNA transcripts but depleted at 5'UTRs and introns, ii) miRNA target sites are located in conserved regions of higher structural accessibility and are enriched in CU-motifs iv) bound regions in the 3' UTRs display a higher pairing capacity to 5' ends of miRNAs (seed) than exonic regions v) there is a strong correlation between the presence of ALG-1 binding sites at the 3' UTR or coding exons of a gene and its upregulated or downregulated expression, respectively, in *alg-1* (-) animals, vi) genes involved in the miRNA pathway are enriched in Argonaute-binding sites. In summary, we provide a global map of ALG-1 interactions with endogenous mRNAs at nucleotide level resolution, analyze prominent characteristics of the bound sequences and limit the sequences that need to be computationally analyzed to predict targeting miRNAs.

7

Environmental programming of gene expression in *C. elegans*. **Sarah E. Hall**[1], Matthew H. Beverly[1], Carsten Russ[2], Chad Nusbaum[2], Piali Sengupta[1]. 1) Dept Biol, Brandeis Univ, Waltham, MA; 2) Broad Institute, Cambridge, MA.

Environmental programming describes a phenomenon in which early environmental and developmental experience can affect adult health and behavior through long-lasting changes in gene expression. Increasing evidence suggests that affected genes are 'programmed' by an environmental stimulus through changes in chromatin states. *C. elegans* makes an environmentally-regulated developmental decision in early larval stages to continue developing into a reproductive adult, or to enter the alternative dauer stage. When environmental conditions improve, animals exit the dauer stage and continue normal development. Although adults that have passed through or bypassed the dauer stage appear grossly identical, post-dauer adult worms have been reported to retain a cellular memory of their developmental decision. To identify the gene set whose expression may be regulated by a memory of passage through the dauer stage, we compared the transcription profiles of postdauer adult animals and control adult animals that bypassed the dauer stage. We found that the expression of 2,126 genes is predicted to be significantly up or down-regulated in postdauer as compared to control animals. Genes predicted to play roles in reproduction, sensory signal transduction, transcriptional regulation, RNAi, and chromatin remodeling were enriched in the affected gene set. In addition, we quantified altered reproductive and aging phenotypes in postdauer adults compared to controls. To determine whether chromatin remodeling plays a role in maintaining the altered gene expression patterns, we performed chromatin immunoprecipitation followed by Illumina Genome Analyzer sequencing (ChIP-Seq) of genomic regions using antibodies against the common histone modifications H3K4me3, H3K9me3, H3K27me3, and H4panAc. Analysis of the ChIP-Seq data in control and postdauer animals showed a direct correlation of gene expression levels and histone modification enrichments in gene regulatory regions for modifications associated with active transcription. However, we found that H3K9me3 and H3K27me3 modifications were independent of gene expression levels. Furthermore, genes whose expression was altered due to passage through the dauer stage exhibited predictable changes in histone enrichment profiles in postdauer animals. Preliminary evidence suggests that a subset of these gene expression changes are dependent on a chromatin remodeling factor and proteins implicated in RNAi, suggesting a RNAi-dependent transcriptional gene silencing mechanism of environmental programming. We expect that this work will allow us to understand how early environmental experiences affect adult phenotypes, and generate phenotypic diversity in an otherwise isogenic population.

8

Cell cycle re-entry in terminally differentiated body wall muscle cells. **Jerome Peter Korzelius**[1], Vincent Portegijs[1], Marian Groot Koerkamp[2], Frank Holstege[2], Inge The[1], Sander Van den Heuvel[1]. 1) Developmental Biology, Utrecht University, Utrecht, Netherlands; 2) Department of Physiological Chemistry, UMCU, Utrecht, The Netherlands.

It is poorly understood why cells lose the ability to enter the cell cycle upon terminal differentiation. Insight into this process would be of critical importance for regenerative medicine and cancer biology. We use the *C. elegans* body wall muscle (bm) as a model system to study cell cycle re-entry in the context of animal development. Previous work has demonstrated that knockdown of the negative G1/S regulators *lin-35 Rb* and *cki-1* Kip1 causes overproliferation in certain blast cell lineages but it does not affect proliferation of differentiated cells like the body wall muscle. We set out to test whether re-activation of G1 Cyclin/Cdk complexes in differentiated bm cells can trigger cell cycle re-entry. We expressed combinations of the positive G1/S regulators CYD-1/CDK-4 and CYE-1/CDK-2 in the body wall muscle. Interestingly, a combination of CYE-1 together with mutant CDK-2AF (lacking the negative regulatory phosporylation sites) stimulated cell cycle re-entry in the bm lineage. Specifically, first stage larva showed the normal number of body wall muscle nuclei, while subsequent larval stages showed expression of a degradable S-phase marker, mitosis-specific histone H3 phosphorylation, condensed chromosomes and extra nuclear division. However, these bm cells did not incorporate EdU, a thymidine orthologue, suggesting that these cells have an altered cell cycle program without S-phase. To gain insight into the transcriptional changes that take place in a body wall muscle cell re-entering the cell cycle, we performed tissue-specific microarray analysis (Roy et al., 2002). Genes required for S-phase, mitosis and DNA damage response were highly enriched among the upregulated genes in dividing bm cells. These results suggest that although the transcriptional program for DNA-replication is activated, the cell still cannot undergo S-phase. In contrast, we did not observe any changes in genes known to be required for body wall function and we witnessed no changes in muscle structure or function in these animals. In summary, this work demonstrates that terminally differentiated bm cells can activate cell cycle gene transcription and go through mitosis upon G1/S Cyclin/Cdk overexpression. However, the absence of DNA synthesis upon re-entry suggests that there are additional controls that act to prevent re-entry in differentiated bm cells. These findings open up avenues for forward and reverse screens to identify additional genes that act in cell cycle re-entry.

9

Electron tomography: a new tool for exploring C. elegans cellular anatomy. **David H. Hall**[1], Kristin A. Politi[1], KD Derr[2], William J. Rice[2], Chris Crocker[1], Kevin Fisher[1], Leslie Gunther[1], Ken C.Q. Nguyen[1]. 1) Ctr C Elegans Anatomy, Albert Einstein Col Med, Bronx, NY; 2) New York Structural Biology Center, 89 Convent Ave, New York, NY.

C. elegans has been studied intensively using serial section reconstruction for several decades, so that every cell type is now known in considerable detail. Nevertheless, limitations of the serial section technique (notably the poor resolution of detail within the depth of each 50 nm "thin" section) have made it difficult to model the shapes of fine details in many organelles, such as the cristae of a mitochondrion, the canaliculi of the excretory canal, membrane ruffles in many cell types, or the constituents at a chemical synapse. High pressure freeze fixation and freeze substitution (1) are a required element in preserving smaller structures that generally escaped our notice in conventional TEM imaging. Modern electron microscopes using higher energy electrons now offer much better resolution by collecting multiple images in a tilting series through comparatively thick sections (150 nm) using the SerialEM program (2). The Protomo software package (3) is used to compute a 3D tomographic reconstruction that offers the same level of detail in any dimension (roughly 2 nm resolution). Annotation and modeling is done using IMOD (4) and/or Amira. This permits us to take a new look at many familiar objects in the anatomy of C. elegans, to identify missing parts of the whole anatomy, and potentially, to detect smaller anatomical defects in a variety of mutant backgrounds. Here we will introduce the tomographic procedure, and share finished 3D models of some typical intracellular organelles at high resolution. Supported by NIH RR 12596 to DHH. 1.Weimer, R.M. (2006) Methods Mol. Biol. 351: 203-221. 2.Mastronarde, D.N. (2005) J. Struct. Biol. 152: 36-51 3.Winkler, H. and Taylor, K.A. (2006) Ultramicroscopy 106: 240-254. 4.Kremer, J.R., Mastronarde, D.N. and McIntosh, J.R. (1996) J. Struct. Biol. 116: 71-76.

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Correlative microscopy in *C. elegans*. **Irina Kolotuev**, Yannick Schwab, Michel Labouesse. Dev and Cell Biology, IGBMC, Illkirch, France.

Correlative light and electron microscopy (CLEM) approach is aimed to bridge the data acquired with different imaging media. It is successfully applied in cell culture systems, however, the use of this approach in multicellular organisms raises technical problems due to the addition of a third dimension and of their motility, which makes difficult the retrospective location of the region of interest (ROI). We are interested in cellular processes associated with cytoskeletal rearrangements, tubulogenesis and secretion, and have realized the need to use CLEM to address a wide range of questions impossible without a precise correlation. We developed a CLEM technique that enables easy processing of all stages and is adequate both for morphology and immuno EM specimen preparations. Using it enables observation of single animals in real time, HPF fixation and flat embedding. Our major improvement over previous protocols has been the development of a precise mapping system that considerably simplifies and speeds up the retrospective location of the ROI within 1μm. Our method allows us to acquire thin sections of the ROI in about one hour starting from the in-block specimen. We chose to illustrate the technique and its potential through two examples. a)*exc-4* is a chloride intracellular channel mutant affecting the excretory system. The terminal defect has been previously shown to result in a huge vacuole in the excretory canal. We were interested to see the nature of damage in embryonic and early larval stages and could follow the same specimen from the location of the vacuolized region in DIC to exactly the same region in TEM. We found that the mutation results in hyperfusion of the inner lumen with the canal composing canaliculi and further deposition of agglomerates in the oversized inner lumen. b)*che-14*, a homolog of the transmembrane Dispatched protein, was previously proposed to be involved in secretion and tube formation. Its subcellular localization had only been inferred based on GFP reporter fluorescence. Using animals with CHE-14::GFP construct and GFP antiserum, we found by immuno-EM gold particles at the apical surface of epithelial and sensory support cells, in accord with GFP reporter studies. Moreover, we could locate precisely the seam cells that showed mosaic expression of GFP construct, side by side with non-expressing cells, confirming the powerful ability of the technique to locate the exact ROI. We believe that this technique has a potential to contribute to correlative studies in any small animal (we could successfully apply it to fly embryos) and will facilitate the use of the time demanding procedure of specimen preparation for TEM in different developmental stages.

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The posterior connectome of the C. elegans male. **Scott W. Emmons**[1], Yi Wang[1], Travis Jarrell[1], Meng Xu[1], David H. Hall[2], Donna G. Albertson[3], Nicole Thomson[3]. 1) Dept Genetics, Albert Einstein Col Medicine, Bronx, NY; 2) Dept Neuroscience, Albert Einstein Col Medicine, Bronx, NY; 3) MRC Laboratory of Molecular Biology, Cambridge, UK.

For many biological systems knowledge of structure is key to understanding function. It was Sydney Brenner's insight that the structure of the C. elegans nervous system could be determined and analyzed by means of genetics that provided the inspiration for C. elegans research (Brenner, 1974). For over 20 years, the completed wiring diagram of the C. elegans hermaphrodite has afforded a unique basis for genetic studies of worm behavior. Among C. elegans behaviors, the most complex motor program is the multi-step mating behavior of the male. Reconstruction of the male nervous system was initiated along with that of the hermaphrodite in the 1970's (Sulston, Albertson and Thomson, 1980), but its completion has awaited development of the modern PC. Using electron micrographs from the MRC we digitized and analyzed using a software platform for annotation of electron micrographs from the computer screen, we have determined the connectivity among the neurons and muscles in the male tail, the posterior connectome. Reconstruction of the anterior nervous system is underway. The male posterior connectome consists of the interconnections among the processes of 175 neurons (85 male-specific and 90 shared with the hermaphrodite) and 65 muscles (41 male-specific and 24 shared). These cells are joined together in a complex network by some 8000 synapses, 4000 chemical and 4000 electrical, more than are contained in the entire hermaphrodite nervous system. The networks of chemical and electrical synapses are largely overlapping, suggesting parallel routes of information transfer and processing. Male-specific and shared neurons and muscles are fully integrated together in the network. Many of the shared neurons are sexually dimorphic, not only in having a more branching architecture and having synapses with male-specific neurons and muscles, but also in lacking some and gaining other synaptic interactions amongst themselves. In spite of its complex network architecture, potential circuits for the various steps of the mating program can be discerned in the connectivity diagram, in some cases revealing previously unsuspected roles for individual neurons or classes of neurons. The results provide an unprecedented opportunity not only to understand how a decision-making, multifunctional neural network processes sensory information in a coherent manner, selecting a choice among alternate behavioral outputs in a goal-oriented behavior, but also an opportunity and a challenge to understand how this incredibly complex structure, the connectome, is specified by the genome.

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C. elegans Gene Knockout Consortium Report: Progress, New Technologies and Funding. **Mark Edgley**[1], Don Moerman[1,2], Stephane Flibotte[3], Jeff Holmes[4], John Rummage[4], Bob Barstead[4]. 1) Michael Smith Labs, Univ British Columbia, Vancouver, BC, Canada; 2) Zoology Department, Univ British Columbia, Vancouver, BC, Canada; 3) Michael Smith Genome Sciences Centre, BCCA, Vancouver, BC, Canada; 4) Oklahoma Medical Research Foundation, Oklahoma City, OK.

The *C. elegans* Gene Knockout Consortium (http://www.celeganskoconsortium.omrf.org/) has for many years pursued systematic targeted gene disruption in the nematode, both upon request from the research community and in the larger context of the whole set of genes for which human orthologs exist (more than 7,000 genes). Our primary approach uses a PCR detection method to generate single-gene deletions. More recently we have incorporated array Comparative Genome Hybridization (array-CGH; Genome Research 17: 337-347) and whole-genome sequencing for efficient detection of mutations. CGH has proven valuable and cost-effective for deletion discovery and SNP mapping (Genetics 181: 33-37; see abstract by Flibotte et al., this meeting). With deep sequencing we have generated the first comprehensive catalog of all mutations in a genetic background after EMS mutagenesis. The combined strategies of directed and non-directed approaches improve throughput, broaden the types of mutations we can identify, and in the long term should lower the cost per allele. All Consortium deletions are stabilized as homozygous or balanced heterozygous strains, breakpoints are sequenced, and the strains and data are placed immediately into the public domain through our collaborators at the Caenorhabditis Genetics Center and WormBase. Distribution of Consortium strains accounts for a significant proportion of distributions from the CGC and has stood at 20% for the past two calendar years. To date the Consortium has generated deletions in more than 3,300 genes (not including about 700 genes deleted in natural isolates), and most of these have been stabilized and archived at the CGC. Together with those from the Mitani lab, deletion alleles represent over 5,000 genes. In the last two years we have been concentrating on genes encoding transcription factors and kinases, and we hope to complete the knockout coverage for these gene families in the next year. Of 934 total transcription factor genes, 720 are currently identified by gk, ok or tm deletions, with approximately another 50 identified by point mutation. Of 438 total kinase genes, 42 are represented by such deletions with another 150 identified by point mutation. Both our labs are currently in the process of renewing funding to continue this work. We plan to stay with the targeted PCR approach as funding allows, while continuing to develop CGH and deep sequencing and shifting resources to those methods as the technologies improve.

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WormBase or Worm**S**Base? **Anthony S. Rogers**, The WormBase Consortium. Dept Informatics, Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

The scale of data produced from biological experiments has grown enormously over recent years. Genome-wide experiments are common and the capacity to sequence whole worm genomes widespread. Both of these provide huge challenges to data providers like WormBase. We have already incorporated the genomes of the five sequenced *Caenorhabditis* species into WormBase and provide a synteny viewer for visualising regions of genome similarity. We plan to expand this list by adding more distantly related nematode species as they are sequenced allowing a wider community to benefit from the vast amount of knowledge on *C. elegans*. The addition of data from OMIM about worm orthologs of human disease-associated genes has already made WormBase useful to a wider audience. Researchers working on nematodes of medical or economical importance will also benefit from the infrastructure that WormBase can provide. Working closely with the modENCODE project WormBase will continue integrating large studies such as transcriptome sequencing and CHiP-seq experiments and to develop improved ways to present this to users. Alongside these developments WormBase continues the curation of published scientific literature describing detailed functioning of individual genes. We have developed novel ontologies to describe the anatomy and phenotypes of *C. elegans* and its mutants and are working to update these to accommodate the other species included in the database.

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Caenorhabditis Genetics Center. **Ann E. Rougvie**, Theresa Stiernagle. Dept Gen, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN.

The Caenorhabditis Genetics Center (CGC), supported by the National Institutes of Health–National Center for Research Resources (NIH-NCRR), supplies *Caenorhabditis* strains and information to researchers throughout the world. The CGC continues to be housed at the University of Minnesota, but will see changes in the next year as Theresa Stiernagle retires as Curator and pursues other interests. The CGC will continue its duties of acquiring, maintaining and distributing worm stocks. The CGC now has over 11,499 different strains. 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 green fluorescent protein (GFP) reporter fusions. 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-NCRR, a small yearly user fee and charge per strain is assessed with each order. The CGC strongly encourages use of credit cards for these charges. We provide quarterly reports to the NIH with statistics that reflect our services to the worm community. We especially like to be acknowledged in papers for providing strains. We also like to receive pdf files of such papers, copies of which we provide to NIH.

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zyg-8 promotes microtubule stability in touch neurons for proper mechanotransduction. **Jean-Michel Bellanger**, Anne Debant. CRBM-CNRS, Montpellier, France.

zyg-8 encodes a microtubule (MT)-associated protein (MAP) that is required maternally for embryonic survival and proper anaphase spindle positioning in one-cell stage embryos (Gönczy et al., 2001). Due to the lethality of *zyg-8* mutant embryos, putative later functions of the gene have not been investigated to date. Using conditional gene inactivation, we showed that *zyg-8* functions throughout the animal development to ensure full embryonic viability, proper morphogenesis, fertility and egg-laying. In addition, we noticed that mutant animals are lethargic and impaired in a gentle body touch assay, suggesting a defect in mechanotransduction. This process that converts light mechanical stimuli into electrical signals in the so-called touch neurons (also referred to as MT cells) requires bundles of large-diameter MTs that are unique to these cells. We therefore postulated that ZYG-8, as a potent MT stabilizer in early embryos, may promote or maintain the integrity of these structures in adults. Consistent with such a hypothesis, we established that a *zyg-8* regulatory sequence drives GFP expression in touch neurons, from embryonic to adult stages. The analysis of the morphology of these neurons in *mec-4::GFP;zyg-8* mutant animals revealed defects in cell body shape as well as abnormal sprouting along some processes. Low doses of the MT depolymerizing drug colchicine lead to similar defects, indicating that *zyg-8* may control the morphology and process outgrowth of touch neurons by promoting MT stability. Our phenotypic analysis indicated, however, that *zyg-8* loss-of-function might lead to MT unstability through a mechanism partially distinct from that of colchicine. We thus asked whether *zyg-8* may contribute to the bundling or the caliber of the atypical MTs. Strikingly, ultrastructural analysis of neuronal MTs revealed that, in contrast to wild-type animals, no large-diameter MTs can be found in touch neurons of *zyg-8* mutant adults. Instead, these cells are filled with bundles of MTs whose diameter is indistinguishable from that of regular MTs found in ventral cord (VC) neurons. In addition, *zyg-8* mutation had no effect on the diameter of these VC MTs, indicating that *zyg-8* acts selectively on touch neuron MTs, by promoting or maintaining their large-diameter.

Together, our results indicate that *zyg-8* (i) ensures proper cell divisions and overall development beyond the one-cell embryo stage and (ii) promotes MT stability and architecture in touch neurons that, in turn, maintain normal cell shape, process outgrowth and physiological activity of these cells.

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Regulatory logic of dopaminergic neuron differentiation. **Nuria Flames**, Oliver Hobert. Dept Biochem/Molec Biophysics, Columbia Univ, New York, NY.

Vertebrate dopaminergic neurons play a central role in the coordination of movement, attention, memory and reward. In addition, defects in the synthesis or function of the neurotransmitter dopamine underlie several pathological conditions such as Parkinson's disease, schizophrenia and drug addiction. The dopamine synthesis pathway is extremely well conserved from worms to human. However, the factors that directly activate the expression of the dopamine synthesis pathway genes remain only poorly understood in both organisms. Caenorhabditis elegans hermaphrodite contains eight dopaminergic neurons that are involved in locomotory control and several aspects of neuronal plasticity. These eight neurons fall into 4 distinct classes, each composed of a pair of bilaterally symmetric neurons. All 4 classes display very distinct developmental histories, but nevertheless express the core pathway genes that define the dopaminergic cell fate (*dat-1*, *cat-2*, *cat-1*, *cat-4* and *bas-1*). In this work, we show that in C. elegans, a simple cis-regulatory element controls the expression of all dopamine synthesis pathway genes in all dopaminergic cell types (including also the male dopaminergic neurons). We show that an ETS transcription factor, AST-1, is the trans-acting factor responsible for the activation of the dopaminergic neuron cis-regulatory element. Loss of the *ast-1* gene results in loss of specification of all different dopaminergic neuron classes and ectopic expression of *ast-1* is sufficient to activate the dopamine production pathway in several additional neurons. We also show that AST-1 is expressed in all the dopaminergic neurons throughout the life of the animal and its function is constantly required to maintain the dopaminergic fate. In conclusion, our studies reveal an astoundingly simple regulatory logic of dopaminergic neuron differentiation.

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Antagonistic Wnt pathways define motor neuron-specific inputs. **Judsen Schneider**[1], Rachel Skelton[1], Steve Von Stetina[2], David Miller[1]. 1) Dept Cell & Developmental Biol, Vanderbilt Univ, Nashville, TN; 2) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

Wnt signals have been shown to function as either positive or negative determinants of connectivity during neural development. Here we describe a novel mechanism in which a specific set of synapses in the *C. elegans* motor circuit is defined by the integration of both positive and negative Wnt-dependent pathways. In the wildtype motor circuit, interneurons AVA, AVD, and AVE synapse with VA and DA motor neurons to drive backward locomotion whereas interneurons AVB and PVC make connections with VB and DB motor neurons to mediate forward movement. Mutations in the UNC-4 homeodomain transcription factor disrupt backward locomotion by miswiring VA motor neurons with inputs (i.e. gap junction with AVB, synapse from PVC) normally reserved for their VB motor neuron sisters. We have shown that UNC-4 functions post-synaptically in VAs to turn off VB genes that specify these aberrant connections. One of these UNC-4 targets, the homeodomain protein, CEH-12/HB9 is selectively de-repressed in posterior VAs in *unc-4* mutants where it directs the creation of ectopic gap junctions with AVB. *ceh-12* expression in posterior VAs depends on interaction of EGL-20/Wnt from cells in the tail region with the frizzled receptors MOM-5 and MIG-1 in *unc-4* mutant VA motor neurons. In the wild type, UNC-4 antagonizes the function of this Wnt signaling pathway. The MOM-5/MIG-1 dependent pathway is also antagonized by the frizzled receptor LIN-17 and its Wnt ligand, LIN-44, to promote VA-type inputs. Thus, our results indicate that the specificity of synaptic inputs, onto VA motor neurons, is defined by a balance between positive and negative Wnt-dependent signals. The conservation of longitudinal Wnt gradients in insect and vertebrate axial nerve cords points to a potentially similar mechanisms of Wnt-dependent specification of motor circuit architecture in defined regions of the mammalian spinal cord.

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The transcription factor *hbl-1* controls the timing of DD neuron synaptic remodeling. **Katherine L. Thompson-Peer**[1,2,3], Jihong Bai[1,2,3], Joshua M. Kaplan[1,2]. 1) Dept of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept of Genetics, Harvard Medical School, Boston, MA; 3) These authors contributed equally.

During early development many neurons form synapses exuberantly, which are later refined during precise time windows often referred to as critical periods. Dynamic synapse formation and elimination occurs during these critical periods in an activity-dependent manner. The DD GABAergic motor neurons provide a model to study a developmentally programmed form of synaptic remodeling (1, 2). The DD neurons are born in the embryo and initially form neuromuscular junctions (NMJs) with ventral body muscles. At the end of the L1, the ventral DD NMJs are eliminated and dorsal NMJs are formed. In contrast, the VD neurons, GABAergic motor neurons born post-embryonically, do not undergo this remodeling process. VD-specific expression of the UNC-55 transcription factor is responsible for inhibiting synapse remodeling, as *unc-55* mutants undergo ectopic VD remodeling. Therefore, among the genes that are regulated by UNC-55, we expect to find some that are required for synapse remodeling.

We show that UNC-55 represses expression of the hunchback-like transcription factor HBL-1 in the VD neurons, and that *hbl-1* is required for ectopic VD remodeling in *unc-55* mutants. Moreover, HBL-1 also promotes the normal remodeling of DD synapses, as *hbl-1* mutants exhibit significant delays in the timing of DD synapse remodeling. *hbl-1* is negatively regulated by the *let-7* family of microRNAs: animals lacking these microRNAs express greater levels of *hbl-1* and undergo DD synaptic remodeling precociously. Similarly, mutations that enhance synaptic transmission, and which consequently increase overall neuronal network activity, have increased *hbl-1* expression and precocious DD remodeling.

Together, our data suggest that *hbl-1* is an important regulator of the critical period of synapse remodeling. The timing of DD remodeling is controlled by pathways regulating HBL-1 expression. These results may provide a genetic model for how the critical period of a specific form of synaptic remodeling is determined.

1. White, J. G., Albertson, D. G. & Anness, M. A. R. (1976) Nature 271, 764-766.
2. Hallam, S. J. & Jin, Y. (1998) Nature 395, 78-82.

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An ER-resident membrane protein complex regulates nAChR subunit composition at the synapse. R. Almedom[1], **J. Liewald**[1], G. Hernando[2], D. Rayes[2], J. Pan[3], H. Hutter[3], C. Bouzat[2], A. Gottschalk[1]. 1) J. W. Goethe-University, Institute of Biochemistry, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany; 2) Instituto de Investigaciones Bioquimicas, Universidad Nacional del Sur-CONICET, Bahia Blanca, Argentina; 3) Simon Fraser University, Department of Biological Sciences, 8888 University Drive, Burnaby, BC, V5A 1S6, Canada.

Nicotinic acetylcholine receptors (nAChRs) are pentameric ion channels that mediate excitatory neurotransmission and muscle activation. Regulation of subunit assembly is only partially understood. Previously, we identified proteins associated with the L-AChR, one of 2 nAChRs acting at the neuromuscular junction (NMJ), after tandem affinity purification and RNAi-based functional screening. We characterized 2 evolutionary conserved transmembrane proteins: NRA-2 & NRA-4. In paralysis assays loss of either protein led to resistance to cholinergic agonists. Double mutants showed no exacerbation of the effects, indicating that NRA-2 and NRA-4 act in the same pathway. Sensitivity to GABAergic agonists was unaffected, verifying that the proteins affect muscle nAChRs, but not LGICs in general. By bimolecular fluorescence complementation we could show that NRA-2 and NRA-4 form a complex in the ER of muscle cells, where they may interact with nAChRs during biogenesis. Cell-surface expression of certain L-AChR subunits was differentially affected in NRA-2 or NRA-4 mutants. In particular, the α-subunit UNC-38 was reduced, while UNC-29 levels were increased in one nra-4 allele. In patch-clamp experiments of adult muscle, NRA-2 mutants expressing ChR2 in cholinergic neurons responded to light-stimulated ACh release similar to UNC-38 mutants. However, after ligand application sensitivity to cholinergic agonists was altered differently: While levamisole and nicotine sensitivity were reduced, ACh induced normal currents. Single channel recordings from cultured embryonic muscle cells showed differences in the frequency and duration of open events in nra-2 mutants. Both observations indicate altered nAChR properties, e.g. caused by altered subunit composition. In line with this assumption, nra-2 mutant phenotypes were reverted by mutations in the L-AChR subunit acr-8, indicating that NRA-2 may normally prevent ACR-8 from assembling with other L-AChR subunits. Our results show that NRA-2 and NRA-4 influence nAChR properties and cell-surface expression in *C. elegans* muscle. We suggest that these proteins, though not essential for formation of functional nAChRs, act in a complex to either influence choice of subunits for assembly in the ER or the extent to which pentamers of a particular subunit composition are allowed to leave the ER.

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USP-46 is a Deubiquitinating Enzyme that Regulates the Synaptic Abundance of the Glutamate Receptor GLR-1. **Jennifer R. Kowalski**, Peter Juo. Dept. of Physiology, Tufts University School of Medicine, Boston, MA.

Regulated protein turnover by the ubiquitin system is critical for synapse development and function. Deregulation of ubiquitin-mediated degradation correlates with several neurological and neurodegenerative disorders [1]. Hundreds of enzymes exist with the ability to add (ubiquitin ligases) or remove (deubiquitinating enzymes; DUBs) ubiquitin from proteins. However, the functions of these enzymes and their relevant substrates in neurons are largely unknown. Ubiquitination controls the synaptic abundance of the *C. elegans* glutamate receptor GLR-1 and mammalian glutamate receptors [1,2]. Regulation of glutamate receptor abundance at the postsynaptic membrane is critical for modulating the strength of synaptic signaling. Ubiquitination of receptors at the plasma membrane provides a signal for their endocytosis and subsequent degradation in the multivesicular body (MVB)/lysosome. Deubiquitination of receptors in the endosome is thought to prevent their degradation and promote their recycling to the plasma membrane. Although ubiquitin ligases that control GLR-1 synaptic abundance have been identified, no DUBs are known that regulate any neurotransmitter receptors [3-6]. We show here that the DUB R10E11.3 acts in interneurons to regulate the abundance of GFP-tagged GLR-1 (GLR-1::GFP) in the ventral nerve cord (VNC). R10E11.3 is the *C. elegans* homolog of a human DUB of unknown function called ubiquitin specific protease-46 (*usp-46*). Loss-of-function of *usp-46* using RNAi or the deletion mutant *ok2232* leads to decreased GLR-1::GFP abundance at VNC synapses. *usp-46* mutants also have defects in *glr-1*-dependent behaviors, consistent with reduced glutamatergic signaling. The decrease in synaptic GLR-1::GFP in *usp-46* mutants is not due to defects in synapse development or *glr-1* transcription. However, we observe a decline in total GLR-1::GFP protein levels, consistent with a role for USP-46 in regulating GLR-1 protein stability. Furthermore, expression of DN-VPS-4, which inhibits GLR-1 degradation by the MVB/lysosome [7], blocks the decrease in synaptic GLR-1::GFP seen in *usp-46* mutants. Finally, the synaptic abundance of GLR-1(4KR)::GFP, a non-ubiquitinatable version of GLR-1, is unchanged in *usp-46* animals, suggesting that USP-46 promotes the deubiquitination of GLR-1. These data identify a novel role for USP-46 in regulating the synaptic abundance of GLR-1::GFP. We propose a model in which USP-46 promotes GLR-1 recycling to the plasma membrane by deubiquitinating GLR-1 in the endosome and thus preventing its degradation in the lysosome.

1. Yi and Ehlers, 2005. 2. Burbea, *et al.*, 2002. 3. Juo and Kaplan, 2004. 4. Dreier, *et al.*, 2005. 5. Schaefer and Rongo, 2006. 6. Park, *et al.*, 2009. 7. Chun, *et al.*, 2008.

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MOLECULAR MECHANISMS ACTIVATED BY THE UNC-40/DCC RECEPTOR TO DIRECT SYNAPTIC TARGET SELECTION. **Daniel A. Colon-Ramos**. Dept Cell Biol, Yale Sch Med, Ne Haven, CT.

Neural circuits are assembled through the coordinated innervation of pre- and postsynaptic partners. We recently showed that in C. elegans connectivity between two interneurons (AIY and RIA) is orchestrated by glial cells that express UNC-6 (netrin). In the postsynaptic neuron RIA, UNC-6/Netrin is detected by the Netrin receptor, UNC-40/DCC, which plays a conventional guidance role in directing outgrowth of the RIA process ventrally towards the glia. In the presynaptic neuron AIY, UNC-40/DCC plays an unexpected and previously uncharacterized role, cell-autonomously promoting the assembly of presynaptic terminals in the immediate vicinity of the glia. We now find that MIG-10/lamellopodin is required for the Netrin receptor, UNC-40/DCC, to direct presynaptic localization in the AIY interneuron. MIG-10/lamellopodin localizes to presynaptic sites in an UNC-40/DCC dependent manner. Moreover, MIG-10/lamellopodin is required for presynaptic localization in AIY, but is dispensable for UNC-40/DCC dependent guidance in RIA. These results indicate that UNC-40/DCC-mediated presynaptic localization is genetically separable from UNC-40's guidance role, and suggest that UNC-40/DCC directs presynaptic localization through the MIG-10/lamellopodin pathway.

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zig-5 and *zig-8* regulate sax-7/L1 CAM in maintaining nervous system architecture. **Claire Bénard**, Oliver Hobert. Dept Biochem, Columbia Univ, New York, NY.

Brain function requires that neuronal structures established early in development persist throughout life. How is nervous system architecture maintained as an animal increases its body size, remodels parts of its anatomy and incorporates new neurons? Dedicated mechanisms maintain the precise position of axons in fascicles and of soma in ganglia. To date, four molecules have been implicated in neuronal maintenance: the two-Ig domain protein ZIG-4, the FGF receptor EGL-15(5A), the L1-like SAX-7 protein, and the giant protein DIG-1 that contains multiple cell adhesion and cell-cell interaction domains.

Here we report the identification of two new mediators of neuronal maintenance, *zig-5* and *zig-8*, which function by regulating the activity of *sax-7*. *zig-5* and *zig-8* encode secreted two-Ig domain proteins. *sax-7* encodes an L1-like cell adhesion protein that functions within neurons to maintain neuronal position. *sax-7* is expressed as two isoforms that differ in the number of extracellular Ig domains and are differentially adhesive: a more adhesive short form (4 Ig domains) that is active in maintaining neurons, and a less adhesive long form (6 Ig domains). We find that *zig-5 zig-8* double mutants, but not single mutants, phenocopy the maintenance defects of *sax-7* mutants. In order to address whether *zig-5* and *zig-8* might be functionally related to *sax-7*, we have analyzed their genetic interactions. In particular, a mutation of *sax-7*, which depletes only the long isoform and displays no maintenance defects on its own, suppresses the defects of *zig-5 zig-8*. In addition, overexpression of the short form of *sax-7* fully suppresses the defects of *zig-5 zig-8* double mutants. Previous work from the Mori and our own labs suggests that the long isoform of SAX-7 may adopt an autoinhibitory conformation, in which the activity of Ig domains #3 and #4 (crucial for adhesion) are inhibited by association with Ig domains #1 and #2, resulting in a reduction of adhesiveness. We speculate that ZIG-5 and ZIG-8 may help transform the autoinhibited form into an activated form, for example, by binding to Ig domain #1 or #2, thereby allowing Ig domains #3 and #4 to interact. Genetic removal of the long isoform would therefore abrogate the need of *zig-5* and *zig-8*, thereby explaining our interaction data. We will present biochemical data that test this model. We have further identified that loss of *zig-1*, another two-Ig domain protein, fully suppresses the defects of *zig-5 zig-8*. Our current view is that *sax-7* and *zig-5/zig-8* promote cellular adhesion, which is opposed by *zig-1*, creating a balanced adhesiveness of neurons to their environment to bring about robust maintenance.

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A G-Protein Coupled Receptor (GPCR) Mediates UNC-6/Netrin Signaling in Axon Guidance. **Jasmine T. Plummer**, Joe Culotti. Department of Molecular Genetics, University of Toronto, Samuel Lunenfeld Research Institute, Toronto, ON, Canada.

The UNC-6/Netrin and its receptors UNC-40/DCC and UNC-5 is a highly conserved signaling pathway, which in *C.elegans*, guides growing axons and migrating cells circumferentially along the dorsoventral axis. Ectopically expressing UNC-5 in the touch neurons *(mec-7p::unc-5)* causes axons to be misdirected towards the dorsal nerve cord. To discover novel components of the UNC-6 guidance system, a sensitized genetic background was used previously to perform a screen for suppressors of this dorsal axonal rerouting and identified *seu* (*S*uppressor of *E*ctopic *U*NC-5) mutants (Colavita and Culotti, 1998). We have cloned *seu-2(ev523)* and report that it encodes a seven transmembrane G-protein coupled receptor (GPCR). *seu-2(ev523)* allele contains a mutation in the 1st transmembrane domain which causes a H→Y change and is likely a null allele. We isolated a *seu-2* deletion mutant, which is also able to suppress the dorsalward projections caused by ectopic UNC-5. *seu-2::GFP* is expressed neuronally in both sets of motorneurons, a subset of the touch neurons, a few tail neurons and in various neurons of the nerve ring including the amphid neurons.

A specific Rax (*R*ay *Ax*on defective) phenotype has been previously observed in *unc-40, unc-6* and *rax-1* mutants (Jia and Emmons, 2006). We demonstrate that not only is SEU-3 indeed RAX-1, but that both *seu-2* and *seu-3* also share the Rax phenotype similar to that seen in mutants of *unc-6*. Genetic analysis of *seu-2; seu-3* double mutants reveal they appear to act within the same pathway, downstream of UNC-6 in an UNC-40 dependent manner. Furthermore, given SEU-2 is a GPCR, we attempted to determine whether other downstream components of G-protein signaling also played a role in UNC-6 signaling. Preliminary data reveals certain G-protein alpha subunit mutants exhibit UNC-6 like phenotypes. Downstream targets of GPCR signaling, such as intracellular calcium, have been shown to regulate Netrin signaling in vertebrate studies. However, the GPCR responsible for this mechanism has yet to be identified. We propose that SEU-2 may function in the UNC-6-UNC-40 dependent pathway to mediate axon guidance. Characterizing genes, such as *seu-3*, may provide further insight into the other signaling components governing axon guidance.

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Watching Neurons Grow: The Rac GTPases, UNC-34 and the Arp2/3 complex are Important for Growth Cone Structure and Dynamics. **Adam Norris**, Erik Lundquist. Molecular, Cellular and Developmental Biology, University of Kansas. 1200 Sunnyside Ave. Lawrence, KS.

Developing neurites have growth cones at their tips, providing both a mechanism for sensing the extracellular environment and for powering the forward motion of the neurite. Growth cones consist of a sheet-like mass of branched actin filaments forming a lamellipodium, as well as multiple spiky protrusions of bundled actin filaments forming filopodia. UNC-34/Enabled, the Arp2/3 complex, and the Rac GTPases have been implicated in neurite pathfinding, but the effects of these molecules on the growth cone during outgrowth are unclear. Here we ask what effect these genes have on growth cone structure and dynamics. We show that mutants for each of these genes cause a decrease in the number of filopodia in the PQR dendrite growth cone. Despite the decrease in filopodia, the PQR dendrite extended to its normal position. However, the dendrites did exhibit an increase in improper turning, thereby making the path from cell body to final position longer and more circuitous. We show a strong correlation between a decrease in filopodial number and an increase in total dendrite length. To determine whether the observed decrease in filopodia was due to a decrease in filopodia formation or maintenance we took time-lapse images of migrating VD axon growth cones at two-minute intervals. Using these methods we show that the decrease in filopodia in *unc-34*, *unc-115* and Arp2/3 mutants is due primarily to decreased *de novo* filopodia formation and not maintenance. These studies agree with previous studies that showed UNC-34 being necessary for filopodia in the growth cone. These studies also expand the number of known actin cytoskeleton regulatory genes involved in filopodia, and show that the filopodial defects observed are due primarily to a decrease in *de novo* filopodia formation.

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C. elegans glia require the thermotaxis gene *ttx-1* for temperature and dauer dependent functions. **Carl Procko**, Yun Lu, Shai Shaham. Department of Developmental Genetics, Rockefeller University, New York, NY.

An animal senses stressful stimuli within its environment and responds appropriately. The nematode *Caenorhabditis elegans* responds to stresses, including high temperature and nutrient availability, by becoming a developmentally-arrested dauer larva. Many of these stresses are detected through a pair of bilateral amphid sensory organs in the head of the animal. Previous studies have revealed roles for the AFD amphid neuron in temperature sensation. Like other amphid neurons, AFD is closely associated with a glial sheath cell that surrounds the specialized dendritic ending of the neuron. It has been shown that in dauer larvae, the bilateral amphid sheath glia expand and fuse with one another at the nose tip. In addition, the dendritic endings of another pair of sensory neurons expand and overlap within the fused glia. To understand how stress signals from the environment regulate sheath glia fusion in dauers, we have characterized a sheath cell-specific GFP reporter for the *ver-1* gene, and have shown that it exhibits temperature- and dauer-dependent activity. Surprisingly, activity of *ver-1::gfp* depends on the LIM homeobox gene *ttx-1*. *ttx-1* has been shown to be required to establish AFD thermosensory identity, and, in a thermal gradient assay, *ttx-1* mutants demonstrate a constitutive cryophilic behavior. Ablation of AFD did not, however, affect temperature-dependent *ver-1::gfp* expression in glia. Using cell-specific promoters we demonstrated that expression of TTX-1 specifically within amphid sheath cells could restore *ver-1::gfp* reporter activity in *ttx-1* mutants. In contrast, AFD neuron-specific expression of TTX-1 restored wild type thermotaxis behavior, suggesting that the glial temperature response does not contribute to this process. Using an electrophoretic mobility shift assay, we have identified a direct TTX-1 binding site within the temperature- and dauer-dependent *ver-1* promoter, and used this to find other TTX-1 regulated genes. These results may indicate that within the nervous system, glia as well as neurons can possess sensory functions. As described, high temperature is also one of a few stresses that regulates dauer entry. We further find that in *ttx-1* mutants the sheath glia fail to fuse in dauer larvae, resulting in aberrant extension of the dendritic ending of an amphid sensory neuron. Preliminary studies using cell specific promoters to restore TTX-1 function indicate that TTX-1 is required within the glia for this process. Collectively, these results may suggest that amphid sheath glia can respond to environmental stresses independently of sensory neurons, and in turn this may facilitate the remodeling of the sensory neurons and glia in response to stress in the dauer larva.

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The Na^+/Cl^--dependent betaine transporter *snf-3* or the phospholipase Cβ *egl-8* blocks neurodegeneration. **Aude S. Ada-Nguema**[1], Randi L. Rawson[1], Guoliang Jiang[3], You-Jun Fei[3], Vadivel Ganapathy[3], Erik Jorgensen[1,2]. 1) Department of Biology, University of Utah, Salt Lake City, Utah USA 84112; 2) Howard Hughes Medical Institute, University of Utah, Salt Lake City, Utah USA 84112; 3) Departments of Biochemistry and Molecular Biology, Medical College of Georgia, Augusta, GA USA 30912.

We have found that a loss of the betaine transporter in the hypodermis and a loss of PLCβ in the neurons leads to a synthetic neurodegenerative phenotype. We isolated a recessive allele of *snf-3(ox354)* from a clonal enhancer screen aimed at identifying genes that redundantly function with *egl-8* (PLCbeta). *snf-3 egl-8* double mutants are uncoordinated, dumpy, and exhibit adult-onset degeneration of axons, whereas single mutants of either gene do not show these defects. In addition, *snf-3 egl-8* double mutants have a mild defect (25% penetrant) in DD/VD dorsal axon guidance. These phenotypes derive from a defect in hypodermis-neuron signaling: *snf-3* expression in the hypodermis or *egl-8* expression in neurons is sufficient to rescue to the double mutant phenotypes; but not the converse. We conclude that SNF-3 functions non-cell autonomously to regulate metabolic or intercellular signaling pathways, which function in axonal outgrowth and maintenance. SNF-3 (Sodium Neurotransmitter Family) is a member of the Na^+/Cl^--dependent GABA subfamily of neurotransmitter transporters. Ion-coupled transporters control the levels of extracellular transmitters and thereby limit their signaling. Unlike its mammalian homolog BGT-1, which transports GABA and betaine, the *C. elegans* SNF-3 specifically transports betaine. Betaine is associated with osmoregulation and lipid and protein methylation. However, our data suggest a novel function for betaine in cell signaling. One possibility is that the accumulation of betaine in the extracellular environment in the mutant strain antagonizes a signaling pathway that is crucial for axon guidance and neuronal survival. To identify this signaling pathway, we performed a suppressor screen of the double mutant subviable phenotype and isolated 40 triple mutants that restored neuronal function. We can divide these suppressors into *egl-8* and *snf-3* dependent suppressors based on the phenotype of the double mutant. Characterization of these genes may unravel a novel betaine signaling or metabolic pathway.

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New *Caenorhabditis* species: phylogeny and evolution. **Karin C. Kiontke**[1], Marie-Anne Félix[2], David H. A. Fitch[1]. 1) Deptartment of Biology, New York University, New York, NY; 2) Institut J. Monod, CNRS-University of Paris 7, Paris, France.

Recently, nine new *Caenorhabditis* have been discovered, bringing the number of *Caenorhabditis* species in culture to nineteen, eleven of which are undescribed. To elucidate the relationships of the new species to the five species with sequenced genomes, we have used sequence data from two rRNA genes and several protein-coding genes for reconstructing the phylogenetic tree of *Caenorhabditis*.

Four new species (spp. 5, 9, 10 and 11) group within the so-called *Elegans* group of *Caenorhabditis*, with *C. elegans* being the first branch. Although none of them is the sister species of *C. elegans*, *C.* sp. 5 and *C.* sp. 9 are close relatives of *C. briggsae*. *C.* sp. 9 can hybridize with *C. briggsae* in the laboratory. Of the remaining new species, *C.* sp. 7 branches off between *C. elegans* and *C. japonica*. Three of these species, *C.* sp. 7, *C.* sp. 9 and *C.* sp. 11 have been chosen for genome sequencing. Four further new species branch off before *C. japonica* within a monophyletic clade which also comprises *C.* sp. 3 and *C. drosophilae*.

Only one of the new species, *C.* sp. 11, is hermaphroditic. The position of *C.* sp. 11 in the phylogeny suggests that hermaphroditism evolved three times within the *Elegans* group.

Two of the new species were isolated from rotting leaves and flowers, and seven from rotting fruit. Rotting fruit is also the habitat in which *C. elegans* has been found to proliferate (Barrière and Félix, Genetics 2007) and from which *C. briggsae*, *C. brenneri* and *C. remanei* were repeatedly isolated. This suggests that the habitat of the stem species of *Caenorhabditis* after the divergence of the earliest branches (*C. plicata, C. sonorae* and *C.* sp. 1) was rotting fruit.

Other characters, like the shape of the stoma and the male tail, introns, susceptibility to RNAi and genome size are being evaluated in the context of the phylogeny.

The rate of discovery of new *Caenorhabditis* species has steadily increased since the description of *C. elegans* in 1899, with a leap in the last few years. There is no indication that we are even close to knowing all species in this genus.

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The secret life of *E. coli* OP50. Joe Hedden, Nick Loman, Claudia Boehnisch, Mark Pallen, **Robin May**. School of Biosciences, University of Birmingham, Birmingham, United Kingdom.

Throughout the half a century that *C. elegans* has been a model organism, it has been fed on lawns of *E. coli* OP50. However, unlike its predator, OP50 has been neglected as an object of study. To set the record straight, and to investigate *C. elegans* nutrition from the perspective of its prey, we have begun to investigate the biology of *E. coli* OP50.

We have used Solexa sequencing to produce a draft genome sequence for OP50. In parallel, we have investigated the influence of growth media on OP50/*C. elegans* interactions and used high-resolution microscopy to study the behaviour of the bacterium within worm intestine. Our data suggest that, far from being an innocent prey item, OP50 is a pathogen of *C. elegans* that retains the ability to turn the tables and kill its predator in several situations. In addition, the long history of *C. elegans* (and thus OP50) culture provides a powerful resource with which to study microevolutionary processes in a bacterium closely related to, and yet distinct from, other laboratory strains of *E. coli*.

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Eating organic: effect of wild microbes on *C. elegans'* metabolism. **Buck S. Samuel**[1,2], Christian Braendle[3], Marie-Anne Felix[4], Gary Ruvkun[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Institute of Developmental Biology and Cancer, CNRS, University of Nice Sophia-Antipolis, Nice, France; 4) Institut Jacques Monod, CNRS, Universities of Paris 7 and 6, Paris, France.

Animals have evolved in a microbial world. Microbes exert influence on a broad range of physiologic processes, from shear survival to development to regulation of energy balance. *C. elegans* is highly tuned to microbial cues as potential food. While *E. coli* is fed often to lab strains, such associations are not likely to occur in the wild. Indeed, culture-based assessments of natural *C. elegans* habitats, such as compost and rotting fruits, indicate the presence of a broad range of bacterial phylotypes (M.-A. Felix, unpublished). To more broadly examine the spectrum of microbes encountered by wild *C. elegans* populations, we performed culture-independent sequencing of bacterial 16S rDNA from eight habitats harboring wild *C. elegans* populations. The data indicates that these animals most commonly encounter bacteria belonging to four phylogenetic divisions (phyla), Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria.

Our goal is to use this natural association to explore conserved endocrine responses to microbes in *C. elegans*. To this end, we selected a panel of ten bacteria that were identified in at least three independent habitats and assayed various measures of energy balance. Growth rates, brood size and feeding rates of *C. elegans* are coarse assessments of the food quality and potential modulation of energy store partitioning. We found that *C. elegans* (N2) exhibited a spectrum of growth rates on these wild microbes compared to *E. coli*. These differences cannot solely be attributed to bacterial cell size or wall thickness, as two closely related gram-positive Firmicutes have opposite impacts on growth rates, suggesting additional factors are involved. Decreased brood sizes were also observed in animals grown on three diverse bacteria. Feeding rates were diminished on four microbes, with a Bacteroidetes being the most dramatic. Two of these microbes exhibit decreased Nile Red staining, though biochemical analyses of lipid content are ongoing. To allow for better classification of these growth responses by food quality-based measures, we are employing additional assays of *C. elegans*' activity and behavior.

These results suggest that exposure to commonly encountered wild microbes distinctly alters *C. elegans* energy metabolism, which may represent co-evolved endocrine responses to microbes.

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Quantitative genetic analyses of *C. elegans* intraspecies variation in responses to *Pseudomonas aeruginosa* and *Staphylococcus aureus*. **Erik C. Andersen**[1], Zachary Okhah[2], Kirthi Reddy[3], Dennis H. Kim[3], Leonid Kruglyak[1,2,4]. 1) Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ USA 08544; 2) Ecology and Evolutionary Biology Department, Princeton University, Princeton, NJ USA 08544; 3) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA USA 02139; 4) Howard Hughes Medical Institute.

The nematode *Caenorhabditis elegans* responds to pathogenic bacteria with conserved innate immune responses and pathogen avoidance behaviors. We investigated natural variation in *C. elegans* responses to different pathogen infections, using a collection of advanced intercross recombinant inbred lines (RILs) between the laboratory wild-type strain N2 and a wild isolate from Hawaii CB4856. Initially, we chose to focus on the opportunistic human pathogen *Pseudomonas aeruginosa*, a gram-negative, aerobic bacterium. Quantitative trait mapping of the susceptibilities of 126 RILs led to the identification of a polymorphism in the *npr-1* gene as causal in the difference in susceptibility to *P. aeruginosa* between these two strains. We showed that the mechanism of NPR-1-mediated pathogen resistance is through oxygen-dependent behavioral avoidance rather than through direct regulation of innate immunity. Because the *npr-1* allele present in the N2 strain is a lab-derived gain-of-function allele (1), we sought natural variants involved in pathogen resistance by scoring susceptibility to *P. aeruginosa* in only those RILs with the ancestral *npr-1* allele (found in CB4856). Through quantitative genetic analysis of 89 additional strains, we identified a second quantitative trait locus (QTL) on LGV. We will discuss our progress in determining the gene underlying this novel QTL.

Subsequently, we investigated differences in the susceptibilities of N2 and CB4856 to *Bacillus thuringiensis*, *Candida albicans*, *Cryptococcus neoformans*, *Enterococcus faecalis*, *Serratia marcescens*, and *Staphylococcus aureus*. Assays using the gram-positive bacterium *S. aureus* had the most robust survival difference between the two parental strains. Quantitative trait mapping of the susceptibilities of 73 RILs led to a causal polymorphism in the *npr-1* gene, indicating that behavior determines the susceptibility to this pathogen as well. We will present our progress on the identification of additional QTL controlling susceptibility to *S. aureus*.

(1) McGrath PT *et al.* Neuron 2009; 61(5):692-699.

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Life is not fair: larger chromosomes are transmitted to males in *C. elegans*. **John Wang**[1], Pei-Jiun Chen[2], George Wang[3], Winship Herr[2], Laurent Keller[1]. 1) DEE, University of Lausanne, Lausanne, Switzerland; 2) CIG, University of Lausanne, Lausanne, Switzlerland; 3) Dept of Biological Sciences, Stanford University, Stanford, CA.

The genome is typically viewed as a collection of cooperating genes whose alleles, in sexual eukaryotes, are segregated fairly during sexual reproduction. However, underneath the veneer of the harmonious and egalitarian genome, conflicts among genes exist and the lottery of gene transmission is sometimes biased. Aside from transposable elements and sperm competition, these topics have been underexplored in *C. elegans*. We decided to investigate if genetic inheritance in *C. elegans* is also biased. We tested whether chromosomal aberrations in a heterozygous state are transmitted unequally to offspring using strains carrying transgene insertions or deficiencies. For 4/4 integrated transgene strains tested, we found that heterozygote males (GFP/+) transmitted the insertion-bearing chromosome preferentially to male progeny. The magnitude of the transmission skew was large and differed among the lines (ranging from 2:1 to 6:1 ratio of GFP:non-GFP males). In parallel, we observed a skew of equivalent magnitude in transmission of the non-GFP bearing chromosome to hermaphrodites. We also tested two large deficiencies (*tDf1* and *nDf24*). For both, heterozygote males transmitted the wild-type and deficiency alleles preferentially to the male and hermaphrodite progeny, respectively. These results show that the genetic inheritance in C. elegans is indeed biased, with the larger chromosomes preferentially transmitted to males. To examine the sensitivity of the mechanisms responsible for this transmission skew, we then tested if smaller chromosomal aberrations would exhibit the same phenotype. Thus far, we have tested single gene deletions in *unc-47* (*gk192*) and *unc-63* (*gk234*). For both we observed small (~5%) but significant biases in transmission. This suggests that the skewing mechanism may be able to detect very small chromosomal differences in the range of a few kb. As an entry to understanding transmission skew, we first asked if the skew is sex-specific. We examined transgene transmission in XO *her-1* mutants and found that sperm, but not oocytes, display transmission skew. This suggests that transmission skew may happen during spermatogenesis. Transmission skew has important implications and poses interesting questions. This effect could play an important role in genome evolution and reveals an unexpected genetic linkage between autosomes and the X chromosome. More interestingly, the insertion-bearing chromosomes could serve as a model for birth of Y-chromosomes and for understanding how rapid evolution of sex determination systems occurs.

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The genomes of gonochoristic versus hermaphroditic *Caenorhabditis* species. **Erich M. Schwarz**, *Caenorhabditis* Genome Analysis Consortium. Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125.

To identify candidate regulatory elements and possible genetic determinants of hermaphroditism, we have undertaken comparative analysis of three newly determined genomes from gonochoristic (dioecious) relatives of *C. elegans* (*C. remanei*, *C. brenneri*, and *C. japonica*) versus two published genomes of hermaphroditic *C. elegans* and *C. briggsae*. Core analyses include refined prediction of protein-coding genes using tools empirically optimized with control analyses of *C. elegans* (nGASP), predictions of small and non-coding RNA genes, repeat predictions, and scans for unresolved heterozygosity found in outbreeding species even after incrossing. Protein-coding genes are being examined for novel protein domains, lineage-specific domains, domain architectures, nematode-specific genes, and accelerated evolution or expansion of protein families. Whole-genome alignments allow global searches for conserved non-coding DNA and reveal syntenic gene order: these are being produced by the well-validated PECAN/ENREDO/GERP pipeline, the newly devised FSA algorithm, and other tools such as OrthoCluster and CYNTENATOR. Finally, detailed analysis of orthologous protein groups and sex-determination genes will be aimed at uncovering traces of the parallel evolution of hermaphroditism in the *C. elegans* and *C. briggsae* genomes.

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Evolutionary Comparisons of Nematode Dosage Compensation and Sex Determination. **Te-Wen Lo**[1], Qinwen Liu[2], Eric Haag[2], Barbara Meyer[1]. 1) Dept of MCB, UC Berkeley/HHMI, Berkeley, CA; 2) Dept of Biology, University of Maryland, College Park, MD.

Dosage compensation (DC), is an essential process by which expression of X-linked genes is equalized between males(XO/XY), and females (XX). Comparison of flies, worms, and mammals indicates that DC mechanisms differ, suggesting the process is rapidly evolving. Thus, insights into DC evolution are more readily gained from comparisons over shorter time scales, those between closely related taxa, as shown recently by studies of the *Drosophila* genus.

Although *C. elegans(Ce)* and *C. briggsae(Cb)* have diverged 30-100 Myr, our analysis has shown that dosage compensation complex (DCC) components are conserved between species, as is the pivotal role of *xol-1* in determining sexual fate and the level of X gene expression. We have thus set the stage for new insights into the evolution of X binding sites, DC, and sex determination. By isolating chromosomal deletion alleles of *Cb xol-1*, *dpy-27*, and *mix-1*, raising an antibody to the *Cb* homolog of DPY-27, a DCC subunit, and developing FISH probes to X and chromosome I, we showed that the *Cb* DPY-27 associates with *Cb* X in a hermaphrodite-specific manner that requires the *Cb* homolog of the *Ce* DCC subunit MIX-1. Furthermore, we have shown that *Cb* MIX-1 immunoprecipitates with *Cb* DPY-27, indicating an association in the *Cb* DCC. As predicted, deletion of *Cb dpy-27* causes XX-specific lethality and rescues the XO-specific lethality caused by *Cb xol-1* deletions. *Cb* males lacking *xol-1* die because DPY-27, and presumably all DCC subunits, bind to the single X chromosome and reduce gene expression. Despite the conserved role of *xol-1*, the X signal elements that communicate the primary sex determination signal (X:A) by repressing *xol-1* activity appear to have diverged, demonstrating the rapid evolution of the primary sex determination signal.

While DCC subunits appear conserved, DCC binding sites appear diverged. The *Ce* consensus motif (MEX, motif enriched on X) pivotal for *Ce* DCC recruitment to X is only enriched .6-2-fold on *Cb* X compared to autosomes, in contrast to the 3.8-24-fold enrichment on the *Ce* X chromosome. Ongoing ChIP-seq experiments to define the *Cb* DCC binding sites will reveal the degree of divergence. Our analysis will provide further insights into the evolution of sex chromosomes and dosage compensation.

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The role of *gld-1* in *C. briggsae* germline sex determination. **Alana V. Doty**, Eric S. Haag. Program in Behavior, Evolution, Ecology, and Systematics, and Dept. of Biology; Univ of Maryland College Park; College Park, MD.

Though many developmental pathways are conserved across wide phylogenetic distances, sex determination mechanisms are often hyperdivergent. To understand the rapid evolution of sex determination we can examine closely related species, between which sex determination changes may still be interpretable. We conducted genetic screens in *C. briggsae* for Mog (masculinization of germline) mutants. Our goal is to compare the identity and regulation of these genes to those of the well-characterized *C. elegans* sex determination pathway to see how hermaphroditism has evolved independently in the two species. We isolated three *C. briggsae* masculinizing mutant alleles: two from forward screens, *nm41* and *nm64*, and one from a deletion screen, *nm68*. All are alleles of *Cbr-gld-1*, which encodes the ortholog of the C. elegans germline RNA-binding protein and translational repressor, GLD-1. As judged by DIC microscopy and Hoechst staining, *gld-1*(lf) hermaphrodite germlines never undergo oogenesis and often contain excess mature sperm and spermatocytes reaching into their distal gonad arms. Other mutant animals make germline tumors or have arrested pachytene cells. The published work of Nayak et al. (2005) and our results show that *gld-1* has an opposite major sex determination role *C. elegans* vs. *C. briggsae*: in *C. elegans*, it is necessary to allow spermatogenesis in hermaphrodites, but is instead needed for the switch to oogenesis in *C. briggsae* hermaphrodites. Because GLD-1 is well conserved between species, we hypothesize that GLD-1 has different cofactors and/or has come to repress different messenger RNA targets in the two species. To test this, we created transgenic lines via bombardment in which the null *C. elegans gld-1* allele *q*485 is rescued by the wild-type *C. briggsae gld-1* locus. This demonstrates that *Cbr*-GLD-1 is capable of acting as a translational repressor as GLD-1 does in C. elegans, and supports the idea that differential mRNA target acquisition is the mechanism by which GLD-1 has come to have opposite sex determination roles in *C. briggsae* and *C. elegans*. Next, to identify these different targets in *C. briggsae*, we immunoprecipitated mRNAs bound by wild-type *Cbr*-GLD-1 *in vivo*. We will report results from microarray analysis using chips containing probes for all predicted *C. briggsae* protein-coding genes. By comparing a validated set of *Cbr*-GLD-1 targets to what is know of GLD-1 targets in *C. elegans*, we can determine which, if any, are unique to the *C. briggsae* sex determination pathway and thus may have been important in the evolution of hermaphroditism in *C. briggsae*.

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The Tip60/NuA4 HAT complex promotes spermatogenesis in *C. briggsae*. **Yiqing Guo**, Ronald E Ellis. Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ.

In a general screen for female mutants in *C.briggsae*, we found two alleles of a new gene required for spermatogenesis in both hermaphrodites and males. We named this gene *cb-fog-4* because of its Fog phenotype. Epistasis studies showed that *fog-4* acts downstream of *tra-2*, and upstream of or in parallel to *tra-1*.

Genetic mapping located *cb-fog-4* on Chromosome II, and fine scale mapping placed it in a 160kb region between the SNPs cb43333 and cb43262. There are only 25 predicted genes in this region. One of these genes, *cb-trr-1*, is *fog-4*, since *cb-trr-1*(*RNAi*) animals are Fog, and both mutants have lesions in *cb-trr-1*. TRR-1 is a homolog of TRRAP in humans. Since both mutations are missense alleles, we set up a non-complementation screen using TMP/UV to isolate null alleles, and found 3 Fog alleles that have deletions in *cb-trr-1*.

TRRAP is highly conserved. It is a common subunit of several HAT complexes, including Tip60/NuA4 and PCAF/GCN5. RNAi against *cb-mys-1*, the catalytic subunit of Tip60/NuA4, also causes a Fog phenotype, but RNAi against *cb-pcaf-1*, the catalytic subunit of PCAF1/GCN5, does not. Furthermore, RNAi targeting four other components of the Tip60/NuA4 complex, *gfl-1*, *yl-1*, *epc-1*, and *ssl-1*, also causes a Fog phenotype. Thus, we propose that the Tip60/NuA4 complex controls spermatogenesis in *C. briggsae*. In *C. elegans*, *trr-1* mutations cause a weakly penetrant vulval defect on their own, as well as slow growth and sterility, but do not create Fog animals.

RNAi against either *cb-trr-1* or *cb-mys-1* in a *fem-2* mutant background caused embryonic lethality and L1 arrest. Moreover, RNAi showed that *epc-1* and *ssl-1* are also essential for the viability of embryos. By contrast, *cb-trr-1*; *cb-fem-3* animals produce normal oocytes. These results suggest that the Tip60/NuA4 complex is essential for embryonic development and is redundant with FEM-2.

We propose that *cb-trr-1* might provide a model for the recruitment of chromatin regulators into a development pathway.

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Inactivation of TRA-2 and SWM-1 Creates *C. remanei* Hermaphrodites. **Christopher C Baldi**, Ronald E Ellis. Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ.

We are studying how novel traits arise during evolution. The phylogeny suggests that hermaphroditism evolved independently in *C. briggsae* and *C. elegans*, whereas related species like *C. remanei* make females. How did this happen? We used RNAi to show that *fog-1* and *fog-3* regulate sperm production in nematodes. Furthermore, *fog-3* transcripts are expressed in hermaphrodite larvae, while they are making sperm, but not in females. This expression pattern is controlled by *tra-2* and other sex-determination genes.

In *C. elegans*, *fog-2* lowers *tra-2* activity to cause hermaphrodite development. When we used RNAi to lower *tra-2* activity in *C. remanei*, many *XX* animals still developed female bodies, but produced sperm as well as oocytes. Surprisingly, these 'pseudo-hermaphrodites' were not self-fertile. Their oocytes were normal, since they could be fertilized by male sperm. However, their own sperm were not active, although they produced MSP, stimulated ovulation in virgin animals, and could be activated by pronase *in vitro*.

These sperm can be activated *in vivo* by seminal fluid, and fertilize oocytes. First, we crossed pseudo-hermaphrodites with *fog-3(RNAi)* males, which make seminal fluid but not sperm. These crosses produced an average of 2 eggs per mother, all of which failed to hatch. Second, we mated pseudo-hermaphrodites with *C. elegans* males, since their sperm cannot fertilize *C. remanei* oocytes. These crosses produced an average of 3 eggs. Furthermore, 7 of these eggs hatched, with 4 developing into females and 3 dying as larvae. Thus, mutations that lower *tra-2* activity could have created hermaphrodites with inactive sperm.

How could pseudo-hermaphrodites become self-fertile? In *C. elegans*, SWM-1 prevents premature sperm activation by inhibiting proteases. We found a single *swm 1* homolog in *C. remanei*. Furthermore, many *tra 2(RNAi); swm-1(RNAi) XX* animals became self-fertile hermaphrodites, producing an average of 4 eggs. Ten of these eggs hatched, and 6 became adult females. Thus, mutations that alter *swm-1* could activate sperm.

Thus, the evolution of hermaphrodites required two steps. (1) Mutations that altered *tra-2* activity in *XX* animals, allowing them to produce sperm and oocytes. (2) Mutations that affected *swm-1*, allowing the activation of *XX* sperm. The order in which these steps occurred is unclear. But once both had taken place, selection could improve this novel mode of reproduction.

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Germline RNAi insensitivity exhibits genetic complexity and heterogeneity in wild *C. elegans*. **DA Pollard**, MJ Kramer, MV Rockman. Biology, NYU, New York, NY.

C. elegans has played a central role in the elucidation of the general mechanisms of RNA interference (RNAi), but natural populations of *C. elegans* vary in their sensitivity to RNAi in the germline. The Hawaiian strain (CB4856) is resistant to germline RNAi and a single gene in the PIWI/RDE-1/Argonaut family, *ppw-1*, accounts for most but not all of the variation segregating in a cross of CB4856 and N2 (Tijsterman et al, Current Biology, 2002). We sought to expand the characterization of the loss of RNAi sensitivity in *C. elegans* to identify new genes involved in the pathway and better understand the genetics underlying this natural variation. Using feeding RNAi assays targeting the essential embryonic polarity gene *par-1*, we surveyed germline RNAi sensitivity in SNP-genotyped natural isolates and recombinant inbred lines from a cross of N2 and CB4856. We found germline RNAi resistance is common and geographically widespread in natural populations, with nearly one in four wild isolates showing resistance. Association mapping with the natural isolates implicates a 250kb region of chromosome IV that contains 43 genes. Our analysis of recombinant inbred lines from a cross of N2 and CB4856, however, revealed only one large-effect QTL, centered over the *ppw-1* locus on chromosome I. No other significant QTL were mapped despite the presence of resistant strains with the N2 allele of *ppw-1*. The mapping of distinct loci from association mapping across strains and QTL mapping between two strains suggests that germline RNAi resistance may have been gained multiple times through separate mechanisms and that epistatic interactions among alleles at multiple loci are involved. We conclude that germline RNAi resistance is a widespread and complex trait in *C. elegans* with potentially novel underlying molecular mechanisms.

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Evolutionary Conservation of Cell Fusion in Nematodes. **Ori Avinoam**, Benjamin Podbilewicz. Department of Biology, Technion–Israel Institute of Technology, Haifa, Israel.

Membrane fusion is the process by which membranous compartments unite to form a single compartment. Virtually all membranes can fuse, ranging from small intracellular vesicles and organelles to entire cells. Thus, membrane fusion is critical for many biological processes such as intracellular trafficking and exocytosis, fertilization and viral infection, embryonic and post embryonic development. With the exception of some viral fusogens, SNARE proteins, and FF proteins (AFF-1 and EFF-1), the large majority of fusogens remain unidentified or uncharacterized. The fusogenic activity of EFF-1 and AFF-1 was demonstrated by the finding that their ectopic expression in *C. elegans* and in Sf9 insect cells is sufficient to fuse cells that normally do not fuse. Fusion of heterologous cells by FF proteins indicates that they are *bona fide* fusogens. Thus, FFs can be used to understand the principles of eukaryotic cell-cell fusion machineries. Using sequence comparison of 18 nematode species, 31 FF putative orthologs were identified and analyzed. We found that FF proteins show similar domain architecture; all members are type I membrane proteins with an extracellular portion 520-540aa long and a variable cytoplasmic tail. In addition all members harbor the same regions of high conservation along with 16 conserved Cysteines in their extracellular portion. We expressed FFs from *C. elegans* and *Trichinella spiralis*, in Baby hamster kidney cells (BHK-21) and observed that the proteins are distributed both in intracellular compartments and at the cell surface. In addition, we determined that FF proteins from these species can fuse BHK-21 mammalian cells. Furthermore, expression of the *Pristionchus pacificus* EFF-1 ortholog in *C. elegans* embryos resulted in ectopic fusion. These results suggest that FFs are functionally conserved in nematodes, that they may be interchangeable between species, and that they can fuse insect and mammalian cells. It has been previously shown that expression of EFF-1 is required on both fusing membranes in order for them to fuse. Surprisingly, while this homotypic mode of action is conserved for AFF-1, expression of AFF-1 and EFF-1 leads to heterotypic fusion in tissue culture. Taken together these results suggest that FF proteins are folded in a similar three-dimensional structure which may be essential for their fusogenic activity. To test this hypothesis and to study the molecular mechanism of FF proteins we perform structure function analysis of AFF-1, we generated mutations and tested their effect on surface expression and protein function in tissue culture and in worms. We will discuss insights on the mechanism of AFF-1 and their evolutionary implications.

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cye-1 and *cdk-2* may link the proliferation versus meiotic entry decision with the mitotic cell cycle. **Paul M. Fox**[1], Valarie Vought[2], Min-Ho Lee[3], Eleanor Maine[2], Tim Schedl[1]. 1) Dept Genetics, Washington Univ Sch Medicine, St Louis, MO; 2) Dept Biology, Syracuse Univ, Syracuse, NY; 3) Dept Bio Sci, University at Albany, SUNY, Albany, NY.

In the C. elegans germline, proliferative cells balance self renewal with entry into meiotic prophase. This decision must normally be coordinated with progression through the mitotic cell cycle in order to prevent a conflict between these two exclusive developmental processes. Currently, it is thought that cells must pass through a specialized meiotic S-phase prior to initiation of meiosis. In order to better understand how cell cycle progression is coordinated with meiotic entry, we analyzed the occurrence of mitotic cell cycle events after inducing meiosis in proliferative cells with the temperature sensitive *glp-1(bn18)* loss-of-function mutant. During this process we observed reproducible kinetics for proliferative cells reaching meiotic prophase that appeared to depend, in part, on mitotic cell cycle position. After initiating mitotic S-phase, proliferative cells appear committed to completing mitosis and loss of *glp-1* did not interfere with completion of mitosis. Our results are consistent with germ cells deciding to enter meiosis during the G1 phase of the cell cycle, allowing cells to proceed through meiotic S-phase prior to meiosis.

Initiating meiotic entry at a particular part of the mitotic cell cycle may involve regulation of specific cell cycle factors. To investigate this possibility, we performed an RNAi screen of a panel of cell cycle factors in the *glp-1(bn18)* background at the permissive temperature to identify genes that also regulate the decision to proliferate or enter meiosis. The overall results of this screen indicate that premature meiotic entry is not caused simply by a failure in cell cycle progression since RNAi depletion of the majority of cell cycle regulators and machinery caused cell cycle defects but did not induce premature meiotic entry. In contrast, reduction of *cye-1* or *cdk-2* by RNAi causes germ cells to prematurely enter meiosis in the *glp-1(bn18)* strain. Though *cye-1* is required for mitotic cell cycle progression, lower levels did not appear to interfere with meiotic S-phase. We propose that in the presence of low GLP-1 activity the decision to enter meiotic or mitotic S-phase is regulated by the level of CDK-2/CYE-1 activity with high CDK-2/CYE-1 promoting mitotic S-phase and low CDK-2/CYE-1 activity promoting meiotic S-phase.

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GLD-1-mediated repression of cyclin E/Cdk2 maintains totipotency during meiosis. Bjoern Biedermann[1], **Mathias Senften**[1], Jane Wright[1], Irene Kalchhauser[1], Gautham Sarathy[2], Min-Ho Lee[2], Rafal Ciosk[1]. 1) Friedrich Miescher Inst, Basel, Switzerland; 2) University at Albany, NY, USA.

The germ line is the only cell lineage that maintains the potential to recreate, through the fusion of sperm and egg, all cell types in a new individual. This wide developmental potential, or totipotency, of the germ line is manifested in unusual tumors called teratomas. In these tumors, germ cells that have lost their totipotent character differentiate into various somatic cell types. Although recent studies have implicated RNA regulation, the mechanism by which totipotency is normally maintained during germ cell development remains unexplained. In C. elegans, a germline teratoma can be induced through the loss of a single RNA binding protein, GLD-1. To understand the origin of teratomatous cells, we examined gld-1 gonads in a time-course experiment. By using various cell cycle markers, we found that gld-1 germ cells re-enter the cycle through an abnormal M phase, which is driven by Cyclin E/Cdk2. We found that, in the wild-type germ line, Cyclin E/Cdk2 activity is repressed through GLD-1-mediated translational repression of CYE-1/cyclin E. Importantly, we found that de-repression of CYE-1/CDK-2 activity in gld-1 germ cells promotes not only reentry into the cell cycle, but also teratomatous differentiation. Our findings demonstrate a mechanism by which GLD-1 controls the cell cycle and suggest that GLD-1 maintains totipotency, at least in part, by inhibiting cyclin E/Cdk2 -induced embryonic-like differentiation during progression through meiosis.

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The Puf RNA-binding Proteins FBF-1 and FBF-2 Inhibit the Expression of Structural Components of Meiotic Chromosomes in Germline Stem Cells. **Chris Merritt**, Geraldine Seydoux. Dept Molec Biol & Genetics, Johns Hopkins Univ Sch Med, Baltimore, MD.

FBF-1 and FBF-2 (collectively known as FBF) are two highly similar PUF domain RNA-binding proteins required for stem cell maintenance in the *C. elegans* germline[1]. To maintain germline stem cells, FBF inhibit the expression of positive regulators of meiosis, including the two RNA binding proteins GLD-1 and GLD-3[2]. We have obtained evidence that FBF also inhibits the expression of HIM-3, a component of meiotic chromosome axes (homolog of S. cerevisiae Hop1p[3]). We found that the *him-3* promoter is active in germline progenitors, even in immature gonads that have not yet initiated meiosis. Proper regulation of HIM-3 depends on its 3'UTR: a GFP:H2B:*him-3* 3'UTR fusion driven by a pan-germline promoter is repressed in progenitors and activated only in cells that have initiated the meiotic program. This regulation depends on FBF: depletion of FBF-1/2 by RNAi or by mutation activates the GFP:H2B:*him-3* 3'UTR reporter in progenitors. Endogenous HIM-3 protein is also mis-expressed in progenitors in *fbf-1/2* mutants. FBF-1 and FBF-2 bind in vitro to a sequence motif termed FBF-binding element (FBE)[4]. There are two putative FBEs in the *him-3* 3' UTR and mutations in one of these activates the GFP:H2B:*him-3* 3'UTR reporter in progenitors, suggesting that FBF may regulate *him-3* directly.

To test whether FBF might regulate the expression of other meiotic proteins, we created 3'UTR fusions for 14 other genes coding for structural components of meiotic chromosomes. We found that the 3' UTRs of *syp-2*, *syp-3* and *htp-1* are down-regulated in progenitors in a pattern similar to *him-3*. Depletion of FBF and mutation in a putative FBE both activate the *syp-3* 3'UTR reporter in progenitors. Similar experiments are in progress for *syp-2* and *htp-1*.

To determine whether FBF-1/2 affect RNA levels, we compared the level of endogenous *him-3, syp-2*, and *syp-3* mRNAs in L2 larvae depleted/not depleted of *fbf-1/2* by RNAi. We found that depletion of FBF causes a 2-3 fold increase in mRNA levels compared to controls. We are currently testing if these mRNAs exist in a complex with FBF *in vivo*. Our data suggest that FBF maintain progenitors by directly silencing mRNAs coding for structural components of meiotic chromosomes.

1. Crittenden, *et al.*, *Nature* **417** (6889), 660 (2002). 2. Kimble and Crittenden, WormBook, 1 (2005); Reinke, *WormBook*, **1** (2006). 3. Zetka, *et al.*, *Genes Dev.* **13** (17), 2258 (1999). 4. Bernstein, *et al.*, *RNA* **11** (4), 447 (2005).

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Germline Chromatin Structure is Regulated by XND-1. Cynthia Wagner, **Judith Yanowitz**. Dept Embryology, Carnegie Institution, Baltimore, MD.

We are investigating the role of chromatin modifications on meiotic recombination and germline development. Mutations in *xnd-1*(*X nondisjunction factor-1*) lead to defects in crossover formation on the X as well as a change in the global distribution of crossovers. We show that *xnd-1* directly regulates the formation of double strand breaks (which are required for the first step in recombination) since crossovers can be rescued by exogenous breaks caused by ionizing radiation and since repair proteins fail to localize properly to the X. Surprisingly XND-1 protein is found on autosomes, suggesting that it affects the X chromosome *in trans*. We will present data that suggests that XND-1 shapes the genomic crossover landscape by inhibiting crossover formation near active genes on the autosomes and allowing for crossovers to occur on the heterochromatic-like X chromosome. Supporting a direct role of XND-1 in germline chromatin organization, the lack of crossovers on the X is strongly suppressed by removing MES protein function (which is required for X chromosome silencing). Thus it appears that XND-1 and the MES proteins are acting antagonistically in the germline to coordinate X chromosome behaviors.

We also show that loss of *xnd-1* leads to progressive sterility—both as animals age and as populations are propagated, suggesting this gene is required to maintain germline immortality. We have identified a heterochromatic histone mark that is upregulated in *xnd-1* mutants, suggesting that XND-1 directly controls X chromosome behavior, recombination, and germline maintenance through a unique set of chromatin modifications.

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LAB-1 cooperates with cohesin to ensure accurate homolog segregation during meiosis I. **Yonatan B. Tzur**, Carlos E. de Carvalho, Ivo van Bostelen, Monica P. Colaiácovo. Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.

Achieving accurate chromosome segregation is a critical outcome for meiotic cell divisions. Errors in correctly separating homologs during meiosis I, and sister chromatids during meiosis II, may lead to aneuploidy, with dramatic consequences such as congenital defects, miscarriages and infertility. It has therefore been of critical importance to understand the regulation involved in coordinating loading, stability and dissociation of cohesins from DNA. We have recently reported the finding of LAB-1, a protein which plays a key role in late prophase I where it localizes to the long arms of the bivalents during diakinesis and restricts the spreading of the Aurora B Kinase, AIR-2, thereby preventing the premature loss of sister chromatid cohesion. Here we identify a distinct and earlier role for LAB-1 in promoting the establishment and maintenance of sister chromatid cohesion during early/mid-prophase I.

Utilizing fluorescence in situ hybridization we find that *lab-1(RNAi)* reduces homologous pairing levels on both autosomes and the X chromosome compared to wild type. Levels of RAD-51 foci, a marker for DNA double-strand break repair, dramatically increase following *lab-1* depletion, and the foci persist throughout the late stages of prophase I. Further evidence of an impaired progression in DSB repair stems from the increased germ cell apoptosis observed in *lab-1(RNAi)* in late pachytene. Synapsis is also impaired in the *lab-1* depleted gonads, as exemplified by the mixture of synapsed and unsynapsed chromosomes observed in pachytene, and the aberrant polymerization of SYP-1, a synaptonemal complex central region component, detected along unsynapsed tracks. Interestingly, SYP-1 can also partially polymerize along chromosomes in mutants for the meiosis specific cohesin *rec-8*, but it fails to do so in *lab-1(RNAi);rec-8(ok958)* mutants. A role for LAB-1 in the establishment and maintenance of sister chromatid cohesion is also implied by the delayed deposition of LAB-1 following depletion of *smc-3*, another member of the cohesin complex. Whereas SMC-3 localization during early prophase is not impaired in either *lab-1(RNAi)* or *rec-8* mutants, SMC-3 signal is drastically reduced or completely absent in *lab-1(RNAi);rec-8* mutants. We therefore propose that LAB-1 cooperates with the cohesin complex to bind sister chromatids, thereby promoting proper pairing, synapsis and recombination in *C. elegans* meiosis.

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A High-Content Screen to Functionally Classify Proteins Required for Cell Viability and Division. **Rebecca A. Green**[1], Anjon Audhya[2], Karen Oegema[1], Arshad Desai[1]. 1) Ludwig Institute for Cancer Research, La Jolla, CA; 2) Biomolecular Chemistry, University of Madison, WI.

Systems biology analyzes the role of molecular assemblies within functional networks. The success of this approach relies upon the availability of systematic and functional information about the components of these molecular assemblies. RNA-mediated interference (RNAi) is currently the method of choice for linking genes to their cellular functions. In addition to relatively 'low content' single reporter assays, RNAi has been used to perform high-content microscopy-based assays, integrating spatial and/or temporal information. This approach has been used to characterize the set of genes required for cell viability and division in *C. elegans*. Of the 20,000 genes, ~10% are required for embryo production or viability; these 2000 genes were previously screened by filming the first two embryonic divisions following individual RNAi depletion. This screen provided high quality data that allowed for the functional classification of ~400 genes, however it did not provide high quality functional information on the ~560 genes whose inhibition blocks embryo production (the sterile collection). Here, we functionally characterize the sterile collection by performing a second high-content screen. We use two-color fluorescence confocal microscopy to examine gonad structure in anesthetized worms after individual depletion of each of the 560 sterile gene products. The gonad-morphology data was analyzed by binary scoring for 94 potential defects. A clustering algorithm was used to group genes with similar phenotypic profiles, and phenotypes within the major clusters were then re-examined by eye for accuracy and identification of subclasses within each broad phenotypic group. Our live-imaging data, annotation and analysis will be integrated with the data from the prior DIC imaging based screen of the embryonic lethal collection in an online database that will be made available upon publication. Our analysis placed genes into ~20 broad classes that could be partitioned into ~100 different phenotypic sub-classes, which typically corresponded to the subunits of a specific protein complex. In addition to characterizing the 390 genes in the sterile collection, for which there was some prior functional information, our screen has placed the ~50 unknown and 120 previously uncharacterized genes in the sterile collection into functional groups. Cumulatively, our data doubles the number of genes for which we have high quality systematic functional information and provides an important platform for systems biology based analysis of the pathways contributing to embryo production and development.

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P granule-nuclear pore complexes are principal sites of mRNA export in *C. elegans* germ cells. **Ujwal Sheth[1,2]**, Jason Pitt[1], James R Priess[1,2]. 1) FHCRC, Seattle, WA; 2) HHMI.

Germline-specific P granules contain numerous RNA-binding proteins, but little detectable mRNA except under quiescent states. P granules are perinuclear during most of development, but are cytoplasmic in oocytes and early embryos. Although functions of cytoplasmic P granules have not been determined, loss of perinuclear P granules is associated with inappropriate expression of at least some "masked" maternal mRNAs and transformation of germ cells into somatic cells [1]. The perinuclear P granules are associated specifically with clusters of nuclear pore complexes (NPCs), representing about 75% of the total NPCs. Here, we wanted to determine whether the NPCs associated with P granules (Pg-NPCs) are active sites of mRNA export, and found that mRNA export factors such as NXF-1 are enriched at the base of the Pg-NPCs under normal growth conditions. We discovered that heat shock could be used to induce expression of transgenic mRNA in late pachytene nuclei, and used this assay to examine mRNAs containing several types of 3'UTRs. These include 3'UTRs from mRNAs that are (a) normally translated in the gonad, (b) not translated in the gonad, or (c) targeted for degradation by RNAi. None of these nascent mRNAs were retained in P granules, and instead each showed a similar, transient accumulation in P granules before entering the general cytoplasm. This transient accumulation depended on P granule proteins such as GLH-1, and was not seen in somatic cells. Thus, Pg-NPCs appear to contain a dynamic population of mRNAs. Although in time-lapse movies Pg-NPCs appear to be stable (>20 mins), we found in photobleaching experiments that P granule components such as PGL-1 are highly dynamic (<20 sec half-recovery times). We conclude that, under normal growth conditions, the Pg-NPCs do not appear to act as storage sites for masked mRNAs although it remains possible that cytoplasmic P granules have a role in storage. Instead, our results support the view that Pg-NPCs are sites of dynamic interactions between proteins and nascent mRNAs. We noted previously that the GLH family of P granule proteins contains numerous FG repeats [2], analogous to FG repeats in core nucleoporins that function in the export of mRNA. Interestingly, we found that a gonad-specific isoform of DDX-19 is highly enriched in FG repeats, and that DDX-19 and a novel *C. elegans* protein with FG repeats are enriched in P granules. Thus, we hypothesize that these repeats function in the extended export of mRNA/protein past the NPC and through P granules.

[1] Ciosk et al., Science 311, 851(2006) [2] Schisa et al., Development 128, 1287 (2001).

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Nup98/NPP-10: a Link between Nuclear Pores and Germ Granules in *C. elegans* and Mouse. **Ekaterina Voronina**, Geraldine Seydoux. Dept of Molecular Biol & Genetics, Johns Hopkins SOM/HHMI, Baltimore, MD.

P granules (or germ granules) are cytoplasmic ribonucleoprotein complexes unique to germ cells. During most of germ cell development, P granules localize to the cytoplasmic face of the nuclear envelope, often close to nuclear pores (Strome and Wood, 1982; Pitt et al., 2001). To investigate a possible link between nuclear pore components and P granules, we systematically depleted all annotated *C. elegans* nucleoporins (Galy et al., 2003) by RNAi and found that Nup98/NPP-10 is required for germ granule integrity and perinuclear localization. Nup98/NPP10 is a dynamic component of nucleopores implicated in mRNA export from the nucleus (Pritchard et al., 1999).

Depletion of Nup98/NPP-10 in embryos and larvae causes germ granules to disperse into the cytoplasm. The maternal *nos-2* mRNA, which is enriched in P granules and translationally repressed in wild-type, is released from P granules and translated prematurely upon NPP-10 depletion. Depletion of NPP-10 also causes premature expression of certain 3' UTR reporters normally silenced in the larval germline stem cells (Merritt et al., 2008). *Interestingly, similar effects are seen when the constitutive P granule component GLH-1 is inactivated.* These results suggest that NPP-10 is essential for P granule integrity and contributes to the *P granule-dependent* mechanisms that silence mRNAs in the germline.

An antibody specific to NPP-10 recognizes both nuclear pores and germ granules in the worm. Immunoprecipitation experiments suggest that NPP-10 exists in a complex with *nos-2* mRNA, raising the possibility that NPP-10 interacts directly with P granule components. To investigate whether this association is conserved beyond *C. elegans*, we examined the mouse homolog Nup98 in testes. We found that immunoprecipitation of Nup98 from mouse testes extracts brings down MVH, a mouse germ granule protein related to GLH-1. We conclude that Nup98/NPP-10 functions as an evolutionarily conserved link between nuclear pores and germ granules, perhaps to facilitate mRNA transport from the nucleus to the germ granules.

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Self-aggregation of PGL proteins plays a crucial role in P granule assembly. Momoyo Hanazawa[1], Masafumi Yonetani[2], **Asako Sugimoto[1,2]**. 1) Lab. for Developmental Genomics, RIKEN CDB, Kobe, Japan; 2) Dept. of Biological Sciences, Grad. School of Science, Osaka Univ., Osaka, Japan.

In many organisms, germ cells have regions of specialized cytoplasm, generally referred to as "germ granules". They are large RNA-enriched nonmembranous organelles, and believed to play roles in germ cell specification and differentiation. Germ granules in *C. elegans* are called P granules, which are specifically segregated into the germ lineage during early embryogenesis and present in germ cells throughout development. Although ~20 protein (mostly RNA binding proteins) and several RNA components of P granules have been identified, it is still unclear how they are assembled and segregated into germ cells.

To dissect the assembly mechanism of P granules, we have developed an assay system to test the granule formation ability of each P granule component using cultured mammalian cells. We have found that, among the 14 P granule components examined, only PGL-1 and PGL-3 proteins have the ability to form cytoplasmic granules autonomously in mammalian cells. Interestingly, these granules formed by PGL proteins exhibited a layered architecture and encapsulated endogenous RNA and poly(A) binding protein, and some co-expressed P granule components. A high resolution microscopy revealed that some P granules in *C. elegans* embryos and adult gonads also show a similar layered architecture. When PGL proteins were ectopically expressed in somatic cells in adult worms, they formed cytoplasmic granules, confirming that PGL proteins can form granules in the absence of other germline-specific components. Structure-function analyses of the PGL-3 protein in mammalian cells and *C. elegans* embryos indicated that RGG box, an RNA-binding motif, at the C-terminus of the protein is dispensable for granule formation, but required for recruiting RNA and other P granule components.

Based on these results, we propose that the ability of the PGL proteins to autonomously form RNP granules play a crucial role in the assembly and architecture of P granules in *C. elegans*. We speculate that the layered architecture formed by PGL proteins may be advantageous to sequester specific maternal mRNAs that are to be delivered to and/or stabilized in the germ lineage.

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An EGG complex that is required for the oocyte-to-embryo transition. **Jean M Parry**[1], Nathalie V Velarde[2], Matthew H Zegarek[3], Marina Druzhinina[1], Lindsay K Kelly[1], Ariel J Lefkovith[1], Julie Hang[1], Jon Ohm[1], Barth D Grant[3], Fabio Piano[2], Andrew Singson[1]. 1) Waksman Institute and Department of Genetics, Rutgers University, Piscataway, NJ; 2) Center for Comparative Functional Genomics, Department of Biology, New York University, New York, NY; 3) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ.

Oogenesis produces an egg that once fertilized, must transition to a developing embryo. The molecular underpinnings of this oocyte-to-embryo transition are poorly understood. After fertilization, major events of this transition to a developing embryo typically include the resumption of meiosis, formation of polar bodies, remodeling of the egg surface, rearrangements of the cytoskeleton and the block to polyspermy. These events must also be tightly coordinated with cell cycle progression.We have identified a group of psudo-phosphatases (EGG-3, EGG-4, EGG-5) that define an emerging protein complex that is required for sensing sperm entry and directing many of the events required to convert the egg into a developing embryo. In animals lacking EGG-3 or EGG-4 and EGG-5 function, we observe defects in meiosis, polar body formation, F-actin dynamics, eggshell deposition and/or the block to polyspermy. During oogenesis, EGG-3, EGG-4 and EGG-5 assemble at the oocyte plasma membrane with the egg activation effectors MBK-2 and CHS-1. All of these molecules share a complex interdependence with regards to their subcellular localization during oogenesis. Furthermore, shortly after fertilization, EGG-4 and EGG-5 are required to properly coordinate a redistribution of CHS-1 and EGG-3 away from the plasma membrane at meiotic anaphase I. Therefore EGG-4 and EGG-5 are not only required for critical events of the oocyte-to-embryo transition but also link the dynamics of the regulatory machinery with the advancing cell cycle. Finally, we have identified candidate molecules that could be additional components of this “EGG complex” and we are working to understand how they could be involved in regulating the events of the oocyte-to-embryo transition.

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Males *try-5* to signal sperm activation. **Joseph Smith**, Gillian Stanfield. Dept of Human Genetics, University of Utah, Salt Lake City, UT.

We are interested in how spermatids differentiate into activated spermatozoa capable of directional migration and fertilization of oocytes. Male sperm are stored in the seminal vesicle as immature spermatids and activate after transfer to a hermaphrodite. Prior to transfer, activation is inhibited by *swm-1*, which encodes a protein with a secretion signal and two trypsin inhibitor-like domains. A suppression screen of *swm-1* identified *try-5*, which encodes a trypsin-like serine protease. Whereas *swm-1* males contain prematurely activated sperm, *swm-1 try-5* males contain non-activated sperm. We propose a model in which *try-5* acts as the primary extracellular activating signal for male sperm. This pathway functions in parallel to a second pathway primarily used in hermaphrodites and defined by the *spe-8* group, a group of five genes (*spe-8*, *spe-12*, *spe-19*, *spe-27*, and *spe-29*) necessary for self-sperm activation. Either pathway is sufficient for activation of male or hermaphrodite sperm.

This model makes a number of predictions. First, TRY-5 should act extracellularly. This prediction is supported by the presence of a secretion signal on the putative TRY-5 protein and by *rrf-1* RNAi mosaic experiments, which show that *try-5* is functionally expressed in somatic tissue. Additionally, *try-5* does not suppress the Activated phenotype caused by mutations in *spe-6*; SPE-6 is believed to act as an intracellular brake protein for activation, so a finding that *try-5* does not act downstream of *spe-6* is consistent with an extracellular function. A second prediction is that mating should rescue the defects caused by loss of either the male or hermaphrodite activation pathway. In the case of *spe-8* group hermaphrodites, which are self-sterile, sperm are trans-activated upon mating with a male. Likewise, *swm-1 try-5* males contain non-activated sperm, but are fertile when mated to hermaphrodites. A third prediction is that if both signals are lost, then both males and hermaphrodites should be sterile. Indeed, a *spe-27; swm-1 try-5* strain is both hermaphrodite self-sterile (as expected due to the *spe-8* class *spe-27* mutation) and male-sterile. However, these hermaphrodites are capable of producing self-progeny when mated to wild-type males, indicating that the activation machinery is intact, and the defect is likely in signaling. We are currently testing a fourth prediction of this model: *try-5* should be required for trans-activation. This prediction thus far has been supported. Therefore, our results currently suggest that *try-5* acts as the male sperm activating signal and trans-activating factor.

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DAF-16 regulates prostaglandin signaling and sperm guidance. **Wes Edmonds**[1], Jeevan Prasain[2,3], Dixon Dorand[1], Michael Miller[1]. 1) UAB Dept of Cell Biology; 2) UAB Dept of Pharmacology and Toxicology; 3) UAB Mass Spec Facility, Birmingham, AL.

The eicosanoids, which include prostaglandins (PGs), leukotrienes and thromboxanes, are signaling molecules derived from polyunsaturated fatty acids, or PUFAs. In mammals, eicosanoids are implicated as regulators of inflammation, immunity, reproduction, and central nervous system function. We have shown that *C. elegans* oocytes generate PUFA-derived sperm-attracting signals. An RNAi screen for non-autonomous regulators of sperm guidance identified two predicted PG synthases, K08F4.7 and R11A8.5. Wild-type sperm in K08F4.7 null hermaphrodites have reduced velocity and directional velocity and increased reversal frequency. Indomethacin, an inhibitor of mammalian PG synthesis, causes mild sperm guidance defects. Microinjecting human D, E, and F-series PGs, which are structurally similar, into PUFA-deficient gonads stimulates sperm velocity. These results suggest that PGs mediate sperm guidance.

To identify worm eicosanoids, we developed a profiling method using 27 eicosanoid standards and liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS). Analysis of retention times, parent ions, and collision-induced decomposition patterns from wild-type extracts shows that *C. elegans* produces a wide range of novel PGs, mostly of the F-series. *fat-3* mutants, which are deficient in C20 PUFA production, have decreased, but not completely absent PG levels. Comparative profiling of several targeting-defective mutants suggests that multiple F-series PGs control sperm motility.

Mutation of the delta-12 desaturase *fat-2* causes a major reduction in C18 and C20 PUFAs. However, LC-ESI-MS/MS analysis of *fat-2(wa17)* extracts shows that these mutants generate significant amounts of many PGs. We show that *fat-2* mutants upregulate a compensatory mechanism dependent on the FOXO transcription factor DAF-16, which controls the expression of *fat* genes and numerous other genes implicated in eicosanoid metabolism (Murphy et al. 2003). Loss of *fat-2* causes increased DAF-16 activation, which is reversed by PUFA supplementation. Basal DAF-16 activity in the germ line is critical for correct sperm targeting, yet increased DAF-16 activity, as observed in *daf-2* insulin receptor mutants, *fat-2(wa17)* mutants, and starved animals, causes severe sperm guidance defects. Collectively, we have evidence for two classes of DAF-16-dependent targets, those that mediate the synthesis of eicosanoids important for survival and germline development and those that inhibit the F-series PG function required for sperm guidance.

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Transcriptome profiling of 7 *Pristionchus* species: Horizontal gene transfer, novel gene families and gene turnover. **Christoph Dieterich**[1], Werner Mayer[2], Lisa Schuster[2], Matthias Herrmann[2], Ralf Sommer[2]. 1) Berlin Institute for Medical Systems Biology, Max Delbrück Centre for Molecular Medicine, Berlin, Germany; 2) Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Tuebingen, Germany.

Horizontal gene transfer (HGT), the acquisition of novel genes and gene turnover are of utmost importance for genome evolution. The analysis of these processes requires genome-level studies of related species in a well-established phylogenetic framework. The nematode *Pristionchus pacificus* is a model system in evolutionary biology with genetic and transgenic tools that in nature is found in a necromenic association with scarab beetles. We have isolated and keep more than 160 P. pacificus ecotypes and 24 different Pristionchus species from around the world in the lab. In addition, we have isolated strains from 13 other genera of the family of the Diplogasteridae and their phylogenetic relationship has been revealed. *P. pacificus* constitutes a good starting point for the analysis of HGT. For example, seven members of the glycosyl hydrolase 5 family are found in its genome and cellulase activity has been observed in the supernatant of its cultures. Likewise, thousands of novel protein-coding genes were discovered in the genome sequence, that do not show any sequence similarity to the known protein universe yet are transcribed and cluster into gene families. To study the longevity and evolution of novel genes and those acquired by HGT, we used the 454 next-generation sequencing platform, to index the transcriptomes of 10 species (7 Pristionchus and 3 species of other genera). This approach is a rapid and cost-effective method to study nematode transcriptome complexity and the gene birth/death process within the genus Pristionchus. First, we could verify most of our initial discoveries in the P. pacificus genome sequence. For example, cellulase and diapausin genes are present in Pristionchus, but not in the outgroup species. We assigned HGT events to the most probable source organism and dated them to their respective last common ancestor. The dynamics of gene family evolution (e.g. gene turnover in the Cytochrome P450 family) was assessed in detail. The speed of arrival and departure of truly novel gene inventions could be estimated. We also speculate on the dependency of transcriptome complexity on nematode lifestyle or mode of sexual reproduction.

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Of operons and outrons: a genomic and experimental analysis. **Tom Blumenthal**[1], Jason Morton[1], Mary Ann Allen[1], Peg MacMorris[1], LaDeana Hillier[2], Bob Waterston[2]. 1) Dept Molec, Cellular, Dev Biol, Univ Colorado, Boulder, CO; 2) Dept Genomic Sciences, Univ Washington, Seattle, WA.

An estimated 55% of *C. elegans* genes are trans-spliced by SL1 (Zorio et al. Nature 372:270, 1994). The vast majority of these genes contain an outron, with a promoter just upstream of the trans-splice site, but how far upstream? We present evidence that outron lengths vary widely, but average ~200-250 bp. An estimated 15% of genes are SL2 trans-spliced and these are mostly or entirely downstream in operons. We have analyzed the *C. elegans* transcriptome over development by next generation sequencing (Hillier et al., Genom.Res. March 19, 2009), examining the frequency of SL1 and SL2 trans-splicing across the genome. The results provide evidence for 7,074 SL1- and 2,128 SL2-trans-spliced transcripts, consistent with the earlier estimates based on far less data. We demonstrate that SL1 and SL2 trans-splicing are indeed two separate phenomena, with most gene transcripts trans-spliced to either >95% SL1 or >95% SL2. As expected, SL2 trans-splicing occurs almost entirely at downstream genes in operons. We show that length matters: >50% of intercistronic lengths are ~100 bp, and there is a very strong tendency for downstream genes following this apparently optimal gene spacing to be trans-spliced almost entirely to SL2. Some downstream genes in operons have longer spacing, sometimes much longer. These genes generally are trans-spliced to a mixture of SL1 and SL2, and many or all of these operons contain internal promoters as judged by the presence of the histone variant H2A.Z (Whittle et al., PLOSGenet.4:1,2008), and thus represent what has been termed hybrid operons (Huang et al., Genom.Res.17:1478, 2007). The ratio of SL2 to SL1 usage varies with stage, with a greater frequency of SL1 trans-splicing in L2 larvae, trending to greater SL2 trans-splicing in later stages. A small fraction of SL2 trans-spliced genes are not (apparently) downstream in operons. Our results suggest that some of these actually represent operons with long spacing, whereas others may be due to a non-operon mechanism that results in preferential usage of SL2.

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Germline expression influences operon organization in the C. elegans genome. **Asher D. Cutter**[1], Valerie Reinke[2]. 1) Dept Ecology/Evolutionary Biol, Univ Toronto, Toronto, ON, Canada; 2) Yale University School of Medicine, Yale Univ, New Haven, CT 06520.

Operons are curiously pervasive in the *C. elegans* genome, containing roughly 15% of its encoded genes, more than 95% of which have been preserved in the *C. briggsae* genome over the course of evolution. However, determination of the common forces that promote the origin and/or maintenance of these genic structures has proved elusive. Here we demonstrate that nearly all operon-encoded genes are expressed in germline tissue, based on microarray and large-scale in situ hybridization data. Genes expressed during spermatogenesis, however, are almost completely excluded from operons. We also find that operons cluster non-randomly along chromosomes and that monocistronic genes that are expressed in the germline aggregate near operons. Nevertheless, the molecular functions of operon-encoded genes largely are independent of germline expression. Thus, gene expression in germline tissue is likely to have played an integral role in the evolution of *C. elegans*' operons, and consequently in the evolution of genome organization in this and related species.

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Quantitative screen for alternative splicing regulation factors *in vivo*. **Karine G.M. REBORA**[1], Ilyass ZNIBER[1], Genta OHNO[2], Hidehito KUROYANAGI[2], Laura FONTRODONA[3], Julian CERON[3], Simó SCHWARTZ Jr[4], Denis DUPUY[1]. 1) Institut Europeen de Chimie et Biologie, Pessac, France; 2) School of Biomedical Science, Tokyo Medical and Dental University, Tokyo 113-8510, Japan; 3) Catalan Institute of Oncology, IDIBELL, Gran via s/n, l'Hospitalet de Llobregat 08907, Barcelona, Spain; 4) CIBBIM-Nanomedicine, Vall d'Hebron University Hospital, Passeig Vall d'Hebron 119-129, Barcelona 08035, Spain.

Alternative splicing of pre-mRNAs is a widespread mechanism that participate to spatiotemporal diversity in gene expression in metazoans. It has been estimated that ~10% of *C. elegans* genes are submitted to alternative splicing. To date, there is very limited information about the mechanisms of spatial regulation of alternative splicing during worm development. The aim of this our study is to systematically identify the *trans*-acting elements involved in the alternative splicing regulation. Fluorescent reporters provide the opportunity to observe functional effects of a gene knock-down that may have a subtle phenotype that is undetectable with traditional observations. We are using a transgenic strain carrying a two-color reporter system in which two fluorescent reporters (GFP and RFP) are respectively fused to mutually exclusive alternatively spliced exons (Ohno, et al 2008) of the *let-2* gene (exons 9 and 10). Expression of the constructions is driven under the control of the body wall muscles specific *myo-3* promoter. This strain has been subjected to a genome-wide RNAi screen for modifiers of the alternative splicing ratio. While previous RNAi screens have mostly consisted of visual observation of the worms by microscopy, we use the COPAS-Profiler to perform multidimensional quantitative analysis on the knocked-down worm populations in 96-well format. This enables us to detect not only variation in growth and fertility but also any modification of the relative expression of the two reporters i.e. modification of the alternative splicing balance. In this set up we are able to measure, for each individual RNAi experiment, the total number of worms, their sizes distribution, as well as collect longitudinal expression profiles in two fluorescent channels. Importantly, we are able to compare expression patterns within individual animals, thus providing an endogenous control for stochastic variations in overall expression level. We then plan to extend this study to the regulation of the alternative splicing of some more genes, by performing the same screen after generating similar dual color constructions. This will allow us to distinguish between general splicing regulators and specific ones and provide the first large-scale overview of alternative splicing regulation in vivo in *C. elegans*..

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Genomic Strategies to Map the *C. elegans* Transcriptome. **William Clay Spencer**[1], Joseph Watson[3], Rebecca McWhirter[1], Kathie Watkins[1], Ashish Agarwal[2], Mark Gerstein[2], Shenglong Wang[4], Nurith Kern[4], David Miller III[1]. 1) Dept Cell & Developmental Biol, Vanderbilt Univ Sch Medicine, Nashville, TN; 2) Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT; 3) Program in Molecular Biology and Biotechnology, UNC Chapel Hill, Chapel Hill, NC; 4) NuGEN Technologies, Inc. San Carlos, CA.

The *C. elegans* genome is completely sequenced yet many predicted genes lack biological evidence for transcription. Additionally, a substantial number of cryptic protein-coding genes and ncRNAs (miRNAs, snoRNAs, etc.) are likely to have been overlooked by gene prediction software. To identify these transcripts, we are isolating RNA from specific *C. elegans* cells and tissues for tiling array analysis and for high throughput cDNA sequencing (RNA-Seq). This approach ensures detection of rare RNAs from small populations of cells while also providing clues to their in vivo functions. Our data sets will be merged with complementary results from other laboratories in the modENCODE consortium (modENCODE.org) to provide a detailed picture of the *C. elegans* transcriptome. We use specific promoters to mark cells for isolation by FACS or for mRNA extraction by the mRNA tagging method. The small amount of RNA obtained by these methods (<25 ng) is amplified to generate a labeled ds cDNA target for hybridization to the Affymetrix *C. elegans* Tiling 1.0R array. To date, we have generated tiling array profiles of >20 different cells and tissues including neurons, muscle, intestine, hypodermis, excretory cell, coelomocytes, etc. Threshold analysis detects transcripts from established gene models as well as from candidate transcriptionally active regions (TARs) in intergenic and intronic domains. Biased detection of known tissue and cell-specific transcripts validates these data sets and suggests that other differentially expressed TARs may exercise cell-specific functions. In addition to detecting novel transcripts, our approach is expected to produce gene expression maps that match the single cell resolution of the *C. elegans* anatomy.

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Comparative Functional Analysis of the *Caenorhabditis elegans* and *Drosophila melanogaster* Proteomes. Sabine Schrimpf[1,2], Manuel Weiss[1,2,3], Lukas Reiter[1,2,3,4], Christian H. Ahrens[2,5], Marko Jovanovic[1,2,3], Johan Malstroem[4], Erich Brunner[2], Sonali Mohanti[2,4], Martin J. Lercher[6], Peter E. Hunziker[5], Ruedi Aebersold[4,7], Christian von Mering[1,2,8], **Michael O. Hengartner[1,2,9]**. 1) Institute of Molecular Biology, University of Zurich, Zurich, Switzerland; 2) Center for Model Organism Proteomes, University of Zurich, Zurich, Switzerland; 3) PhD Program in Molecular Life Sciences, University of Zurich, Zurich, Switzerland; 4) Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland; 5) Functional Genomics Center, University of Zurich and ETH, Zurich, Switzerland; 6) Institute of Informatics, University of Duesseldorf, Duesseldorf, Germany; 7) Institute for Systems Biology, Seattle, Washington, USA; 8) Swiss Institute of Bioinformatics, University of Zurich, Zurich, Switzerland; 9) michael.hengartner@molbio.uzh.ch.

The nematode *Caenorhabditis elegans* is a popular model system in genetics, not least because a majority of human disease genes are conserved in *C. elegans*. To generate a comprehensive inventory of its expressed proteome, we performed extensive shotgun proteomics and identified more than half of all predicted *C. elegans* proteins. This allowed us to confirm and extend genome annotations, characterize the role of operons in *C. elegans* gene expression, and semi-quantitatively infer abundance levels for thousands of proteins. Furthermore, for the first time to our knowledge, we were able to compare two animal proteomes (*C. elegans* and *Drosophila melanogaster*). We found that the abundances of orthologous proteins in these two metazoans correlate remarkably well, better than transcript abundances across the two organisms, or even than protein abundance versus transcript abundance within each organism. These observations suggest that the key evolutionary pressure is exercised at the level of protein abundance, and that significant flexibility is allowed in how this final abundance is achieved. Thus, changes in transcript levels may for example be offset during evolution by opposing changes at other steps in gene expression, such as translation efficiency or protein stability.

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The landscape of *C.elegans* 3'UTRs. **Marco Mangone**[1], Arun Prasad Manoharan[2], Han Ting[2], Oliver Attie[1], Emily Mis[1], Philip MacMenamin[1], Charles Zegar[1], Vishal Khivansara[2], Kevin Chen[1,3], Wei Chen[3], Nikolaus Rajewsky[3], Kourosh Salehi-Ashtiani[4], Marc Vidal[4], Yuji Kohara[6], Jean Thierry-Mieg[5], Danielle Thierry-Mieg[5], Fabio Piano[1], John Kim[2], Kristin Gunsalus[1]. 1) Center for Genomics and Systems Biology, Department of Biology, New York University, 1009 Silver Center, New York, USA; 2) University of Michigan, Department of Human Genetics, 6183A LSI210 Washtenaw Ave.Ann Arbor, MI 48109 -2216; 3) Max Delbrück Centrum für Molekulare Medizin, Robe Rössle-Str. 10, Berlin-Buch, 13092 Berlin, Germany; 4) Center for Cancer Systems Biology (CCSB), Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Genetics, Harvard Medical School, Boston, MA; 5) National Center for Biotechnology Information (NCBI). NIH Bldg 38A8600 Rockville PikeBethesda, MD 20894; 6) National Institute of Genetics, 411-8540 Yata 1111, Mishima, Shizuoka 411-8540, JAPAN.

Three-prime untranslated regions (3'UTRs) contain sequence elements used by RNA-binding proteins and regulatory RNAs such as microRNAs (miRNAs) to influence mRNA stability, translation and localization. However, the annotation of these regions within transcripts is generally incomplete. In *C.elegans*, which has a well annotated genome, about half of the ~20,000 curated genes in WS190 are not annotated with any 3'UTR and less than 10% are annotated with multiple or alternative 3'UTR isoforms. As part of the modENCODE project, we have developed a pipeline targeting ~7,000 genes using 3' RACE and found at least one 3'UTR for around 90% of this targeted set. To analyze these data we have used different sequencing technologies that resulted in the identification of multiple distinct 3'UTR isoforms for over half of the targeted genes. In addition to 3'RACE, we have developed a technique to capture polyA ends followed by deep sequencing. This polyA-capture approach has resulted in over 2,000,000 polyA tags that map to ~12,000 genes across all major developmental stages and males, with the majority of these sequences mapping to full-length 3'UTRs. The depth of these data shows the remarkable complexity in the distribution of polyadenylation events in vivo. We have also manually curated 3'UTR boundaries using all available cDNAs, derived mostly from the 200,000 staged ESTs, and obtained 3'UTRs boundaries for ~11,500 genes. For about half of these (~5,000 genes), the data connect 3'UTRs to specific transcripts with known trans-spliced leader sequences at the 5' end. Combining the results of these analyses has increased our knowledge of 3'UTR structures significantly, identifying novel 3'UTR isoforms for about half of all *C.elegans* protein-coding genes.

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A Genetic Interaction Map of Early Embryogenesis in *C. elegans*. **Patricia Giselle Cipriani**, Huey-Ling Kao, Amelia White, Kristin Gunsalus, Fabio Piano. Center for Genomics and Systems Biology, Biology Dept, New York University, New York, NY.

Close to 1,000 genes have been identified as necessary for proper early embryonic processes in *C. elegans*. Yet many more genes are known to be expressed that could contribute to these processes. To uncover the structure of the underlying genetic networks during early embryogenesis and to identify new components and pathways that participate in these key events, we set up a system to systematically test for enhancing and suppressing interactions using RNAi. We have used 24 available temperature sensitive *(ts)* alleles whose strong loss-of-function phenotype affects the early embryo. To select the genes to test against these mutants, we used a bioinformatics approach to enrich for genes that are likely to play a role in early embryogenesis, but have not been identified by feeding RNAi screens. In addition, we enriched our list for homologs of human disease genes. Overall, we have RNAi-tested an average of 500 genes against each *ts* allele. We have acquired and analyzed over 100,000 RNAi experiments and identified over 560 high confidence genetic interactions. On average, we found ~18 enhancers and ~5 suppressors per essential gene. The combined genetic network connects all the tested essential genes through at least one non-essential gene. Analyzing each set of interactions separately, the network of suppressor interactions is less connected than the enhancer network, in addition to the lower number of interactions overall. Interestingly, many of our positive interaction pairs include at least one human disease gene ortholog that had not been known to work in the embryo before, opening new ways to study these genes. To investigate the interaction network more closely, we used the network navigation tool N-Browse to help navigate these data and integrate them with other available functional data. These analyses revealed that most of the observed genetic interactions we identified were not previously predicted or known and, as expected, they do not overlap with the available protein-protein interaction data. The preliminary analysis of our data has revealed new functions of previously uncharacterized genes, uncovered pathways previously not known to operate in the embryo, and shown connections between pathways that are potentially buffering each other. Some of these new pathways implicate functions such as RNA turnover as potential phenotypic capacitors in the early embryo. In addition, we see peroxisome biogenesis as a critical function not previously identified in early embryogenesis. We have confirmed some of our results from high-throughput screening by detailed phenotypic analysis and quantification, and we have also made substantial progress on the automatic scoring of the images produced.

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An Intricate Network of Nuclear Hormone Receptors Regulates Energy Balance in the Nematode *C. elegans*. **H Efsun Arda**[1], Stefan Taubert[2], Colin Conine[1], Marc van Gilst[3], Reynaldo Sequerra[4], Lynn Doucette-Stamm[4], Keith Yamamoto[5], Albertha JM Walhout[1]. 1) Gene Function and Expression, Univ Massachusetts Med Sch, Worcester, MA; 2) Department of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 3) Fred Hutchinson Cancer Research Center, Basic Sciences Division Seattle, WA, USA; 4) Agencourt Bioscience Corporation, Beverly, MA, USA; 5) Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA, USA.

Obesity is a growing epidemic and results from an imbalanced energy homeostasis. Most metabolic genes are transcriptionally regulated, therefore deciphering the complex regulatory networks that govern energy homeostasis is crucial to understand the mechanisms underlying obesity. The nematode *C. elegans* is a powerful model organism to study metabolic transcriptional regulatory networks. Already ~400 genes have been identified that alter the body fat of the worm when inactivated, and an additional ~25 genes were shown to give transcriptional response upon fasting. In order to elucidate the transcriptional regulation of these "fat" and "fasting" genes, we mapped a transcription regulatory network using high-throughput, gene-centered yeast one-hybrid assays. This network contains numerous nuclear hormone receptor (NHR) type transcription factors, which are involved in fat metabolism throughout the Metazoa. We found that most of these previously uncharacterized NHRs partition into different modules based on the targets they share in the network. We show that several of them are necessary to maintain normal levels of body fat. Finally, we also found that many of the transcription factors identified in the fat network can interact with a mediator subunit, MDT-15, which is required for the regulation of fat metabolism both in *C. elegans* and mammalian systems. Taken together, our results provide an integrated network of transcriptional regulation of energy balance of *C. elegans*.

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Temperature and light-entrained circadian rhythms in *C. elegans*. **Alexander M. van der Linden**[1,2], Sebastian Kadener[1], Michael Rosbash[1], Piali Sengupta[1]. 1) Dept Biol. and National Center for Behavioral Genomics, Brandeis Univ, Waltham, MA (slinden@brandeis.edu); 2) Dept Biol., University of Nevada, Reno, NV (from 9/09).

Circadian control of feeding is critical for animals to properly adapt to their environment, and for optimal development. However, the nature of this interaction and its relationship to metabolism is poorly understood. *C. elegans* has proved to be an extremely powerful model for understanding the molecular basis of feeding behavior, but it is unknown whether *C. elegans* feeding is under circadian control. Although circadian locomotory rhythms were described in *C. elegans* previously (1), these observations have been difficult to replicate, and no rhythmically expressed clock or clock-output genes in *C. elegans* have been identified. We set out to not only investigate the yet unidentified circadian clock in *C. elegans*, but to also explore the interaction between circadian clocks, metabolism and feeding behaviors. Since circadian transcripts exhibit rhythmic oscillations, genome-wide expression profiling experiments are a powerful approach to identify clock and clock-output genes. We used microarrays and a series of data analysis algorithms to identify circadian-regulated genes under photo- and thermocycling conditions in *C. elegans*. We identified subsets of candidate clock-output genes that exhibit robust rhythmic expression with 24 hr periodicity under either light-dark or warm-cold conditions, as well as in constant dark-dark or cold-cold conditions following entrainment. Our results indicate that thermocycles directly evoke a global expression response broader than photocycles, and that a subset of genes undergo cycling in a circadian manner. Verification of microarray data via qRT-PCR suggests that these genes may represent *bona fide* cycling genes. These sets include molecules predicted to regulate metabolic processes, suggesting that feeding–an activity closely related to energy metabolism–may be under circadian control. Preliminary results suggest that *tax-2*, which is important for thermo- and phototaxis (2) is necessary for the cycling of these genes. We have also developed a reporter assay system to monitor rhythmic circadian gene expression, and we are planning to use this system in genetic screens to define the core clock components. Moreover, we are determining whether feeding behavior is under circadian control, and whether food signals can entrain the clock. Since it is likely that the molecular components of the *C. elegans* clock are distinct from those of other organisms, this work is also expected to provide information regarding the evolution of circadian clocks. 1) Saigusa et al, 2002; Simonetta & Golombek, 2007; 2) Coburn et al, 1996; Ward et al, 2008.

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Searching for signaling balance through the identification of genetic interactors of the Rab Guanine-nucleotide Dissociation Inhibitor, *gdi-1* in *Caenorhabditis elegans*. **Anna Y-W. Lee**[1], Richard Perreault[2], Sharon Harel[2], Elodie Boulier[2], Matthew Suderman[1], Michael Hallett[1], Sarah Jenna[2]. 1) McGill Centre for Bioinformatics, McGill University, Montréal, Québec, Canada; 2) Department of Chemistry, PharmaQÀM, Biomed, Université du Québec Montréal (UQÀM), Montréal, Québec, Canada.

Many biological mechanisms depend on a state of signaling homeostasis maintained by the appropriate integration of synergistic and antagonistic signaling pathways. Accordingly, the symptoms of numerous diseases result from genetic mutations that disrupt this homeostasis. The relationships between signaling pathways suggest avenues through which homeostasis can be restored and disease symptoms subsequently alleviated. Specifically, disruptions caused by a loss-of-function mutation in a particular pathway may be compensated by concomitant perturbations in an antagonistic pathway. We use here a prediction-based approach to identify genetic interactions that allow such pathway relationships to be mapped. In particular, we identified genetic interactors for the Rab-specific guanine-nucleotide dissociation inhibitor *gdi-1* in the nematode *Caenorhabditis elegans*. *gdi-1* is a close homolog of *GDI1*, a gene encoding a major cell signaling protein associated with non-syndromic forms of mental retardation in human (αGDI). Our genome-wide approach predicts over 800,000 novel genetic interactions in *C. elegans* with an estimated accuracy ranging from 42% to 67%. Our novel methodology of data integration using the guilt-by-association principle is likely responsible for the improved genome coverage and accuracy of the predicted interactions when compared to previous studies. By validating a subset of interactions predicted for *gdi-1*, we confirmed five novel genetic interactors for this gene. More specifically, we demonstrated that *gdi-1*activity is antagonistic with phosphorylated myosin-light chain and *dyb-1* activities, during gonad morphogenesis and ovulation in *C. elegans*. *dyb-1* encodes the *C. elegans* ortholog of dystrobrevin, a component of the dystroglycan complex associated with Duchenne Muscular Dystrophy (DMD). We showed that *gdi-1* antagonism with *dyb-1* is conserved at neuromuscular junctions, where *gdi-1(RNAi)* suppresses *dyb-1*-associated muscle degeneration. Our data suggest that *GDI1* would be a potent therapeutic target for DMD. In addition, it is of interest to investigate whether *dyb-1* would be a potent therapeutic target for cognitive disabilities associated with mutations in *GDI1*. In conclusion, we have shown that our methodology has the potential to streamline the therapy development process for monogenic disorders that involve genes and signaling pathways conserved between human and *C. elegans*.

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Rapid high resolution SNP-CGH mapping in *C. elegans*. **Stephane Flibotte**[1], Mark Edgley[2], Jon Taylor[2], Iasha Chaudhry[2], Robert Waterston[3], Donald Moerman[4]. 1) Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, B.C., Canada, V5Z 4S6; 2) Michael Smith Laboratories, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4; 3) Department of Genome Sciences, University of Washington, Seattle. WA, USA, 98195-7730; 4) Department of Zoology, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4.

Recently we published a manuscript on a significantly improved and simplified method for high-resolution mapping of phenotypic traits in *Caenorhabditis elegans* using a combination of Single Nucleotide Polymorphisms (SNPs) and oligonucleotide array Comparative Genome Hybridization (array CGH) (Genetics 181:33-37 2009). We designed a custom oligonucleotide array using a subset of confirmed SNPs between the canonical wild-type Bristol strain N2 and the Hawaiian isolate CB4856, populated with densely overlapping 50-mer probes corresponding to both N2 and CB4856 SNP sequences. SNPs were selected from the published set in WormBase and expanded using new SNPs identified from the whole-genome sequence of CB4856. Using this method a mutation can be mapped to a resolution of about 200 kb in a single genetic cross for a few hundred dollars in reagent costs. Six mutations representing each of the *C. elegans* chromosomes were detected unambiguously and at high resolution using genomic DNA from populations derived from as few as 100 homozygous mutant segregants of mutant N2/CB4856 heterozygotes.

We will discuss refinements we have made in microarray design, data analysis procedures and animal husbandry since the publication of the original paper. For example, we have evaluated the effects of varying the oligonucleotide length on data quality. The oligonucleotide length was varied between 35 and 50 and as expected the SNP signal tends to be larger but noisier for shorter oligonucleotides, which translates into only small differences in mapping accuracy and we are therefore still recommending our usual length of 50. Furthermore, as expected the magnitude of the signal differs slightly between various transition/transversion types and it can be useful take such information into account both at the design and analysis stages. Our current chip design interrogates nearly 13,000 SNPs scattered across the genome with an average of about thirty 50-mer probes per SNP. Our mapping method will be particularly powerful when applied to difficult or hard to map low penetrance phenotypes. It should also be possible to map polygenic traits using this method. We will illustrate the method with several examples.

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A New Role for the Anchor Cell in Vulval Development: Initiating Dorsal Lumen Formation. **Kathleen Estes**[1], Wendy Hanna-Rose[2]. 1) University of California, San Diego, La Jolla, CA; 2) The Pennsylvania State University, University Park, PA.

Tubulogenesis, the creation of tubular organs during development, is a complicated process involving multiple developmental events including fate specification, cell shape changes and cell-cell interactions. Little is known about initiation of tubulogenesis, particularly how a lumen is established and how two developing tubes connect to form a network. We use the *C. elegans* vulva and its connection with the uterus to study these complex events. The gonadal anchor cell (AC) functions as the signaling center that induces vulval development, a role that has been well studied. Here we demonstrate that the AC also promotes initiation of lumen formation of the dorsal vulva. In the absence of proper invasion and penetration of the dorsal vulval cells by the AC, the dorsal lumen of the vulva is absent. Using mutants that exhibit aberrant AC invasion of the dorsal vulva, including *fos-1*, *unc-6* netrin and *unc-40* netrin receptor, we found that dorsal vulval tubulogenesis is incomplete when the AC fails to invade the vulva. Interestingly, the ventral vulva develops normally in these mutants, suggesting the mechanisms for lumen formation differ between the ventral and dorsal vulva. By examining fate specification and polarity of the dorsal vulval cells, we determined that neither fate specification defects nor polarity defects can account for the aberrant vulF shape. We hypothesize that the absence of a dorsal vulval lumen in these AC invasion mutants is due to lack of the physical presence of the AC within the dorsal vulva as those cells are dividing and undergoing morphogenesis. This demonstrates yet another role of the anchor cell in vulval tubulogenesis and a new mechanism of lumen formation in development.

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LET-4 is a transmembrane protein required for *C. elegans* excretory system development. **Vincent P. Mancuso**, Meera Sundaram. Dept Genetics, Univ Pennsylvania, Philadelphia, PA.

Tubes are required in organisms for a variety of functions, including ingestion and excretion, gas exchange, and transportation of nutrients. Many tubes are multicellular, but the simplest are unicellular. For a single cell to form a tube requires a few achievements: adopting the correct fate, correct positioning and overall morphology of the cell, formation and elongation of a hollow interior lumen, and connection of the ends of the tube to other cells. To address the most basic questions regarding the formation and maintenance of tubes, we study the *C. elegans* excretory system. This 'primitive renal system' includes three tandem unicellular tubes: the excretory canal cell, the duct cell, and the pore cell. Fluid is passed from the canal cell to the duct cell and then pore cell through a continuous lumen.

To better understand the development of the excretory system, we are investigating the role of *let-4*, which causes an excretory system defect. The previously uncloned mutant *let-4(mn105)* is a recessive zygotic-effect mutation with a >99% penetrant lethal excretory system defect. *let-4* mutants begin to accumulate fluid in the canal cell just before hatching, and die as fluid-filled L1s. We have cloned *let-4*, and found that it encodes a predicted transmembrane protein with an extracellular leucine-rich repeat(LRR) domain and a conserved intracellular domain.

We generated transcriptional and translational reporters to determine where *let-4* is expressed. *let-4* is expressed in the canal cell, duct cell, and pore cell, as well as in epithelial cells of the vulva, rectum, and hypodermis. A functional LET-4::GFP fusion protein localizes to the apical side of the hypodermal, vulval and rectal cells. The protein is present in the canal cell, duct cell, and pore cell, but apical localization hasn't yet been confirmed in these cells.

We hypothesize that *let-4* mutants have a defect in excretory system lumen connectivity. Our phenotypic characterization so far suggests that all three unicellular tubes of the excretory system are present and properly positioned; however, fluid does not appear to pass from the canal cell through the duct and pore. This phenotype is similar to that seen in *lpr-1* mutants (see abstract from Stone, Hall and Sundaram). To visualize the lumen, we are performing transmission electron microscopy analysis in collaboration with David Hall. We are also performing immunohistochemistry to label the lumen of the duct cell and pore cell in *let-4* mutants, and to test for possible mislocalization of apical cytoskeletal factors.

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daf-6/Patched, *che-14*/Dispatched and Wnt Components Interact To Direct Amphid Lumen Morphogenesis. **G. Oikonomou**, E. Perens, Y. Lu, S. Shaham. The Rockefeller University, New York, NY.

Sensory structures often consist of neurons that extend their dendrites through a lumen formed by ensheathing epithelia or glia. We have been studying the development of the C. elegans amphid as a model system for sensory organ morphogenesis.

Previously, we showed that *daf-6* is required for lumen formation by the amphid glia. In *daf-6* mutants, a lumen is not generated and the ciliated sensory dendrites become trapped in large vacuoles within the glia. *daf-6* encodes a Patched-related protein that localizes to the luminal surfaces of the amphid channel as well as other tubes, including the excretory cell, vulva and intestine. Whereas *daf-6* animals are defective only in amphid lumen morphogenesis, animals mutant for both *daf-6* and the Dispatched gene *che-14* exhibit defects in many tubular structures. Furthermore, in mutants with defective sensory cilia, DAF-6 localization in the amphid is also disrupted and the amphid channel is malformed. Thus, lumen formation in the amphid may be orchestrated by neuronal cues that regulate the localization and function of DAF-6 and CHE-14.

To further elucidate the role of *daf-6* in amphid lumen morphogenesis, we performed a genetic screen for suppressors of the dye-filling defect of *daf-6* animals. One of these suppressors is an allele of the NLK/MAPK homologue *lit-1*. *lit-1* is part of a MAPK module involved in the regulation of non-canonical Wnt signaling pathways that govern various cell fate decisions; however, mutations in *lit-1* do not affect glial cell specification. Other components of this MAPK module, including *wrm-1*/beta-catenin and *mom-4*/MAP3K, also suppress *daf-6*. *lit-1* is expressed in many cells, including amphid glia, vulval cells and glial cells of other head sensilla. Heat shock studies and serial section EM of *daf-6* embryos revealed that *daf-6* functions during amphid channel formation. Consistent with this, expression of the *lit-1* cDNA by an embryonic amphid glia promoter rescues the suppression of *daf-6*. A rescuing GFP::LIT-1 fusion protein expressed in glia shows nuclear and reticular localization, but surprisingly also lines the amphid channel, suggesting a direct role for LIT-1 in tubulogenesis. Interestingly, *lit-1* enhances the amphid and vulval defects of *che-14* mutants, consistent with a model in which opposing activities of *daf-6* and *che-14* impinge on *lit-1* to promote optimal *lit-1* activity.

Our results suggest a novel interaction between Patched, Dispatched and components of the Wnt pathway in directing lumen formation in the amphid sensillum.

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Up- and down-regulation of TOR-Raptor and TOR-Rictor complexes by semaphorin in *C. elegans*. **Akira Nukazuka**[1,2], Shusaku Tamaki[2], Hajime Fujisawa[2], Yoichi Oda[2], Shin Takagi[2]. 1) National Institute for Basic Biology, Okazaki, Japan; 2) Div Biological Sci, Nagoya Univ, Nagoya, Japan.

Semaphorins regulate a wide range of developmental events. In *C. elegans*, the signal controls morphogenesis of epidermal cells, as shown by the finding that semaphorin mutants display aberrant arrangement of epidermis-derived tissues, rays. To gain insight into semaphorin signaling, we conducted a screen and isolated a loss-of-function mutation in Rictor, which suppressed the ray phenotype in semaphorin mutants.

Rictor is known to make a complex TORC2 with TOR. Independently of TORC2, TOR also makes a complex TORC1 with Raptor. Signaling via TORC1 promotes mRNA translation, whereas TORC2 stabilizes F-actin formation. Consistently with previous reports on an antagonistic relationship between two TORC pathways, we found that Raptor overexpression, similarly to Rictor deficiency, suppressed the ray phenotype in semaphorin mutants. Conversely, TORC1 inhibition and TORC2 activation phenocopied semaphorin mutants. Thus, our data indicate that TORC1 cooperates while TORC2 antagonizes with semaphorin signaling. In addition, knockdown of 4EBP, which is a TORC1 substrate and a translation repressor, suppressed the semaphorin mutant phenotype, suggesting that TORC1 mediates the signal by repressing 4EBP and thus activating translation. Likewise, deficiency of PKCα, which is a TORC2 substrate, also suppressed the mutant phenotype, implicating that TORC2 inhibition, which probably results in actin destabilization, also mediates the signal. Therefore, our data imply that TOR serves to integrate the semaphorin input and subsequently transduce it into divergent downstream cascades.

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Identification of an essential component of the subapical intermediate filament anchorage machinery in *C. elegans*. **O. Bossinger**[1,2], K. Hüsken[1], T. Wiesenfahrt[1,2], H. Gerhardus[1,2], F. Geisler[1,2], D. Ueberbach[2], R. Leube[1]. 1) Institute of Molecular and Cellular Anatomy, RWTH Aachen University, D-52074 Aachen, Germany; 2) Institute of Genetics, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany.

The mechanically resilient endotube surrounds the intestinal lumen of nematodes. It is characterized by a prominent electron-dense filamentous meshwork which is connected to the circumferential apical junction and contains abundant intermediate filaments (Karabinos et al., 2001; Bossinger et al., 2004; Hüsken et al., 2008). To identify factors that are responsible for the formation and positioning of this exquisite structure IFB-2::CFP reporter strains were subjected to chemical mutagenesis. Among the resulting mutants we found two strains in which the IFB-2::CFP-labelled subapical intermediate filament network was completely absent, except for the apical junction that was strongly labelled. Large juxtajunctional aggregates around the apical junction were observed by electron microscopy which also revealed loss of the electron-dense meshwork. By combined snp-mapping and RNAi-analyses the mutation was localized in both instances to a single gene that we refer to as ifo-1 (intermediate filament organizer). Remarkably, ifo-1 is transcribed exclusively in the intestine and IFO-1 is restricted to the endotube where it co-localizes with IFB-2, IFC-2 and F-actin. Taken together, IFO-1 qualifies as an essential determinant of polarized intermediate filament network formation in *C. elegans*.

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The Conserved Glc7/PP1 Phosphatases GSP-3 and GSP-4 Regulate Sperm Morphogenesis. **Aiza Go**, Jui-ching Wu, Mark Samson, Hongkang Zhou, Susan Mirsoian, Diana Chu. Biol, San Francisco State Univ, San Francisco, CA.

Sperm have evolved widely distinct modes of motility. Mammalian sperm are flagellar and rely on actin for cytoskeletal function, whereas *C. elegans* sperm are ameboid and utilize MSP for motility. Nevertheless, they share developmental events important for sperm function, like meiosis and the tight compaction of DNA that results in transcriptional inactivity. Thus, sperm rely heavily on post-translational regulation for morphogenesis into migration of competent cells. Interestingly, Glc7/PP1 phosphatases are required for male fertility in mouse, with roles in both sperm meiosis and sperm morphogenesis. We identified two Glc7/PP1 phosphatases, GSP-3 and GSP-4 that are 98% identical and function redundantly in hermaphrodite and male fertility. Cytological and biochemical analyses have confirmed that GSP-3 and GSP-4 are sperm specific. Though we have identified defects in chromosome segregation in *gsp-3gsp-4* mutants, we also investigated the role of these proteins in sperm morphogenesis. Our studies revealed that the sterility of *gsp-3 gsp-4* mutants is indeed caused by a failure of sperm morphogenesis and activation. First, in sterile *gsp-3gsp-4* hermaphrodites, immotile hermaphrodite sperm block the spermatheca and prevent exogenous sperm from accessing the oocyte. However, when *gsp-3gsp-4* hermaphrodites are manipulated to not produce sperm, they could be fully fertilized by wild type male sperm. Furthermore, we examined the morphology of *gsp-3gsp-4* mutant male sperm and found they exhibit abnormal sizes and shapes. The *gsp-3gsp-4* sperm also show severe defects in morphogenesis. Instead of growing pseudopods on one side of the cell, random protrusions form all over *gsp-3gsp-4* sperm. Upon artificial activation, these protrusions can undergo such extreme extension that sperm occasionally explode. Thus GSP-3 and GSP-4 play an important role in regulating pseudopod formation and sperm motility. Further supporting this, we found that GSP-3 and GSP-4 localize as a crescent shape on the periphery of mature but inactive sperm, reminiscent of the asymmetric localization of the pseudopod. In activated sperm, GSP-3 and GSP-4 then shifts to colocalize with MSP only in pseudopods. Our current model is that GSP-3 and GSP-4 regulates sperm activation and pseudopod formation through modulating MSP dynamics. We are now conducting coimmunoprecipitation experiments to find interacting partners of GSP-3 and GSP-4 that target them to specific subcellular domains to function in either sperm meiosis or morphogenesis. Our studies will provide insight on how these evolutionarily conserved PP1 phosphatases are adapted as a core regulator for sperm formation and activity by otherwise morphologically different sperm.

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The role of Glycogen Debranching Enzyme during *C. elegans* development and aging. **Jeffrey S. Simske**. Rammelkamp Ctr, Cleveland, OH.

In a screen for enclosure defective mutants, the partially penetrant, maternal-effect cold-sensitive allele *jc11* was identified. 82 and 19 percent of *jc11* animals display mutant phenotypes at 15 and 25 degrees, respectively, and a single maternal or zygotic copy of wild type *jc11* is sufficient for gene function. Using 4D microscopy *jc11* embryos are observed to arrest at various developmental stages including pre-morphogenesis, enclosure, and elongation. Animals that successfully complete elongation often have morphogenetic defects including tail and snout abnormalities. *jc11* was genetically inseparable from the *lin-7* allele used in the genetic screen and a minimal genomic region containing the neighboring gene R06A4.8 was sufficient for the rescue of *jc11* phenotypes. RNAi against R06A4.8 phenocopies *jc11*. R06A4.8 is predicted to encode glycogen debranching enzyme (*agl-1*). The R06A4.8 locus in *jc11* animals was completely sequenced and a four base pair deletion was found in the c-terminus of *agl-1*. This deletion is predicted to result in a frame shift and early termination, resulting in the truncation the final 253 residues of the protein, adding 22 extraneous residues prior to a stop codon. The *jc11* mutation is predicted to eliminate the glycogen binding domain of debranching enzyme. *jc11* was also phenocopied by treatment with MOR-14 (N-Methyl-1-Deoxynojirimycin), an inhibtor that specifically targets the α-1,6-glucosidase activity of glycogen-debranching enzyme. Overexpression of AGL-1::GFP results in increased debranching activity based on reduced glycogen staining with carminic acid in both embryos and adults expressing AGL-1::GFP. Recently, debranching enzyme was shown to interact with the β1 subunit of the energy sensor AMP-activated kinase, while binding of AMPK β1 directly to glycogen depends on the α-1,6-branch; AMPK binding to glycogen branches in turn functions to inhibit AMPK activity [1, 2]. Loss of *agl-1* function in *C. elegans* is predicted to result in the formation of a 'limit dextrin' glycogen molecule in which outer glucose chains are digested by phosphorylase to within four glucose residues of the α-1-6 branch, resulting in a glycogen molecule likely to bind to and inhibit AMPK activity. The prediction that AMPK activity is inhibited in *agl-1* mutants is supported by initial experiments indicating that *jc11* animals surviving to adulthood have shorter life spans, similar to animals lacking AMPK activity. The role of glycogen debranching enzyme in the integration of energy and lifespan signals via AMPK is being further investigated. 1.Sakoda, H., et al.,. Am J Physiol Endocrinol Metab, 2005. 289(3): p. E474-81. 2.McBride, A., et al., Cell Metab, 2009. 9(1): p. 23-34.

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Investigating the role of PAR-3 in *C. elegans* embryonic epithelial cells. **Annita Achilleos**, Jeremy Nance. Skirball Institute, NYU Medical Center, New York, NY.

Epithelial cells are required for animal development, barrier formation and nutrient uptake. These cells are highly polarized along their apical-basal axis, and form junctions near their apical surfaces that provide adhesion with neighboring epithelial cells. The PAR proteins PAR-6, PAR-3, and PKC-3 are required for junction formation and polarization of mammalian and fly epithelial cells. We showed previously that PAR-6 is required for maturation of junctions but not for polarization of *C. elegans* embryonic epithelial cells. Depleting PAR-3 by RNAi results in defects in the polarity of a subset of larval epithelial cells (Aono et al., 2004), suggesting that PAR-3 might have an earlier role than PAR-6 in polarizing epithelial cells. To determine if PAR-3 is required at the initial stages of epithelial cell polarization, we used a combination of transgenes and a putative null mutation in *par-3* to remove both maternal and zygotic PAR-3 from the embryo before epithelial cells formed. Mutant embryos arrested during elongation and developed lesions that exposed internal cells. Intestinal and other internal epithelial cells that formed in the absence of PAR-3 had disrupted polarity, and junction proteins that normally accumulate at the apical surface (DLG-1, HMR-1) showed a dispersed and often clustered localization. Surprisingly, polarization and junction formation appeared superficially normal in epidermal epithelial cells lacking PAR-3. These findings highlight the different roles that PAR proteins play in regulating epithelial cell polarization and junction formation, and indicate that internal and external epithelial cells utilize at least partially different mechanisms to develop apico-basal polarity.

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Circumferential Actin and Elongation. **Christopher Allen Vanneste**, Paul Mains. Department of Biochemistry and Molecular Biology, University of Calgary. Calgary, Alberta.

Our work is focused on *C. elegans* embryonic elongation, which involves a smooth muscle like, actin-mediated contraction within the lateral epidermal (seam) cells. This contraction results in the embryo undergoing the four-fold lengthening into the vermiform worm shape without an increase in cell size or number. Our lab has previously shown that elongation involves the contractile cassette of *let-502* (Rho-binding kinase), *mel-11* (myosin phosphatase) and *nmy-1/2* (non-muscle myosin heavy chains) (Piekny *et al.,* 2003). MEL-11/myosin phosphatase inhibits elongation by dephosphorylating myosin while LET-502/Rho-binding kinase triggers elongation by inhibiting MEL-11 via sequestering it to the membrane.

Priess and Hirsh (1986) showed that the cytoskeletal networks in the worm epidermis are organized differently in the lateral vs. the dorsal/ventral cells and work suggests that only the microfilaments in the lateral cells contract during elongation. However, the mechanism by which the highly organized actin networks are set up and how these differ in lateral vs. dorsal/ventral cells has yet to be elucidated.

Here we describe FHOD- 1, a highly conserved Formin Homology Domain protein that may contribute to the spatial differences in the microfilament networks. Formins are actin nucleators, acting as progressive caps that form long unbranched actin filaments that are bundled into stress fibres. There are contradictory reports of human FHOD-1's interaction with the small GTPase RAC and with Rho-binding Kinase (Gasteier *et al.,* 2003. Westendorf *et al.,* 1999 & 2001. Takeya *et al.* 2008). We found that *fhod-1*(RNAi) enhances the lethality of *let-502* and suppresses that of *mel-11*, indicating that *fhod-1*(+) acts to induce elongation. We have generated antibodies to FHOD-1 and shown that it forms a filamentous pattern similar to that of actin. Significantly, FHOD-1 is only found in the lateral seam cells, consistent with the findings of Priess and Hirsh that the actin network is organized differently in the lateral cells compared to their neighbours. A deletion allele (*tm2363*) generated by the National Bioresource Project (which we believe is a complex rearrangement) shows defects in that actin network restricted to the seam cells.

Currently we are trying to determine the *fhod-1*'s null phenotype and narrowing down FHOD-1's timing of expression to see if it is the sole formin involved in organizing the seam cells actin fibres.

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The ROCK homolog LET-502 is temporally and spatially regulated by LIN-12 Notch and LIN-1 ETS during vulval morphogenesis. **Sarfarazhussain Farooqui**, Ivo Rimann, Alex Hajnal. Dept Zoology, Univ Zurich, Zurich, Switzerland.

Cell fate specification and morphogenesis are two important aspects of vulval development. After the vulval precursor cells (VPCs) have acquired their fates, the VPCs divide thrice and their descendants undergo migration, elongation and fusion at the vulval midline to form seven toroidal rings stacked one over the other in an invariant fashion (Ranjana Sharma-Kishore et. al, 1999). Recently, genetic approaches have revealed that two parallel pathways regulate vulval morphogenesis. The first pathway requires the SMP-1 Semaphorin ligand, the PLX-1 Plexin receptor and the CED-10 Rac small GTPase, while the second pathway utilizes the MIG-2 Rac small GTPase and its activator UNC-73 GEF (Dalpe et al. 2005; Ranjana S Kishore et al. 2002). Here, we show a role for the ROCK homolog LET-502 during vulval morphogenesis. Maternally rescued *let-502(ok1283)* animals display defects in vulval toroid formation, with evidence of abnormal cell migration and elongation. The expression of the vulval fate markers was not affected in the *let-502(ok1283)* animals, suggesting that *let-502* does not affect VPC fate specification. Genetic epistasis reveals redundancy of LET-502 with both the SMP-1/PLX-1/CED-10 and UNC-73/MIG-2 signaling pathways. During vulval morphogenesis, *let-502* is specifically expressed in the secondary vulval cells. Analysis of the *let-502* promoter reveals spatial and temporal regulation during morphogenesis by LIN-12 Notch signaling and the transcription factor LIN-1 ETS. Accordingly, *lin-12* notch loss-of-function mutants display defects in toroid formation similar to the defects observed in *let-502(ok1283)* mutants. Hence, Notch signaling may regulate vulval morphogenesis through the activation of *let-502* transcription.

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Screening for essential muscle genes using high throughput RNAi identifies a copine involved in early sarcomere assembly. **Adam D. Warner**, Teresa Rogalski, Aruna Somasiri, Don Moerman. Cell Biol Group–Zoology, Univ British Columbia, Vancouver, BC, Canada.

The sarcomere is an impressively organized array of interdigitated actin and myosin filaments, anchored by dense body and M-line attachment complexes, respectively, to the sarcolemma of a muscle cell. Prominent proteins in Z-disc analogs and homologs known as dense bodies are required for body wall muscle in *C. elegans* to develop and function properly. Without these essential attachment complex proteins such as α and β-integrin (PAT-2, PAT-3), vinculin (DEB-1), kindlin (UNC-112), PINCH (UNC-97), ILK (PAT-4), and actopaxin (PAT-6), embryos arrest at the two-fold stage of embryonic development[1]. Early work on β-integrin/PAT-3[2] and kindlin/UNC-112[3] in the worm first implicated these proteins as being essential in muscle, and the worm has continued to be a valuable model organism for identification and study of novel essential muscle genes.

Focusing on the paralyzed and arrested at two-fold stage (Pat) phenotype that occurs with loss of essential muscle genes in worms, we have carried out a muscle transcriptome-wide RNAi screen to identify genes with essential roles in muscle. Four genes without a previously annotated Pat phenotype have been uncovered. One of the positive hits from our screen is F31D5.3, which codes for a transmembrane protein with a C-terminal integrin-like A domain, and bears homology to the copine family of proteins in higher organisms including humans. To date, very little research has been carried out on copines in other organisms, although some copines have been found present in human heart and skeletal muscle[4]. We have received a gene knockout for F31D5.3 (*gk266*) from the *C. elegans* Gene Knockout Consortium (Vancouver) that is lethal, arresting at the two-fold stage of embryogenesis just as our aforementioned RNAi study found. Furthermore, when F31D5.3 RNAi is administered to worms after they hatch from embryos, myofilaments in body wall muscle become severely disorganized. Lastly, expression data from SAGE experiments (Moerman lab, unpublished) shows highly elevated expression of F31D5.3 in embryonic body wall muscle compared to other tissue types, and a ~3.5 fold enrichment in expression levels compared with whole embryos. Further experiments are ongoing to identify where F31D5.3 is localized in body wall muscle, and to attempt placing F31D5.3 in the sarcomere assembly pathway.

[1]Williams BD and Waterston RH, (1994). JCB: 124(4):475-90. [2]Gettner et al (1995). JCB: 129(4):1127-41. [3]Rogalski et al (2000). JCB: 150(1):253-64. [4]Cowland et al (2003). J. Leukocyte Biology: 74(3) 379-88.

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Molecular interactions reveal multiple roles for UNC-53 in cell migration, intracellular trafficking, and innate immunity. Kristopher Schmidt[1,2], **Eve Stringham**[1,2]. 1) Biology, Trinity Western University, Langley, BC, Canada; 2) MBB, Simon Fraser University, Burnaby, BC, Canada.

Previous studies have shown that UNC-53 is required for the migration of cells and the outgrowth of several neuronal processes. UNC-53 binds the adapter protein SEM-5 (Stringham et al., 2002) as well as ABI-1, a regulator of Arp 2/3 mediated actin filament assembly (Schmidt et al., 2009). A two hybrid screen identified the T12G3.1 gene product as a strong interactor of UNC-53. RNAi and mutant analysis of T12G3.1 revealed a partial overlap in cell migration phenotypes with *unc-53*. While the outgrowth of the posterior excretory canals is truncated in both T12G3.1 and *unc-53* mutants, the latter has a guidance phenotype in mechanosensory neurons that does not appear to be shared with T12G3.1. T12G3.1 encodes a protein of 693 amino acids that contains a zinc finger of the ZZ type, a SH3 binding domain, and a UBA (ubiquitin associated) domain. T12G3.1 is 38 % identical to murine p62/sequestosome-1, especially around the ZZ and UBA domains. In mammals, p62 has been shown to shuttle polyubiquitinated proteins to the proteasome for degradation and to bind autophagocytic vesicles, suggesting a role in intracellular trafficking. Consistent with this hypothesis, a pT12G3.1::gfp fusion is expressed in cells that have specialized roles in secretion or endocytosis including the excretory gland cell, motorneurons, coelomocytes, intestine, and spermatheca. A translational pT12G3.1::T12G3.1::GFP fusion shows localized expression to cell bodies but is excluded from neuronal processes. We observe persistent expression of UNC-53 in neurons and in coelomocytes, specialised cells that endocytose pseudocoelomic fluid suggesting that UNC-53 may also have a role in vesicle trafficking. Using an assay where GFP produced in body muscle is secreted into the pseudocoelom and then endocytosed into coelomocytes, we have demonstrated a coelomocyte-uptake (cup) defect in worms depleted of T12G3.1, *unc-53*, or *arx-2/Arp2* by RNAi and in genetic nulls. Similarly, null alleles of *unc-53* are defective in receptor mediated endocytosis of yolk protein in oocytes. p62/sequestosome-1 is a physical interactor and regulator of the *pmk-1*/p38 MAPK involved in innate immunity to microbial pathogens. We find that, like *pmk-1* mutants, null alleles of *unc-53* and T12G3.1 are sensitive to *Pseudomonas aeruginosa* and are both expressed in intestine, the primary site of *pmk-1* activity. Thus UNC-53 appears to have a multi functional role in cell migration, protein trafficking, and may be involved in innate immunity. We propose a model where UNC-53 may mediate all of these activities by modulating cytoskeletal dynamics.

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The anticonvulsant ethosuximide disrupts sensory function to extend *C. elegans* life span. James J. Collins[2], Kimberley Evason[1], **Christopher L. Pickett[1]**, Daniel L. Schneider[1], Kerry Kornfeld[1]. 1) Developmental Biology, Washington University, Saint Louis, MO; 2) School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

Ethosuximide is a medication used to treat seizure disorders in humans. We previously demonstrated that ethosuximide could delay age-related changes and extend the life span of *C. elegans*. The mechanism of action of ethosuximide in life span extension is unknown, and elucidating how ethosuximide functions is important for defining endogenous processes that influence life span and for exploring the potential of ethosuximide as a therapeutic for age-related diseases. To identify factors that mediate the activity of ethosuximide, we conducted a genetic screen and identified mutations in two genes, *che-3* and *osm-3*, that confer resistance to ethosuximide-mediated toxicity. Mutations in *che-3* and *osm-3* cause defects in an overlapping set of chemosensory neurons, resulting in defective chemosensation and life span extension. These findings suggest that ethosuximide extends life span by inhibiting the function of specific chemosensory neurons. This model is supported by the observation that ethosuximide treated animals displayed numerous phenotypic similarities with mutants that have chemosensory defects. Furthermore, long-lived *osm-3* animals were resistant to the life span extension caused by ethosuximide, suggesting the drug extends life span by inhibiting chemosensation. These studies demonstrate a novel mechanism of action for a life span extending drug and indicate that sensory perception has a critical role in controlling life span. Sensory perception also influences the life span of *Drosophila*, suggesting that sensory perception has an evolutionarily conserved role in life span control. These studies highlight the potential of ethosuximide and related drugs that modulate sensory perception to extend life span in diverse animals.

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Relating cellular and organismal aging phenotypes in *C. elegans*. **Javier Apfeld**, Walter Fontana. Department of Systems Biology, Harvard Medical School, Boston, MA.

Aging appears to be a universal phenomenon in living systems leading to functional changes affecting the onset of disease and the timing of death. Living systems comprise multiple structural and organizational levels, from sub-cellular to whole organism. Aging may occur at any of these levels. In the past two decades aging research has begun to map how organismal lifespan depends on genetic and environmental factors. Yet, the relationship between cellular and organismal aging phenotypes is rarely investigated and poorly understood. We have developed and deployed indicators of cellular state to probe causality between processes leading to failure in individual neurons and processes that determine organismal lifespan in *C. elegans*. We perform longitudinal measurements in individual neurons utilizing fluorescent probes in both wild-type animals and transgenic animals commonly used as models of neurodegenerative disease. We find that the states of certain neurons become more uncertain as animals get older. In addition, time-dependent correlations between some pairs of neurons develop from initially uncorrelated states. The existence of correlations between specific states of different cells suggests the existence of processes of inter-cellular communication mediating those dependencies. Notably, these neuronal aging processes are independent of processes that determine organismal lifespan. We find that mutations in components of the insulin signaling pathway that increase lifespan promote neuronal aging processes in some neurons yet inhibit them in others. The action of insulin signaling in these neuronal processes is independent of its influence on organismal lifespan. Together, these results show that *C. elegans* undergoes independent aging processes at different organismal levels.

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Strongyloides ratti: An unusual nematode with extraordinary plasticity in aging. **David Gems[1]**, Fiona Thompson[2], Michael Gardner[2], Mark Viney[2]. 1) Inst of Healthy Ageing and GEE, Univ Col London, London, United Kingdom; 2) School of Biological Sciences, University of Bristol, Bristol, UK.

Parasitic nematodes often evolve greater longevity than free-living species, which likely reflects different extrinsic mortality rates in their respective niches1. *S. ratti* has distinct parasitic and free-living adults, living in the rat small intestine and the soil, respectively. We have documented the biology of aging in this organism, for the first time in a parasitic nematode. Both adult morphs show clear reproductive and demographic aging. For free-living nematodes, the mean and maximum lifespan is 3.0 ± 0.1 days and 4.5 ± 0.8 days respectively, making this the shortest-lived nematode identified to date. By contrast, the maximum lifespan of parasitic adults is 403 days2,3. Thus, the two adult forms have evolved strikingly different rates of aging, this despite being genetically identical (females). The 80-fold difference in their lifespans, the greatest plasticity in aging yet reported, is therefore likely to reflect evolved differences in gene expression. This suggests that inter-specific differences in lifespan may evolve via similar mechanisms3. To try to gain some insight into the genetic basis of lifespan evolution, we have now compared gene expression in long- and short-lived *S. ratti* adults. DNA microarray analysis of long- and short-lived adults identified 32 genes that were up-regulated in long-lived adults, and 96 genes up-regulated in short-lived adults4. Among the 32 longevity-associated genes there was little enrichment of genes linked to cellular maintenance. Strikingly, 38.5% of the genes expressed more in short-lived morph adults encode predicted ribosomal proteins, compared with only 9% in long-lived adults. Thus, overall, there is a negative correlation between expression of ribosomal protein genes and longevity in *S. ratti*. Interestingly, it has been reported that engineered reduction of expression of ribosomal protein genes increases lifespan in *C. elegans*. Our findings therefore suggest that differences in levels of protein synthesis could contribute to evolved differences in animal longevity. 1. *Biogerontology* 1, 289 (2000). 2. *Exp Gerontol* 39, 1267 (2004). 3. *Aging Cell* 5, 315 (2006). 4. *Mech Ageing Dev* 130, 167 (2009).

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GENETIC INTERACTIONS BETWEEN *apl-1*, A GENE ENCODING AN AMYLOID PRECURSOR-RELATED PROTEIN, AND *daf-16*, A REGULATOR OF LIFESPAN. **Collin Ewald**, Chris Li. The City University of New York, New York, NY.

Alzheimer's Disease (AD) is an age-dependent, neurodegenerative disease. Aggregation of the β-amyloid peptide is toxic to neurons and may be the main cause of neurodegeneration in AD. The β-amyloid peptide is a cleavage product of the amyloid precursor protein (APP). Although the processing of APP has been elucidated, the physiological function of APP remains unclear. In *C. elegans*, APL-1 has high sequence similarity to human APP, but does not contain the β-amyloid peptide. Similarly to APP, APL-1 is also cleaved to produce a large extracellular fragment and a small intracellular stub. Null mutations of *apl-1* lead to an L1 lethality, which can be rescued by neuronal expression of only the extracellular fragment of APL-1 (APL-1EXT), suggesting the importance of *apl-1* for development and viability. Duplication of the human APP locus can lead to familial AD. Hence, we examined how APL-1 overexpression can affect later stages. Overexpressing APL-1 resulted in a shortened body length and decreased mean lifespan in a dose-dependent manner. Overexpression of only the extracellular domain of APL-1 was also sufficient to decrease lifespan and body length. Since APL-1 is expressed in neurons and other cell types, we hypothesized that APL-1 overexpression in neurons was responsible for the decreased lifespan. Surprisingly, driving APL-1 expression with a predominantly neuronal promotor (P*snb-1::apl-1*) increased the mean lifespan by 20%. Overexpressing only the extracellular domain of APL-1 under the control of the *snb-1* promotor (P*snb-1*::APL-1EXT) was also sufficient to enhance lifespan. To determine whether this lifespan effect is due exclusively to neuronal overexpression, we drove APL-1 expression with the *rab-3* promotor (P*rab-3::apl-1*), which only expresses in neurons. Although the P*rab-3::apl-1* construct is sufficient to rescue the *apl-1* L1 lethality, these animals do not have an enhanced lifespan. Hence, the APL-1-dependent lifespan effect must be due to overexpression in a different tissue. To further characterize the tissue-specific lifespan effect of APL-1, we tested whether APL-1 overexpression effects were dependent on *daf-16*, a transcription factor regulating lifespan in a tissue-specific manner. The increased lifespan of animals carrying P*snb-1::apl-1* was abolished in a *daf-16* null background, suggesting the requirement of DAF-16 for lifespan extension. Furthermore, while overexpression of APL-1 decreases body length, the normal length of the animal can be restored in a *daf-16* null mutant background. Hence, DAF-16 is required for two APL-1 overexpression phenotypes, the tissue-specific lifespan effects and the short body length, suggesting a genetic interaction between *daf-16* and *apl-1*.

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Attenuated immune response during aging in *C. elegans*. **Matthew J. Youngman**, Odile Kamanzi, Annie R. Inman, Dennis H. Kim. Dept Biol, MIT, Cambridge, MA.

A decline in immune function, termed immunosenescence, leads to increased susceptibility to infection during aging. Using infection of *C. elegans* with *P. aeruginosa* as a model for host-pathogen interactions, here we demonstrate an age-dependent enhanced susceptibility to pathogen in worms. Previous studies have shown that the intestine of adult worms becomes packed with *E. coli*, a species considered to be relatively innocuous in younger animals, and that bacterial colonization of the gut leads to death from infection. We examined colonization of the intestine of older worms by pathogenic and innocuous bacteria expressing GFP. The rate of accumulation of *P. aeruginosa* and *E. coli* is accelerated in adult animals during aging, and older worms are killed from infection by *P. aeruginosa* more rapidly than late larval stage animals, thus validating *C. elegans* as a system for studying immunosenescence. Whereas accumulation of *P. aeruginosa* in the intestine is always associated with mortality, we found that accumulation of *E. coli* is not necessarily indicative of imminent death, as a bolus of labeled *E. coli* persists in the intestine of older worms and is either maintained or cleared over the course of several days. We are currently testing whether the relative amount of *E. coli* within the intestine may predict absolute lifespan or the survival rate upon challenge with pathogen. The gradual clearance of accumulated bacteria from the intestine of older worms suggests that the immune response is attenuated yet intact later in life. To determine the basis of host defense during aging, we tested whether insulin signaling, which regulates lifespan and has been shown to influence resistance to pathogen in L4 worms, plays a role in host defense later in life. Our results suggest that the insulin signaling pathway is required for host defense in an age-dependent manner. Adult *daf-16* mutants accumulate *P. aeruginosa* and *E. coli* more rapidly and to a greater extent than age-matched wild type animals, and *daf-16* mutants are unable to clear the bolus of GFP-labeled *E. coli*. Loss of DAF-16 function exacerbates the enhanced susceptibility to pathogen of adults, but L4 stage *daf-16* mutants are killed by infection with *P. aeruginosa* at a rate similar to wild type worms, suggestive of a role for DAF-16 in host defense specifically during aging. Accordingly, overexpression of DAF-16 confers resistance to pathogen in adult worms but not larval stage animals. Our data implicate microbial colonization of the *C. elegans* intestine as an indicator of an age-related decline in immune function, and suggest that increased DAF-16 activity later in life is necessary but not sufficient to confer resistance to infection.

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An age-dependent switch in the functional balance of mitogen-activated protein kinase pathways affects infection susceptibility and lifespan. Kwame Twumasi-Boateng[1], Man-Wah Tan[2], **Michael Shapira[1,3]**. 1) Graduate Group in Microbiology, UC Berkeley, CA; 2) Genetics Department, Stanford University, CA; 3) Integrative Biology, UC Berkeley, CA.

Orchestrating responses to environmental perturbations of homeostasis relies on the action of various signal transduction pathways, with mitogen-activated protein kinase (MAPK) pathways being prominent representatives. Three main MAPK pathways, the p38, JNK and ERK, employ kinase activation cascades to induce protective responses during infection, wounding, oxidative stress and heavy metal exposure, to name a few. The consequences of MAPK pathway activation in response to various conditions are context-dependent. Age is one such context, but is rarely considered when studying these signaling pathways. Here we describe a switch occurring in *C. elegans* between the fourth larval stage (L4) and the two-day-old adult, in which the effect of MAPK activation changes from being protective to detrimental. The switch is manifested following the knockdown of *vhp-1* expression. *vhp-1* encodes a phosphatase that dephosphorylates and deactivates both the p38 MAPK homolog PMK-1 and the JNK-like MAPK KGB-1 [1, 2]. Exposing worms from the egg stage until L4 to RNAi directed against *vhp-1* resulted in increased resistance to infection with *Pseudomonas aeruginosa*. However, worms exposed for the same duration to *vhp-1* RNAi beginning at the L4 stage, or for a twice-as-long duration starting at the egg stage, became more sensitive to the pathogen and showed a significant shortening of their general lifespan when grown on dead *E. coli*. Genetic analyses supplemented with Western blots to follow MAPK phosphorylation showed that both pathways are activated in either age. However, whereas pathogen resistance following *vhp-1* knock-down at the young age is dependent on *pmk-1* alone, increased pathogen susceptibility and the shortening of lifespan two days later is dependent solely on *kgb-1*. Our results demonstrate a shift in dominance between two MAPK pathways, where beneficial effects surmount detrimental ones early in life, but this balance overturns later in life.

1. Mizuno, T., et al., *EMBO J* 2004. **23**: 2226-2234.
2. Kim, D. H., et al., *Proc Natl Acad Sci U S A* 2004. **101**: 10990-10994.

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TGF-β Sma/Mab Signaling is a Novel Regulator of Reproductive Aging and the Maintenance of Germline and Oocyte Quality in *C. elegans*. **Shijing Luo**, Gunnar Kleemann, Wendy Shaw, Jasmine Ashraf, Coleen Murphy. Department of Molecular Biology, Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ.

Reproductive cessation is a common phenotype of aging. It has become an important issue today, as more women have children later in life, despite a dramatic decline in reproductive capacity and increased incidence of birth defects and miscarriages with maternal age. While much has been done to understand the general aging process, little is known about the regulation of reproductive aging. Work in our lab and others suggests that *C. elegans* is a good model to study reproductive aging. It was reported that the longevity regulator the insulin/IGF-1 signaling pathway reguates reproductive span in *C. elegans* as well (Hughes et al.). We have also found that a body size regulator, the TGF-β Sma/Mab pathway is a novel pathway that regulates reproductive span independently of lifespan regulation. To understand the mechanisms of reproductive aging regulation, we tested the effect of several factors on reproductive span. We found that reproductive aging in *C. elegans* depends on oocyte quality, and is independent of progeny number, ovulation rate, or body size. While oocyte morphology, fertilizability, and developmental competence become defective with age in wild type, both TGF-β Sma/Mab and insulin/IGF-1 signaling mutants maintain oocyte quality with age. To understand the mechanisms of oocyte quality control, we examined the germline from the distal mitotic germ cells to the proximal mature oocytes in the gonad pipeline. We found that oocyte quality decline is partly correlated with reduced intergrity of the mitotic germline, and both TGF-β Sma/Mab and insulin/IGF-1 signaling mutations prevent such degradation. Furthermore, these mutations also increase the DNA damage repair capacity of the oocytes. Mosaic analysis of the TGF-β Sma/Mab pathway indicates that reproductive span and oocyte quality are regulated in the soma, suggesting that communication between the soma and germline is required to affect the reproductive aging process. To find the oocyte-autonomous effectors downstream of TGF-β Sma/Mab signaling, we took a genomic approach and found that many genes upregulated in TGF-β mutant oocytes are involved in embryonic development, cell cycle regulation, chromosome seggregation, apoptosis, and DNA repair. Our data suggest that *C. elegans* maintains reproductive capacity through regulation of oocyte quality, as in humans, and the TGF-β Sma/Mab pathway normally promotes reproductive aging. The mechanisms of reproductive aging regulation are distinct from longevity regulation mechanisms that maintain the soma and mechanisms by which the TGF-β Sma/Mab pathway regulates body size.

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HCF-1 modulates lifespan and stress responses in *C. elegans* by regulating the transcriptional activities of DAF-16 and SIR-2.1. **Gizem Rizki**[1], Ji Li[1], Max Jan[2], Coleen T. Murphy[2], Siu Sylvia Lee[1]. 1) Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Molecular Biology, Princeton University, Princeton, NJ.

DAF-16, the *C. elegans* ortholog of the evolutionarily conserved FOXO transcription factors, is a key mediator of longevity. DAF-16 acts as a central integrator of diverse environmental and cellular stimuli, and mounts a transcriptional response to modulate stress responses and lifespan. Although multiple pathways and factors that affect the function of DAF-16 have been identified, the precise molecular mechanisms determining the specific transcriptional responses by DAF-16 have yet to be discovered. We recently identified the *C. elegans* host cell factor 1 (*hcf-1*) as a novel longevity determinant and as a transcriptional co-repressor of DAF-16 (Li J. *et al*, 2008, PLoS Biol). While not much is known about *C. elegans* HCF-1, the mammalian ortholog of HCF-1 is known to regulate the activities of several transcription factors by assembling appropriate transcriptional complexes. Loss of *C. elegans hcf-1* results in a robust lifespan extension and resistance to oxidative and UV-stress that is dependent on *daf-16*. HCF-1 antagonizes the transcriptional activity of DAF-16 in the nucleus by physically associating with DAF-16 and limiting its access to select target gene promoters. To test if *hcf-1* also requires the activity of *sir-2.1*, another key longevity determinant that acts as a *daf-16* cofactor, we performed epistasis analysis between *hcf-1* and *sir-2.1*. Our preliminary results indicate that loss of *sir-2.1* suppressed the longevity and UV-stress-resistance phenotypes of *hcf-1* mutants. In addition, *sir-2.1* was necessary for the elevated expression of superoxide-dismutase 3 (*sod-3*), a DAF-16 target gene, in *hcf-1* mutants. Co-immunoprecipitation experiments revealed that HCF-1 and SIR-2.1 proteins physically associate. Using gene-expression microarray analysis, we identified genes regulated by DAF-16 in response to HCF-1. In agreement with our hypothesis that HCF-1 modulates specific functions of DAF-16, we found that the HCF-1/DAF-16 target genes constituted only a subset of known DAF-16 target genes altered in response to insulin/IGF signaling (Shaw WM *et al*, 2007, Curr Biol). Interestingly, comparison of *daf-16*-dependent gene expression changes in long-lived *hcf-1* mutants to those in long-lived *sir-2.1* overexpressing strains (CTM, unpublished results) revealed a >90% overlap of similarly regulated genes. Therefore, our results suggest that HCF-1 modulates longevity and stress responses through antagonizing the transcriptional activities of DAF-16 and SIR-2.1.

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HSF-1 is an Essential Downstream Mediator of Resveratrol-induced, SIR-2.1-dependent Longevity in C. elegans. **Marton L. Toth**, Balazs Dancso, Peter Csermely, Csaba Soti. Medical Chemistry, Semmelweis University, Budapest, Hungary.

Major longevity assurance mechanisms include the heat-shock response governed by the heat-shock transcription factor (HSF-1) and the metabolic stress response, exemplified by the Sir2 deacetylase. Sir2, and recently HSF-1 have been implicated in the life-span extending effect of dietary restriction. Resveratrol, a polyphenol from red wine, is a dietary restriction mimetic that induces longevity and displays an impressive therapeutic potential against various degenerative diseases via Sir2. Resveratrol has also been shown to induce the heat-shock response in mammalian cells. In this study, we have asked how the heat-shock response is involved in resveratrol-induced/Sir2-related longevity in C. elegans. We find that resveratrol specifically induces chaperone expression, thermotolerance and longevity depending on both SIR-2.1 and HSF-1. Moreover, we show that SIR-2.1 is a potent activator of HSF-1-dependent thermotolerance. Finally, we identify HSF-1 as an essential downstream effector of resveratrol-induced, SIR-2.1-dependent longevity in C. elegans. Thus, our results demonstrate a direct interaction of metabolic and proteotoxic stress responses in life-span regulation and indicate that the robustness of the heat-shock response may determine the therapeutic response to resveratrol. Marton Toth's current address: Rutgers, The State University of NJ, Molecular Biology & Biochemistry, Piscataway, NJ.

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A soma-to-germline transformation in long-lived *C. elegans* mutants. **Sean P. Curran**[1,2], Xiaoyun Wu[1,2], Christian G. Riedel[1,2], Gary Ruvkun[1,2]. 1) Dept. Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept. Genetics, Harvard Medical School, Boston, MA.

Unlike the soma which ages during the lifespan of multicellular organisms, the germ line traces an essentially immortal lineage across the generations. We report here that *C. elegans* insulin-like signaling and other extraordinarily long-lived mutants exhibit a soma-to-germline transformation that supports the view that the increased health of these mutants is modulated in part by preventing the natural decline in somatic integrity with age. Mutations that decrease insulin-like signaling cause the somatic misexpression of germline-limited *pie-1* and *pgl* family of genes, and dramatically disrupt the regulation of the germline restricted *pie-1p::gfp::pgl-1* reporter within intestine and hypodermal tissues. DAF-16/FoxO, the major transcriptional effector of insulin-like signaling, specifically regulates *pie-1* expression by directly binding to the *pie-1* promoter and is required for the somatic misexpression of germline genes in insulin-like signaling mutants. The somatic tissues of these long-lived insulin-like signaling mutant strains are more germline-like and protected from genotoxic stress. Loss of the longevity-modulating cytosolic chaperonin complex also leads to somatic misexpression of PGL-1. These results suggest that the acquisition of germline characteristics by the somatic cells of *C. elegans* longevity mutants may contribute to their increased health and survival.

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Mutations in *egl-1* and *daf-2* improve oocyte quality in aging females. **Qing Wei**[1,2], Sara Audux[1,2], Ronald E Ellis[2]. 1) GSBS, UMDNJ-SOM, Stratford, NJ; 2) Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ.

As women age, the quality and quantity of their oocytes declines, resulting in fewer pregnancies and an increased chance of having a child with birth defects. We are using the nematode C. elegans to study how oocyte quality is regulated during aging. To assay quality, we use *fog-2*(*q71*) females mated at various ages, and determine the fraction of oocytes that produce viable eggs after fertilization. Our previous results showed that oocyte quality declines in older nematodes, as in humans. Furthermore, apoptosis helps alleviate this problem, since mutations in *ced-3* and *ced-4* prematurely lower oocyte quality during aging.

Surprisingly, a loss-of-function mutation in *egl-1*, which normally prevents germ cells from undergoing cell death in response to DNA damage, appeared to improve oocyte quality in older animals. Normally, CEP-1 acts through EGL-1 and CED-13 to control CED-9 activity and regulate germ cell deaths. As expected, we found that EGL-1 promotes oocyte quality in young irradiated animals. However, *egl-1* mutants, *ced-13* mutants and *egl-1*; *ced-13* double mutants produced high quality oocytes when old, which implies that EGL-1 and CED-13 are harmful to oocytes in older animals. Furthermore, *egl-1*(*lf*) mutations lowered the number of dying germ cells in older animals. Finally the harmful effects of EGL-1 depended on apoptosis, since *ced-3* mutants and *ced-3*; *egl-1* double mutants behaved similarly. Thus, we suspect that EGL-1 lowers the quality of oocytes in older animals by inappropriately stimulating apoptosis.

We also noted that *egl-1*(*lf*) animals reproduced for a longer period of time than wild-type animals. Since oocyte quality declines with age, it might be influenced by genes that regulate lifespan. To test this idea, we studied *daf-2*(*e1370*ts) mutants and found that they showed improved oocyte quality when old. Furthermore, the effect of *daf-2* on oocyte quality depended on *daf-16* but was not affected by mutations in *ced-3*.

Thus, we suspect that selection optimized the production of high quality oocytes in young animals, during their peak reproductive period. Genes like *egl-1* and *ced-13*, which control some germ cells death, and *daf-2*, which regulates the response to certain forms of stress, might not have optimal activity in older females.

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A sperm signal downstream of DAF-16/FOXO induces vitellogenin gene expression in C elegans. **Ana S. DePina**, Wendy B. Iser, Catherine A. Wolkow. Laboratory of Neurosciences, Intramural Research Program, National Institute on Aging, Baltimore, MD.

The insulin/IGF-I receptor-like protein, DAF-2, and the PI3K p110 catalytic subunit AGE-1, regulate development, fertility and lifespan in C. elegans. The major downstream target of DAF-2/insulin receptor signaling is the FOXO transcription factor, DAF-16. DAF-2 and AGE-1 negatively regulate DAF-16 by preventing its translocation to the nucleus. In the absence of DAF-2/insulin receptor signaling, DAF-16 promotes increased expression of stress response genes and reduced expression of metabolically costly genes, resulting in lifespan extension. This suggests that organisms can control the allocation of metabolic resources to influence longevity. One group of genes reported to be down-regulated in long lived *daf-2* mutants are the vitellogenin (*vit*) genes, which encode yolk proteins that transport lipids into developing oocytes. To investigate the molecular pathways required for resource allocation to reproduction, we assessed *vit* expression levels after mating with wildtype males and in sterile mutants. We found that introduction of sperm to *fem-1* mutants that lack sperm rescues fertility and induces *vit* expression, while introduction of sperm to *daf-2* mutants induces *vit* expression without affecting fertility. In *fem-3*, a mutant that only makes sperm, and in *spe-11*, a sperm mutant that affects embryonic divisions after fertilization, *vit* expression levels were comparable to WT. However, in *spe-9*, a fertilization defective sperm mutant, *vit* expression remained low throughout adulthood. These results suggest that sperm induces *vit* expression in the intestine of adult hermaphrodites in a *spe-9* dependent manner. This induction is not dependent on gamete production, presence of oocytes, or the early embryogenesis factor, *spe-11*. We propose that in long lived *daf-2* mutants DAF-16 acts to inhibit germline functions, which subsequently affects sperm, and specifically *spe-9*, leading to *vit* repression. These findings provide new insight about mechanisms that regulate the balance between resource allocation to reproduction and longevity.

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Starvation-induced adult reproductive diapause protects germline stem cells and extends reproductive longevity in *C.elegans*. **Giana Angelo**, Marc Van Gilst. Basic Sci Division, FHCRC, Seattle, WA.

We present the characterization of a starvation induced adult reproductive diapause in *C. elegans*. When a dense population of wild-type worms is acutely starved during the L4 larval stage, a fraction of animals transition into adults and cease reproductive activities, hence the name "Adult Reproductive Diapause". Reproductively arrested adult worms are most often distinguished by the presence of two arrested or deceased embryos *in utero*. Optimal establishment of the ARD requires the nuclear receptor NHR-49, a close relative of mammalian HNF4α. Further study revealed that animals in the adult reproductive diapause survive starvation for at least 30 days and still recover to produce offspring and age normally when food is restored, providing more evidence that this state is a truly effective reproductive diapause. There are two extraordinary features of the ARD: (1) although starvation leads to the progressive loss of almost the entire germline, a small population of germ cells is consistently preserved, and (2) upon restoration to food, these surviving germ cells faithfully regenerate a functional germline, demonstrating that authentic germline stem cells are protected for at least a month of complete starvation. We propose a "disposable germline" hypothesis for ARD maintenance and recovery, whereby meiotic germ cells are sacrificed as a means of sustaining somatic tissues and residual germ cells during starvation. Upon restoration to food, protected germline stem cells are reactivated in order to regenerate a fully functional germline and restore fecundity.

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Motor-Driven Motion Associated with Meiotic Chromosome Pairing. **David Wynne**[1], Pete Carlton[2], Abby Dernburg[1,3,4,5]. 1) MCB Dept, UC Berkeley, Berkeley, CA; 2) Biochemistry and Biophysics Dept, UCSF, San Francisco, CA; 3) Howard Hughes Medical Institute; 4) California Institute for Quantitative Biosciences; 5) Lawrence Berkeley National Lab, Berkeley, CA.

Accurate segregation of homologous chromosomes during meiosis is necessary for the faithful transmission of the genome from parent to progeny. To segregate properly, homologs must first undergo pairing, synapsis, and recombination. The mechanism of homologous chromosome pairing remains a major mystery of meiosis. In *C. elegans*, as in many other eukaryotes, pairing is accompanied by a global rearrangement of chromosomes. We have found that this rearrangement is driven through the association of special chromosome regions known as Pairing Centers (PC) with nuclear envelope proteins and cytoskeletal motors (Phillips et al. 2005, Sato A. unpublished). Using fluorescent markers for nuclear envelope attachment sites and Pairing Centers, we are analyzing chromosome dynamics through real-time imaging and quantitative motion tracking. Our results reveal a dramatic increase in chromosome motion at the onset of chromosome pairing that persists after homologous loci are paired. We show that this increased mobility is dependent on the conserved cell cycle checkpoint kinase, CHK-2, and that motion slows following knockdown of cytoplasmic dynein. Our data supports a model in which homolog pairing is promoted by a small number of fast, motor-driven movements that augment the smaller, Brownian motions seen prior to meiosis. The observation that fast motions persist well after pairing is completed suggests additional roles in chromosome synapsis or recombination.

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Toward a Unified-Field Theory of the Adult Hermaphrodite Gonad. **J. Amaranath Govindan**, Saravanapriah Nadarajan, Seongseop Kim, Todd Starich, David Greenstein. Dept Gen, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN 55455 USA.

A sperm-sensing mechanism regulates oocyte meiotic maturation and ovulation, tightly coordinating sperm availability and fertilization. Sperm release the major sperm protein (MSP) to trigger meiotic maturation and to promote gonadal sheath cell contraction. We present evidence that MSP signaling organizes key steps by which germ cells generate zygotes when sperm are available for fertilization. Genetic mosaic analysis establishes that all described MSP-dependent meiotic maturation events in the germline require GSA-1 ($G\alpha_s$)-ACY-4 (adenylate cyclase) signaling in the gonadal sheath cells, including oocyte microtubule reorganization, MAP kinase activation, recycling of the VAB-1 receptor, and chromatin-localization of AIR-2::GFP. In the absence of GSA-1-ACY-4 signaling, specific and saturable MSP-binding sites cluster on the gonadal sheath cells. Further GSA-1::YFP and ACY-4::CFP exhibit sperm-specific FRET in the sheath cells (see abstract by S. Kim). MSP signaling promotes actomyosin-dependent cytoplasmic streaming that drives oocyte growth and is sufficient to trigger phosphorylation of the regulatory myosin light chain (rMLC-4) throughout the germline. GSA-1-ACY-4 signaling is necessary and sufficient to drive cytoplasmic streaming and rMLC-4 phosphorylation. Thus, the gonadal sheath cells ensure that oocyte production and growth, as well as meiotic maturation, occur efficiently in response to MSP. We performed a genetic screen for mutations that cause oocytes to grow abnormally large only when MSP is present (see abstract by S. Nadarajan). Surprisingly, we recovered reduction-of-function *glp-1/Notch* alleles in this screen. *glp-1*(rf) mutations cause oocytes to grow abnormally large in a manner that is dependent on GSA-1-ACY-4 signaling in the gonadal sheath cells. By contrast, *glp-1*(gf) mutations result in the production of small oocytes. Proper MSP-dependent oocyte growth depends on DTC signaling and the downstream function of the FBF-1/2 RNA-binding proteins. *glp-1* affects two processes needed for oocyte growth, cytoplasmic streaming and cellularization. *glp-1*(rf) mutants exhibit elevated rates of cytoplasmic streaming and delayed cellularization. Interestingly, abnormal oocyte growth in *glp-1* mutants, but not premature meiotic entry of germline stem cells, requires the function of the cell death pathway and germline innexins. Thus, the two major signaling centers in the adult hermaphrodite gonad, GLP-1 distally and MSP proximally, while individually functioning in a separate process-germline proliferation and meiotic maturation, respectively-work in concert to regulate the differentiation of female germ cells into functional oocytes.

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VAP MSP domains are secreted metabolic factors that bind to Eph and Roundabout receptors. **Sung Min Han**[1], Youfeng Yang[1], Hiroshi Tsuda[2], Chao Tong[2], Claire Haueter[2], Hugo J. Bellen[2], Michael A. Miller[1]. 1) Dept. of Cell Biology, University of Alabama at Birmingham, Birmingham, Alabama; 2) HHMI, Dept. of Molecular and Human Genetics, Baylor College of Medicine.

In C. elegans, MSPs function as secreted hormones that bind to Eph receptors and unidentified receptors to trigger oocyte maturation. Human VAPB, Drosophila VAP33, and C. elegans VPR-1 are homologous proteins called VAPs with an N-terminal MSP domain and a C-terminal transmembrane domain. A missense mutation (P56S) in the VAPB MSP domain is associated with amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), but the function of VAP proteins is not understood. Recently, we showed that VAP MSP domains are cleaved and secreted ligands for Eph receptors (Tsuda et al. 2008). The human VAPB MSP domain is present in blood serum and the P56S mutation prevents secretion in fly cells. These data suggest that VAPB proteins have a signaling function that is relevant to ALS pathogenesis. There is increasing evidence that ALS is a systemic disorder, as patients and mouse models can exhibit mitochondrial abnormalities inside and outside of the CNS, insulin resistance, dyslipidemia, and defective energy homeostasis. We show that in C. elegans and Drosophila loss of VAPs causes muscle mitochondrial defects, which can be rescued by VAP expression specifically in neurons. Genetic, biochemical, and molecular studies demonstrate that mitochondria from vap mutants have impaired respiration. These data support the hypothesis that VAP proteins are secreted signals that regulate mitochondrial function. However, Eph receptor mutants do not have mitochondrial defects. To identify new MSP domain receptors, we used a fluorescent binding assay. We show that MSP and VAP MSP domains bind to Roundabout (Robo) receptors, which are broadly expressed in neurons, muscle, and likely oocytes. sax-3 Robo is required for muscle mitochondrial function and vpr-1 and sax-3 act in the same genetic pathway. In addition to mitochondrial defects, loss of C. elegans vpr-1 causes defects consistent with impaired insulin signaling, including fat accumulation in the intestine and body wall muscle that is dependent on DAF-16/FOXO activity. The increased DAF-16 activity in vpr-1 mutants appears to be part of a compensatory mechanism. Loss of the vab-1 Eph receptor, a VPR-1 receptor, causes mild fat metabolism defects. Finally, vpr-1 mutants are sterile and neuronal overexpression can partially rescue these defects in some animals and cause germline tumors in others. Collectively, our data support a model in which VAPs are components of a neuroendocrine signaling mechanism that controls metabolism and energy homeostasis.

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MPK-1 ERK regulates NOS-3, FEM-3/E3 ubiquitin ligase complex and TRA-1 cascade during oogenesis to execute cell membrane organization and morphogenesis. **Swathi Arur**, Mitsue Omachi, Amanda Hay, Tim Schedl. Department of Genetics, Washington University School of Medicine.

Active MPK-1 ERK (dpMPK-1) displays a spatially restricted and dynamic pattern in the *C. elegans* hermaphrodite germline. It is inactive in the distal germline and first activated in pachytene germ cells. Once active, MPK-1 executes various cell biological processes such as pachytene cell membrane organization *via* activating or inactivating substrates upon phosphorylation. Absence of *mpk-1* signaling results in severe loss of cell membranes from pachytene germ cells. We find that a null allele of *nos-3*, a member of the NANOS family of translational repressors, partially suppresses the severe loss of membranes from the *mpk-1* null germ cells. NOS-3 is likely a direct target of MPK-1 as it is a robust *in vitro* substrate of activated ERK2 and *in vivo* MPK-1 dependent phospho-NOS-3 accumulates in pachytene stage germ cells overlapping with dpMPK-1. Together, this suggests that MPK-1 mediated phosphorylation likely inactivates NOS-3 during pachytene to promote membrane organization. Previous studies show that during sex determination NOS-3 represses FEM-3 translation. FEM-3 in turn functions as part of an E3 ubiquitin ligase complex to degrade nuclear TRA-1/Gli which acts as a transcriptional repressor. The sex determination function of this pathway likely acts in the distal germline. However, in pachytene germ cells, where MPK-1 mediated phosphorylation likely inactivates NOS-3, the resulting high FEM-3 levels should lead to decreased TRA-1. Consistent with this hypothesis, we find a higher accumulation of TRA-1 in distal germ line nuclei, but low/no accumulation where phosphorylated NOS-3 and dpMPK-1 accumulate. Also, we find that absence of *nos-3* results in lower accumulation of TRA-1 throughout presumably because of high FEM-3. Examination of *fem-3* null germlines reveals that indeed TRA-1 accumulates at a higher level in pachytene. Most interestingly, loss of *mpk-1* activity also results in higher level of TRA-1 suggesting a model where control of NOS-3 activity by MPK-1 dependent phosphorylation spatially regulates the NOS-3/FEM-3/TRA-1 cascade for two distinct biological outputs: female germ cell fate and membrane organization during oogenesis. In distal germline, where there is no active MPK-1, NOS-3 mediated repression of FEM-3 translation leads to high TRA-1 activity likely assisting in specification of the female germ cell fate. However, in pachytene, active MPK-1 through phosphorylation of NOS-3 switches pathway activity leading to inactivation of TRA-1, which presumably allows transcription of genes that function in membrane organization during oogenesis.

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EFA-6 induces microtubule catastrophe in early *C. elegans* embryos. **Sean O'Rourke**, Sara Christensen, Ronald Kwong, Bruce Bowerman. Inst Molec Biol, Univ Oregon, Eugene, OR.

During mitotic spindle assembly, microtubules polymerize outwards from centrosomes and reach the cell cortex. Microtubule-cortical contacts are important for spindle positioning and function. In early *C. elegans* embryos, microtubules contact the cortex briefly before undergoing catastrophe. Cortical molecules that regulate the switch from growing to shrinking microtubules have not been described. Here we show that a cortically-localized, putative GTPase exchange factor called EFA-6 promotes microtubule catastrophe, as embryos lacking this protein display long microtubules at the cell cortex. We have found that microtubule plus ends reside at the cortex greater than 5 times longer in *efa-6* mutant embryos. However, astral microtubules in embryos lacking EFA-6 are nucleated and grow at identical rates, when compared to wild-type embryos. The abnormally long microtubules cause centrosomes to detach from the male pronucleus, abolish anaphase spindle oscillations, and can suppress the embryonic lethality associated with partial loss of cytoplasmic dynein. EFA-6 is widely conserved and is known to exchange GDP for GTP on ARF6 GTPases. However, ARF-6 and EFA-6 exchange factor activity are not required for this regulation of microtubule dynamics. Instead, the N-terminus of EFA-6 is essential for this activity, as is a pleckstrin homology domain that presumably mediates cortical localization. Thus, EFA-6 has novel, exchange factor-independent function(s) during early embryonic cell divisions. We are currently testing whether the EFA-6 N-terminal domain is sufficient to promote microtubule catastrophe when it is targeted to the cortex through a CAAX motif, independent of its own PH domain.

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Establishment of PAR polarity in *C. elegans* through a reaction-diffusion mechanism. **Nathan W. Goehring**[1], Debanjan Chowdury[2], Philip Khuc-Trong[2], Ernesto Nicola[2], Stephan W. Grill[1,2], Anthony A Hyman[1]. 1) Max Planck Institute for Cell Biology and Genetics, Dresden, Germany; 2) Max Planck Institute for Physics of Complex Systems, Dresden, Germany.

A conserved network of PAR polarity proteins is required for establishment of cellular polarity in a wide variety of systems. These proteins are typically segregated into mutually exclusive, functionally distinct membrane domains which define the axis of polarity. In the one-celled *C. elegans* embryo, discrete sets of PAR proteins are partitioned into roughly equal sized anterior and posterior domains, which are essential for the proper inheritance of cell fate determinants during the asymmetric first cell division. The formation of PAR domains has been shown to depend on a highly contractile cortical actomyosin network that is itself polarized and is required for establishing PAR polarity. PAR domains also depend on mutual antagonistic interactions between PAR proteins, which are essential for maintaining their stable, mutually exclusive distribution within discrete domains. However, despite progress in understanding the molecular activities involved in these processes, we lack a basic physical mechanism for explaining how the activities of the actomyosin cortex and the PAR proteins are coupled in order to give rise to the stable, reproducibly sized PAR domains that are observed in the embryo. In order to provide insight into the types of mechanisms that could underlie the formation of PAR domains, we undertook a quantitative description of the dynamics of PAR proteins in C. elegans embryos. The results of this analysis indicate that PAR proteins diffuse extensively on the membrane of embryos, exchange between membrane-associated and cytoplasmic states, and are subject to advection by actomyosin-dependent cortical flow. We find that these observed behaviors, when coupled to the documented antagonism between PAR proteins, are sufficient to generate a reaction-diffusion driven system for establishing PAR polarity. The theoretical model we propose provides a single, rather simple physical mechanism that explains the actomyosin-dependent polarization of the embryo, the maintenance of mutually exclusive PAR domains, and the reproducible determination of domain size. Importantly, in this model the stable partitioning of the embryo into domains is a function of intrinsic properties of the PAR proteins and does not appear to depend on an underlying scaffold function of the actomyosin cortex. Rather, this work suggests that the actomyosin cortex acts primarily through the generation of cortical flow which provides a robust trigger to ensure that polarization of the PAR system occurs with the proper timing and geometry.

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PAR-2 is a microtubule-binding protein that links microtubules and cortical polarity in *C. elegans* zygotes. **Fumio Motegi**, Seth Zonies, Geraldine Seydoux. Molecular Biology and Genetics, Johns Hopkins University, HHMI, Baltimore, MD.

The *C. elegans* zygote becomes polarized shortly after fertilization by the sperm-donated microtubule-organizing center (MTOC), which induces PAR polarity on the cortex. How the MTOC communicates with the cortex is not known. Possible signals include proteins that accumulate on the MTOC's pericentriolar material (PCM) and the microtubules themselves (Motegi and Seydoux, 2007 for review). We have obtained evidence for a direct role for microtubules by studying PAR-2.

PAR-2 is a RING finger domain protein that accumulates on the cortex nearest the MTOC during polarization (Boyd et al, 1996). PAR-2 also accumulates on the MTOC itself, most prominently during mitosis. We have found that PAR-2 has a strong affinity for microtubules in vitro (KDapp<0.2 μM). This affinity depends on three separate microtubule-binding domains, which overlap with the domain in PAR-2 required for localization to the cortex (Hao et al., 2006). Mutations in one of the domains reduce PAR-2 affinity for microtubules by 5 fold. When expressed in zygotes depleted of endogenous PAR-2, this PAR-2 mutant was defective in localizing to MTOCs, but was able to localize to the posterior cortex and rescue the *par-2* embryonic lethality as efficiently as wild-type, suggesting that microtubule binding is not essential under normal conditions. The microtubule-binding defective mutant, however, was not able to localize to the cortex in zygotes that lack or delay PCM assembly or have reduced cortical actin contractility. In those zygotes, wild-type PAR-2 can still accumulate on the cortex nearest the MTOC, but PAR-2 defective in microtubule binding cannot and remains in the cytoplasm. We conclude that microtubule binding contributes to PAR-2 localization under conditions when the MTOC signal is compromised. Along with previous findings, these data suggest that polarity establishment in the zygote depends on two redundant mechanisms: a first mechanism dependent on efficient PCM assembly and actin contractility, and a second mechanism dependent on microtubules and PAR-2.

Boyd, L., Guo, S., Levitan, D., Stinchcomb, D.T., Kemphues, K.J. (1996). *Development* 122, 3075-3084.
Hao, Y., Boyd, L., Seydoux, G. (2006). *Developmental Cell* 10, 199-208.
Motegi, F., Seydoux, G. (2007). *JCB* 179, 367-369.

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PPH-6 Regulates AIR-1 Distribution to Modulate Cortical Pulling Forces during Spindle Positioning in One-cell Stage Embryos. **Katayoun Afshar**, Pierre Gönczy. Institute for Experimental Cancer Research (ISREC),School of Life Sciences, Swiss Federal Institute of Technology(EPFL), Lausanne, Switzerland.

Accurate spindle positioning is key for generating cellular diversity during development. In the *C. elegans* one-cell stage embryo, the mitotic spindle is positioned asymmetrically toward the posterior, resulting in unequal division. Spindle displacement follows from an imbalance of net pulling forces on the spindle poles, with more active force generators acting on the posterior side. The Gα subunits GOA-1 and GPA-16, the Goloco proteins GPR-1/2 and the Coiled-coil protein LIN-5, along with microtubule dynamics and dynein function are known to be required for generation of pulling forces. However, the mechanisms underlying modulation of the pulling forces in space and time are largely unknown. We have investigated the role of the Serine-Threonine phosphatase PPH-6 as a modulator of pulling forces. Depletion of PPH-6 or of its regulatory subunit causes notably defects in spindle behavior during anaphase. Spindle severing experiments indicate that PPH-6 acts as a positive regulator of pulling forces during anaphase. To understand the role of PPH-6, we performed mass spectrometry that led us to uncover that PPH-6 associates with the Aurora A homologue AIR-1. Interestingly, upon depletion of PPH-6, AIR-1 accumulates at the cell cortex, suggesting that PPH-6 negatively regulates cortical AIR-1 distribution. Upon double inactivation of *pph-6* and *lin-5*, AIR-1 still localizes to the cortex, suggesting that PPH-6 regulates AIR-1 distribution upstream of LIN-5. The cortical localization of AIR-1 in *pph-6(RNAi)* embryos is independent of TPXL-1, which is known to be required for AIR-1 astral distribution. To address whether excess AIR-1 at the cortex is responsible for the reduction of pulling forces in *pph-6(RNAi)* embryos, we inhibited AIR-1 using chemical inhibitors, and depleted its cortical localization during anaphase. Strikingly, pulling forces were rescued by such a drug treatment in *pph-6(RNAi)* embryos. Altogether, we have uncovered that PPH-6 is a novel regulator of AIR-1 that limits its cortical localization, which is necessary for proper generation of cortical pulling forces during spindle positioning in one-cell stage *C. elegans* embryos.

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A tug-of-war model for the centrosome centering in *Caenorhabditis elegans* early embryo. **K. Kimura**, A. Kimura. Cell Arch Lab, Center for Frontier Research, National Institute of Genetics, Mishima, Shizuoka, Japan.

The centrosome is a major microtubule organizing center in animal cells. The centrosome is generally positioned in the cell center by driving forces through the microtubules. Such positioning is critical for proper microtubule-dependent cellular activities such as cell division and organelle distribution. It has been widely assumed that the centering is powered by the microtubule pulling force, which depends on the minus-end directed motor protein, cytoplasmic dynein. To pull the centrosome via microtubules toward the center, DHC (dynein heavy chain), the subunit responsible for the motor activity, should be anchored to some intracellular structures. However, such structures remain unknown. Here, we propose a tug-of-war model that intracellular organelles associated with DHC have anchoring function to pull microtubules. Three lines of examinations support our model. We used *C. elegans* embryo at a pronuclear migration stage, a typical example of centrosome centering. First, we performed screening for genes involved in the anchoring function of the structures and identified *dyrb-1* encoding a light chain subunit of dynein. Live cell imaging of GFP fused DYRB-1 during the centering suggested that substantial fraction of DYRB-1 with the structure move along astral microtubules. Second, we analyzed organelle transport along astral microtubules to verify the model, and found that intracellular organelles such as early endosomes move along astral microtubules in a DHC and DYRB-1 dependent manner. Moreover, we found a strong correlation between organelle movement along astral microtubules and centrosome centering. Third, to test whether the organelle movements are required for centrosome centering, we attempted to decrease the organelle movements by knockdown of membrane traffic regulators and observed whether the centering is impaired. We found that the centering was delayed in embryos with reduced organelle movement. We confirmed that other events such as spindle rocking movement that are dependent on microtubules and DHC function are not impaired in these embryos. These results suggest that centrosome-directed movements of intracellular organelles produce a counteracting force against the movement that pulls the centrosome toward each moving organelles.

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A "Notch + Forkhead" code in the *C. elegans* pharynx. **Jeffrey P Rasmussen**[1,2], Kathryn English[2,3], James R Priess[1,2,3]. 1) Molecular and Cellular Biology Program, University of Washington, Seattle, WA; 2) Division of Basic Sciences, FHCRC, Seattle, WA; 3) HHMI, Seattle, WA.

We are studying the morphogenesis and differentiation of two adjacent, single-cell tubes in the digestive tract called pm8 and vpi1. These cells are born in the dorsal sector of the digestive tact primordium. pm8 and vpi1 partially delaminate from their neighbors, and invade ventral cells to wrap around the midline of the primordium to form tubes. These cells are born in identical sublineages, but show several differences. For example, each cell uses a distinct fusogen to self-fuse into a tube (thereby avoiding cross-fusion), and pm8 becomes a pharyngeal muscle while vpi1 is a non-muscular valve cell. We previously found that many, if not all, of these differences result from Notch signaling in pm8, and we here investigate how Notch controls the fate of pm8. While Notch interactions can involve only a few direct targets, such as genes encoding the REF-1/Hairy/Enhancer of Split proteins, we have thus far found at least six different Notch targets in the pm8 interaction. pm8 expression of the Notch target *pax-1* appears to require synergy between Forkhead box-containing transcription factors and the Notch transcriptional effector, LAG-1/Su(H). Predicted binding sites for both proteins are essential for pm8 expression, and are conserved in *pax-1* orthologs in the five sequenced *Caenorhabditis* genomes. Using comparative genomics, we identified additional *C. elegans* genes with similar, conserved sites. Thus far, three of the candidate genes tested are expressed in pm8, but not vpi1. We found that the predicted enhancer from one of these, *inx-11*, is necessary and sufficient for pm8 expression, and LAG-1/Su(H) binds the *inx-11* enhancer in vitro. *inx-11* encodes an innexin, a component of invertebrate gap junctions, which may function to coordinate the contraction of pm8 with other pharyngeal muscles. Gap junctions may be particularly important for pm8 function because, unlike the other pharyngeal muscle groups, pm8 is not directly connected to the nervous system. These results suggest that "Notch + Forkhead" is a combinatorial code that can drive pm8-specific expression, in contrast to the previously described "Notch + GATA" code that can drive intestine-specific expression. There appear to be at least two additional codes that regulate expression of the Notch targets *ref-1* and *ceh-24* in pm8, as enhancers for these genes contain critical sequences that do not resemble the Forkhead consensus motif. We are using a similar, comparative genomics approach to decipher how these additional networks may regulate pm8 morphogenesis and/or myogenesis.

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A Tension-dependent Pathway Promoting Fibrous Organelle Maturation During Embryonic Morphogenesis. **Huimin Zhang**, Hala Zahreddine, Marie Diogon, Frédéric Landmann, Yasuko Nagamatsu, Michel Labouesse. Cell & Developmental Biology, IGBMC, ILLKIRCH, France.

C. elegans body-wall muscles are required for embryonic development. Loss of muscle tension results in embryonic elongation arrest at the 2-fold stage. However how muscle contraction promotes hypodermal extension still remains unknown. To address this issue, we focused on the attachment complex fibrous organelles(FO), the only structure connecting muscles to the hypodermis. We present evidence that a mechanotransduction pathway might exist between muscles and FOs, contributing to hypodermal elongation. C. elegans FOs are composed of two hemidesmosome-like complexes at both sides of the hypodermis, connected by intermediate filaments(IF) in between. Intact FOs are very important for transmitting muscle tension to the cuticle. Consistent with previous reports, our results showed that the FO organization changes when muscles start to contract, and their integrity requires muscle tension. To analyze FO functions in C. elegans, we performed a genome-wide RNAi screen, searching for genes that decrease the viability of a weak VAB-10/plakin mutant, a key component of the FO complex. We identified 14 candidate genes. In particular, we showed that the p21-activated kinase homologue PAK-1 is a novel FO component. Strong mutations in this gene combined with the viable vab-10 allele destabilize FOs, resulting in severe epidermal defects. By 2-dimensional electrophoresis and antibody staining, we found that PAK-1 controls phosphorylation of IFs and their organization. More interestingly, hypodermal localized PAK-1 activity requires tension exerted by muscles, suggesting that PAK-1 is downstream of a novel mechanotransduction pathway. Combining biochemical and genetic approaches, we propose that this mechano-sensing machinery does not involve PAK-1 protein delocalization or phosphorylation. Instead, preliminary results showed that constitutively active CDC-42 can rescue the loss of IF phosphorylation observed in muscle mutants, indicating that muscle tension more likely activates PAK-1 through Rac/Cdc-42 signaling. Altogether, we suggest that C.elegans muscle contraction generates a mechanical response in the hypodermis through FOs. Possibly by turning on CED-10/CDC-42 signalling, two classic upstream activators of PAK-1, the muscle tension triggers a chemical signal that finally results in phosphorylation of IFs. This process enables FO maturation into a junction able to resist mechanical stress. Our finding suggests that the C.elegans fibrous organelles are not only an attachment structure, but also a mechanosensor transducing physical tension into chemical signals.

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DRAG-1 is a cell type specific modulator of the Sma/Mab TGF-beta pathway in *C. elegans*. **C. Tian**, D. Sen, H. Shi, M. Foehr, Y. Plavskin, A. Lindy, A. Al-Barwani, J. Liu. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Members of the transforming growth factor β (TGF-β) superfamily play important roles during metazoan development, including cell fate specification, cell proliferation, cell migration and cell death. Mutations in the TGF-β pathway can lead to tumorigenesis and many other somatic and hereditary disorders. In *C. elegans*, the Sma/Mab TGF-β pathway regulates body size and male tail patterning. Previous studies from our lab have found that the Sma/Mab TGF-β pathway also regulates mesoderm development. In particular, mutations in the C2H2 zinc finger protein SMA-9 cause a dorsal to ventral fate transformation in the postembryonic mesodermal lineage, the M lineage, and this defect can be suppressed by mutations in all the core components of the Sma/Mab TGF-β pathway, suggesting that SMA-9 normally antagonizes the function of Sma/Mab TGF-β signaling in patterning the M lineage (Foehr et al., 2007). In the same *sma-9* suppressor screen as described in Foehr et al. (2007), we identified a single locus, recessive *sma-9* suppressor mutation *drag-1(jj4)*. Like the Sma/Mab core pathway mutants, *jj4* mutants are small and suppress the M lineage defects of *sma-9* mutants. However, unlike the Sma/Mab pathway mutants, *jj4* mutants do not have male tail defect. Genetic epistasis studies between *jj4* and various mutations in the Sma/Mab pathway placed DRAG-1 in the Sma/Mab TGF-β pathway at the level of the ligand-receptor. We mapped and cloned the *drag-1* gene and found that *drag-1* encodes a putative GPI-anchored protein that is a homolog of the vertebrate BMP co-receptor Dragon. Dragon is a member of the RGM (Repulsive Guidance Molecule) family that is conserved in vertebrates and *C. elegans*, but not in Drosophila. We found that *drag-1* is expressed in the same cells that express *sma-6*, one of the Sma/Mab TGF-β receptors, and that a functional DRAG-1::GFP fusion is localized outside of the nucleus. Deleting the putative GPI anchor region partially disrupted the function of *drag-1*, while artificially tethering DRAG-1 to the plasma membrane still allowed DRAG-1 to function. Taken together, our results suggest that DRAG-1 likely functions as a co-receptor in the Sma/Mab TGF-β pathway. We are currently determining whether or not DRAG-1 interacts with the ligand and/or receptors of the Sma/Mab pathway and assaying the functional significance of the interactions *in vivo*.

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A single immunoglobulin domain protein required for the localization of acetylcholine receptors at the *C.elegans* neuromuscular junction. **Georgia Rapti**[1], Janet Richmond[2], Jean-Louis Bessereau[1]. 1) Ecole Normale Superieure- INSERM U789, Paris, France,; 2) University of Illinois, Chicago, USA.

Acetylcholine receptors sensitive to the nematode-specific cholinergic agonist levamisole (L-AChRs) form clusters at the *C. elegans* neuromuscular junctions (NMJs) and their proper localization is critical for efficient excitatory neurotransmission. Here we demonstrate that *oig-4* (one Ig domain-4) is a new gene required for L-AChR synaptic clustering. We have previously shown that L-AChR clustering requires two proteins, LEV-9 and LEV-10, which form an extracellular synaptic scaffold. LEV-9, a CCP-domain rich secreted protein, physically interacts with the ectodomain of LEV 10, a CUB-domain rich type 1 transmembrane protein. Interestingly, none of the proteins of the L-AChR/LEV-9/LEV-10 complex forms clusters at the synapse by itself, indicating that additional components are necessary for synaptic localization of this complex.

lev-10 and *lev-9* mutants show partial resistance to levamisole: they sense levamisole and hypercontract but do not die on drug concentrations lethal for wild-type animals. To identify new components of the L-AChR clustering machinery we performed a screen for mutants with partial resistance to levamisole after EMS mutagenesis and we isolated a new mutant allele, *kr39*. Using classical genetic mapping we identified a mis-sense point mutation in the predicted gene *oig-4*. We isolated a second *oig-4* mutant allele in a non-complementation screen. In both mutants, levamisole-resistance is rescued by providing an *oig-4* genomic fragment. Immunostaining of L-AChR subunits and in vivo imaging of knock-in strains with fluorescent L-AChR subunits demonstrate that L-AChRs do not localize properly at the NMJs of *oig-4* mutants. Moreover, the LEV-10 protein is no longer detected at synapses of these mutants. However, presynaptic boutons are properly formed. OIG-4 is specifically required for the L-AChR clustering as the UNC-49 GABA receptor and the ACR-16 N-AChRs are clustered normally at NMJs of *oig-4* mutants.

oig-4 is predicted to encode a secreted protein with a single immunoglobulin (Ig) domain. We demonstrated that OIG-4 is expressed predominantly in the body wall muscle. Its muscle expression is sufficient to rescue the partial levamisole-resistance phenotype. A functional OIG-4-GFP protein is secreted when expressed by the muscle and it forms clusters at NMJs. Altogether, these results suggest that we have identified a new extracellular synaptic protein that might be part of the L-AChR/LEV-9/LEV-10 scaffold and may link this complex to local synaptic determinants.

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Synaptic vesicle acidification is a checkpoint for vesicle fusion. **Glen G. Ernstrom**, Mark T. Palfreyman, Shigeki Watanabe, Erik M. Jorgensen. Department of Biology and the Howard Hughes Medical Institute, University of Utah, Salt Lake City, UT.

The fusion of a single synaptic vesicle releases a quantum of neurotransmitter that evokes a corresponding quantal electrical event in the postsynaptic cell. Some perturbations such as repetitive stimulation or overexpression of vesicular neurotransmitter transporters can alter quantal size. However, under normal circumstances quantal events are strikingly uniform in size. The mechanisms that maintain this characteristic size are unknown. We have used hypomorphic mutations in genes that are involved in the loading of neurotransmitter into synaptic vesicles to investigate quantal size homeostasis. Our readout was the frequency and size of spontaneous postsynaptic currents at the neuromuscular junction. As expected, a weak mutation in the glutamic acid decarboxylase enzyme *unc-25(sa94)* and a weak mutation in the vesicular GABA transporter *unc-47(ox135)* significantly decreased GABA quantal size.

A vesicular proton gradient is also required for neurotransmitter loading. Thus, we also examined the effects of V-ATPase proton pump mutations on neurotransmitter loading. Surprisingly, we found that a weak mutation in the *B* subunit [*vha-12(n2915)*] and the *a* subunit [*unc-32(e189)*]of the V-ATPase did not decrease quantal size. Rather, the frequency of the events was reduced. By targeting the pH-sensitive GFP, phluorin, to the lumen of synaptic vesicles we confirmed that both *vha-12(n2915)* and *unc-32(e189)* synaptic vesicles were more alkaline than wild-type vesicles. *In situ* FM dye uptake studies demonstrated that synaptic vesicle cycling was impaired in the V-ATPase mutants. This result suggests that the change in event frequency was due to a defect in neurotransmitter release rather than the fusion of empty vesicles. Finally we demonstrated that the effect of the V-ATPase mutants could be phenocopied by pharmacological inhibition of the V-ATPase (i.e. 15 minute exposure to bafilomycin A1). Our results are consistent with an acidification checkpoint model whereby only fully acidified synaptic vesicles are competent to fuse. The uniform size of quantal events may be controlled not by a direct mechanism that monitors neurotransmitter content in a vesicle, but instead by an indirect mechanism affected by the acidification state of the vesicle.

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Dysferlin/*fer-1* promotes cholinergic synaptic transmission in *C. elegans* and mice. **P. Krajacic**[1,2], M. Mosqueira[1,2], J. Hermanowski[1,2], O. Lozynska[1,2], J. Snitzman[3], X. Shen[3], P. Arratia[3], T.S. Khurana[1,2], T. Lamitina[1]. 1) Physiology, University of Pennsylvania, Philadelphia, PA; 2) Pennsylvania Muscle Institute, University of Pennsylvania, Philadelphia, PA; 3) Dept of Mechanical Engineering, University of Pennsylvania, Philadelphia, PA.

The autosomally inherited disease Limb Girdle Muscular Dystrophy 2B (LGMD2B) is a progressive and incurable muscle disorder. Dysferlin gene mutations cause enigmatically disparate phenotypes in LGMD2B, but Dysferlin's function and the molecular mechanisms underlying disease pathogenesis are not fully understood. To better understand the pathogenesis of this disease, we studied the *C. elegans* Dysferlin homolog *fer-1*. Previous studies have characterized *fer-1* only with regards to its roles in spermatogenesis. We found that *fer-1* is also expressed in muscle and that genes involved in locomotion are mis-regulated in *fer-1* mutants, suggesting that *fer-1* might alter muscle functional properties. To examine this possibility, we studied aspects of muscle organization in *fer-1* mutants. We found that *fer-1* mutants exhibit diminished post-synaptic localization of acetylcholine receptors (AChRs) at the neuromuscular junction. The localization of both GABA receptors (*unc-49*) and pre-synaptic vesicles was unaffected in *fer-1* mutants, suggesting a specific role for Dysferlin as a regulator of cholinergic synaptic transmission. Consistent with these data, three independently derived *fer-1* mutants were resistant to drugs that increase cholinergic signaling, and this phenotype could be rescued with a *fer-1*+ transgene. Electrophysiological approaches confirm that *fer-1* mutants have a defect in acetylcholine-dependent synaptic signaling that mimics phenotypes observed in an AChR mutant. Acute administration of a cholinesterase inhibitor improved the excitability and functional properties of *fer-1* muscle. To determine whether the synaptic functions of *fer-1* are evolutionarily conserved, we examined synaptic signaling in a mouse model of Dysferlin deficiency. Like *C. elegans fer-1* mutants, Dysferlin (-/-) mice also have physiological defects in muscle force generation upon repetitive neuronal stimulation ("rep-stim"). "Rep-stim" force decrement is a classical phenotype of other disease of cholinergic dysfunction, such as Myasthenia gravis. Preliminary studies suggest that the rep-stim decrement of Dysferlin mice is reversed by chronic administration of the cholinesterase inhibitor Pyridostigmine bromide. These data suggest that Dysferlin plays an evolutionarily conserved role in cholinergic synaptic signaling in both C. elegans and mammals and that drugs enhancing cholinergic signaling might be an effective therapeutic strategy for treating LGMD2B.

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BKIP-1 is a Novel Regulatory Protein Critical to SLO-1 Function in vivo. **Bojun Chen**, Qian Ge, Haiying Zhan, Zhao-Wen Wang. Dep Neuroscience, Univ Connecticut Health Ctr, Farmington, CT.

The BK channel is a large-conductance potassium channel gated by both membrane potential and intracellular calcium. It is almost ubiquitously expressed and performs many important physiological functions, including shaping action potential wave form and regulating neurotransmitter release. The C. elegans BK channel SLO-1 is expressed in most, if not all, neurons and several types of muscles (body-wall, vulval, and anal depressor muscles). BK channel functional properties are tuned to specific cellular needs through a variety of mechanisms, including association with auxiliary/regulatory proteins. Several BK channel auxiliary/regulatory proteins have been identified in mammals (known as β1-, β2-, β3, and β4-subunits) and Drosophila (known as Slob and dSLIP1). However, none of them has obvious homologues in C. elegans. It is likely that there are other unidentified BK channel auxiliary/regulatory proteins. In an attempt to identify the putative additional BK channel auxiliary/regulatory proteins, we performed a genetic screen for mutants that suppressed the lethargic phenotype caused by expressing a slo-1 gain-of-function (gf) transgene. This analysis led to the identification of bkip-1 (BK channel interacting protein 1), which encodes a putative integrative membrane protein distinct from any of the known BK channel auxiliary/regulatory proteins. bkip-1 mutants completely or near completely suppressed the lethargic phenotype of slo-1(gf), and were grossly indistinguishable from slo-1 loss-of-function (lf) mutants in locomotion behaviors. Analyses of postsynaptic currents at the neuromuscular junction showed that neurotransmitter release was similarly increased in bkip-1(lf) mutant as in slo-1(lf) mutant, and that bkip-1(lf) eliminated an inhibitory effect of slo-1(gf) on neurotransmitter release. In bkip-1 mutants, slo-1 transcription was normal but SLO-1 protein level in the nerve ring was significantly reduced, suggesting that BKIP-1 may play a role in SLO-1 synthesis, surface expression or localization. The expression pattern of bkip-1 was grossly indistinguishable from that of slo-1. When analyzed in the Xenopus oocyte expression system, BKIP-1 increased SLO-1 membrane current density, and, in a Ca2+-dependent manner, slowed SLO-1 activation and shifted the conductance-voltage (G-V) relationship. These observations establish BKIP-1 as an important SLO-1 regulatory protein in C. elegans.

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A Genetic Suppressor Screen with Whole Genome Sequencing identifies novel effectors of RHO-1 in the nervous system. **Andrew Porter**, Rachel McMullan, Stephen Nurrish. MRC Laboratory of Molecular Cell Biology, University College, London, United Kingdom.

The RHO-1 GTPase acts cell autonomously to increase neurotransmitter (NT) release by inhibiting a diacylgycerol kinase (DGK-1), causing an increase in diacylglycerol (DAG). UNC-13 and PKC-1 are binding targets of DAG, and known effectors of RHO-1 signalling. However, RHO-1 still modulates NT in *dgk-1* mutants, suggesting DGK-1 is not the only effector downstream of RHO-1. Animals overexpressing constitutively active (CA) RHO-1 in cholinergic neurons have a loopy locomotion and increased rate of paralysis on 1mM aldicarb. After EMS mutagenesis, we selected mutants with more wild-type locomotion. From 2000 haploid genomes we found 120 suppressors. We conducted a secondary screen for suppression of CA RHO-1 expressed from the heat shock promoter of a separate transgene, which causes both neuronal and non-neuronal phenotypes. We identified 12 mutants which suppressed the neuronal effects of CA RHO-1 (loopy locomotion) but not the non-neuronal effects. Suppressor *nz94* had an additional 'fainter' phenotype, where the animals stop abruptly. It failed to complement the known fainter mutation *unc-80*, and we identified a premature stop codon in the *unc-80* gene in *nz94*. UNC-80 (along with UNC-79) forms a complex with NCA-1, a novel neuronal ion channel. Mutations in these genes suppress the loopy phenotype of CA RHO-1 but have varying effects on the aldicarb response. This raises interesting questions about the relationship between the behaviour and absolute amounts of NT, previously thought to be strongly correlated. NCA-1 channels and their regulators are highly conserved. Loss of function of the Drosophila equivalents causes uncoordinated locomotion; loss in mice causes death from apnoea 24hrs after birth. It appears this complex has a conserved role in regulating state changes (moving/not moving, breathing/not breathing). We are currently investigating whether the NCA-1 channel is a direct target of RHO-1 and will discuss the site of action of the NCA-1 complex in mediating RHO-1 driven changes in locomotion, and NCA-1's role in established pathways which alter locomotory behaviour. Three additional suppressor mutants were selected for Illumina whole genome sequencing. We hope to identify other genes downstream of rho-1 in the nervous system, and to test the feasibility whole genome sequencing as an alternative to traditional mapping. We are sequencing a completely unbackcrossed mutant, isolated directly from the screen, which will push the limits of the current technology. By the time of the meeting we hope to have identified the mutant genes in these CA RHO-1 suppressors.

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Neuroligin deficient mutants of *C. elegans* are hypersensitive to oxidative stress and some heavy metals. **Jim Rand**[1,2,3], Jerrod Hunter[1,2], Greg Mullen[1], Jessica Heatherly[1,3], John McManus[1], Angie Duke[1]. 1) Genetic Models of Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104; 2) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; 3) Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Neuroligins are postsynaptic cell adhesion molecules that bind specifically to a set of presynaptic membrane proteins called neurexins. Mutations in the human neuroligin genes NLGN3 and NLGN4 are associated with a subset of cases of autism spectrum disorders (Jamain et al., 2003; Laumonnier et al., 2004; Yan et al., 2005). *C. elegans* has a single neuroligin gene (*nlg-1*), and approximately one-sixth of *C. elegans* neurons, including some sensory neurons, interneurons, and a subset of cholinergic motor neurons, express a neuroligin transcriptional reporter (see abstract by Hunter et al.). Null *nlg-1* mutants are viable, with a grossly normal nervous system, and although they are not deficient in any major motor functions, they are defective in a subset of sensory behaviors and sensory processing (see abstract by Heatherly et al.). In addition, *nlg-1* mutants are hypersensitive to oxidative stress (i.e., paraquat treatment); this is a completely unexpected phenotype for a synaptic protein mutant. Like many other stress-sensitive mutants, *nlg-1* mutants have a reduced lifespan and an increased level of oxidatively damaged proteins. *nlg-1* mutants are also hypersensitive to inorganic ($HgCl_2$) and organic (thimerosal) mercury compounds and copper compounds, but not to cadmium ($CdCl_2$). There is a body of literature documenting in individuals with autism the presence of biomarkers associated with oxidative stress, and it is striking that a mutation which, in humans, is associated with autism produces a similar oxidative stress phenotype in nematodes. (Supported by a grant from Autism Speaks).

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Glial DEG/ENaC channel ACD-1 functions in odor sensation in *C. elegans*. Ying Wang, **Laura Bianchi**. Dept. of Physiology and Biophysics, University of Miami, Miami, FL.

DEG/ENaC channel subunits are two-transmembrane domain proteins that trimerize to form voltage-independent Na+ or Na^{+}/Ca^{2+}-selective ion channels. Neuronally expressed DEG/ENaC channels have been implicated in sensory perception across species including touch sensation, pain sensation and proprioception. We recently reported that *C. elegans* DEG/ENaC channel ACD-1 participates in acid avoidance behavior and chemotaxis to lysine-acetate. Surprisingly, we found that ACD-1 is expressed in amphid glia rather than neurons where it acts to orchestrate these behaviors. More specifically, we found that *acd-1* knock-out does not cause sensory deficits on its own but that it exacerbates mild defects in acid avoidance and chemotaxis to lysine-acetate caused by mutations in *deg-1*, another DEG/ENaC channel gene expressed sensory neurons. Because acidic solutions and lysine-acetate cause extracellular and intracellular acidification respectively, we tested if ACD-1 is sensitive to protons. In the heterologous expression studies, we found that ACD-1 is inhibited by both extracellular and intracellular acidification. We thus hypothesized that exacerbation of sensory defects by *acd-1* knock-out may occur if ACD-1 channel is sensitive to the cue that acts on the specific sensory neuron whose function has been compromised by mutations in neuronal genes. To test this hypothesis, we assayed ACD-1 sensitivity to odors and tastants in *Xenopus* oocytes. We found that ACD-1 channels are inhibited by the odor isoamyl alcohol, which attracts *C. elegans*. We thus acquired a *C. elegans* mutant strain with no detectable abnormalities in sensory perception, but that is mutant for the gene *tax-2* (a cyclic nucleotide gated channel) required for sensing isoamyl alcohol and expressed in AWC sensory neurons, among others. *tax-2(p694)* mutation deletes part of the promoter region of *tax-2* reducing TAX-2 channel expression level. We built *tax-2(p694);acd-1* double mutants and tested attraction to the odor isoamyl alcohol. Consistent with our hypothesis, we found that *tax-2(p694);acd-1* double mutant animals are defective in attraction to this odor. Notably, *acd-1* and *tax-2(p694)* single mutants detect this odor just like wild type animals. Functional imaging studies are under way to determine the consequences of *acd-1* and *tax-2* mutations on AWC neurons responses to odors.

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AWC sensory neurons release two neurotransmitters to regulate exploratory behavior upon removal from food. **Sreekanth H. Chalasani**[1], Dirk Albrecht[1], Saul Kato[2], Cornelia I. Bargmann[1]. 1) Laboratory Neural Circuits and Behavior, Box 204, HHMI/Rockefeller Univ, New York, NY 10065; 2) Center for Theoretical Neuroscience Columbia University College of Physicians and Surgeons 1501 Riverside Dr., New York, NY 10032.

Animals increase their pirouette frequency in response to removal from food stimulus for a period of 15 min. The AWC and ASK sensory neurons and the AIB interneurons stimulate pirouettes immediately after removal from food, while the AIY and AIA interneurons inhibit pirouettes (Wakabayashi *et al* 2004, Gray *et al* 2005).

We have found that AWC sensory neurons become active in response to removal of stimulus, releasing two neurotransmitters (glutamate and a neuropeptide NLP-1). The released glutamate acts to activate AIB and inhibit AIY interneurons, promoting reversals (Chalasani *et al* 2007). In contrast to glutamate, AWC-released NLP-1 acts on AIA interneurons to suppress reversals, suggesting that reversal frequencies are regulated by at least two opposing signaling systems. AWC calcium responses are modulated in these neurotransmitter mutants, suggesting that feedback pathways affect AWC neuronal activity.

References:

Chalasani, S. H., Chronis, N., Tsunozaki, M., Gray, J. M., Ramot, D., Goodman, M. B., and Bargmann, C. I. (2007). Dissecting a circuit for olfactory behaviour in *Caenorhabditis elegans*. Nature **450**, 63-70.

Gray, J.M., Hill, J.J., and Bargmann, C.I. (2005). A circuit for navigation in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. **102**, 3184-3191.

Wakabayashi, T., Kitagawa, I., and Shingai, R. (2004). Neurons regulating the duration of forward locomotion in *Caenorhabditis elegans*. Neurosci. Res. **50**, 103-111.

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Dopaminergic neuronal cell-fate mutants retrieved through automated screening. **Maria Doitsidou**, Nuria Flames, Oliver Hobert. Columbia University Medical Center, New York, NY.

We are interested in dopaminergic neuronal specification and we have utilized an automated method to isolate *C. elegans* mutants, in which these neurons fail to be appropriately specified. A fluorescence activated sorting mechanism implemented in the COPAS Biosort machine allowed the isolation of mutants with subtle alterations in the cellular specificity of gfp expression(1). This methodology was significantly more efficient than comparable manual screens. We present the various mutants that were isolated from the screens. Some of these mutants are alleles of known transcription factors previously not implicated in dopaminergic cell differentiation, while others define novel genetic loci. Particular focus will be given in the phenotypic characterization of two mutants recently cloned using deep sequencing technology (2)(*), namely a homeobox protein of the Distal-less class and a zing finger transcription factor.

(1): Doitsidou *et al*., Nature Methods, October 2008

(2): Sarin *et al*., Nature Methods, October 2008

(*): see ‘whole genome sequencing for mutant identification’ workshop, and Bigelow H. poster.

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Development of a tetracycline controllable expression system for *C. elegans*. **U. Schaeffer[1,2]**, A. Feldmann[1,2], R. Baumeister[1,2]. 1) ZBSA, University of Freiburg, Freiburg, Germany; 2) Bio3/Bioinformatics and Molecular Genetics, University of Freiburg, Germany.

Spatial and temporal control of gene expression is an important tool for many studies in multi-cellular model organisms.

One of the most prominent inducible expression systems is based on a modified bacterial transcription factor called “rtTA”. This transcription factor is only active in the presence of tetracycline, thus the timing of expression of a gene of interest can be controlled by the addition or removal of tetracycline. Binding of “rtTA” to tetracycline results in its interaction with the DNA-response element “tetO”, and activates the expression of genes under the control of this response element. Using an appropriate promoter to drive the expression of rtTA the induction of gene expression can also be spatially restricted to a specific tissue.

The inducible “Tet-on” expression system has already been used in a variety of model organisms such as *Drosophila*, Zebrafish or mice. We have focused our efforts to adjust this system for *C. elegans*. Until now we already have generated several transgenic “driver” strains and “reporter” strains. The “driver” strains contain a recombinant rtTA under control of tissue specific promoters. The “reporter” strains harbor a Tet-response element that controls one of several reporter genes, such as GFP or Venus.

We will present driver strains in which gene expression can be induced specifically in e. g. the muscles, in all neurons or only in a subset of neurons. The reporter strains that were generated contain the coding sequences for nuclear localized GFP or cytosolic Venus protein under control of the tetO element and a minimal promoter. We have already used these strains to investigate the kinetics of the system with fluorescence microscopy and qPCR. The background expression of the system was also tested using Western blot analyses.

Additionally, we plan to provide Gateway-compatible expression vectors in which the rtTA response element is placed upstream of the Gateway-cassette. These plasmids can be used to introduce any cDNA from the ORFeome library and also contain the *unc-119* rescue element to allow the selection of transgenic animals. Currently, we are testing the potency of the system in several applications e. g. in rescue experiments with *unc-54* mutants or by the expression of toxic gene products.

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Microfluidic *in vivo* screening for compounds affecting neural regeneration in *Caenorhabditis elegans*. **Chrysanthi Samara[1]**, Christopher Rohde[1], Cody Gilleland[1], Stephanie Norton[2], Stephen Haggarty[2], Mehmet Yanik[1]. 1) Research Laboratory of Electronics, Massachusetts Institute of Technology, Cambridge, MA; 2) Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA.

Therapeutic treatment of central nervous system pathologies, such as spinal cord injuries, brain trauma, stroke, and neurodegenerative disorders, will greatly benefit from the discovery of small molecules that enhance neuronal growth after injury. Identification of a diverse repertoire of such molecules and of their cellular targets can also provide important tools for fundamental investigations of the mechanisms involved in the regeneration process. Currently, small-molecule screens for factors affecting neuronal regrowth can only be performed using simple *in vitro* cell culture systems. However, these systems do not truly represent *in vivo* environment. Importantly, off-target, toxic or lethal effects of chemical compounds can only manifest in model organisms with multiple tissue types. Thus, the thorough investigation of neuronal regeneration mechanisms requires *in vivo* neuronal injury models. *In vivo* neuronal regeneration studies have been performed mainly in mice and rats. However, their long developmental periods, complicated genetics and biology, and expensive maintenance limit large-scale studies in these animals. We previously demonstrated femtosecond laser microsurgery as a highly precise and reproducible injury method for studying axonal regeneration mechanisms in *Caenorhabditis elegans*[1,2]. Wild type nematodes move constantly, and to perform precise laser axotomy or imaging at the cellular level, animals must be immobilized. Therefore, we developed microfluidic on-chip technologies that allow automated and rapid manipulation, orientation, and non-invasive immobilization of *C. elegans* for sub-cellular resolution imaging and femtosecond-laser microsurgery[3,4]. These technologies can be used for high-throughput genetic and compound assays. We report here the results of the *in vivo* small-molecule screens for compounds affecting axonal regeneration after laser-induced axotomy in *C. elegans*. Using the technologies described above, we screened a library of small molecules. A number of compounds with a wide variety of cellular targets, such as cytoskeletal components, vesicle trafficking, and protein kinases were found to affect mechanosensory neuron regeneration following laser axotomy. REFERENCES: 1.Yanik M.F. et al., (2004). Nature. 432, 822 2.Yanik M.F. et al., (2006). IEEE J Sel Top Quant Electron. 12, 1283-91 3.Rohde C.B. et al., (2007). Proc Natl Acad Sci U S A. 104, 13891-5 4.Zeng F. et al., (2008). Lab Chip. 8, 653-6.

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A microfluidic chip enabling high-throughput in vivo femtosecond laser nanoaxotomy for nerve regeneration studies. Frederic Bourgeois[1], Samuel Guo[1], Navid Ghorashian[1], Trushal Chokshi[2], Massimo Hilliard[3], Nikos Chronis[2], **Adela Ben-Yakar[1]**. 1) Mechanical Engineering, University of Texas at Austin, Austin, TX; 2) Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI; 3) Brain Institute, University of Queensland, St. Lucia, Australia.

The nematode Caenorhabditis elegans is an ideal organism for studying one of the present challenges in neuroscience: nerve regeneration and degeneration. A thorough understanding of these highly dynamic processes requires experimental conditions that minimally affect the animal: no chemicals for immobilization, a reduced number of manipulations, and a reconstituted environment suitable for the animal’s development. We developed a microfluidic device, the ‘nanoaxotomy’ chip that fulfills all these criteria and can easily be automated to enable high-throughput genetic and pharmacological screenings. Using this chip, we performed in vivo nanoaxotomy and subsequent time-lapse imaging of regrowing axons in the absence of anesthetics, with the same precision and accuracy as we previously achieved on agar pad with anesthetics. The nanoaxotomy chip is designed in such a way that the worms are serially operated, minimizing the time during which they are immobilized and possibly improving their well-being. Notably, we observed that without anesthetics, axons of both motor and touch neurons can regrow much faster. The severed axons of the touch neurons reconnect to their distal stumps within 1-2 hours whereas those of the motor neurons regrow all the way to their target within 6-8 hours. The advantages of this microfluidic chip over the immobilization techniques previously used in studies of C. elegans, such as anesthesia on agar pads or glue, are: (i) the use of no chemicals other than the liquid growth medium to interfere with the physiological processes of the worms, possibly increasing nerve regeneration success, (ii) the adaptive deflection of the membrane allows the immobilization of the worms from L4 to adult size, (iii) the worms do not need a recovery period after surgery, permitting immediate behavioral study of the post-axotomy functionality, (iv) the sample population is well contained, and experiment conditions are easily reproducible because the trap for surgery and the environment for recovery are on the same chip and finally, (v) the design of the chip is simple enough to be adapted to other organisms or many other kinds of experiments, including ablation, irradiation, stimulation or simply observation, widening the possibilities of high-throughput biological investigations.

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Trimethylation of lysine 36 of histone H3 is a chromatin mark for expressed exons in C. elegans, humans, and mice. **Paulina M. Kolasinska-Zwierz**[1], Thomas Down[1], Isabel Latorre[1], Tao Liu[2], Shirley Liu[2,3], Julie Ahringer[1]. 1) The Gurdon Institute and Department of Genetics, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, United Kingdom; 2) Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, 44 Binney Street, Boston, Massachusetts 02115, USA; 3) Department of Biostatistics, Harvard School of Public Health, Boston, Massachusetts 02115, USA.

Chromatin regulation has been studied in a variety of systems, but most extensively in unicellular yeasts and mammalian cells. *C. elegans* is an excellent alternative system for studies of chromatin regulation due to its well-annotated genome, the ease of RNAi, and the rich resource of chromatin mutants. Importantly, *C. elegans* has a complement of chromatin factors very similar to that of humans, in contrast to yeast. Because modifications to histone tails are correlated with and can regulate chromatin structure, their patterns of localization can provide a framework for studying chromatin function. Thus, as part of the modENCODE consortium aimed at identifying functional elements in the *C. elegans* genome (http://www.modencode.org/), we generated a genome-wide map of histone H3 tail methylations.

We find that *C. elegans* genes show similarities in distributions of histone modifications to those of other organisms, with H3K4me3 near transcription start sites, H3K36me3 in the body of genes, and H3K9me3 enriched on silent genes. Unexpectedly, we also observe a striking novel pattern: exons are preferentially marked with H3K36me3 relative to introns. H3K36me3 exon marking is dependent on transcription and its level is lower in alternatively spliced exons, supporting a splicing related marking mechanism. We further show that the difference in H3K36me3 marking between exons and introns is evolutionarily conserved in human and mouse. We are currently investigating the mechanism and function of H3K36me3 exon marking, which we propose provides a dynamic link between transcription and splicing.

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Novel proteins bound to SL1 and Sm Y RNAs: possible roles in recycling Sm proteins. Peg MacMorris, Tassa Saldi, **Tom Blumenthal**. Dept Molec, Cellular, Dev Biol, Univ Colorado, Boulder, CO.

C. elegans codes for two classes of SL RNA, SL1 RNA and SL2 RNA, that donate their 5' 22 nt to mRNAs in trans-splicing. A two-polypeptide complex, SNA-2/SNA-1, unique to nematodes, is bound to the SL1 RNA but not to SL2 RNA. Surprisingly however, a different class of snRNA, whose function is unknown, also unique to nematodes, Sm Y RNA, contains a SNA-2/SUT-1 complex, where SUT-1 is a paralog of SNA-1. Deletions of *sna-1* or *sut-1* are viable, but 15° sterile. They can also phenocopy a variety of morphological abnormalities at 15°. Both the *sna-2* deletion and the *sna-1/sut-1* double deletion are lethal. Thus SNA-1 and SUT-1 are functionally redundant, even though they are bound to different classes of snRNP. Why does SL2 RNA have neither of these proteins? How can these results be rationalized? The answer may lie in the issue of how the Sm ring proteins are recycled after trans-splicing. SL1, SL2 and Sm Y snRNPs all contain the Sm proteins, which are normally components of stable spliceosomal snRNPs, U1, U2 etc. We think the SNA and SUT-1 proteins may collaborate with Sm Y RNA to recycle the Sm proteins after splicing. The Evans lab showed that Sm proteins are present in P granules and are required for fertility. We hypothesize that at 15°, when either SUT-1 or SNA-1 is missing, there is a failure to recycle some of the Sm protein used in trans-splicing, which could result in splicing problems, producing morphological defects, or failure to make active P granules, producing sterile worms. In support of this idea, we have found a strong genetic interaction between *prmt-5*, whose protein product modifies Sm proteins, and both *sut-1* and *sna-1*: whereas the *prmt-5* deletion alone is without detectable phenotype, this mutation suppresses the *sut-1* and enhances the *sna-1* deletion phenotypes. Interestingly, these genes also interact with mutations in genes encoding P granule proteins, *pgl-1* and *glh-1*.

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Spatiotemporal analysis of promoter functions implicates spindle assembly checkpoint genes in polyploidization in *Caenorhabditis elegans*. **Maja Tarailo-Graovac**, Jun Wang, Jeffrey Shih-Chieh Chu, Domena Tu, David L. Baillie, Nansheng Chen. Simon Fraser University, Burnaby, Canada.

The importance of spindle assembly checkpoint (SAC) genes for genome stability and healthy cell function has been clearly established. It is known that SAC delays anaphase onset by inhibiting the activity of the APC/C until all of the kinetochores have achieved proper attachment. However, functional roles of the checkpoint components beyond division, as well as transcriptional regulation of the SAC genes during development of a multicellular organism remain largely unexplored. To start investigating these, we studied spatiotemporal expression patterns of the checkpoint genes using the SAC gene promoters driving the expression of GFP. Specifically, we generated transgenic strains carrying extrachromosomal transgenes corresponding to eight SAC genes, five widely conserved core components (*mdf-1/MAD1*, *mdf-2/MAD2*, *san-1/MAD3*, *bub-1/BUB1* and *bub-3/BUB3*) and three SAC components only conserved in higher eukaryotes (*hcp-1/CENP-F*, *hcp-2* and *rod-1/ROD*). As expected, we found that SAC promoters drive mainly ubiquitous GFP expression during early embryonic stages. However, we also detected SAC gene promoters driven GFP expression in all developmental stages, including tissues and cell types not undergoing division. Importantly, we observed that most of the checkpoint genes are expressed in four or fewer tissues, with a striking overlap in expression in polyploid tissues. Namely, all of the assayed SAC genes show promoter activities in gut, while six of them are also expressed in hypodermal cells. To confirm the observed patterns and to identify expression at the cell level, we have also successfully utilized Mos1-mediated single-copy insertion (MosSCI)[1] method to generate single-copy transgenes of SAC gene promoter driven GFP expression at endogeneous levels. The discovery that genes contributing to the surveillance mechanism that prevents aneuploidy in one cell type may be required in promoting polyploidy in a different cell type is an important one. Gut localization of MDF-2/Mad2 checkpoint component was observed previously but its significance has not been explored.[2] Taken together, we have identified and analyzed for the first time common SAC gene expression pattern in polyploid tissues, which together with the new strains we have generated will be useful for understanding novel roles of SAC genes. Currently, we are assaying the consequence of the absence of the SAC components on development of gut and hypodermis, polyploid tissues in *C. elegans*.. [1] Frøkjaer-Jensen C., et al. 2008 Nat Genet. 40:1375-1383. [2] Kitagawa, R., and A. M. Rose, 1999 Nat. Cell Biol. 1: 514-521.

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DNA adenine methyltransferase identification (DamID) analysis implies that DAF-16 is a master regulator. **Eugene F. Schuster**[1], Joshua McElwee[2], Jennifer Tullet[1], Ryan Doonan[1], Filip Matthijssens[3], Jacques R. Vanfleteren[3], David Gems[1]. 1) Institute of Healthy Ageing, Univerisity College London, London, UK; 2) Rosetta-Merck, Seattle, WA, USA; 3) Dept. of Biology, Ghent University, Ghent, Belgium.

The DAF-16 FoxO forkhead transcription factor regulates lifespan in response to changes in the insulin/insulin-like growth factor signalling (IIS) pathway. Microarray studies in long-lived *daf-2* insulin/IGF receptor mutants have implied a role for DAF-16 in many biological processes (*e.g.* somatic maintenance) that regulate ageing [1,2]. However, it is unknown if DAF-16 directly regulates these processes. We therefore set out to identify direct targets of DAF-16, to try to bridge the gap between a single transcription factor and the many downstream genes and processes it regulates. DAF-16 binding *in vivo* was surveyed using an approach not previously tried in *C. elegans*: DNA adenine methyltransferase (Dam) identification[3]. We expressed a chimeric DAF-16::Dam protein in *C. elegans*, and treated them with *daf-2* RNAi to activate DAF-16. Dam then acts to methylate adenosines in GATC sequences near its binding site. Methylated DNA was then isolated and hybridized to whole genome tiling arrays. We found an enrichment of DAF-16 binding to promoter regions and in genes containing DAF-16 binding elements (DBEs), including genes previous identified using chromatin immmunoprecipitation (ChIP) [4]. In total, we identified 907 genes with DAF-16::Dam methylation peaks and interestingly these were significantly enriched for genes up-regulated in daf-2 mutants (p = 1.4e-11), but not for down-regulated genes. To create a list of higher confidence DAF-16 targets for further study (*e.g.* ChIP-PCR, longevity assays), we chose 65 genes that were both DAF-16 bound and up-regulated in long-lived *daf-2* mutants. These include several genes where the mammalian orthologs are FoxO regulated (*e.g.* PEPCK). Against expectation, somatic maintenance genes (*e.g.* detoxification enzymes, chaperonins) are not over-represented among these 65 genes and the data suggests that DAF-16 does not directly regulate these processes. Instead, we find genes linked to sugar homeostasis and IIS itself (*e.g. ist-1* , *akt-1* and *akt-2*), and regulators of stress resistance, including *skn-1*, *sek-1* and AMP kinase. This implies that DAF-16 may act as a master regulator of other regulatory pathways, acting as a central node within several positive feedback loops.

1. McElwee et al. J. Biol. Chem. 279, 44533 (2004). 2. Murphy et al. Nature 424, 277 (2003). 3. van Steensel, Henikoff, Nat Biotechnol 18, 424 (2000). 4. Oh et al., Nat Genet 38, 251 (2006).

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elt-2 and *sbp-1* are the only two transcription factors required in the intestine for viability after endoderm specification. **John M. Kalb**[1], Renee A. Larson[1], Jessica Nowak[1], James D. McGhee[2]. 1) Biology Dept., Canisius College, Buffalo, NY; 2) Dept. of Biochemistry and Molecular Biology, Univ. of Calgary, Calgary, AB Canada.

Our current model is that the GATA-factor ELT-2 is the predominant transcription factor controlling gene expression in the *C. elegans* intestine following endoderm specification. We are interested if other transcription factors besides ELT-2 have critical roles in the intestine. We have identified roughly 300 different transcription factors in SAGE libraries prepared from FACS-sorted cells of the embryonic intestine. The loss of function of 32 of these transcription factors results in embryonic lethal or larval lethal phenotypes, but their specific requirements in the intestine are difficult to ascertain because these genes may also be expressed in non-intestinal tissues. In order to examine their intestinal function, we used a strain of worms that is RNAi sensitive only in the intestine (OLB11, kindly provided by Olaf Bossinger). OLB11 worms are deficient in RNAi due to a mutation in the *rde-1* gene, but have intestinal RNAi rescued by an integrated array containing *rde-1* cDNA controlled by the intestinal-specific promoter of the *elt-2* gene. Control experiments show the strain behaves as expected: embryos and larvae of injected mothers are completely resistant to RNAi effects of genes expressed exclusively or predominantly outside of the intestine but are sensitive to RNAi against genes expressed exclusively or predominantly in the intestine. Double stranded RNA corresponding to each of the 32 intestinally expressed transcription factors were injected into OLB11 worms and their progeny were scored for viability and growth rates. Intestinal-specific RNAi against only two transcription factors, *elt-2* and *sbp-1*, results in larval lethality, with injected progeny arresting as L1 larvae, matching the RNAi effects of these genes in wild type worms. Intestinal RNAi against four genes, F57B10.1 (*let-607*), C16A3.4, F23B12.7 and *dve-1* causes a slow growth phenotype; however, these worms develop into fertile adults with no apparent defects in the structure of the intestine. Intestinal RNAi against the other 26 transcription factors does not affect viability or growth rates. Currently we are using the OLB11 worms to assess the intestinal function of some of these transcription factors in regulating aging. So far we have determined loss of intestinal *skn-1* (RNAi performed by feeding) results in a shortened life span, and we are presently looking at other genes, such as *pha-4*, in this process.

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Transcriptional Regulation by the conserved regulatory complex Mediator in *C. elegans*. **Stefan Taubert**[1], Jess Porter[2], T. Keith Blackwell[2]. 1) Centre for Molecular Medicine and Therapeutics, Department of Medical Genetics, University of British Columbia, Vancouver BC; 2) Joslin Diabetes Center, Department of Pathology, Harvard Medical School, Boston MA.

The multi-subunit complex Mediator is a conserved transcriptional regulatory complex for transcription factors (TFs) such as Nuclear Hormone Receptors (NHRs). Although Mediator is globally required for PolII-dependent transcription, some of its subunits confer specific biological functions. Others and we found that the *C. elegans* Mediator subunit MDT-15 cooperates with the TFs SBP-1/SREBP [1] and NHR-49 [2] to control certain aspects of lipid biology such as the fasting response, and fatty acid desaturation; through the latter, MDT-15 also impinges on global worm health and life span. Recently, we found that MDT-15 is also involved in systemic detoxification, as *mdt-15* depletion or mutation abrogates the induction of (i) Phase II detoxification genes in response to a hydrophobic toxin, (ii) metal responsive genes in response to heavy metals [3], and (iii) genes that respond to oxidative stress (unpublished). As many of these physiological processes are under control of the Insulin/IGF-1 signaling (IIS), we are currently testing whether MDT-15 is involved in IIS signaling, potentially as a cofactor of the IIS target TFs DAF-16 and SKN-1. Thus, we propose that MDT-15 is a key component of a regulatory network that monitors the availability and quality of ingested materials. Moreover, the fact that individual components of the Mediator complex selectively confer regulatory action has prompted us to discover the targets and biological functions of other Mediator subunits such as the Mediator kinase CDK-8. Preliminary data suggest that this protein affects processes such as worm development and host defense. In summary, Mediator is a highly dynamic and versatile regulatory complex that serves to integrate and fine-tune diverse biological processes. 1. Yang et al, 2006, Nature. 2. Taubert et al, 2006, Genes & Development. 3. Taubert et al, 2008, PLoS Genetics.

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Targeting the Condensin-like Dosage Compensation Complex to X. **Rebecca Pferdehirt**, Barbara Meyer. HHMI/UC Berkeley, Berkeley, CA 94720.

X-chromosome dosage compensation is a chromosome-wide regulatory mechanism that equalizes levels of X gene products between males (1X) and females (2X). In *C. elegans*, dosage compensation is executed by a multi-protein complex that resembles condensin, a conserved complex involved in chromosome compaction, resolution and segregation. This dosage compensation complex (DCC) binds both X chromosomes of XX animals to decrease X transcript levels by about two-fold. The mechanisms by which the DCC is targeted to X are not well understood. Our recent work has revealed that multiple inputs are important for orchestrating proper DCC localization and function, ranging from DNA sequence motifs to post-translational protein modification.

The DCC binds to two types of sites on X. *rex* sites recruit the DCC in an autonomous manner using a 12 bp consensus motif that is enriched on X, is clustered within *rex* sites, and is critical for DCC binding. *dox* sites fail to bind the DCC when detached from X, lack the X-enriched motif variants and, unlike *rex* sites, are enriched in promoters of genes. ChIP-chip of the DCC in embryos mutant for other DCC components has revealed differential requirements for binding *rex* versus *dox* sites and for loading of specific DCC subunits. These results refine our understanding of the hierarchy of DCC assembly and binding.

We have discovered that post-translational modification by the small ubiquitin-like molecule SUMO (*smo-1* in *C. elegans*) is essential for proper targeting of the DCC to X. Reduction of sumoylation by RNAi causes a redistribution of DCC binding from X to autosomes such that DCC binding sites are significantly reduced on X and increased on autosomes. Although all DCC components tested show defects in binding by ChIP-chip, they do not all behave in precisely the same manner with respect to *rex* and *dox* site binding, suggesting sumoylation may be important for proper assembly of the DCC on X. *smo-1* RNAi also causes significant over-expression of genes on X, as measured by expression microarrays. Finally, preliminary data suggests that one or more members of the DCC are targets of sumoylation. Together these results indicate that sumoylation is one of multiple inputs required for proper targeting of DCC assembly on X. Sumoylation, perhaps of one or more DCC components, may direct DCC localization by either repelling the DCC from autosomes or by actively directing its preferential binding to X. Dosage compensation requires cumulative action of multiple discrete, yet common mechanisms to direct sex-specific localization and function of a universal complex involved in genome-wide chromosome dynamics.

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Automatic Gene Expression Profiling at Single Cell Resolution in both *C. elegans* and *C. briggsae*. **Zhongying Zhao**[1], John Murray[1], Zhirong Bao[1], Thomas Boyle[1], Max Boeck[1], Dan Blick[1], Matthew Sandel[1], Elicia Preston[1], Dionne Vafeados[1], Stephane Flibotte[2], Don Moerman[3], Robert Waterston[1]. 1) Dept Genome Sci, Univ Washington, Seattle, WA.USA, 98195; 2) Canada's Michael Smith Genome Sciences Centre, BC Cancer Agency, Vancouver, B.C., Canada, V5Z 4S6; 3) Department of Zoology, University of British Columbia, Vancouver, B.C., Canada, V6T 1Z4.

We have developed technology that allows automatic determination of the expression profile of fluorescent reporter genes in *C. elegans* embryos through time with single-cell resolution. The technology is routinely used to trace the expression patterns through the 350-cell stage. Recent advances extend its tracing power to 450 cells and beyond and allow characterization of either GFP or mCherry reporters. We have applied the technology to promoter fusions reporters for more than 100 genes, most of which encode transcription factors differentially expressed in the embryo. We have also analyzed protein-fusion reporters expressed from fosmid transgenes for more than 25 genes, allowing the characterization of posttranscriptional regulation. We will present examples of interesting patterns, including some comparing promoter and protein fusions. We have extended the technology to work in *C. briggsae* with appropriate strains to allow both GFP and mCherry reporters to be used with results comparable to those obtained in *C. elegans*. The technology could be readily adapted to the newly identified sister species of *C. briggsae*, allowing detailed investigation of the effect of the differing degrees of sequence variation on gene expression. We will present comparisons of expression patterns of the same constructs in *C. briggsae* and *C. elegans*. To improve the utility of *C. briggsae* in comparative studies of gene function, we have extended several standard *C. elegans* methods into *C. briggsae*. We built a frozen EMS mutagenized library for knock-out deletion screening and utilized it to isolate a cbr-*unc-119* deletion allele, which works efficiently as a transgenic selection marker for ballistic bombardment. To facilitate forward mutagenesis studies, we developed a customized SNP-based oligo-array CGH chip for rapid and inexpensive mapping of unknown mutants, using bulk segregant analysis from AF16/HK104 hybrids. We have used the system to map a series of both known and unknown mutants on all six chromosomes. Using these mapping results, we have been able to identify candidate genes in the interval with similar phenotypes in *C. elegans* and test them through injection-rescue. These tools extend many of the experimental advantages of *C. elegans* to *C. briggsae*, which will facilitate future comparative studies.

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A new model of *C. elegans* embryogenesis with cell contacts and spatio-temporal gene expressions generated with VW-Base and Endrov. Jurgen Hench[1,2], **Johan Henriksson**[1,2], Martin Lüppert[1,2], Akram Abou-Zied[1,2,4], Konstantin Cesnulevicius[1,2], Krai Meemon[1,2], David Baillie[3], Thomas Burglin[1,2]. 1) Dept Biosciences & Nutrition, Karolinska Institutet, Stockholm, Huddinge, Sweden; 2) Södertörns högskola, Dept of life sciences, Huddinge, Sweden; 3) Simon Frasier University, Burnaby B.C., Canada; 4) King Abdull-Aziz University, Faculty of Medical Sciences, Saudi Arabia.

In *C. elegans* about 150 different cell types are generated during ontogeny. To understand development, ideally, one would like to obtain precise levels of expression for each developmental control gene in each type of cell. We have developed a multi-channel spatio-temporal (4D) microscopy framework (VW-base, Endrov, see abstract by J.Henriksson et al) to monitor live GFP expression throughout embryogenesis. We have applied this to homeobox gene transcription factors and have so far recorded 60 homeobox gene expression patterns in 200 recordings using DIC and DIC/mCherry::Histone timelapse microscopy. Our recording workflow allows for the automatic processing into thumbnail movies and we can extract the expression data from the image archive using various algorithms. We have lineaged some expression patterns. We also used our 4D image database to assess the normal development of embryos. We have found that agarose-mounted embryos are not suitable representatives for 4D models due to compression artifacts (agarose mounted embryos are 20μm in thickness while uncompressed embryos are 30μm.) and random rotational movements. Therefore we generated a new model using embryos mounted with polylysine and spacer beads.These have low variability with respect to cell position and cell movement, and–critically–do not rotate during development. Embryos mounted in this fashion are oriented randomly with respect to the dorsal-ventral axis. We have constructed a high-quality model by fitting 6 manually annotated recordings. From the model we have found that the average number of cell contacts increases over time to reach a maximal number of contacts of about 14. Cell contacts are approximated by Voronoi decomposition. 3500 contacts last 2.5 minutes or longer. Cell division variance is less than 10% of the lifespan. Most cells travel according to an approximate χ^2-distribution with an average around 4μm. This new 4D model is suitable to map the spatio-temporal gene expression patterns onto. We propose a standard way of submitting 4D expression patterns to Wormbase for retrieval using Endrov as a client.

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Combination of *cis*-regulatory modules for temporal regulation of gene expression in *C. elegans*: Towards promoter design. **Akihiro Mori**[1,2,3], Y. Kohara[1,2]. 1) Dept Genetics, Grad. Univ. Advanced Studies, Japan; 2) NIG, Japan; 3) JSPS Research Fellow.

It is essential for multi-cellular organisms to regulate tissue-/stage-specific transcriptional processes adequately for proper development. These processes are mostly regulated by binding of proteins (transcription factor or TF) to specific regions (*cis*-regulatory modules or CRM) of genes. Thus, it is important to reveal combinations of TF-CRM for understanding gene regulatory network. Although *in silico* approaches predicted tissue-specific CRMs, *in vivo* results often showed existence of such CRMs is insufficient for proper gene expression. Here, we hypothesize there exist independent mechanisms for stage-specific initiation of gene expression besides tissue-specific regulatory; the combinatory regulations can be required for gene expressions. First, we investigated CRMs with developing a two-step algorithm, Filtering-Esteeming system, for *in silico* prediction. This system extracts short-sequence motifs specific to positive dataset (PD) (upstream region of genes that show the same expression patterns) by comparing with negative datasets (ND) (upstream regions of other genes besides the PD genes). After confirmation of the system, we applied the system to the gene sets that show the same *in situ* hybridization patterns in NEXTDB, and we identified several CRM candidates in the early intestinal lineage. Previous studies of this lineage showed many genes were activated by multiple GATA factors stage-specifically; however, it is still unrevealed how these factors recognize initiation timing of gene expressions properly. We focused on three consecutive stages of the lineage; E2 (two E-cells), E4, and E8 stages, to verify the CRM candidates *in vivo*. The candidates were analyzed by GFP reporter assay. We examined various deletion/substitution constructs for both our candidates and GATA sites. These results suggest; (i) There exist distinct CRMs at each stage, (ii) the candidate CRMs near GATA sites may be required for regulating the initiation timing of proper gene expressions, and (iii) the timings can be controlled by replacing the stage-specific CRMs. Next, we investigated TFs for our target CRMs. We again applied the system to datasets of yeast-one hybrid (Deplancke *et al.*, 2006) and predicted transcriptional factor binding sites (TFBS). As a result of comparing predicted TFBS with our target CRMs, we found DAF-12 is a good candidate as a temporal regulator at E8 stage. We confirmed that GFP expression patterns of E8-stage genes in *daf*-12 (m20) is similar to that of the deletion/substitution constructs of E8-stage CRM. Taken together with the previous results of reporter assay, these results strongly suggest DAF-12 regulates the initiation of gene expression with GATA factor at E8 stage in intestine.

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The *C. elegans* TransgeneOme: a fosmid transgene resource for genome scale protein function analysis. **Mihail Sarov**[1], John Murray[2], Mei Zhong[3], Valerie Reinke[3], Stuart Kim[4], Robert Waterston[2], Michael Snyder[3], Anthony Hyman[5]. 1) TransgeneOmics, MPI-CBG Dresden; 2) University of Washington School of Medicine; 3) Yale University School of Medicine; 4) Stanford University Medical Center; 5) Hyman lab, MPI-CBG Dresden.

Our ultimate goal is to understand the molecular principles of development through systematic description of the physical interactions and localization of all proteins encoded in the *C. elegans* genome. To that end we have developed a transgene-based platform for protein tagging with fluorescent/affinity epitopes [1]. We use *in vivo* homologous recombination based DNA engineering (recombineering) in *E. coli* to seamlessly insert a tag coding sequence into genomic fosmid clones containing the gene of interest in its native genomic environment. Stable integration of these large constructs into the worm genome result in reliable, near endogenous levels and patterns of gene expression. Through evaluation of multiple tags we found a cassette that is well tolerated by most proteins and works well for both affinity purification and localization. We have now scaled up the construction of fosmid transgenes using an efficient 96 well format liquid culture recombineering, which delivers a extremely high throughput without compromise in quality.

As part of the NIH funded modENCODE project [2] we have applied this approach to the transcription factor proteins, coupled with high resolution protein localization [3] and chromatin immunoprecipitation to map binding sites in the genome. We are currently focusing on extending this approach to further functional sets (Chromatin, cell division and G protein coupled receptors) and eventually to the rest of the genome. The TransgeneOme resource will be another powerful tool for the *C. elegans* research community. The clones will be made available on the condition of sharing the stable worm lines. We believe that this would be an efficient way of solving the bottleneck step of strain generation through distributed community effort.

[1] Sarov et al. A recombineering pipeline for functional genomics applied to *Caenorhabditis elegans*. Nat Methods (2006)
[2] http://www.modencode.org/
[3] Murray et al. Automated analysis of embryonic gene expression with cellular resolution in *C. elegans*. Nature Methods (2008).

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Using Mos1 elements to modify the genome. **Christian Frøkjær-Jensen**[1,2], M. Wayne Davis[1], Christopher E. Hopkins[1], Blake Newman[1], Rachel Lofgren[1], Morten Grunnet[2], Søren-Peter Olesen[2], Erik M. Jorgensen[1]. 1) HHMI, Dep. Biology, University of Utah, Salt Lake City, UT; 2) The Danish National Research Foundation Centre for Cardiac Arrhythmia, University of Copenhagen, Denmark.

Transgenic *C. elegans* are routinely generated by injecting DNA into the germline. Injected DNA assembles into complex, yet repetitive, extrachromosomal arrays. As such, these minichromosomes are not stably inherited. Moreover, the genes are typically overexpressed in somatic cells and silenced in the germline. Alternatively, transgenes can be inserted by biolistic transformation. The bombarded DNA is stably integrated which solves many of the limitations of DNA injection. However germline expression is still infrequent. Also, DNA copy number and insertion site is essentially random. As a result, transgene expression is variable and comparison between different transgenic lines is difficult.

Based on recent advances[1] we developed a technique we call MosSCI[2] (Mos1-mediated Single Copy Insertion) that circumvents many of these problems. Briefly, the technique functions by mobilizing a Mos1 transposon from a defined locus in non-coding DNA, thus introducing a double strand break. The break is healed by copying from an extrachromosomal template in which DNA that is homologous to the breakpoint flanks the transgene. Thereby a single copy of a transgene is inserted into the chromosomal site. We have shown that insertions can be generated either directly by injection or from stable extrachromosomal arrays. We have successfully inserted transgenes as long as 17 kb and verified that single copies are inserted at the targeted site. Inserted transgenes are expressed at endogenous levels and can be expressed in both the hermaphrodite and male germlines.

We have recently expanded the technique to manipulate genomic regions adjacent to Mos1 inserts. By shifting the homology regions on the targeting vectors we have engineered defined deletions of genes adjacent to Mos1 inserts. Because unc-119 rescue is used as the positive selection marker for targeting homozygous lethal deletions are automatically balanced perfectly. The technique works efficiently by injection and deletions can be recovered in less than two weeks.

1. Robert, V. & Bessereau, J.-L. (2007) EMBO 26: 170-83 2. Frøkjær-Jensen et al., (2008). Nat. Genet. 40: 1375-83.

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A polyQ-repeat protein promotes the novel, morphologically conserved death of the linker cell. **Elyse S. Blum**, Shai Shaham. Rockefeller University, NY, NY 10065.

Programmed cell death is an essential process during metazoan development. During wild-type *C. elegans* development, nearly all cells slated to die activate caspases and undergo stereotypical morphological changes including chromatin compaction and cell shrinkage. The male-specific linker cell (LC), however, is an exception. The LC leads the migration of the developing gonad, and once migration is complete at the L4-Adult transition, the LC dies. LC death is independent of *ced-3*, *ced-4*, *ced-9*, and *egl-1*, indicating that LC death is controlled by a novel program. Indeed, electron micrographs of dying LCs reveal non-apoptotic features, including nuclear crenellation and organelle swelling. Remarkably similar features are also seen in normally dying cells of the vertebrate spinal cord and ciliary ganglion, suggesting that LC death is morphologically conserved.

To understand the molecular mechanism of LC death, we performed a genome-wide RNAi screen to identify genes whose loss prevents LC death. RNAi against *pqn-41*, a gene predicted to encode a polyQ-repeat protein, blocks LC death in 20-30% of animals examined. Similar defects are seen in *pqn-41*(*ns294*) mutants we generated. A transgene containing *pqn-41* genomic DNA fused to GFP is nuclearly expressed in many cells in the animal. Strikingly, expression in the LC is only visible as the cell begins to die, and we identified a *pqn-41* promoter region required for LC expression. PQN-41 protein is localized to nuclei and nuclear puncta in all cells, except for the dying LC, where PQN-41 is also cytoplasmic.

Previous studies revealed that LC death requires the heterochronic genes *let-7* and *lin-29*, and that these genes act within the LC to promote its death. We have now shown that a MAPK module containing the TIR-1 adapter protein and the SEK-1 MAPKK also regulate LC death: 30% *tir-1*(RNAi) and 49% *sek-1*(*ag1*) adult males exhibit LC survival. The heterochronic and MAPK pathways likely function in parallel to regulate LC death, as double-mutants exhibit additive effects, and expression of components of one pathway does not depend on the other. Since both pathways impinge on transcriptional regulators, it is possible that both pathways converge on the promoters of key LC death genes. We are testing this hypothesis with regard to *pqn-41*.

Several human neurodegenerative disorders result from polyQ expansions within endogenous proteins, however, the mechanisms promoting cell death in these diseases is not known. Our studies raise the intriguing possibility that these aberrant proteins activate an endogenous cell death program similar to that driving the death of the LC.

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The life versus death decision of the sexually dimorphic CEM neurons: a story beyond *egl-1* BH3-only. **Ralda Nehme**[1], Phillip Grote[1,2], Tatiana Tomasi[1,2], Stefanie Loeser[2], Heinke Holzkamp[2], Barbara Conradt[1]. 1) Dartmouth Medical School, Department of Genetics, Norris Cotton Cancer Center, 7400 Remsen, Hanover, NH 03755, U.S.A; 2) Max-Planck-Institute of Neurobiology, Am Klopferspitz 18a, 82152 Martinsried, GERMANY.

The majority of the cell death events that take place during the development of a *C. elegans* hermaphrodite occur within 30 min after the cell is generated. The death of the CEMs (cephalic companion neurons) in hermaphrodites is an exception. The CEMs are four sexually dimorphic neurons located bilaterally between the two bulbs of the pharynx. The CEMs are sexually dimorphic as a result of programmed cell death. They are born at around 320 min after the first cell division in both males and hermaphrodites and show signs of differentiation before being specifically removed in hermaphrodites at around 470 min. Whereas in most dying cells the pro-caspase proCED-3 is inherited from progenitors and the transcriptional activation of the BH3-only gene *egl-1* is thought to be sufficient for apoptosis induction, we found that, in the CEMs, most probably as a result of proCED-3 turn over during the 150 min, the transcriptional activation of not only *egl-1* but also *ced-3* is necessary for apoptosis induction. We also found that the Bar homeodomain transcription factor CEH-30 represses *egl-1* and *ced-3* transcription in the CEMs in masculinized *sel-10(n1077*gf*)* hermaphrodites in which the CEMs survive. Thus, *egl-1* and *ced-3* transcription are co-regulated in the CEMs. In addition, we identified three genes, *unc-86*, *lrs-1* and *unc-132*, which encode a POU homeodomain transcription factor, a leucyl-tRNA synthetase and a novel protein with limited sequence similarity to the mammalian proto-oncoprotein and kinase PIM-1, respectively, that are required for CEM specification and the transcription of the *ceh-30* gene in the CEMs. Finally, we have evidence that the life versus death decision of the CEMs is also affected by cell migration and/or cell attachment. Specifically, the inactivation of genes encoding for example UNC-52 perlecan, the integrin-linked kinase PAT-4, the integrins PAT-2 or INA-1, CED-10 RAC-1, or UNC-73 Trio results in the inappropriate death of the CEMs in masculinized hermaphrodites. This result provides the first evidence that cell non-autonomous signals can control the programmed death of neurons in C. elegans. We propose that the life-versus-death decision of the CEMs is determined by the concerted action of different pathways, namely the sex determination, CEM specification, and cell migration/attachment pathways. We are currently testing whether these pathways act to fine tune *ceh-30* transcription in the CEMs.

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Bilaterally asymmetric deployment of alternative apoptotic pathways is determined by a novel left-right symmetry breaking mechanism. **Shin Sik Choi**, Joel H. Rothman, Tim A. Bloss. NRI, University of California Santa Barbara, Santa Barbara, CA.

The left-right (L-R) anatomical asymmetry of *C. elegans* is initiated at the four-cell stage by skewing of initially left-right oriented mitotic spindles along the anteroposterior axis. This event is responsible for most of the known left-right differences in the animal. We have identified a second handedness-defining mechanism that is independent of anatomical handedness and that controls alternative pathways for apoptosis. The adult male tail contains nine bilateral pairs of sensory rays required for successful mating behavior. Each ray consists of two neurons and a structural cell. It was reported many years ago by John Sulston that rays are commonly absent in wildtype males. Using a ray structural cell marker, we confirmed that the absence of rays is accompanied by the absence of a structural cell. We found that this natural loss of rays requires core apoptotic regulators. *ced-3* and *ced-4* loss-of-function and a *ced-9* gain-of-function mutation partially eliminate ray loss, whereas virtually no ray losses occur in an *egl-1(lf)* or a *ced-3(lf);ced-49lf)* double mutant. These results imply that the absence of rays is the result of stochastic cell death in the ray lineages and that natural CED-3-independent and CED-4-independent cell death occur during normal development. We found that the loss of rays shows a clear handedness bias: rays on the right side are absent twice as frequently as those on left. Remarkably, mutations in ced-3 eliminate asymmetric loss of only the left rays, whereas ced-4 mutants show asymmetric loss only on the right. These results suggest that CED-3-independent and CED-4-independent cell death occur during male tail development on the right and left respectively. We were surprised to find that this L-R bias and the differential utilization of CED-3 and -4 in ray loss persist in anatomically handedness-reversed worms carrying a *spn-1/gpa-16* mutation and in *nsy-4* mutants, in which left-right asymmetry of certain olfactory neurons is eliminated. However, we found that heat-shock stress during embryogenesis reversed the handedness of ray loss, resulting in a reverse in the ratio of missing rays on left and right sides. Further, we found that CSP-1 is required for the CED-3-independent cell death on the right side, implicating this caspase in natural apoptosis. Our findings suggest that a second L-R handedness-determining mechanism may exist that activates alternative apoptotic pathways and possibly other developmental events.

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Induction of Germ Cell Apoptosis by Multiple Isoforms of IFG-1 are CED-4/Apaf-1 Dependent in *C. elegans*. **Vince Contreras**, Melissa Henderson, Enhui Hao, Brett Keiper. Dept Biochem, East Carolina Univ, Greenville, NC.

Apoptosis causes the coordinated dismantling of the protein synthesis machinery. In mammals, the protease caspase-3 cleaves a factor of the translation complex called eIF4G. This modification induces the synthesis of pro-apoptotic proteins such as Apaf-1, while general protein synthesis is suppressed. In the *C. elegans* gonad, apoptosis occurs naturally to maintain the viability of sibling germ cells that will mature as oocytes. We demonstrate that worms express multiple isoforms of eIF4G from a single gene (*ifg-1*). The isoforms of IFG-1 (p170 and p130) differ structurally and in association with mRNA cap complexes. Loss of the long p170 isoform induced the expression of the Apaf-1 ortholog CED-4 early in dying oocytes. We have depleted IFG-1 p170 in worms containing ced-1:gfp, which decorates apoptotic corpses, and either *ced-3* or *ced-4* loss-of-function alleles. Upon depletion of IFG-1 p170, there was virtually complete suppression of germ cell death in worms lacking CED-3 or CED-4. While the number of fertile oocytes increased, embryonic lethality was still observed as previously described. This indicates that there are non-apoptotic functional roles for the short IFG-1 p130 that can be exercised in the absence of p170 for late oogenesis. These results also suggest that germ cells can use a component of the translational apparatus as a novel mechanism to alter their fate by upstream activation of the caspase cascade. Concomitantly, during apoptosis Apaf-1 has been shown to be preferentially translated through cap-independent synthesis by short isoforms of human eIF4G. We are currently exploring similar translational control mechanisms involving CED-4 during the cap-independent (p130) induction of physiological germ cell death. These studies address changes in the availability and/or translational efficiency of *ced-4* mRNA. We have also used a conditional mutant strain that alters *ifg-1* splicing resulting in nonsense mRNA. IFG-1 p170 and p130 were markedly depleted in these worms, which were viable at 20°C. Growth at 25°C, however, resulted in gonad degeneration, arrested embryonic development, and F1 sterility. At this non-permissive temperature, we will determine if the protein synthesis mechanism is similarly altered, and its influence on germ cell proliferation and death. Ultimately, our findings support a model in which a balance between p170 and p130 isoforms maintain growth-promoting protein synthesis while preventing the induction of CED-4 in order to preserve oocytes destined to mature. (Supported by grants MCB-0321017 and MCB-0842475 from the NSF and IRG 5-89812 from the American Cancer Society).

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C. elegans Rab GTPase Activating Protein, TBC-2, Promotes Cell Corpse Degradation by Regulating the Small GTPase RAB-5. Weida Li[1,3], **Wei Zou[2,3]**, Dongfeng Zhao[3], Jiacong Yan[3], Zuoyan Zhu[1], Jing Lu[3], Xiaochen Wang[3]. 1) College of Life Science, Peking University, Beijing, China; 2) College of Biological Sciences, China Agricultural University, Beijing, China; 3) National Institute of Biological Sciences, No. 7 Science Park Road, Zhongguancun Life Science Park, Beijing, 102206, China.

During apoptosis, dying cells are quickly internalized by neighboring cells or professional phagocytes and are enclosed in phagosomes that undergo a maturation process to generate phagoslysosome, in which cell corpses are eventually degraded. It is not well understood how apoptotic cell degradation is regulated. In a forward genetic screen for additional genes involved in cell corpse clearance, we identified *C. elegans tbc-2* gene, which is required for efficient degradation of cell corpses. *tbc-2* encodes a Rab GTPase activating protein (GAP) and its loss-of-function affects several events of phagosome maturation including RAB-5 release, PI(3)P dynamics, phagosomal acidification, RAB-7 recruitment and lysosome incorporation, which leads to many persistent cell corpses at various developmental stages. Intriguingly, the persistent cell corpse phenotype of the *tbc-2* mutants can be suppressed by reducing gene expression of *rab-5*, while overexpression of a GTP-locked RAB-5 caused similar defects in phagosome maturation and cell corpse degradation. We propose that TBC-2 functions as a GAP to cycle RAB-5 from an active GTP-bound to an inactive GDP-bound state, which is required for maintaining RAB-5 dynamics on phagosomes and serves as a switch for the progression of phagosome maturation.

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The role of F58G11.6 in apoptotic corpses degradation in C.elegans. Cristina Nieto[1], Johann Almendinger[2], Stephan Gysi[2], Andres Kaech[2], Ralf Schnabel[3], Michael Hengartner[2], Sergio Moreno[1], **Juan Cabello[1]**. 1) Instituto de Biologia Molecular y Celular del Cancer. Centro Investigacion Cancer, Salamanca, Spain; 2) Institute of Molecular Biology. University of Zurich. Zurich. Switzerland; 3) Institute of Genetic. TU Braunschweig. Braunschweig. Germany.

In a screening for maternal effect lethal mutations on chromosome V of C.elegans, we found three alleles (named t2070, t2129 and t2170) of the same gene showing accumulation of persistent apoptotic corpses in embryos and in the hermaphrodite adult gonad. We cloned the three mutations using three factor mapping followed by SNP mapping. They define three alleles of the gene F58G11.6. Persistent corpses in the gonad of t2070, t2129 and t2170 mutant worms were positively stained with Acridine Orange (AO) indicating that its accumulation is not due to a defect in engulfment. Instead it suggested that, in these mutants, corpses were efficiently engulfed and accumulated within phagosomes as undigested corpses. Electron microscopy further confirmed that these persistent corpses were completely engulfed within the sheath cells. Consistent with this result F58G11.6::YFP localizes in halos around the apoptotic corpse containing phagosomes. The results suggest that F58G11.6 plays a role in phagosome maturation, the process that allows other vesicles, such as lysosomes, to bind and fuse to the phagosome first acidifying and second digesting the apoptotic corpse. Our genetic analysis indicates that F58G11.6 acts at the level of the HOPS complex in the phagosome maduration pathway during the digestion of the apoptotic corpses.

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How do necrotic cells expose „eat me“ signals to attract engulfing cells? **Victor Venegas**, Zheng Zhou. Dept Biochem & Molec Biol, Baylor Col Medicine, Houston, TX.

During animal development, cells undergoing apoptosis, or programmed cell death, are recognized and internalized by other living cells via phagocytosis, a highly conserved process from nematodes to humans. Phagocytosis removes dying cells before they release harmful cellular contents, actively preventing tissue damage, inflammation, and auto-immune responses. Eight genes (*ced-1, 6, 7, dyn-1* and *ced-2, 5, 10, 12*) are known to act within two partially redundant pathways to promote engulfment of apoptotic cell corpses. Among them, CED-1 has been found to act as a phagocytic receptor that recognizes cell corpses and initiates engulfment. Recently, CED-1 was also found to initiate the degradation of engulfed apoptotic cells. The recognition of cell corpses by CED-1 is dependent on CED-7, a C. elegans homolog of mammalian ABC transporters. Our lab has shown CED-7 is necessary for the efficient exposure of phosphatidylserine (PS) on apoptotic somatic cells. Furthermore, we provide evidence to indicate that PS may act as an “eat-me” signal and a ligand for CED-1.

Cells injured by external factors such as trauma or diseases undergo necrosis, a type of caspase-independent cell death, and must also be cleared for the protection of surrounding tissues. In *C. elegans*, the dominant *mec-4* (*dm*) mutants provide a model for necrotic-like cell death similar to mammalian necrosis. MEC-4 is the core subunit of a multimeric, mechanically gated Na+ channel complex. The *mec-4* (*dm*) mutations lead to enhanced ion conductivity and subsequent degeneration of six touch receptor neurons. Chung *et al* [1] have shown that necrotic cells are engulfed through functions of the apoptotic-cell engulfment machinery. However, the mechanism by which necrotic cells are recognized by engulfing cells still remains unclear. In this study we look at the role of putative “eat me” signals in the engulfment of necrotic cell corpses. Using a reporter for the presence of phosphatidylserine (PS), we found that PS was present on the surface of *mec-4* (*dm*)-induced necrotic cell corpses. In additon, we have also observed CED-1 recognition and localization around necrotic corpses. As is observed in apoptotic cell death, our work has demonstrated that necrotic cell corpses present “eat me” signals for active recognition. This work thus suggests two distinct cell deaths use a similar mechanism for recognition and subsequent engulfment of cell corpses. Previous studies and work in our lab suggest that CED-7 function is required in both the engulfing and apoptotic cell. We are further addressing the cell-specific role of CED-7 in the engulfment of necrotic cells.

[1]Chung *et al*. Nat Cell Biol 2, 931-7 (2000).

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The RhoGAP SRGP-1 regulates cell killing and clearance through CED-10 in *C. elegans*. **Lukas J. Neukomm**[1], Andreas P. Frei[1,2], Juan Cabello[3], Xhong Ma[4], Lisa B. Haney[4], Marko Jovanovic[1], Bernd Wollscheid[2], Kodimangalam S. Ravichandran[4], Sergio Moreno[3], Michael O. Hengartner[1]. 1) Institute of Molecular Biology, University of Zurich, Zurich, Zurich, Switzerland; 2) Institute of Molecular Systems Biology, ETH Zurich, Zurich, Switzerland; 3) Centro de Investigacion del Cancer, Campus Miguel de Unamuno, Salamanca, Spain; 4) Beirne B. Carter Center for Immunology Research and the Department of Microbiology, University of Virginia, Charlottesville, Virginia, USA.

The proper removal of apoptotic cells is critical during embryonic development and in tissue homeostasis to prevent inflammation and autoimmunity. Phagocytes recognize apoptotic cells and start to re-orchestrate their cytoskeleton and membranes towards the corpse ensuring its clearance. However, the molecular details of the “engulfment machinery” are not fully understood. The powerful genetics of *C. elegans* has been used to identify genes involved in the clearance of apoptotic cells. Ten genes were isolated which act in two partially redundant pathways. One group is composed of five genes: the small GTPase MIG-2/RhoG and its nucleotide exchange factor UNC-73/TRIO, as well as the adaptor protein CED-2/CrkII and the bipartite RacGEF complex CED-5/Dock180 and CED-12/ELMO. In the second pathway, CED-1/SREC/EATER functions as a transmembrane receptor which might recognize the apoptotic cell. The CED-7/ABCA1 transporter is likely important for membrane dynamics, and plays an additional critical role in dying cells. CED-6 and its human homologue GULP encode a signaling adaptor molecule which physically interact with CED-1. DYN-1/Dynamin2 regulates the vesicular traffic downstream of CED-6. Recent work also uncovered a pathway required for phagosome maturation and subsequent degradation of ingested cell corpses. Using the combined approach of the vital dye Acridine Orange (AO, which visualizes engulfment activity) and *RNAi*, we identified *srgp-1*, a RhoGEF which re-allows AO staining of *ced-6* or *ced-5* germ cell corpses and suppress persistent corpse numbers during development. *srgp-1* acts in the engulfing cell, which is supported by SRGP-1::GFP localization. *srgp-1(lf)* results in increased engulfment kinetics and *srgp-1* over-expression enhances engulfment defects. Epistasis suggest that *srgp-1* might act onto *ced-10*, which is supported by a physical interaction of SRGP-1 with CED-10 and by the modulation of the GTPase activity. Taken together *srgp-1(lf)* resembles an increased engulfment signaling background–which can promote apoptosis of cells that fail to fully undergo apoptosis. This “promotion” of engulfment might be a prerequisite for therapeutics designed to induce the clearance of unwanted cells.

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A mechanism for exchange of RAB-5 for RAB-7 on the surface of the phagosome. **Jason M. Kinchen**, Kodi S. Ravichandran. Center for Cell Clearance, University of Virginia, Charlottesville, VA 29908.

The efficient engulfment (phagocytosis) of apoptotic cells is essential for the physiological well being of the organism. Engulfment can be broadly broken down into a series of steps, comprising recognition, internalization, phagosome maturation and finally lysosomal degradation of the apoptotic cell by the phagocyte. Genetic studies to date have identified two evolutionarily conserved signaling pathways involved in the recognition and internalization of apoptotic cells. However, while there has been much study of signaling during uptake of other particles (e.g. bacteria or IgG-opsonized beads) in mammalian models, signaling during apoptotic cell degradation remains poorly understood. We had recently used both targeted and reverse genetic screens to identify a conserved pathway for the removal of apoptotic cells, a process termed phagosome maturation. Key to this process are the GTPases RAB-5 and RAB-7, which are sequentially recruited to the phagosome during corpse degradation, ultimately leading to phagosome fusion with the lysosome. However, the steps involved in the transition between the RAB-5(+) and RAB-7(+) stages during phagosome maturation are not defined. To identify genes that might be required for the exchange of RAB-5 for RAB-7 on the phagosome, we have used a targeted reverse genetic screen for players that might bind either protein. We performed in-depth analysis of two candidate genes, whose disruption resulted in the accumulation of undegraded (but internalized) corpses within abnormal phagosomes. Using a series of genetic, cell biological and biochemical studies, we propose a mechanism for linking RAB-5 activation to the recruitment of RAB-7 to the phagosome.

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The neural protein PKC-2 suppresses Duchenne Muscular Dystrophy. **A. Reedy**, C. Lecroisey, MC Mariol, H. Salter, L. Ségalat, K. Gieseler. Centre de Genetique Moléculaire et Cellullaire, Université Claude Bernhard Lyon-1, Villeurbanne, Rhône-Alpes, France.

Duchenne muscular dystrophy (DMD) is a disease that is characterized by progressive degeneration of the skeletal, cardiac and smooth muscles, as well as neurons. DMD is caused by mutations in the Dystrophin gene, however the pathogenesis for DMD is not yet clear. Mutations in the *C.elegans* Dystrophin homolog (*dys-1*), lead to hyper-contraction, hyperactivity and an increased Acetylcholine (Ach) level. They also have infrequent body wall muscle degeneration, that can be greatly enhanced in the background of a weak mutation affecting the myogenic factor MyoD (Harte *et al,* 1998 ; Gieseler *et al,* 2000 ; comments in Chamberlain and Benian, 2000). We use this d*ys-1; CeMyoD* double mutant as a model for DMD, as it exhibits progressive muscle degeneration. Studies in numerous model organisms, including *C.elegans*, indicate that loss of dystrophin or associated proteins lead to defects in Acetylcholine (Ach) transmission at the neuromuscular junction, and this may play a role in the pathophysiology of DMD. Previous genetic studies using our DMD worm model have shown that muscle degeneration is suppressed by mutations that inhibit cholinergic transmission (*unc-13*) or calcium influx (*egl-19n582*) (Mariol *et al,* 2001; 2007); because these mutations lead to muscle inactivity, it is impossible to conclude whether the suppression of muscle degeneration is due to muscle inactivity and/or to the suppression of another Ach induced effect. We have recently shown a mutation of *pkc-2* reduces muscle degeneration in the DMD worm by almost 50%. There is currently some question as to PKC-2 localisation, and therefore the role of *pkc-2* mutations in DMD pathology. A *pkc-2* transgene, under a *lac-Z* promoter, has been shown to localise in both muscles and neurons; however, the PKC-2 antibody was observed only in neurons. (Islas-Trejo *et al*, 1997). *GFP::pkc-2* transgenes, expressed as extrachromosomal arrays or integrated with MOSTIC have been observed in the neurons, neural cell bodies, vulva, and spermatheca, with no signal in muscles. The *pkc-2* mutant was shown to have increased resistance to the Ach esterase inhibitor aldicarb, indicating it may affect Ach neurotransmission. Interestingly, *pkc-2* mutants do not exhibit any obvious muscle activity phenotype. Loss of function mutations in both *pkc-2* and *unc-13* have been shown to suppress the DMD worm phenotype, yet no suppression was seen with RNAi. As neuronal expressed genes are relatively resistant to RNAi (Kamath *et al*, 2001), we think this is further evidence to suggest that the role of PKC-2 is in fact neural vs. muscular.

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Use of *C. elegans* to Identify and Characterize VPS41 as a New Therapeutic Target for Parkinson's Disease. **A. Harrington**[1], Q. Ruan[2], S. Hamamichi[1], J. Schieltz[1], D. G. Standaert[2], K. A. Caldwell[1,2], G. A. Caldwell[1,2]. 1) Biological Sciences, Univ Alabama, Tuscaloosa, AL; 2) Dept Neurology, Univ Alabama at Birmingham, Birmingham, AL.

Parkinson's disease (PD) is the second most common neurodegenerative disorder. Studies of familial forms of PD have identified genes that when mutated or over-expressed, result in enhanced susceptibility to dopamine (DA) neurodegeneration. One of these proteins, alpha-synuclein (α-syn), has been shown to aggregate and form protein inclusion bodies. Overexpression of human α-syn in *C. elegans* resulted in accumulation of this protein and was used to screen for effectors of age-dependent aggregrate formation *in vivo*. Using RNAi, our lab has identified several genes that, when knocked down, increase aggregate formation within the body wall muscles. Notably, overexpressing several of these effector proteins in worm DA neurons led to protection from α-syn-induced neurodegeneration (Hamamichi et al. 2008, PNAS). Of these neuroprotective proteins, a lysosomal trafficking protein, VPS-41, was the most effective at preventing neurodegeneration. VPS41 is a highly conserved protein expressed in mammalian DA neurons. It has metal ion binding, clathrin CLH, RING finger, and AP3 interaction domains, and has been shown to be involved in lysosomal trafficking in yeast. To identify domains of VPS41 responsible for its neuroprotective actions, we truncated different domains of human VPS41 and overexpressed these truncates in α-syn-expressing DA neurons of worms. Select truncated forms of VPS41 exhibited DA neuroprotection against α-syn-induced degeneration, with the clathrin CLH and AP3 interaction domains being the common domains between the protective isoforms. This neuroprotection by VPS41 was not seen in an AP3 mutant background or when the clathrin CLH domain was mutated with a SNP (C647R). Furthermore, VPS41 overexpressing worms also showed resistance to 6-OHDA induced DA degeneration, indicating VPS41 can protect against different forms of cellular stresses associated with modeling PD *in vivo*. Importantly, the neuroprotective capacity of VPS41 extends to mammalian cellular models where SH-SY5Y neuroblastoma cells transfected with human VPS41 exhibit protection from a broad kinase inhibitor, staurosporine; the PD-related neurotoxin, rotenone; and the oxidative stress inducer, 6-OHDA. The presence of VPS41 in these cells significantly reduces the sensitivity of the lines to all three toxins. These data demonstrate that VPS41 is part of an evolutionarily conserved mechanism that is neuroprotective in both mammalian cells and *C. elegans*. Thus, the VPS41 protein and, by extension, the regulation of autophagy, represents a promising target for therapeutic development against PD.

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The Coenzyme Q Synthesis Gene *coq-1* Protects *C. elegans* GABA Neurons from Calcium-Dependent Programmed Necrosis. **Mallory L. Hacker**[1], Laurie R. Earls[2], Joseph D. Watson[3], David M. Miller III[1]. 1) Vanderbilt University, Nashville, TN; 2) St. Jude Children's Research Hospital, Memphis, TN; 3) University of North Carolina School of Medicine, Chapel Hill, NC.

Coenzyme Q is a component of the mitochondrial electron transport chain and essential for energy metabolism. CoQ deficiency in humans causes cerebellar ataxia and myopathy, indicating that selected tissues are especially sensitive to reduced levels of CoQ. To develop a model for these degenerative diseases in *C. elegans*, we used RNAi to knock down expression of *coq-1*, the initial enzyme in the CoQ biosynthetic pathway. RNAi or genetic ablation of *coq-1* resulted in a progressive, uncoordinated, or Unc, phenotype and necrotic degeneration of GABA neurons. Both the Unc and degenerative phenotypes emerge during late larval development and progress in adults. Neurons in motor and sensory circuits that utilize other neurotransmitters and body muscle cells did not degenerate upon RNAi depletion of *coq-1*. The mechanism of GABA neuron cell death depends on the release of intracellular calcium stores and requires the apoptotic genes *ced-4* (Apaf-1) and to a lesser extent *ced-3* (caspase). Our finding that the mitochondrial fission gene *drp-1* (dynamin-related protein) is required for GABA neuron degeneration is consistent with a model in which CED-4 is activated by a signal released from CoQ-depleted mitochondria. Curiously, this pathway is not regulated by the apoptotic genes *egl-1* (BH3-only) and *ced-9* (Bcl-2) and therefore is likely to include novel components. The progressive and selective degeneration of GABA neurons and the role of mitochondrial dysfunction in CoQ-depleted animals parallels salient features of the human neurodegenerative disorder Huntington's chorea in which GABA neurons in the brain that control movement are preferentially affected.

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Heterochronic genes and lateral signaling during vulval precursor cell (VPC) fate specification in *C. elegans*. **Ji Li**[1], Iva Greenwald[1,2]. 1) Columbia University; 2) Howard Hughes Medical Institute.

Timing influences vulval precursor cell (VPC) competence and fate patterning. For example, VPCs respond to LIN-3 produced by the gonad in the L2 stage but are not induced until the L3 stage. LIN-12, the receptor for the lateral signal, is present in the L2 stage, but constitutively active LIN-12 does not specify the 2° vulval fate until the L3 stage.

Heterochronic genes mediate many different timing events. In the canonical pathway, the microRNA *lin-4* downregulates its targets, LIN-14 and LIN-28, to allow stage-specific events to occur. Previous work suggested that VPCs in *lin-4(0)* mutants are immature, as they express *lin-12* but do not display normal 1° or 2° fates (Euling and Ambros, 1996). Using markers and tools that have become available since then, we have re-examined VPC fates in *lin-4(e912)* null mutants.

Our results suggest that there is a defect in lateral signaling. VPCs in *lin-4* respond to the inductive signal, as, a 1° fate marker, *egl-17p::gfp*, is expressed in P6.p in a gonad-dependent manner. In addition, the lateral signal is likely to be produced, as *apx-1p::yfp* and *lag-2p::yfp* are expressed. However, *lin-4* mutants exhibit a phenotype indicative of a failure of LIN-12-mediated lateral signaling: ectopic expression of *egl-17p::gfp* and loss of expression of two 2° fate markers, *lin-11p::gfp* and *lst-5p::yfp*, in P5.p and P7.p. Tissue-specific rescue experiments suggest that *lin-4* functions in the VPCs to promote the 2° fate. Furthermore, loss of *lin-4* can suppress constitutively active mutant forms of LIN-12, including LIN-12(intra). Our results suggest that in *lin-4* mutants, VPCs are not competent to respond to LIN-12 activation or that there is a block downstream of LIN-12 signal transduction.

To understand the nature of the block in lateral signaling, we need to know the relevant lin-4 target(s). Our data suggest that *lin-28* and *hbl-1* are not responsible for the lateral signaling defect in *lin-4(0)*, because *lin-28(n719null)* or *hbl-1(ve18)* cannot restore the 2° fate marker expression in *lin-4(0)*. However, the hypomorphic allele *lin-14(n179)* suppresses *lin-4(0)*, and *lin-14(n355)*, a mutation the removes the binding sites for lin-4, shows a similar phenotype as *lin-4(0)*. These results indicate that the lateral signaling defect in *lin-4* mutants is primarily due to inappropriate *lin-14* activity.

We are currently testing the critical period during which LIN-14 accumulation blocks 2° fate specification. We also plan to do a genetic screen to look for targets or co-factors of LIN-14 that are involved in 2° fate specification.

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lin-28 and lin-46 act at distinct molecular steps to control hbl-1 and stage-specific seam cell fates. **Eric G. Moss**, Bhaskar Vadla, Christian Heine, Kevin Kemper. Dept Molecular Biol, UMDNJ, Stratford, NJ.

hbl-1 is critical for hypodermal cell fates in the larva (Fay, 1999; Abrahante, 2003; Lin, 2003). At least three miRNAs repress hbl-1 at a critical time to permit the transition from L2 to L3 cell fates (Abbott, 2005). We sought to understand how lin-28 and lin-46, which are apparently just upstream of hbl-1, control the same decision. First, we found that lin-28 positively regulates hbl-1 expression. In a strain lacking the three miRs, hbl-1 is constitutive, whereas in a strain also lacking lin-28, it is down-regulated. Therefore, either lin-28 directly supports hbl-1 or it represses a fourth repressor. In investigating mammalian Lin28, we found it associated with mRNPs, ribosomes and P-bodies, suggesting it is an mRNA binding protein (Moss, 2003; Balzer, 2007). Furthermore, it positively regulates the translation of Igf2 mRNA (Polesskaya, 2007). However, several recent studies have shown-remarkably-that mammalian Lin28 specifically binds to and blocks the processing of let-7 family miRNA precursors (Viswanathan, 2008; Newman, 2008, Piskounova, 2008; Rybak, 2008, Heo, 2008). Using a yeast three-hybrid assay, we found that LIN-28 specifically binds precursors of four of seven let-7-family miRNAs: let-7, miR48, miR-84, miR-241, but not miR-793, -794 or -795. It does not bind pre-miRs of miR-1, lin-4, or several other RNA sequences, including portions of the hbl-1 3'-UTR. To determine whether lin-28 affects the accumulation of miRNAs in vivo, we used TaqMan miRNA qRT-PCR assays. Using RNA from approximately 150 wildtype or lin-28(0) larvae in the pre-molt lethargus at each stage, we found, to our surprise, no effect of lin-28 on the level of miR-48 at any stage. By contrast, we saw large changes in let-7 levels at both the L1 and L2 molts. While further assays are being conducted, our preliminary results suggest that lin-28, indeed, specifically binds to and greatly affects let-7 accumulation in C. elegans. We know, however, that blocking let-7 can't be the only thing lin-28 is doing. LIN-46 resembles certain proteins involved in protein-protein interactions (Pepper, 2004). Genetic data suggest lin-46 acts upstream of hbl-1, working with or in parallel to the three miRs. We conducted a two-hybrid screen to identify LIN-46 interactors and found, to our surprise, LIN-46 specifically binds the product of another heterochronic gene, both in yeast and in vitro. LIN-46 interacts with a conserved C-terminal zinc-finger domain of this protein, which, when expressed in wildtype animals, produces a phenotype like that of lin-46(0). Thus, circumstantial evidence suggests that LIN-46 does not act at the level of the miRNA regulation of hbl-1, but rather just downstream of that.

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The Conserved NAB Family Transcriptional Co-factor *mab-10* Acts with the Heterochronic Gene *lin-29* to Regulate Terminal Differentiation in Hypodermal Lineages. **David T. Harris**, Bob Horvitz. HHMI, Dept Biology, MIT, Cambridge, MA 02139 USA.

The study of heterochronic mutants has revealed a complex genetic pathway that regulates the timing of many developmental events in *C. elegans*. Heterochronic mutants fall into two classes: precocious mutants, which prematurely express later developmental fates, and retarded mutants, which reiterate earlier developmental fates. Both classes can alter the timing of the larval-to-adult transition. This transition comprises four events: seam cell fusion, generation of an adult-specific cuticle, exit of seam cells from the cell cycle, and exit from the molting cycle.

Like retarded *let-7* and *lin-29* mutants, *mab-10* males undergo an extra molt approximately 18 hours after the larval-to-adult transition (C. Link, Worm Breeder's Gazette 10, 92, 1988). To understand further the regulation of the larval-to-adult transition, we have analyzed *mab-10* and found that both *mab-10* males and hermaphrodites enter lethargus as adults and often execute an extra molt. While the seam cells of *mab-10* mutants fuse appropriately at the end of the L4 stage and generate a relatively normal adult cuticle, the seam cell nuclei of *mab-10* mutants inappropriately undergo extra rounds of division. We conclude that *mab-10* is required for the prevention of seam cell divisions and for the cessation of molting, but is not required for seam cell fusion or adult cuticle synthesis. By contrast, the C2H2 zinc finger protein LIN-29 is required for all four events during the larval-to-adult transition.

We cloned *mab-10* and found that it encodes the only *C. elegans* member of the conserved NAB (NGFi-Alpha Binding) family of transcription factors. Recent studies of mice have implicated the NAB family of proteins in regulating the terminal differentiation of specific stem cell lineages (Le et al., Nature Neurosci. 8, 932, 2005). NAB proteins are believed to act as co-factors for C2H2 zinc fingers to regulate differentiation. Our genetic analyses and co-localization and *in vitro* binding experiments suggest that MAB-10 functions as a cofactor for the C2H2 zinc finger transcription factor LIN-29 to regulate specifically the exit of the seam cells from the cell cycle and the cessation of molting, but not seam cell fusion or adult cuticle synthesis. We propose that the regulation of developmental stage in *C. elegans* and the regulation of terminal differentiation in mammalian stem cell lineages share a common mechanism controlled by a conserved heterochronic pathway.

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The 3-β-hydroxysteroid dehydrogenase (3βHSD) family member HSD-1 controls dauer arrest via regulation of nuclear DAF-16/FoxO. Xi Wang[1], Kirk Burkhart[2], Chunfang Guo[1], Kathleen Dumas[1], Elizabeth Adams[1], Hena Alam[1], **Patrick J. Hu[1,3,4]**. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Genetics, University of Wisconsin, Madison, WI; 3) Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 4) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

The *C. elegans* insulin receptor ortholog DAF-2 promotes reproductive development and controls lifespan by regulating nuclear translocation of the FoxO transcription factor DAF-16 via a conserved PI 3-kinase/Akt pathway. Nuclear localization of DAF-16/FoxO is necessary but not sufficient for full DAF-16/FoxO activity, suggesting that other inputs regulate the activity of nuclear DAF-16/FoxO. We recently discovered the EAK (enhancer-of-akt-1) pathway, which acts in parallel to AKT-1 to inhibit nuclear DAF-16/FoxO activity. *eak-2* is allelic to *hsd-1*, which encodes a conserved 3βHSD family member thought to participate with the cytochrome P450 DAF-9 in the biosynthesis of steroids known as dafachronic acids (DAs). DAs function as ligands for the nuclear receptor DAF-12. As is the case for other *eak* mutants, *hsd-1* and *daf-9* mutations enhance the dauer arrest phenotype of an *akt-1* null mutation, suggesting that the EAK pathway regulates DA action and that DAs act in parallel to AKT-1 to promote reproductive development. *hsd-1* mutations enhance the dauer arrest phenotype of *akt-1* mutants and increase mRNA levels of the direct DAF-16/FoxO target gene *sod-3* in *akt-1* mutants in a DAF-16/FoxO- and DAF-12-dependent manner. In contrast to *akt-1* mutation, *hsd-1* mutation does not promote DAF-16::GFP nuclear localization. Furthermore, *hsd-1* mutation enhances the dauer arrest phenotype of a strain expressing a constitutively nuclear DAF-16::GFP fusion protein that lacks all consensus AKT-1 phosphorylation sites, suggesting that HSD-1 regulates the activity of nuclear DAF-16/FoxO. Taken together, our results support a model whereby HSD-1 and AKT-1 act via distinct and complementary mechanisms to promote reproductive development by regulating the expression of a subset of DAF-16/FoxO target genes. Although *hsd-1* mutants have normal lifespans, HSD-1 activity is required for lifespan extension in mutants with reduced DAF-2 pathway signaling, suggesting that HSD-1 and DAs may modulate DAF-2 pathway signaling in a context-dependent manner.

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Genetic analysis of dauer formation in *Pristionchus pacificus* reveals conserved and diverse aspects of signaling mechanisms. **Akira Ogawa**, Ralf Sommer. Max-Planck-Institute for Developmental Biology, Tübingen, Germany.

Despite the wealth of knowledge of *C. elegans* dauer formation, regulation of dauer formation in other nematode species is relatively poorly understood. To address how environmental regulation of dauer formation evolved, we are studying dauer formation in the satellite model nematode *Pristionchus pacificus*. *P. pacificus* shares with *C. elegans* most of the advantageous features of experimental organisms and has genetic tools such as forward and reverse genetics, a complete genome, and transgenic techniques. In the wild *P. pacificus* associates with scarab beetles as dauer larvae and occupies a distinct ecological niche from *C. elegans*. Since dauer formation play critical roles in the adaptive strategy of *P. pacificus*, one can expect many evolutionary novelties in *P. pacificus* dauer formation. Also genetic analysis of dauer formation in *P. pacificus* may lead to identification of conserved genetic components of dauer formation that are missed in *C. elegans* studies. This is because multiple genes may be involved in the same step of the regulatory cascade (redundancy) or there may be genes that are involved in both dauer formation and earlier development (pleiotropy). Such genes are hard to identify in conventional genetic and RNAi screens and studies in *P. pacificus* that may have different patterns of redundancy and pleiotropy might help identify such genes. So far we have shown that i) *P. pacificus* uses a distinct pheromone(s) from *C. elegans* to sense the population density ii) An endocrine module involving dafachronic acids (DAs) and DAF-12 controls dauer formation in *P. pacificus*. The latter finding was further extended to infective larva formation of the parasitic nematode *Strongyloides papillosus*. Exogenous administration of Δ7-DA completely suppresses the formation of infective larva in *S. papillosus* suggesting infective and dauer larvae share a common evolutionary origin. To elucidate the genes that control *P. pacificus* dauer formation we screened daf-c and daf-d mutants, and obtained more than 10 strains of each class. In a small-scale screen for daf-c mutants, we obtained several fully penetrant daf-c strains that are relatively rare in *C. elegans*, as well as partially penetrant strains. All the daf-c strains tested could be at least partially rescued by exogenous administration of Δ7-dafachronic acid, suggesting they have a mutation in a gene upstream of DA/DAF-12. Interestingly the fully penetrant daf-c strains we obtained did not respond to up to 250 nM of Δ4-dafachronic acid. Positional mapping of the gene responsible for one of the daf-c strains is ongoing.

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Endocannabinoid signaling has a role in dauer formation. **Mark Lucanic**, Jason Held, Mark White, Ida Klang, Brad W Gibson, Gordon J Lithgow, Matthew Gill. Buck Institute, Novato, CA.

The endocannabinoid system in mammals plays a role in many physiological functions and is an important emerging therapeutic target. Cannabinoid receptors have been identified in many different species, including some invertebrates, but they have not been detected in C. elegans. We have now identified components of an endocannabinoid system in C. elegans and find that it interacts with the insulin signaling pathway to modify dauer formation. In a chemical screen for suppressors of dauer formation we identified a synthetic antagonist of the mammalian cannabinoid receptor as a potent suppressor of the Daf-c phenotype of daf-2(e1368). The activity of this compound, and other synthetic cannabinoids, prompted us to take a biochemical approach to defining a worm endocannabinoid system, with a focus on identifying endogenous cannabinoid ligands (endocannabinoids). Analysis of worm lipid extracts by gas chromatography-mass spectrometry (GC-MS) identified a number of fatty acid ethanolamides that are structurally similar to mammalian endocannabinoid ligands. We find that endocannabinoid levels are reduced in daf-2(e1368) mutants under dauer inducing conditions and that endocannabinoid treatment is sufficient to promote reproductive growth in these animals, similar to the effects of synthetic cannabinoids. We also find that levels of endocannabinoids are elevated following treatment with a chemical inhibitor of fatty acid amide hydrolase (FAAH), an enzyme involved in endocannabinoid degradation and inactivation, and that RNAi of the putative worm FAAH gene has a similar effect. Overexpression of this putative C. elegans FAAH gene in wild type animals results in a profound developmental delay, further supporting a role for endocannabinoids in reproductive growth. Finally, daf-2 mutants carrying extra copies of this FAAH gene under heterologous transcriptional control are defective in dauer exit, suggesting a role for endocannabinoids in dauer recovery. In summary these data suggest that endocannabinoids function in C. elegans to promote reproductive growth either under normal conditions or following recovery from dauer arrest. The lack of obvious cannabinoid receptors in the worm suggest the existence of a novel C. elegans receptor, whose identity may shed light on the novel receptors predicted to exist in mammals. Finally the identification of an endocannabinoid system in nematodes now brings the strengths of C. elegans as a major model system to the study of endocannabinoid physiology.

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Methylation of sterols by *strm-1* as a novel mechanism for regulation of dauer larva formation. **Eugeni V. Entchev**[1], J-Thomas Hannich[1], Fanny Mende[1], Hristio Boytchev[1], René Martin[2], Gabriele Theumer[2], Isabelle Riezman[3], Howard Riezman[3], Hans-Joachim Knölker[2], Teymuras V. Kurzchalia[1]. 1) Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany; 2) Department of Chemistry, Technical University of Dresden, Dresden, Germany; 3) Department of Biochemistry, University of Geneva, Geneva, Switzerland.

A complex system of neuronal and hormonal regulation governs developmental switch between reproductive life cycle and entry into diapause. All signals converge onto the activity of a single nuclear hormone receptor, DAF-12, which when bound to a cholesterol derived hormone, dafachronic acid, supports reproductive development. Concomitantly, the reduction of dafachronic acid leads via DAF-12 to activation of diapause promoting program and worms form stress resistant dauer larvae. Previously, it was found that nematodes can perform a unique biochemical reaction by actively introducing a methyl group at the C-4 position in the A ring of sterol nucleus. However, the mechanism and biological significance of the sterol methylation remained puzzling. We have shown that substitution of cholesterol with 4-methylated sterols leads to dauer larva formation. The C-4 methylation is irreversible and methylated sterols cannot be used to produce dafachronic acid via cholesterol. We have also identified a novel type of methyltransferase, STRM-1, that is responsible for the methylation of the sterol nucleus in the A-ring. *strm-1(tm1781)* deletion mutants completely lack methylated sterols. We also show that by using the same substrates needed for the synthesis of dafachronic acid, STRM-1 can reduce the amount of the hormone. As a result, *strm-1(tm1781)* has elevated levels of dafachronic acid, which leads to inefficiency of dauer larva formation. In fact, these mutants are almost insensitive to a synthetic dauer promoting pheromone(s). Thus, the nuclear methylation of sterols emerges as a novel mechanism to control hormone activity.

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Modulation of Insulin Secretion by DAF-8/R-Smad and NHR-69/HNF4α. **Donha Park**, Donald L. Riddle. Michael Smith Laboratories, Univ British Columbia, Vancouver, BC, Canada.

Tightly regulated insulin secretion is a pre-requisite for maintaining appropriate blood glucose level. Failure to do so results in hypoglycemia or in diabetes. It has been reported that treatment of pancreatic β-cells with TGF-β1 induces insulin secretion. However, the molecular mechanism for TGF-β signaling in insulin secretion is largely unknown. We used immunoprecipitation followed by mass spectrometry to identify partner molecules for DAF-8/R-Smad in TGF-β signaling, including a nuclear hormone receptor (NHR-69), which is a strong ortholog of mammalian hepatocyte nuclear factor 4α (HNF4α). HNF4α is a human MODY (Maturity Onset Diabetes of the Young) gene, and its mutation results in decreased insulin secretion. RNAi against *nhr-69* in a sensitized genetic background enhanced dauer formation. *nhr-69* mutants not only enhanced dauer formation of TGF-β pathway Daf-c (constitutive dauer formation) mutants, but also suppressed dauer recovery. Stably integrated *nhr-69p::nhr-69::gfp* was expressed strongly in the intestinal nuclei, hypodermis, and ASI neurons. Nuclear retention of NHR-69 in the intestine was reduced in pre-dauer (L2d) larvae and barely detectable in dauer larvae. When starvation-induced dauers were exposed to food for 30 mins, ASI expression resumed within 6 hrs even without food. *daf-2/IGF1-R*, but not *daf-7/TGF*-β, was required for expression in ASI neurons. NHR-69 was associated with DAF-8 *in vivo* and *in vitro*, but not with DAF-14/Smad or DAF-3/Co-Smad. *daf-8(m85) nhr-69(ok1926)* double mutants showed defects in neuropeptide secretion and increased expression of marker genes *sod-3* and *gst-10*, which are normally repressed by *daf-2* signaling. A yeast-one hybrid analysis had indicated that NHR-69 targets the promoter of *exp-2*, which encodes a voltage-gated potassium channel. Expression of *exp-2* was synergistically increased in *daf-8 nhr-69* mutants. Loss-of-function (lof) *exp-2* mutants conferred an increased insulin secretion and gain-of-function mutants showed decreased insulin secretion. *exp-2 (gof)* mutants also exhibited upregulated *sod-3* and *gst-10* expression. We propose that DAF-8/R-Smad and NHR-69/HNF4α target the voltage-gated potassium channel gene, *exp-2*, to promote insulin secretion in *C. elegans*. A chromatin IP experiment to examine direct binding of NHR-69 to the *exp-2* promoter is underway.

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Specific insulin-like peptides translate distinct sensory information to regulate C. elegans development. **Astrid Cornils**, Mario Gloeck, Joy Alcedo. Friedrich Miescher Institute, Basel, Switzerland.

Insulin-like peptides (ILPs) can convey environmental cues to regulate diverse biological processes. C. elegans has 40 ILPs (1, 2) that might act as ligands of DAF-2, an insulin/IGF-1 receptor homolog in the worm (3). Although it is unclear how these ILPs regulate worm physiology in response to the environment, downregulation of DAF-2 signaling due to harsh environmental conditions prevents the development of reproductive adults and induces the formation of developmentally arrested dauer larvae (reviewed by refs. 4, 5). Since many ILPs are expressed in sensory neurons and interneurons (1), it is possible that these ILPs translate different sensory information to promote either reproductive development or dauer formation, a process regulated by specific chemosensory neurons (6, 7).

We are focusing on the ILPs DAF-28 and INS-6, which are predicted to act as DAF-2 agonists (2), and the ILP INS-1, which is predicted to act as a DAF-2 antagonist (1). Our genetic analyses of deletion mutants showed that *daf-28* and *ins-6* act together to inhibit dauer entry and to promote dauer exit. At the same time, we also found that the relative importance of *daf-28* and *ins-6* on dauer entry vs. dauer exit are reversed. Together our data suggest that both *daf-28* and *ins-6* ensure reproductive development under good environmental conditions. In contrast, we observed that *ins-1* promotes dauer entry and inhibits dauer exit, which suggests that *ins-1* is required to ensure dauer formation under harsh environmental conditions.

Two of the dauer-regulating chemosensory neurons are ASI and ASJ (6, 7). ASI functions to inhibit dauer entry (6), while ASJ functions to promote both dauer entry and dauer exit (6, 7). *daf-28* is expressed in both the ASI and ASJ neurons and is downregulated in dauer larvae in these neurons (2). On the other hand, we found that *ins-6* expression changes between the ASI neurons in well-fed worms and the ASJ neurons in dauer larvae, which is consistent with the observed functions of *ins-6* in dauer entry vs. dauer exit. Thus, our data suggest that specific ILPs act coordinately and in different neurons to regulate different developmental programs in response to distinct sensory cues.

References: (1) Pierce et al., 2001. Genes Dev. 15:672-686. (2) Li et al., 2003. Genes Dev. 17:844-858. (3) Kimura et al., 1997. Science 277:942-946. (4) Guarente and Kenyon, 2000. Nature 408: 255-262. (5) Nemoto and Finkel, 2004. Nature 429:149-152. (6) Bargmann and Horvitz, 1991. Science. 251:1243-1246 (7) Schackwitz et al., 1996. Neuron. 17:719-728.

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NHR-8, A Nuclear Receptor Influencing Reproduction, Lipid, and Cholesterol Homeostasis. **Daniel Magner**, Dongling Li, Joshua Wollam, Adam Antebi. Department of Molecular and Cellular Biology, Huffington Center on Aging, Baylor College of Medicine, Houston, TX.

Our lab is interested in understanding how animals couple metabolic signals to development, reproduction, and longevity. Nuclear receptors (NR) are transcription factors that respond to lipophilic ligands, which may play a critical role in these processes. From genetic screens, we identified the *C. elegans* nuclear receptor *nhr-8* as an important modulator of sterol and lipid metabolism, dauer formation, gonadal maturation and longevity. NHR-8 is a homolog of vertebrate LXR/FXR/PXR, NRs implicated in sterol, lipid, and xenobiotic metabolism in mammals. Previous work suggested that *nhr-8* plays a role in xenobiotic metabolism (Lindblom 2000). The closest relative in *C. elegans* is DAF-12, a NR that regulates dauer formation and life span in response to its ligands, bile acid-like steroids called the dafachronic acids (DA). We find that *nhr-8* mutants have phenotypes reminiscent of a reduction in DA production: they are Daf-c at elevated temperatures (27°C), and form a high proportion of dauers or have gonadal outgrowth defects (Mig) under cholesterol deprivation. In addition, *nhr-8* mutants exhibit a novel phenotype in which they arrest early in larval development in response to cholesterol deprivation and thermal stress. To better understand *nhr-8* physiology, we performed expression profiling using microarrays and Q-PCR. This analysis indicates that *nhr-8* regulates a variety of genes involved in lipid, fatty-acid, and sterol metabolism. In accord with a role in DA production, levels of the hormone biosynthetic gene *daf-36*/Rieske oxygenase were modestly reduced. We also find that *nhr-8* influences expression of the fatty-acid desaturase *fat-7 in vivo*, suggesting it works in a complex regulatory network with other NRs such as *nhr-49* and *nhr-80* to maintain fatty-acid homeostasis. Finally, transcriptional assays in cell culture indicate that NHR-8 and DAF-12 directly activate the promoter of a cytochrome P450 identified from microarrays, supporting the idea that these NRs could also comprise a transcriptional network. *nhr-8* also shows remarkable interactions with respect to aging in the germ-line longevity pathway. *nhr-8* mutants are 50% shorter-lived relative to wild type. Surprisingly however, *nhr-8* dramatically enhances by 50% the longevity of germ-line deficient *glp-1* animals. Presumably, longevity signals from the germ-line are modulated by one or more transcriptional targets of NHR-8. Collectively, our data suggest that NHR-8 works as part of a network of transcription factors that govern sterol and lipid homeostasis, which impact development, reproduction and aging in *C. elegans*.

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The nuclear hormone receptor *nhr-25* is a heterochronic gene that has dual roles in both promoting and inhibiting *C. elegans* adult programs. **Ryusuke Niwa**[1,2,5], Kazumasa Hada[1,2], Hiroshi Hasegawa[2,3], Masako Asahina[4], Yasunori Kanaho[3], Frank Slack[5]. 1) Grad. School of Life and Environmental Sciences, Univ. Tsukuba, Tsukuba, Ibaraki, Japan; 2) Initiative for the Promotion of Young Scientists' Independent Research, Univ. Tsukuba, Tsukuba, Japan; 3) Grad. School of Comprehensive Human Sciences, Univ. Tsukuba, Tsukuba, Japan; 4) Biology Centre, ASCR, Ceske Budejovice, Czech Republic; 5) Dept. Mol. Cell. Dev. Biol., Yale Univ., New Haven, CT.

Developmental timing in *C. elegans* is controlled by heterochronic genes, mutations in which cause changes in the relative timing of developmental events. During the fourth larval stage to adult transition, the lateral hypodermal seam cells exit the cell cycle, fuse and secrete an adult specific cuticle called lateral alae. The timing of this switch is under the control of a growing set of genes including *let-7*, a founder member of microRNAs (miRNAs). The *let-7* family miRNAs and their targets are evolutionally conserved in many animals, suggesting that similar genetic pathways control developmental timing across phylogeny. Recently we have reported that the expression in seam cells of *apl-1*, a homolog of Alzheimer's *amyloid precursor protein* gene, is regulated by the *let-7* family miRNAs and their target heterochronic genes, such as *hbl-1* and *lin-41* (Niwa et al. *Dev. Biol.* 315: 418-425, 2008). We proposed that the expression analysis of *apl-1* in seam cells is a new approach to elucidate the *let-7*-dependent terminal differentiation pathway in *C. elegans*.

Here we report that the *apl-1* expression analysis reveals the nuclear hormone receptor gene *nhr-25* as a novel heterochronic gene. During the course of an RNAi-based loss-of-function screen, we found that *nhr-25* positively regulated *apl-1* expression in seam cells. Similar to *hbl-1* and *lin-41*, *nhr-25* controlled not only the temporal *apl-1* expression but also other *let-7*-dependent developmental timing functions. In addition to the *let-7*- and *nhr-25*-directing the molting process (Heyes et al. *Development* 133: 4631-4641, 2006), we showed that *nhr-25* also controlled adult-specific collagen gene *col-19* expression, seam cell division, seam cell fusion and alae formation. Loss of *nhr-25* function leaded to the precocious expression of the adult-specific collagen gene *col-19* in the larval stage. However, interestingly, loss-of-*nhr-25* function resulted in retarded phenotypes in seam cell division, seam cell fusion and alae formation. These results suggest that *nhr-25* is a novel heterochronic gene that has dual roles in both promoting and inhibiting *C. elegans* adult programs.

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Identification of New Components of Dauer Regulation using Full-Genome RNAi Screens. **Dhaval S. Patel**[1], Li Fang[1], Shyam Bhansali[1], Gary Ruvkun[2], Weiqing Li[1]. 1) Dept Biol Structure, Univ Washington, Seattle, WA; 2) Dept of Molecular Biology, MGH, Boston, MA.

Multiple signaling pathways, including insulin/IGF-1-like and steroid hormones, regulate dauer arrest. In order to identify new regulators of the dauer decision we conducted full-genome RNAi screens to identify modifiers of insulin signaling. We screened for clones that, via single-generation RNAi, either prevent the prompt recovery of *daf-28(sa191)* transient dauers at 25°C or enhance the dauer formation of *daf-2(e1370)* at 20°C. Potentially, genes that normally prevent dauer entry may also be required for dauer development *per se*; so we also noted genes that cause synthetic larval arrest, in addition to dauer arrest, in our primary list. Two single pass screens yielded 164 gene inactivations. The molecular identities of these hits constitute a large spectrum of genes that may genetically interact with insulin signaling. Our screens did not pull out most of the known dauer genes, many of which function in neurons and are thus resistant to RNAi. Our list does, however, include *daf-21*, *age-1*, and *ftt-2*, three genes known to participate in dauer regulation, as well as several known longevity regulators, suggesting that, although selective, our screens have identified relevant new genes that modulate the dauer and/or insulin pathways. In addition to retesting the candidates with various *daf-28* and *daf-2* alleles, we are also using mutants of *hsd-1*, *daf-1* and cGMP-containing media to investigate how these candidate genes function relative to the steroid, TGF-β and cGMP signaling pathways, respectively, that regulate dauer arrest. Of the initial 164 genes, 23 displayed synergism with *hsd-1* in preventing dauer arrest. These genes include components that are potentially involved in steroid signaling, MAP kinase-mediated stress responses, tyrosine degradation, protein turnover and ribosomal biogenesis. We have also investigated whether these components function to negatively regulate DAF-16, as insulin signaling does. By using *hsd-1 daf-16* double mutants, we have determined that of the 23, the synthetic dauer arrest associated with 3, 7 and 13 of the genes is dependent, semi-dependent and independent of DAF-16 activity, respectively. Among the semi-dependent ones are 2 potential new steroid-signaling components. Further analysis with DAF-16::GFP indicates that such semi-dependence coincides with a mixed distribution of DAF-16 in the cytoplasm and nucleus, suggesting a molecular mechanism for how insulin and steroid signaling may converge *in vivo*. We anticipate that the above approaches will reveal new components and mechanisms of the signaling network that regulates dauer formation.

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Flows and Tension during Cortical Polarization of the *C. elegans* Zygote. **M Mayer[1,2]**, M Depken[1,2], JS Bois[1,2], F Juelicher[2], SW Grill[1,2]. 1) Max-Planck-Institute for Molecular Cell Biology and Genetics, Dresden, Germany; 2) Max-Planck-Institute for the Physics of Complex Systems, Dresden, Germany.

Asymmetric cell divisions are a fundamental prerequisite for generating cellular diversity in developing organisms. During the first division of the *C. elegans* zygote, the symmetry-breaking event that dictates polarity is an anterior-directed flow of the contractile cortical actomyosin meshwork. This flow serves to segregate cytoplasmic and cortical cell fate determinants between the anterior and posterior domains. Although a local weakening of the posterior cortex appears to be the trigger that initiates cortical flows, the underlying physical principles and regulatory mechanisms remain poorly understood. To investigate what forces drive this flow and how they are regulated within the cell, we need to understand the relationship between cortical flows, active contractility and tension. Specifically, we asked whether we can detect tension gradients within the living *C. elegans* embryo and if the cortical flows affect cortical tension. To this end, we developed an assay to measure tension in the *C. elegans* cortex in a location- and direction-dependent manner using UV laser ablation. We find that in the direction perpendicular to flow (across the AP axis) cortical tension differs between the anterior and posterior domain, and is under the control of the Rho-GTP cycle. We furthermore reveal an anisotropy in tension in the anterior domain, with high cortical tension orthogonal to the AP axis, but two-fold lower tension along the AP axis, in the direction of the anterior-directed cortical flows. In contrast, cortical tension is isotropic and high throughout the cortex when polarization and flows are impaired (spd-5 RNAi), demonstrating that tensile anisotropy is a direct consequence of the underlying flow. Surprisingly, cortical tension along the AP axis is the same in the anterior and the posterior domain, implying that flows are not associated with tension gradients. A quantification of antero-posterior flow velocity and myosin density profiles together with a theoretical analysis of this process reveal that cortical flows in the *C. elegans* zygote operate in a regime where the cortex is sufficiently viscous and unyielding to support long-range cortical rearrangements by local contractions. Our results suggest a fundamental mechanism by which the requirement to generate flow along the entire cell length produces tensile anisotropies but does not necessarily involve tension gradients. We propose that these mechanisms are not only central to the establishment of AP polarity in the *C. elegans* embryo, but also to other developmental processes which involve contractile flows.

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PAR-4 regulates actomyosin contractility through ANI-2 during early C. elegans embryonic divisions. **Nicolas T Chartier[1]**, Paulina Salazar[1], Amy S Maddox[2,3], Labbé Jean-Claude[1,3]. 1) Cell Division and Differentiation Laboratory and; 2) Cytoskeletal Dynamics and Cell Division Laboratory, Institute for Research in Immunology and Cancer, Université de Montréal,; 3) Department of Pathology and Cell Biology, Université de Montréal, Québec, Canada.

The *C. elegans* PAR proteins regulates cell polarity and asymmetric divisions during early embryogenesis. As for other *par* genes, *par-4* mutations induce polarity defects in the embryo, including synchronous divisions at the two-cell stage and lack of P-granule segregation, resulting in embryonic lethality. However, the mechanism by which PAR-4 regulates cell polarity is still unclear and information is missing about potential members of the PAR-4 signaling pathway in *C. elegans*. As actomyosin contractility regulation is a critical process for polarity establishment and maintenance in the first steps of embryo development, we investigated whether actomyosin function was affected in *par-4* embryos.

We used confocal videomicroscopy to monitor actomyosin dynamics in control and *par-4(it57ts)* embryos expressing NMY-2::GFP. We measured several contractility defects during the early polarisation phase and the first division of the zygote for *par-4(it57ts)* when compared to control embryos. We found that the velocity of NMY-2 patches that move toward the anterior pole during polarity establishment is reduced in *par-4(it57ts)* embryos. Furthermore, we observed multiple furrow ingressions at the time of cytokinesis during the division of *par-4(it57ts)* 1-cell embryos. These furrowing defects were associated with increased nuclear movements and membrane engulfment at the site of furrowing and led to a delay in cytokinesis completion in *par-4(it57ts)* mutants compared to control embryos. Interestingly we identified *ani-2*, one of the *C. elegans* homologues of the actin-binding protein anillin, as a suppressor of *par-4(it57ts)* embryonic lethality. We hypothesized that PAR-4 and ANI-2 function antagonistically in a common actin-regulation pathway in the early embryo. Our results show that ANI-2 depletion by RNAi can indeed partially revert the actomyosin defects of *par-4(it57ts)* embryos, indicating that an increase of ANI-2 function is responsible for contractility defects observed in *par-4(it57ts)* embryos. Furthermore, immunolocalisation experiments showed that the loss of PAR-4 activity favors membranous ANI-2 localization in early embryos.

Taken together, these results suggest a model in which PAR-4 regulates actomyosin contractility by inhibiting ANI-2 localization at the plasma membrane during the first steps of embryonic development.

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Directional control of PAR-dependent polarization in *C. elegans*. **Yukinobu Arata[1]**, Tetsuya Kobayashi[2], Hitoshi Sawa[1]. 1) Cell Fate Decision, RIKEN, Chuo-ku, Kobe, Japan; 2) Institute of Industrial Science, the University of Tokyo, Japan.

Cell polarity is properly oriented in many developmental processes such as cell migration, axonal projection, and asymmetric cell division. It has been shown that evolutionarily conserved *par* genes play a central role in these polarity-linked processes. In *C. elegans* embryo, the fertilized egg (P0) and germline lineage P cells (P1, P2, P3) undergo a series of asymmetric cell divisions to give rise to somatic cells and germline P cells. In the P0 and P1 divisions, daughter P cells are generated on the posterior side, while in the P2 and P3 divisions, daughter P cells are generated on the anterior side. However, how the polarity in P2 and P3 is established and how the direction of the polarity is determined remain unknown. First, we tested the P2 division in the selected embryos with hypomorphic par mutations where P0 and P1 divide normally. Among the embryos of *par-2*, *par-4* and *par-6* mutants, size asymmetry between the daughter cells was significantly disrupted. In addition, localizations of PAR-2 and PAR-6 were asymmetric in the isolated wild-type P2 cell using the *in vitro* cell isolation technique. Therefore, we conclude that asymmetric division of P2 is regulated by a PAR-dependent mechanism. Next, we examined how the direction of P2 polarity is determined. We found that the P2 polarity was always oriented toward the contact site with the adjacent somatic daughter cell, even when the contact sites were randomized *in vitro*. In addition, we found that this directional control was dependent on the *mes-1* gene encoding a transmembrane protein by blastomere recombination, and that *mes-1* is required to localize PAR-2 at the contact site. Therefore, PAR-2 localization is oriented by extracellular signal MES-1. Finally, to determine how the extracellular signal orients PAR-2 localization, we compared mobility of cortical PAR-2 protein in the presence or absence of the signal-sending cell by FRAP. The mobility was higher in the presence of the signal-sending cell, suggesting that the extracellular signal entrains asymmetric PAR-2 localization to the contact site by increasing the relative anterograde movement. The directional control of PAR-2 localization appears to be accomplished by modifying cell-autonomous polarization process.

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SUMO is essential for IFB-1 function and assembly. **Rachel Kaminsky**[1], Carilee Denison[2], Andrew D. Chisholm[3], Steven P. Gygi[2], Limor Broday[1]. 1) Department of Cell and Developmental Biology, Sackler School of Medicine Tel Aviv University, Tel Aviv, 69978 Israel; 2) Department of Cell Biology, Harvard Medical School, Boston, MA 02115 USA; 3) Division of Biological Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA.

Sumoylation is a reversible post-translational modification that is essential for cell and organismal viability. The cytoplasmic intermediate filament protein IFB-1 is a newly identified target for SUMO modification. IFB-1 is expressed in the epidermal hemidesmosome-like structures and is essential for embryonic elongation as well as for the maintenance of muscle attachment to the cuticle. In the absence of SUMO, IFB-1 formed ectopic filaments and protein aggregates in the lateral epidermis. Further, depletion of SUMO resulted in a reduction of the cytoplasmic soluble pool of IFB-1, leading to a two fold decrease in the exchange rate of IFB-1::GFP molecules within the epidermal attachment structures. We suggest that SUMO negatively regulates cIF assembly by maintaining a cytoplasmic pool of non-polymerized IFB-1, and that this is necessary for normal IFB-1 function.

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The Myotubularin complex MTM-6/MTM-9 and the PX domain containing protein SNX-3 play a crucial role in MIG-14/Wls recycling in Wnt producing cells. **M. Harterink**, M. Silhankova, M. Betist, R.G.H.P. van Heesbeen, H.C. Korswagen. Korswagen group, Hubrecht Institute & University Medical Center, Utrecht, Netherlands.

The Wnt family of secreted signaling proteins is responsible for important developmental and homeostatic processes throughout the animal kingdom. Furthermore, deregulation of Wnt signaling is implicated in several human pathologies, most notably cancer. Although much is known about how the Wnt signal is transduced into different intracellular responses, much less is known about how a functional Wnt protein is produced and secreted. MIG-14/Wntless (Wls), a conserved multi-pass transmembrane protein, can bind Wnt and is required for its secretion. It is proposed to function as a Wnt sorting receptor, transporting Wnt from the Golgi to the plasma membrane. After Wnt release, MIG-14/Wls is recycled back to the Golgi by the retromer, a protein complex required for endosome to Golgi retrograde transport of sorting receptors. In order to better understand this mechanism, we performed a genome wide RNAi screen in different sensitized genetic backgrounds for genes that are required for signaling by the Wnt EGL-20. In this screen, we identified several genes involved in phosphatidylinositol 3-phosphate (PI3P) turnover. This lipid is enriched on endosomal membranes and is important for trafficking, since it recruits key regulators of intracellular transport. We found that mutation of one of the Myotubularin PI3P phosphatase complexes, MTM-6/MTM-9, leads to defects in Wnt signaling. Since mutation or knock down of the other Myotubularins does not significantly affect Wnt signaling, the MTM-6/9 complex may regulate a specific pool of PI3P that is required for Wnt signaling. The mtm-6/9 Wnt phenotype can be rescued by knock down of components of the PI 3-kinase complex, showing the importance of a correct PI3P balance. Membrane tethering of the core-retromer is thought to be mediated via members of the sorting nexin family, which can bind PI3P through a PX domain. Surprisingly, however, mutants for the classical retromer sorting nexins snx-1 and snx-5 do not show any Wnt signaling defects. In the screen we identified another sorting nexin, snx-3. The snx-3 mutant shows strong Wnt signaling defects, which are similar but not identical to retromer mutants. We found that both mtm-6 and snx-3 are required in the Wnt producing cells, and that both are required for proper recycling of MIG-14/Wls. Furthermore, we found that depletion of mtm-6 affects the cellular localization of SNX-3. Therefore, we propose that the MTM-6/9 complex regulates the recruitment of SNX-3 to endosomes, which in turn enables the retromer to recycle MIG-14/Wls.

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A ZYG-12-dynein interaction at the nuclear envelope defines microtubule architecture and germ line nuclear position in the C. elegans gonad. **Kang Zhou**[1], Melissa M. Rolls[1], David H. Hall[2], Christian J. Malone[1], Wendy Hanna-Rose[1]. 1) Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA; 2) Center for C. elegans Anatomy, Department of Neuroscience, Albert Einstein College of Medicine.

Cells often execute changes in microtubule organization as they progress through development, a process critical for embryogenesis and morphogenesis. In the C. elegans embryo, a centrosome-organized microtubule network pulls on the pronuclei to mediate migration, and the nucleus-centrosome attachment requires ZYG-12 (Malone, 2003). We have investigated ZYG-12 function prior to embryo formation in the C. elegans gonad. Surprisingly, ZYG-12 is dispensable for centrosome attachment in the germ line. However, ZYG-12 mediated recruitment of dynein to the nuclear envelope is required to maintain microtubule organization, membrane architecture and nuclear positioning within the syncytial gonad. In wild-type animals, germ line nuclei are orderly organized at the periphery of the tubular gonad, and the microtubule skeleton adopts a non-uniform, crisscross-like structure surrounding the nucleus of each individual germ cell. At the non-permissive temperature, the *zyg-12 ts* allele *ct350*, which cannot recruit dynein to the nuclear envelope, displays various phenotypes including disrupted nuclear position, reorganized germ cell membrane, and rearrangement of the microtubule skeleton. Another *zyg-12 ts* allele *or577*, which has an embryonic detached-centrosome phenotype identical to *zyg-12(ct350)* but still recruits dynein to the nuclear envelope, exhibits normal gonadal and microtubule architecture. Because centrosome detachment does not underlie the microtubule network disorganization of *zyg-12* mutant gonads, we looked for alternative sites of microtubule nucleation by examining γ-tubulin localization. Based on observations of γ-tubulin at the plasma membrane in the distal gonad, we propose that gonad architecture, including membrane and nuclear positioning, is determined by microtubule nucleation at the plasma membrane combined with tension on the microtubules by dynein anchored at the nucleus by ZYG-12.

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UNC-83 is a nuclear-specific cargo adaptor for kinesin-1 mediated and dynein regulated nuclear migration. **Heidi N Fridolfsson**, Marina Meyerzon, Nina Ly, Daniel A Starr. Molecular and Cellular Biology, University of California, Davis, Davis, CA.

Targeting of microtubule motors to specific cargo is an active area of research. Nuclei migrate to specific locations of a cell during many developmental events such as fertilization, establishment of polarity, and cell division. The C. elegans KASH protein UNC-83 specifically localizes to the outer nuclear membrane where it is required for nuclear migration in embryonic hypodermal Hyp7 cells. UNC-83 and its partner SUN protein form a bridge across the nuclear envelope, connecting the cytoskeleton to the nuclear lamina. We show that UNC-83 is a nuclear-specific cargo adaptor to recruit both Kinesin-1 and Dynein to the nuclear envelope. A yeast two-hybrid screen confirmed by GST pull-downs identified the kinesin light chain KLC-2 and three dynein regulators (NudE homolog NUD-2, BicaudalD homologue BICD-1, and dynein light chain DLC-1) as UNC-83 interacting proteins. KLC-2 interacts with the kinesin heavy chain UNC-116 to form conventional kinesin. klc-2 and unc-116 mutant animals had a nuclear migration defect similar to unc-83 mutant animals. Immunofluorescence showed that UNC-116 and KLC-2 co-localize in part with UNC-83 at the nuclear envelope. bicd-1(RNAi) caused a weak nuclear migration defect that was enhanced in bicd-1(RNAi); nud-2(ok949) animals, suggesting that BICD-1 and NUD-2 function in parallel pathways. Further experiments showed that the egalitarian homologue EGAL-1 functions in a complex with BICD-1 and DLC-1. dlc-1(RNAi) is lethal, but in rare escapers, weak defects in nuclear migration were observed. In the second complex, NUD-2 interacts with LIS-1, which is also required for nuclear migration. Yeast two-hybrid results and functional deletion analysis indicate that two separate domains of UNC-83 interact with kinesin and dynein. These data suggest a model where UNC-83 acts as the cargo-specific adaptor between the outer nuclear membrane and the microtubule motors kinesin and dynein. In this model kinesin functions as the major force generator during nuclear migration, while dynein is involved in regulation. This model is supported by our finding that a KLC-2::KASH construct targets to the nuclear membrane and rescues nuclear migration in an unc-83 mutant background. Hyp7 nuclear migration is therefore an excellent model to study the regulation of dynein and kinesin driven cargo movement. Current studies include an analysis of the microtubule cytoskeleton structure in real time during Hyp7 nuclear migration in wild-type and mutant embryos.

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The Distinct Roles of Self-assembly and GTP Hydrolysis in Regulating Dynamin's Association with Target Membranes and the Removal of Apoptotic Cells in *C. elegans*. **Bin He**[1], Xiaomeng Yu[1,3], Moran Margolis[2], Xiaohong Leng[1,4], Yael Etzion[2], Dganit Danino[2], Zheng Zhou[1]. 1) Verna and Marrs McLean Dept Biochem & Molec Biol, Baylor Col Medicine, Houston, TX; 2) Department of Biotechnology and Food Engineering, Technion, Haifa 32000, Israel; 3) Department of Biology, Stanford University, Stanford, CA, 94305; 4) Department of Molecular Pathology, University of Texas M. D. Anderson Cancer Center, Houston, TX 77054.

Dynamin is a multi-domain GTPase that forms polymers along lipid surfaces and acts in multiple vesicular trafficking events. In addition to its well-known membrane fission function in endocytosis (1), C. elegans dynamin (DYN-1) is also known to promote regulate synaptic vesicle transport and cytokinesis (2,3). Recent studies found that DYN-1 is essential for the removal of apoptotic cells (3,4,5). In *C. elegans*, apoptotic cells are swiftly engulfed by surrounded tissues and degraded inside phagosomes. The swift engulfment and degradation of apoptotic cells rely on the efficient transport and fusion of intracellular vesicles to phagocytic cups and maturing phagosomes. DYN-1 is localized to the surfaces of extending pseudopods and nascent phagosomes in response to the signaling from phagocytic receptor CED-1, and promotes vesicle delivery to these regions. To understand the distinct roles of DYN-1's self-assembly and GTP hydrolysis for the removal of apoptotic cells, we analyzed two classes of missense mutations of DYN-1. *In vitro*, Class I mutant DYN-1 fails to bind and/or hydrolyze GTP. In contrast, a Class II mutation specifically affects DYN-1's self-assembly along lipid surfaces. Our genetic and cell biological studies in *C. elegans* generated multiple lines of evidence to indicate that DYN-1 undergoes self-assembly *in vivo*. Furthermore, we have found that self-assembly is essential for DYN-1's association to the surfaces of pseudopods and phagosomes and the apical surface of intestinal cells, whereas the dissociation of DYN-1 from its target membranes is GTP-dependent. We propose that the self-assembly and GTP hydrolysis activities of DYN-1 play important and distinct roles in regulating DYN-1's dynamic association to target membranes, an event known to be essential for DYN-1's activity in apoptotic-cell removal and likely to be so for other cellular functions of DYN-1. References: 1.Grant, B. and D. Hirsh, Mol Biol Cell, 1999.10: 4311-26. 2.Clark, S.G., et al., Proc Natl Acad Sci USA, 1997. 94: 10438-43. 3.Thompson, H.M., et al., Current Biology, 2008.12: 2111-2117. 4.Yu, X., et al., Dev Cell, 2006. 10: 743-57. 5.Yu, X., et al., PLoS Biol, 2008. 6: e61. 6.Kinchen, J.M., et al., Nat Cell Biol, 2008. 10: 556-66.

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A *C. elegans* model of the human disease orotic aciduria reveals enlarged lysosome-related organelles in embryos lacking *umps*-1 function. **Steven Levitte**, Becca Salesky, Maddie Cole, Micah Depper, Greg Hermann. Department of Biology, Lewis & Clark College, Portland, OR.

While much is understood regarding the trafficking of material to various organelles in eukaryotic cells, the mechanisms regulating organelle size is a mystery. In order to study this process, we have identified a collection of mutants that display enlarged gut granules, cell-type specific lysosomal compartments found in *C. elegans* intestinal cells. Disrupting the function of umps-1 that encodes a homologue of human UMPS (uridine-5'-monophosphate synthase), which carries out the two terminal reactions in de-novo pyrimidine biosynthesis, results in enlarged vacuoles within embryonic intestinal cells. These vacuoles are autofluorescent, contain the membrane protein PGP-2, and contain birefringent material, all of which characterize gut granules. Consistent with their identification as gut granules, mutations in genes known to be required for gut granule biogenesis suppress the formation of enlarged compartments by u*mps-1(-)*. The enlarged gut granules in *umps-1(-)* embryos are not acidified and do not appear to contain fat, suggesting that they are not formed properly. The size of other endo-lysosomal compartments are not dramatically altered by defects in *umps-1*. Consistent with a role for *C. elegans umps-1* in UMP synthesis, *umps-1(-)* embryos exhibit partially penetrant lethality that is suppressed by the addition of uracil. In addition, u*mps-1(-)* embryos exhibit pharyngeal morphogenesis defects similar to *pyr-1(-)*, which encodes a protein carrying out the first three steps in de-novo pyrimidine biosynthesis. *umps-1* is expressed in intestinal cells, however a functional UMPS-1::GFP protein is localized in the cytoplasm, suggesting it has an indirect role in controlling gut granule size. The human disease orotic aciduria is caused by mutations in UMPS which leads to the buildup of its substrate orotic acid. We find that the enlargement of gut granules seen in *umps-1(-)* embryos is likely due to the accumulation of orotic acid, as loss of *pyr-1(-)* activity suppresses their formation. In a search for transporters that might mediate the movement of orotic acid across the gut granule membrane, we found that wht-2, which encodes an ABC transporter not obviously required for gut granule formation, is required for gut granules enlargement in u*mps-1(-)*. Our work points to the novel possibility of altered lysosomal function in human orotic aciduria, identifies the first candidate orotic acid transporter in any system, and provides a model system for investigating the cellular and genetic processes that contribute to enlarged lysosomal compartments.

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AMPH-1/Amphiphysin/BIN1: A Novel Regulator of Endocytic Recycling. **S. Pant**[1], M. Sharma[2], K. Patel[1], S. Caplan[2], C. Carr[3], B. Grant[1]. 1) Dept Mol. Biol & Biochem, Rutgers Univ, Piscataway, NJ; 2) Dept. of Biochem. & Mol. Biology,UNMC Omaha, NE; 3) UMDNJ Dept. of Pathology and Laboratory Medicine, Piscataway, NJ.

Receptor-mediated endocytosis is crucial for the internalization of receptor-bound ligands of many types. Cargo-laden internalized vesicles fuse with early endosomes. Endosome maturation allows sorting with degradation in the late endosomes and lysosomes or recycling to the plasma membrane to participate in further rounds of endocytosis. Endocytic recycling maintains cellular homeostasis of membrane and lipid components and in polarized cells allows accurate sorting to maintain distinct apical and basolateral domains. The Grant Lab utilizes *Caenorhabditis elegans* as a model system to uncover the molecular players involved in endocytic recycling. We have previously established that the RME-1 protein is found on the endocytic recycling compartment and mediates endocytic recycling in several *C. elegans* tissues. RME-1 is a conserved protein homologous to four mammalian proteins EHD1-EHD4. The protein is an ATPase with an ATP binding N-terminal P-loop that is critical for membrane association and homo-oligomerization. The recent EHD2 crystal structure reveals that, while being ATPases, EHD/RME-1 family proteins bear structural similarity to the clathrin-coated pit GTPase Dynamin. The C-termini of RME-1 family proteins contain a single Eps15-homology (EH) domain. Classically, EH domains function as protein interaction motifs in endocytosis proteins, binding target proteins through Asparagine-Proline-Phenylalanine (NPF) motifs. Mammalian EHD proteins bind to Syndapin, Rabenosyn5, EHBP1, and Numb in this manner.Utilizing an RNAi based approach in a GFP-RME-1 expressing worm strain, we screened for proteins causing alteration in recycling endosome morphology. We focused on predicted worm proteins bearing multiple NPF motifs. Through this screen we identified *C. elegans*AMPH-1/Amphiphysin as a novel regulator of endocytic recycling. Utilizing genetic analysis we establish a novel role for AMPH-1 in the regulation of recycling endosome morphology and recycling of transmembrane cargo. We demonstrate that endogenous RME-1 colocalizes with and associates with endogenous AMPH-1, and this association has a role in recycling. We show that in vitro recombinant purified RME-1 and AMPH-1 bind and tubulate phosphatidylserine liposomes independently, but their properties are distinct when in complex. We propose that the AMPH-1/RME-1 complex functions at the recycling endosome, promoting tubulation and/or scission of cargo carriers destined for the plasma membrane. We show phylogenetic conservation of the role of Amphiphysin family proteins in endosome function using siRNA analysis in HeLa cells.

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New roles for ciliary kinesins in male-specific CEM sensory neurons. **Natalia Morsci**[1,2], Maureen Barr[2]. 1) Cell and Molecular Biology Program, UW-Madison, Madison, WI; 2) Department of Genetics, Rutgers University, New Brunswick, NJ.

The cilium is a specialized organelle used by a cell to detect and convert extracellular sensory stimuli into appropriate intracellular responses. Although all cilia share the same construction–a protruding microtubule-based axoneme encased in ciliary membrane–their shape, size and function are diverse in both human and *C. elegans*. The current hypothesis is that all cilia are built by a conserved kinesin-II-driven process, termed intraflagellar transport (IFT), but their structural and functional diversity is conferred by cell-specific modulation of IFT by additional motors and/or signaling proteins. The molecular mechanisms that control cilia diversity are poorly understood. Here, we explore the roles of multiple kinesins in specializing a cilium in form and function.

We employed *in vivo* fluorescent microscopy to examine cephalic male-specific CEM cilia morphology in wild-type males and mutants defective in the IFT kinesin-2 proteins KLP-11 and OSM-3 and male-enriched kinesin-3 KLP-6. Previous reports suggest KLP-6 functions in cilia-targeted transport of polycystin channel PKD-2 and male-specific sensory behaviors (Peden and Barr 2005). Cilia axoneme reporter TBB-4::GFP shows that cilia length is similar in wild-type, *osm-3(p802)* and *klp-6(my8)* males (~3.4μm). Surprisingly, *klp-11(tm324)* mutation results in elongation of the CEM cilia to 5±.9μm. Mutation of both IFT kinesins resulted in variable phenotype with missing, short or long CEM cilia of *klp-11 osm-3* males. Additional removal of KLP-6 (in *klp-11 osm-3; klp-6* triple mutants) suppresses the long cilia category and results in stunted or absent CEM cilia. Membrane protein PKD-2::GFP and the IFT polypeptide OSM-5::GFP also localized to stunted CEM cilia of *klp-11 osm-3; klp-6* animals. These results indicate that *klp-6* acts with the IFT motors to regulate CEM ciliary length. The existence of cilia, albeit stunted, in *klp-11 osm-3; klp-6* triple mutants suggests additional motors may compensate for the absence of canonical IFT motors and KLP-6.

These results also provide important insight to mechanisms controlling ciliary length. KLP-11 and OSM-3 act redundantly in CEM cilia biogenesis, as they do in amphid channel cilia, and we propose that KLP-11 plays a CEM cilia-specific role in length restriction. We hypothesize that KLP-6 may regulate ciliary length via sensory input or vesicular transport-mediated delivery of membrane to the ciliary base. We are performing experiments to distinguish between these possibilities.

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A *C. elegans* homologue of Joubert syndrome-associated Arl13B associates with the ciliary membrane and is required for proper targeting and motilities of ciliary transmembrane and IFT proteins. Sebiha Cevik, Oktay Kaplan, Katarzyna Kida, Tiina Toivenon, David Cottell, **Oliver Blacque**. University College Dublin, Dublin, Ireland.

Joubert syndrome (JS) and JS-related disorders (JSRD) are clinically heterogeneous, characterised by cerebellar malformation and hypervariable phenotypes such as cystic kidneys, retinitis pigmentosa and polydactyly. Emerging evidence indicate that JS/JSRD are caused by defects in primary cilia, which serve diverse sensory and signaling roles during chemo/mechano-sensation, phototransduction, and development. Although multiple JS/JSRD proteins are known, the molecular bases of their functions remain poorly understood. Here, we investigate the molecular functions of *C. elegans* ARL-13, a homologue of the small Ras G-protein Arl13b, which causes classical JS and is required for vertebrate cilium formation and sonic hedgehog signaling. First, we find that ARL-13 is mostly restricted to proximal (middle) segments of ciliary axonemes, does not undergo intraflagellar transport (IFT), and requires N-terminal palmitoylation lipid modification for its ciliary targeting/retention. Loss of ARL-13 function disrupts cilium chemosensory function, morphology, and ultrastructure, with defects including truncated axonemes, microtubule misplacement/malformation, enlarged middle segments and ciliary accumulation of matrix/membranous material. Consistent with these phenotypes, *arl-13*mutants are defective in localisation and/or translocation of ciliary proteins, with ciliary transmembrane proteins partially mislocalised and IFT proteins exhibiting anterograde IFT motility defects, indicative of kinesin2 motor uncoupling, differential separation of IFT complex A/B components, and reduced OSM-3 translocation rates. Finally, *arl-13* interacts synthetically with *bbs-8*, *dyf-5* and *nph-4*, with double mutants exhibiting enhanced cilia integrity and IFT defects. Taken together, we propose that ARL-13 associates with the ciliary membrane where it maintains cilium structure/function and the localisation and motilities of ciliary transmembrane and IFT proteins, perhaps by regulating aspects of ciliary membrane biogenesis and/or turnover. We also suggest that defects in this function underlie the molecular basis of Arl13b-associated Joubert syndrome.

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Position and precision of the germline stem cell proliferation boundary are regulated by a miRNA regulatory network. **Pradeep M. Joshi**[1], Jess Porter Abate[1,2], Joel H. Rothman[1]. 1) Neurosci Res Inst/MCDB, Univ California, Santa Barbara, CA; 2) Joslin Diabetes Center, Harvard Univ, Boston, MA.

The switch from self-renewal into differentiation of stem cells is tightly regulated within stem cell niches. GLP-1/Notch-type signaling creates a stem cell niche in the syncytial *C. elegans* germline in which the germline stem cells (GSCs) are maintained in a proliferative state. Cells exiting this niche transition from mitosis into meiosis and gametogenesis. While an evolutionarily conserved role for microRNAs (miRs) in regulating GSC proliferation has been suggested by a variety of observations, the network of miRs and their targets that regulate GSC proliferation have yet to be identified. Here, we report identification of thirteen miRs expressed from three gene clusters (clusters A-C) that coordinately regulate germ cell proliferation in the adult germline. Deletion of each cluster results in premature entry of GSCs into meiosis. Expression of ICD-1 protein (the *C. elegans* Bicaudal ortholog), which represses proliferation outside of the GSC niche, defines a sharp boundary of expression within the germline syncytium; very low levels of ICD-1 are present in the GSC niche, with abruptly higher levels in the immediately adjacent cells just distal to the start of the "transition zone" where meiotic entry is first morphologically apparent. *icd-1* mRNA, which is predicted to contain target binding sites for miRs in all three clusters, is similarly excluded from the GSC niche. We found that deleting cluster A results in inappropriate accumulation of ICD-1 protein and mRNAs throughout the GSC niche. In addition, *gld-1* and *gld-3* mRNAs, which are also predicted to bind cluster A miRs, are similarly inappropriately expressed in the niche in this mutant, suggesting that these miRs function in part by regulating target mRNA stability. The premature meiotic entry seen in the miR deletion mutants is suppressed by decreased activity of GLD-1 and ICD-1, implying that the miRs regulate germline proliferation in part through these factors. In a number of systems, miRs appear to establish sharp boundaries of gene expression within tissues. We found that the cluster A miRs are essential for the precision of positioning of the proliferation boundary; boundary placement becomes highly variable in the absence of each miR cluster, the first evidence that miRs regulate gene expression boundaries within a tissue in *C. elegans*. These findings reveal that a complex network of miRs functions to precisely regulate GSC proliferation in the stem cell niche.

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A dsRNA-specific endocytosis pathway for environmental RNAi. **Deborah L. De Jong**, Craig P. Hunter. Dept Molecular & Cell Biol, Harvard Univ, Cambridge, MA.

In *C. elegans*, ingested double-stranded RNA (dsRNA) triggers environmental RNA interference (RNAi) which spreads systemically throughout the animal and into its progeny. Multiple SID (systemic RNAi defective) proteins are required for the first step, transport of ingested dsRNA across the intestinal lumen. One of these is SID-1, a conserved and broadly expressed transmembrane protein required for importing dsRNA into cells. Expression of SID-1 in *Drosophila* S2 cells, a heterologous system with no known SID homologs, enables rapid energy-independent uptake of dsRNA. However, in *C. elegans*, SID-1 is not sufficient to transport dsRNA across the lumenal membrane as SID-2 is also required. SID-2 is a transmembrane protein that, unlike SID-1, is expressed only in the intestine, localizes nearly exclusively to the lumenal membrane, and functions only in the internalization of ingested dsRNA.

To investigate whether SID-2 acts as an accessory protein to SID-1 or if it can directly internalize dsRNA, we overexpressed SID-2 in S2 cells and measured uptake of radiolabeled dsRNA. We found that SID-2 selectively imports dsRNA independent of SID-1. In contrast to SID-1, however, SID-2-dependent uptake is energy-dependent, sensitive to endocytosis inhibitors, and requires an acidic pH similar to that found in the intestinal lumen. Additionally, fluorescently-labelled dsRNA colocalizes with vesicular-like intracellular SID-2::GFP. In contrast to *C. elegans*, *C. briggsae* is incapable of environmental RNAi and a *C. briggsae* SID-2 homolog, although similarly expressed and localized, does not mediate uptake of dsRNA when expressed in S2 cells.

These results show that SID-1 and SID-2 act by distinct mechanisms and under different conditions and so we propose that they act sequentially. Specifically, we propose that lumenal dsRNA is selectively bound and endocytosed by SID-2 and that SID-1 subsequently transports the dsRNA into the cytoplasm. SID-1 and SID-2 interact in a yeast two-hybrid assay indicating that these internalization steps may be connected by a tissue-specific protein complex.

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siRNAs regulate transcription elongation. **Shouhong Guang**, Aaron Bochner, Kirk Burkhart, Derek Pavelec, Scott Kennedy. Dept Genetics, Univ Wisconsin, Madison, Madison, WI.

siRNAs silence gene expression in the nucleus by recruiting chromatin-modifying factors and targeting nascent transcripts. In *S. pombe* and *A. thaliana*, both transcriptional gene silencing (TGS) and co-transcriptional gene silencing (CTGS) programs have been documented. We have conducted a genetic screen to identify factors specifically required for nuclear RNAi in C. elegans. This screen identified several genes, named nuclear RNAi defective (*nrde*)(1-3). Previously, we reported that *nrde-3* is an Argonaute-like protein, transports secondary siRNAs from the cytoplasm to the nucleus, associates with nascent transcripts harboring sequence homology to the guiding siRNAs, and silences gene expression in the nucleus [1].

nrde-2 is a conserved gene containing a SR domain and a domain of unknown function (DUF) 1740 domain. SR proteins play significant roles in pre-mRNA splicing and transcription elongation, suggesting a connection between nuclear RNAi and transcription elongation mediated by the nrde genes. NRDE-2 predominantly localizes in the nucleus. It is not required for endogenous siRNAs production or NRDE-3 mediated transport of siRNAs from the cytoplasm to the nucleus. NRDE-2 physically associates with NRDE-3, and is recruited to nascent transcripts along with NRDE-3 following RNAi.

We further examined the mechanism of siRNA-mediated nuclear gene silencing in the nucleus. By utilizing chromatin-immunoprecipitation (ChIP), we determined the relative occupancy of RNA polymerase II at the RNAi targeted sites after feeding dsRNA. We found a nrde dependent accumulation of polymerase II at the genomic sites targeted by RNAi, but not promoter regions, suggesting nuclear RNAi in C. elegans regulates transcription elongation. Consistent with this, RNAi silences the pre-mRNA exclusively 3' to the region targeted by the dsRNA, in a *nrde* dependent manner.

Currently, we are investigating how NRDEs regulate RNA polymerase II activity and transcription elongation, and what are the endogenous functions of this NRDE pathway. Progress of these experiments will be reported.

1. Guang, S., Bochner, A.F., Pavelec, D.M., Burkhart, K.B., Harding, S., Lachowiec, J., and Kennedy, S., (2008) An Argonaute transports siRNAs from the cytoplasm to the nucleus. Science 321:537—541.

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The Argonaute CSR-1 interacts with 22G-RNAs targeting germline-expressed genes to promote chromosome segregation in *C. elegans*. **J.M. Claycomb**[1], P.J. Batista[1], K. Pang[3], W. Gu[1], J.J. Vasale[1], D.A. Chaves[1], M. Shirayama[1], S. Mitani[4], D. Conte[1], C.C. Mello[1,2]. 1) Program in Molecular Medicine, Univ Massachusetts Med Sch, Worcester, MA; 2) Howard Hughes Medical Institute, Worcester, MA; 3) Department of Molecular Biology, Beckman Research Institute/City of Hope, Duarte, CA; 4) CREST, Japan Science and Technology Agency and Department of Physiology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan.

RNAi-related pathways mediate diverse functions required for genome maintenance and development. Here we analyze several *C. elegans* factors required for both RNAi and chromosome segregation. We show that the Argonaute, CSR-1, the RNA-dependent RNA polymerase, EGO-1, the Dicer-related helicase, DRH-3, and the Tudor-domain protein, EKL-1, all localize to mitotic chromosomes, and that their wild-type activities are required for the proper alignment of the holocentric kinetochores. In the absence of these factors, the kinetochores appear twisted and disorganized, and fail to present rigid, parallel plates to opposing spindle poles.

In addition to their localization to mitotic chromosomes, CSR-1, EGO-1, and DRH-3 are also found in the germline nuage, known as P granules. Loss of any of these factors leads to a disruption of P granule association with the nuclear envelope of syncytial germline nuclei, suggesting a link between small RNA biogenesis and P granule maintenance.

Deep-sequence analysis reveals that small RNAs depleted in the mutants and enriched in the CSR-1 immunoprecipitation (IP) complex are antisense to thousands of germline-expressed protein-coding genes. Interestingly, in spite of its role in experimentally-induced RNAi, CSR-1 does not appear to down regulate the mRNA or protein levels of its endogenous targets. Instead, our findings support a model in which CSR-1 and its interacting small RNAs target protein-coding regions distributed along the length of all chromosomes to promote the proper organization and alignment of holocentric kinetochores at metaphase. The finding that actively expressed mRNAs are monitored by an Argonaute/small RNA system that, in turn, promotes the formation of higher-order chromosome structure is remarkable, and provides insight to the mechanisms underlying the holokinetic properties of nematode chromosomes which were first described by the pioneering cytologists Theodor and Marcella Boveri more than one hundred years ago.

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The core apoptotic executioner proteins CED-3 and CED-4 promote neuronal regeneration in *Caenorhabditis elegans*. **Berangere Pinan-Lucarre**[1], Christopher Gabel[2], Elizabeth Hulme[2], Sergey Shevkoplyas[3], Daniel Slone[1], Jian Xue[1], Sarah Weisberg[2], George Whitesides[2], Aravinthan Samuel[2], Monica Driscoll[1]. 1) Rutgers U., Piscataway, NJ; 2) Harvard U., Cambridge, MA; 3) Tulane U., New Orleans, LA.

How neurons in their native environments respond to, and recover from, localized physical disruptions such as axon severing is poorly understood. Exciting breakthrough developments in femtosecond laser microsurgery allow precise cutting of individual axons within living *Caenorhabditis elegans*. Fantastically, some severed neurons have the ability to regenerate, making *C. elegans* a powerful model for dissecting the genetic requirements of *in vivo* axonal regeneration. We applied this technology to investigate the role of apoptotic and necrotic genes in the neuronal response to laser severing of ALM touch neurons visualized by $p_{mec\text{-}4}$GFP in the adult *C. elegans*.

Using *ced-3* mutants including *ced-3(n2433)*, which encodes a single amino acid substitution that disrupts the caspase active site, we showed that CED-3 caspase, extensively characterized for its role as the essential core executioner protease in apoptosis, promotes efficient regeneration of ALM as well as D-type motor neurons as assessed 24h following axotomy. Time-lapse studies using a microfluidics device further revealed that CED-3 is needed early. We expressed the caspase inhibitor P35 specifically in touch neurons and observed reduced regenerating capacity, indicating that the caspase activity is required inside the severed neuron for its regeneration. In a complementary genetic approach, we found that the regeneration defect caused by *ced-3(n2433)* was rescued by specific expression of *ced-3* in the touch neurons, demonstrating that CED-3 acts cell autonomously for neuronal regeneration. The apoptotic caspase activator CED-4 is required for efficient axonal regeneration, but the upstream apoptotic regulators CED-9 and EGL-1 are dispensable, revealing regulation mechanistically distinct from developmental apoptosis. Regeneration also depends on caspase-related genes *csp-1, csp-2* and *csp-3* as well as *crt-1*, a critical necrotic gene encoding the Ca^{2+}-storing ER chaperone calreticulin, which appears to act with CED-3 in the same biological pathway.

Our work reveals an unexpected reconstructive role for proteins known to orchestrate cell death. We will present more data about the mechanistic regulation of this regeneration pathway at the meeting.

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Dissecting Calcium/cAMP mediated axon regeneration pathways. **A. Ghosh Roy**[1], Z. Wu[1,2], A. Goncharov[1,2], Y. Jin[1,2], A.D. Chisholm[1]. 1) Division of Biological Sciences, UC San Diego, La Jolla, CA 92093, USA; 2) Howard Hughes Medical Institute.

We are interested in the molecular pathways that promote the regenerative response of axons after injury. Injury of axons of cultured neurons causes a transient elevation of intracellular calcium. Pharmacological elevation of calcium and cAMP are long known to enhance the ability of axons to regenerate *in vitro* and *in vivo*. However the molecular mechanisms that govern the Ca/cAMP mediated axonal regeneration are not clearly understood. We previously reported that the lateral touch neurons ALM and PLM show robust regenerative responses after injury (Wu et al., PNAS, 2007). We report here that calcium and cAMP play critical roles in the regenerative responses of these neurons. Using genetically encoded Ca sensors we have found that laser axotomy triggers a rapid local elevation of intracellular calcium that propagates bidirectionally away from the axotomy site. The amplitude of this calcium transient correlates with the extent of total axonal regrowth. Further, gain of function in the voltage-gated calcium channel, *egl-19* increases the amplitude of the calcium transient and accelerates regenerative growth, suggesting calcium levels are a critical determinant of regrowth kinetics. We find that genetic elevation of calcium enhances regeneration in several ways. The injured axon enters the regeneration phase earlier compared to controls, extends faster, and more often fuses with the severed distal fragment. Our ultrastructural analysis shows that in these cases the regrowing axon has physically fused membranes with the distal fragment. This fusion process appears not to require known fusogens such as EFF-1 or AFF-1. Mutations that chronically elevate neuronal cAMP promote regeneration in a similar manner with the exception that the regenerating process also has an increased tendency to extend ventral synaptic branches. Reduction of neuronal cAMP by overexpression of PDE-4 blocked this ventral branch regrowth, indicating that cAMP functions cell autonomously to promote growth. Activation of protein kinase A has similar effects on regeneration, suggesting PKA is the major effector of cAMP. We further find that different bZip transcription factors are required for distinct aspects of the regenerative response, suggesting calcium/cAMP acts via multiple transcription factors. Our studies indicate that calcium and cAMP have conserved signaling roles and are rate limiting for regeneration. We are currenty exploring the relationship between calcium/cAMP signaling and other regeneration pathways.

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The DLK-1 kinase pathway promotes CEBP-1 mRNA stability and local translation in *C. elegans* synapse maintenance and axon regeneration. **D. Yan**[1,2], Z. Wu[1,2], A.D. Chisholm[1], Y. Jin[1,2]. 1) Div.Biol.Sci.,UC San Diego, CA 92093; 2) HHMI.

The evolutionarily conserved PHR (Pam/Highwire/RPM-1) proteins play distinct roles in axon guidance, axon termination, and synapse formation. Studies in *C. elegans*, *Drosophila*, and *mouse* show that an important function of PHR proteins is to act as E3 ubiquitin ligases for the Dual-Leucine-zipper-bearing MAPKKK (DLK), which results in negative regulation of the DLK MAP kinase cascade. However, the signaling output of the DLK kinase cascade is largely unknown.

Previous screens for suppressors of *rpm-1* neuronal defects uncovered many alleles of the MAPK genes *dlk-1*, *mkk-4*, and *pmk-3* (Nakata et al., 2005). By analyzing additional *rpm-1(lf)* suppressors, we identified two new loci. One is a member of the MAP kinase-activated protein kinase family (MAPKAPK), MAK-2, which is closely related to murine MAPKAPK2 (MK2). The other is CEBP-1, a member of the C/EBP subfamily of bZip domain proteins.

By biochemical studies, we find MAK-2 is directly phosphorylated by PMK-3. Phosphorylation of the conserved threonine residues is required for MAK-2 rescuing activity. Expression of phosphomimetic mutant forms of MAK-2 induces gain of function phenotypes that are dependent on CEBP-1 but not PMK-3. These data establish that MAK-2 acts downstream of PMK-3 and upstream of CEBP-1.

Unexpectedly, functional CEBP-1::GFP protein is present in axons and at synapses,In addition to the nucleus.Using an RNA tagging strategy we find that *cebp-1* mRNAs are also localized to axons and synapses, and that this localization requires the *cebp-1* 3' UTR. We show that activation of the DLK-1 pathway causes stabilization of *cebp-1* mRNAs. The 3' UTR of *cebp-1* is necessary and sufficient for this stabilization. Inappropriate elevation of *cebp-1* mRNA in adult neurons disrupts synapses and axon morphology, showing that the DLK-1 pathway is required in mature neuron maintenance and synapse plasticity.

Furthermore, we find that CEBP-1 and the DLK-1 pathway are essential for axon regeneration after laser axotomy in adult touch neurons. We show that axotomy triggers local synthesis of new CEBP-1 in injured axons. This local synthesis is dependent on DLK-1 and requires the *cebp-1* 3'UTR. Our findings identify the DLK-1 pathway as a regulator of mRNA stability in synapse formation and maintenance, and also in adult axon regeneration.

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Single-Stranded DNA Binding Protein (SSDP) regulates *C. elegans* PLM synaptic branching and synaptogenesis. **Qun Zheng**, Michael Nonet. Department of Anatomy and Neurobiology Washington University, School of Medicine, St. Louis, MO 63110.

Neurite branching and synaptogenesis are important steps in nervous system development. Here, we use the mechanosensory circuit as a model system to dissect the molecular signals regulating these processes. We observed that the PLM anterior neurites consistently extend a branch out posterior to the vulva region in a collateral pattern, and form a compact cluster of synapses (synaptic patch) onto postsynaptic partners in the VNC. When PLM's postsynaptic partners are killed genetically, these synaptic patches become diffuse, indicating the requirement of crosstalk between pre- and postsynaptic partners during synaptogenesis. The stereotypic pattern of PLM branching and synaptogenesis are independent of axon guidance cues tested, like Netrin signaling, WNT signaling, ROBO, and L1CAM. However, in *sam-10 (js94)* mutants, we found that PLM neurites branch out anterior to the vulva region and form diffuse synaptic patches along VNC (similar to the pattern of PLM synaptic patch where postsynaptic partners are killed), without obviously affecting early neuronal differentiation steps, like PLM migration, neurite polarity, and branching frequency. Furthermore, no detectable mechanosensory defects are observed in *sam-10* mutants. Cloning of *sam-10* revealed it encodes a homolog of single-stranded DNA binding protein (SSDP), a component of Lim domain transcription factor co-activator complex in vertebrate systems. SAM-10 functions cell autonomously (presynaptically) in regulating PLM neurite branching and synapse formation. In PLM, SAM-10 protein translocates from the cytosol to the nucleus before synaptic branching occurs. We showed that this nuclear localization is controlled by LDB-1 (Lim Domain Binding Protein), consistent with the observation that loss of LDB-1 function results in almost identical PLM defects as in *sam-10* mutants. Our studies suggest that SAM-10 is an important factor in defining multiple aspects of the late differentiation of PLM neurons, including stereotypic pattern of synaptic branching and presynapse assembly. We are currently screening for molecular components downstream of SAM-10 using cell-specific microarrays.

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Different sensory dendrites use distinct machineries to attain their lengths. **Maxwell G. Heiman**, Shai Shaham. Rockefeller University, New York, NY.

Each embryo encodes a map, with cell shape and cell contacts following set patterns. In *C. elegans*, these patterns are essentially invariant, with a given cell forming the same shape and contacts in every individual. To understand how this developmental map is encoded and read, we have taken a forward genetic approach, isolating mutants in which the map is perturbed.

We focused first on the amphids, a well-characterized pair of sense organs in the head of the animal. Each amphid consists of 12 neurons, which extend unbranched sensory dendrites to the tip of the nose, and two glial cells, the sheath and the socket, which also extend processes to the nose where they surround dendritic endings of the neurons. Using time-lapse imaging to observe the formation of single dendrites, we found that these cells acquire their shapes by an unusual mechanism: the neurons first form contacts with the presumptive nose, and then the cell body migrates away, dragging out the dendrite behind it. This mechanism suggests a developmental map based on the formation of specific cell contacts, for example between dendrites and the nose tip. Indeed, we isolated mutants in which a "map failure" occurs, with the amphid failing to attain its proper length, and these mutants affect a pair of secreted extracellular matrix proteins, DYF-7 and DEX-1, required to anchor dendritic tips at the nose during cell migration.

DYF-7 is a zona pellucida (ZP) domain protein expressed in most sensory neurons in the developing embryo; DEX-1 is a nidogen- and zonadhesin-containing protein expressed widely in the developing head and tail. Yet, the effects of these mutants are limited to the amphids and a similar pair of tail sense organs, the phasmids. The presence of 40 predicted ZP-encoding *C. elegans* genes, and several zonadhesin-like genes, suggests the possibility that part of the developmental map is encoded by combinatorial matchmaking between DYF-7- and DEX-1-like proteins. To test this hypothesis, we have isolated mutants in which sensory dendrites of the URX or CEP neurons fail to attach to the nose. Although URX and dorsal CEP neurons are lineal sisters, the mutants show that these cells attach to the nose using machineries distinct from each other and from that of the amphid. While not yet cloned, these mutants might affect additional ZP or ZP-interacting proteins. Indeed, the presence of ZP proteins in sense organs is highly conserved across species and sensory modalities. The involvement of distinct ZP proteins would support the model of a developmental map partly based on cells forming contacts with specific extracellular attachment sites.

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EFF-1 sculpts neuronal trees via membrane bending, branch retraction and self-cell fusion. **Meital Oren-Suissa**[1], Gidi Shemer[1,3], Millet Treinin[2], Benjamin Podbilewicz[1]. 1) Biology, Technion-Israel Institute of Technology, Haifa, Israel; 2) Department of Physiology, Hebrew University-Hadassah Medical School, Jerusalem, Israel; 3) Department of Biology, UNC- University of North Carolina at Chapel Hill, USA.

Highly branched neuronal processes are typical of numerous neurons such as Purkinje cells in the cerebellum, dendritic arborization neurons in Drosophila, and neurons in the brain cortex. Little is known about the molecular mechanisms of neuronal branching and until recently it was believed that neurons in *C. elegans* have limited branching. To explore how neurons branch we use dynamic live imaging of two mechanosensory neurons that are the most branched neurons described in *C. elegans*. We identify a surprising mechanism by which a homotypic cell-cell fusion protein, EFF-1 fusogen, restricts and maintains a stereotyped pattern of repetitive branching of two PVD neurons essential for reception of strong mechanical stimuli. EFF-1 is the founder member of a family of membrane proteins that are essential and sufficient to fuse eukaryotic cells. Unexpectedly, *eff-1* mutants have ectopic and disorganized branching patterns including twice as many branched structural units reminiscent of multibranched candelabra we term menorahs. In contrast, when expressed in the PVDs, EFF-1 is sufficient to reduce the number of branches and to rescue disorganized menorahs. Extra or fewer branches and disorganized menorahs result in defective sensibility to strong mechanical stimuli. Live confocal imaging reveals that wild-type *eff-1* expression is necessary to remodel and actively trim normal menorahs involving formation of stable branching loops and dynamic retraction of branches. Temporal activation of *eff-1(hy21ts)* by temperature downshifting reveals that *eff-1* activity is required for the generation of loops and FRAP experiments of newly generated loops show that the branches become functionally connected because membrane fluorescent proteins move through the loops. Collectively our results suggest that EFF-1 mediates self-cell fusion, bends nanotubes, and maintains complex stereotyped branched neurons.

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Identification of a novel dense-core vesicle trafficking pathway. **Michael Ailion**, Andrea Pappas, Susan Dalton, Kim Schuske, Patrick Hullett, Erik Jorgensen. Department of Biology, University of Utah, Salt Lake City, UT.

In *C. elegans*, the heterotrimeric Gα subunit Gq is a positive regulator of neurotransmission. By screening for genetic suppressors of an activated Gq mutant, we identified 10 genes that appear to regulate dense-core vesicle trafficking or release. Two of the genes, *unc-31*(CAPS) and *pkc-1* (a protein kinase C isoform), have been previously shown to be required for dense-core vesicle docking and release. In *unc-31* and *pkc-1* mutants, fluorescently tagged peptides are transported to their normal release sites in the dorsal nerve cord but have reduced release. In the other eight mutants, there is reduced transport of tagged peptides to the dorsal cord, indicating that these genes function in an earlier step of dense-core vesicle biogenesis or trafficking. One of the mutants carries a mutation in a splice site of the kinesin *unc-104* and has a movement phenotype unlike other *unc-104* mutants. The other 7 mutants have an unmotivated movement phenotype; they are capable of coordinated movement when stimulated, but show little spontaneous movement on food. Thus, these genes appear to function in the regulation of movement rather than the execution of coordinated movements. One of the unmotivated genes, *rund-1*, encodes a novel conserved protein with a putative small GTPase effector domain (the RUN domain). We also found that mutants in the small GTPase *rab-2* have phenotypes very similar to *rund-1* mutants. Genetic epistasis data suggest that *rab-2* and *rund-1* act in the same pathway. *rund-1* is highly expressed in neurons and neuronal specific expression rescues the movement defect. In neurons, RUND-1::GFP is concentrated in punctate positions in the cell body that may represent some secretory compartment. RUND-1::GFP does not colocalize with ER or Golgi markers though it appears to be immediately adjacent to the Golgi marker mannosidase II. We have also molecularly identified three other genes in this pathway. All three encode novel conserved proteins that act genetically in the same pathway with *rab-2* and *rund-1* and cause similar peptide trafficking defects. These include two novel proteins, one with coiled-coil domains and one with WD40 domains, and *hid-1*, a previously cloned mutant with phenotypes consistent with defects in dense-core vesicle secretion. All three are expressed throughout the nervous system. Thus, these genes define a conserved pathway that regulates dense-core vesicle trafficking in neurons.

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Analysis of Endophilin endocytic function suggests a model for coupling exo- and endocytosis. **Jihong Bai**[1,2], Jeremy Dittman[3], Edward Pym[1,2], Joshua Kaplan[1,2]. 1) Dept Molecular Biol, Massachusetts General Hosp, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Department of Biochemistry, Weill Cornell Medical College, New York, NY.

To maintain synaptic transmission, the rates of synaptic vesicle (SV) exocytosis and endocytosis must remain in balance. When exocytosis rates are reduced, endocytosis rates undergo corresponding changes. Relatively little is known about how these processes are coupled. To begin addressing these questions, we analyzed the mechanism by which Endophilin/UNC-57 promotes (SV) endocytosis. Endophilin has two functional domains: an N-terminal BAR domain and a C-terminal SH3 domain. Prior studies suggest that endophilin functions primarily as a scaffold protein via its SH3 domain, which binds other critical endocytic factors (e.g. synaptojanin and dynamin). Contrary to this hypothesis, we show that expressing the BAR domain alone is sufficient for rescuing the endocytic defect of unc-57 mutants. The BAR domain exists in monomer ↔ dimer equilibrium. The monomer and dimer both bind membranes; however, only the dimer induces negative membrane curvature. Mutations disrupting the membrane binding or bending functions of the BAR domain disrupt its endocytic function. Interestingly, we show that the diffusive mobility of endophilin at synapses is regulated by exocytosis. In mutants with decreased SV exocytosis, Endophilin has significantly decreased mobility at synapses. Regulation of Endophilin by exocytosis requires the BAR domain but not the SH3 domain. However, unlike its role in endocytosis, the membrane bending function of the BAR domain dimer is not required for regulation by exocytosis. Based on these (and other) results, we propose that Endophilin associates with SVs, and this association holds Endophilin in an inactive monomeric state. Following SV fusion, Endophilin is released from this inactive state and is able to perform its endocytic function. These results provide a potential mechanism for coupling endocytosis to exocytosis by altering the availability of endophilin.

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Eyeless but not blind: Phototransduction in C. elegans. **Alex Ward[1,6]**, Jie Liu[1,6], Lijun Kang[1], Jingwei Gao[1,2], Yong Yu[1,3], Nana Nishio[4], Hitoshi Inada[4,5], Di Ma[1], Brandon Decaluwe[1], Ikue Mori[4], Zhixiong Xie[2], X.Z. Shawn Xu[1]. 1) Life Sciences Institute and Dept. of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109 USA; 2) College of Life Sciences, Wuhan University, Wuhan, Hubei 430072, China; 3) Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China; 4) Group of Molecular Neurobiology, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan; 5) Dept. of Mol. & Cell Biol, Harvard University, Cambridge, MA 02138; 6) These authors contributed equally to this work.

It has long been assumed that the nematode C. elegans lacks the sense of light, mainly because it lives in the soil and does not have eyes. However, we have recently reported the surprising observation that C. elegans in fact possesses a simple visual system and engages in phototaxis behavior that is mediated by photoreceptor cells and light-sensitive channels [1]. Here we elucidate the phototransduction cascade in C. elegans photoreceptor cells through a combination of electrophysiological and behavioral analysis. As is the case with vertebrate photoreceptor cell rods and cones, C. elegans phototransduction is also mediated by G signaling and cGMP-sensitive CNG channels. Interestingly, instead of signaling through phosphodiesterases (PDEs), light-activated G proteins appear to be coupled to guanylate cyclases that produce cGMP, thereby resulting in opening of CNG channels. Our studies identify a new sensory modality in C. elegans and suggest that animals living in dark environments (e.g. soil and caves) may not be presumed to be blind. Our data also reveal a surprising conservation in phototransduction between vertebrates and C. elegans, indicating that C. elegans represents a powerful genetic model for the study of phototransduction. [1] Ward, A.*, Liu, J.*, Feng, Z., and Xu, X.Z.S. (2008) Light-sensitive neurons and channels mediate phototaxis in C. elegans. Nature Neuroscience 11, 916-22 *co-first authors.

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Olfactory adaptation is regulated by a peptide signal in *C. elegans*. **K. Yamada[1]**, T. Hirotsu[2], M. Matsuki[1], H. Kunitomo[1], Y. Iino[1]. 1) Dept. Biophys. and Biochem., Univ. of Tokyo, Tokyo, Japan; 2) Dept. Biol. Fac. Sci., Kyushu Univ., Fukuoka, Japan.

To maintain sensitivity to the changing environment, animals adapt to continuous sensory stimuli such as those of odorants. Studies on olfactory adaptation in *C. elegans* have so far revealed several molecules involved. For example, EGL-4(cGMP dependent protein kinase), GOA-1/EGL-30(Goα/Gqα), ARR-1(β-arrestin), and GPC-1(Gγ) were all reported to regulate olfactory adaptation to benzaldehyde in the sensory neurons AWC, which sense the odorant. In contrast the Ras-MAPK pathway was reported to regulate olfactory adaptation by acting in interneurons. Therefore, neural circuit-dependent mechanisms are also important for regulation of olfactory adaptation. Here, we report a novel component of olfactory adaptation, *nep-2*, which was identified through our genetic screen for adaptation-defective mutants. *nep-2* encodes a homolog of mammalian neprilysin, which is a type II integral membrane protein with an extracellular domain that acts as a metallopeptidase. Neprilysin catalyzes peptide hydrolysis at the extracellular face of the plasma membrane. NEP-2 has a conserved HExxH motif, which is indispensable for peptidase activity in neprilysin family proteases, and this motif was essential for olfactory adaptation. Therefore, NEP-2 was also suggested to act as a peptidase. *nep-2* was expressed in muscle cells and several neurons, and the Venus-fused NEP-2 localized at the surface of these cells. However, the cell-specific rescue experiments revealed that the expression of *nep-2* is not required in a specific cell. These results suggest that the substrate of NEP-2 is a broadly diffusing paracrine or endocrine peptide. To further understand the function of NEP-2 in olfactory adaptation, we screened for *nep-2* suppressors, which resulted in identification of a suppressor mutation in the gene *snet-1*. *snet-1* encodes a protein of 101 amino acids similar to the precursor of Aplysia L11 peptide. SNET-1 carries sequences for a signal peptide and dibasic processing sites suggesting it is also a peptide precursor. Indeed, the results of rescue experiments with truncated *snet-1* cDNA suggested that the putative peptide-coding region of the protein is important for the function. snet-1 is expressed in several neurons including ASK. The expression of *snet-1* in any of these neurons rescued the suppressed phenotype in the *nep-2; snet-1* double mutant suggesting that SNET-1 acts as an endocrine or paracrine peptide signal. These results suggest a model in which SNET-1 peptide is a negative regulator of olfactory adaptation and NEP-2 restricts the function by degrading excess SNET-1 peptide.

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Complex properties of C. elegans oxygen sensing neurons shape different behavioural responses to oxygen. **Patrick Laurent**, Karl Emanuel Busch, Robin Murphy, Mario de Bono. Dept Cell Biol, MRC-LMB, Cambridge, United Kingdom.

Aggregating wild C. elegans mount complex behavioural responses to ambient oxygen. These include responses to increases as well as decreases in oxygen, transient responses coupled to d[O2]/dt, persistent behavioural changes coupled to [O2] and changes in tuning of the responses depending on experience, context, or genetic background. Here we combine calcium imaging and mutant studies to link these varied behavioural features to different properties of oxygen sensing neurons. We show that some oxygen-sensing neurons exhibit biphasic calcium responses to a rise in O2. These consist of transient spike of high calcium when O2 levels rise followed by a plateau of elevated calcium. The plateaux are apparently coupled to [O2] and can last > 30 minutes–as long as [O2] remains high. This perduring signaling implies different states for the nervous system of animals kept at different oxygen tensions. Consistent with this, feeding animals kept in 21% O2 continue roaming while [O2] remains high (> 30 minutes) and remain in a dwelling state while oxygen is at 7% (> 30 minutes). In wild aggregating strains tuning of both behavioural and neural responses involve multiple oxygen sensors including soluble guanylate cyclases and a novel neural globin, glb-5, which can bind O2 itself. GLB-5 appears to act antagonistically to the soluble guanylate cylcases GCY-35 and 36 to tune the responses to a narrow range of oxygen close to 21% (1,2,3). Both behavioural and neural responses to O2 can be reprogrammed by experience. When cultivated in 7% oxygen, N2 animals shift the dynamic range of their responses towards lower [O2]. Similarly, the dynamic range of calcium responses in oxygen sensing neurons is shifted to lower [O2]. Interestingly, in strains that bear the glb-5 allele found in aggregating wild strains, this plasticity is substantially reduced. In this way these animals maintain tuning close to 21% oxygen–the O2 tension found at earth's surface–irrespective of experience. We speculate that this tuning constancy may allow C. elegans to recognize the surface. Antagonistically acting sensors may represent a general mechanism to achieve tuning constancy in cases where this is important, such as when monitoring physiological parameters with narrow bounding limits such as [O2], and pH. 1) Soluble guanylate cyclases act in neurons exposed to the body fluid to promote C. elegans aggregation behavior. Cheung BH, Arellano-Carbajal F, Rybicki I, de Bono M. Curr Biol. 2004 Jun 22;14(12):1105-11. 2) Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Gray JM, Karow DS, Lu H, Chang AJ, Chang JS, Ellis RE, Marletta MA, Bargmann CI. Nature. 2004 Jul 15;430 317-22.

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Special Presentation. **Martin Chalfie**. Columbia University.

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RGEF-1b, a Diacylglycerol (DAG)-regulated GTP Exchange Factor, Facilitates Chemotaxis by Activating LET-60 in AWC and AWA Neurons. **Lu Chen**, Charles Rubin. Dept Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

During *C. elegans* development, ligand-activated receptor tyrosine kinases recruit SOS-1, a guanine nucleotide exchange factor (GEF), to membranes. SOS-1 activates membrane-bound LET-60-GDP by catalyzing exchange of GTP for GDP. LET-60-GTP plays critical and indispensable roles in many developmental processes. However, little is known about properties, regulation and functions of LET-60 activators in post-mitotic, differentiated cells. We discovered that *rgef-1* encodes two GTP exchangers, RGEF (654 amino acids) and RGEF-1b (620 amino acids), as a consequence of alternative splicing. The *rgef-1* promoter is active in all neurons and >95% of gene transcripts encode RGEF-1b. Disruption of *rgef-1* yielded a null allele (ok675). RGEF-1 deficient animals are compromised in chemotaxis to odorants detected by AWC (2-butanone, benzaldehyde) and AWA (diacetyl) neurons. An *rgef-1*::RGEF-1b-GFP transgene restored chemotaxis to odorants. RGEF-1b-GFP expression was directed by cell-selective promoters, *odr-1*, *odr-3* and *gpa-3* in transgenic animals (*rgef-1* null background). Accumulation of RGEF-1b-GFP in AWC neurons rescued chemotaxis to 2-butanone and benzaldehyde. Moreover, expression of dominant negative RGEF-1b in AWC neurons of WT animals suppressed chemotaxis. Thus, RGEF-1b is indispensable in AWC neurons. RGEF-1b loads GTP onto LET-60 and RAP-1, another p21 GTP binding protein. Expression of constitutively active LET-60^{val12} in AWC rescued the rgef-1 null phenotype; AWC-specific accumulation of dominant negative LET-60^{ASN17} abrogated RGEF-1b mediated chemotaxis to 2-butanone and benzaldehyde (WT background). Expression of constitutively active or dominant negative RAP-1 in AWC had no effect on chemotaxis in transgenic or WT nematodes. Mutations that disrupt RGEF-1b catalytic activity blocked loading of GTP onto LET-60 and restoration of AWC-mediated chemotaxis in rgef-1 null animals. Thus, RGEF-1b GTP exchange activity is essential for chemotaxis; RGEF-1b does not function as a scaffold protein. The RGEF-1b C1 domain avidly binds the second messenger DAG. Mutations that diminish DAG binding activity prevent translocation of RGEF-1b from cytoplasm to membranes and ablate the ability of an exchanger transgene to restore AWC- or AWA-dependent chemotaxis in RGEF-1 depleted animals. Conclusions: RGEF-1b links external stimuli (odorants) and internal DAG to the control of behavior (chemotaxis) via unique actions in chemosensory neurons. RGEF-1b is a DAG-regulated GTP exchanger that activates LET-60 (and presumably, MPK-1(ERK)) in AWC and AWA neurons. RGEF-1b is essential for AWC-mediated activation of downstream chemosensory circuitry; a classical LET-60 activator SOS-1 does not compensate for RGEF-1b deficiency.

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Modulation of chemosensation in ASH through extrasynaptic dopaminergic and neuropeptide signaling. **Marina Ezcurra**[1,2], Peter Swoboda[2], William Schafer[1]. 1) Cell Biology Division, MRC-LMB, Cambridge, United Kingdom; 2) Karolinska Institute, Department of Biosciences and Nutrition, Södertörn University College, School of Life Sciences, Alfred Nobel Allee 7, S-14189 Stockholm-Huddinge, Sweden.

C. elegans avoids a variety of repellents, of which many are detected by the neuron ASH. Avoidance responses are influenced by modulators, and previous studies have shown that food affects responses to nose touch and dilute octanol by acting on ASH through 5-HT signaling. Since ASH is polymodal, we asked if other ASH modalities are modulated by food. Using behavioral assays and calcium imaging, we investigated behavioral avoidance and ASH neuronal responses to CuCl2. We found that food affects CuCl2 avoidance; responses are higher when food is present than when food is absent. Surprisingly, we could not find evidence supporting that 5-HT is required, suggesting that other molecules are involved. Dopamine has been implicated in behavioral plasticity, so we tested the role of dopamine in modulation of CuCl2 avoidance. We found that ASH responses, measured by behavioural assays and calcium imaging, were not modulated by food in the dopamine-deficient mutant cat-2(e1112); cat-2 mutants on or off food resembled N2 animals off food. Moreover, exogenous dopamine mimicked the effect of food on N2 animals, suggesting that dopamine is involved in enhancing ASH responses on food. Behavioral candidate dopamine (and other monoamine) receptor mutants revealed that dop-3(vs106) and dop-4(tm1393), like cat-2(e1112) have defects in food modulation of repellent avoidance, suggesting that dopamine affects avoidance behaviors through these receptors. We are using rescue and imaging experiments to investigate if dopamine acts on ASH through DOP-3 and DOP-4 directly or via other neurons. Neuropeptides have modulatory effects on the nervous system. Using behavioral and imaging experiments we have shown that in the absence of food, mutants for the neuropeptide receptors NPR-1 and NPR-2 adapt slower to repeated stimulation with CuCl2 compared to N2. However, in the presence of food this difference is abolished, indicating that NPR-1 and NPR-2 are required to promote faster adaptation in ASH in the absence of food, in contrast to dopamine which promotes slower adaptation in the presence of food. Both npr-1 and npr-2 mutant phenotypes can be rescued by sra-6 promoter control, indicating that neuropeptide signaling acts directly on ASH to modulate adaptation. We are investigating how dopaminergic and neuropeptide modulatory pathways interact through double mutant analysis and cell-specific knockdown of candidate signal transduction molecules.

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Exclusively optical interrogation and monitoring of neural circuits in *C. elegans*. **Zengcai Guo**[1], Anne Hart[2], Sharad Ramanathan[1]. 1) Harvard University, Cambridge, MA; 2) Massachusetts General Hospital & Harvard Med. School, Charlestown, MA.

One of the fundamental challenges in neuroscience is to understand how neural circuits control behavior. To understand the signal processing properties of a neural circuit, we need to know the dynamics of signal propagation in the circuit, and whether synapses are excitatory or inhibitory. While most of physical connections between neurons have been deduced through EM reconstruction, the information about the functional connections between neurons is largely unknown. Genetically encoded calcium indicators (Cameleon and GCaMP) have been successfully used to monitor calcium activity in neurons. Light sensitive channels (ChR2, VChR1, and NpHR) have been used to activate or inhibit neurons in conjunction with patch-clamp to interrogate specific synapses. The ability to simultaneously interrogate multiple synapses using these tools will make elucidation of the signal processing capabilities of the neural circuits in C. elegans more tractable.

Here we develop techniques that allow us to simultaneously excite and monitor calcium activity in vivo in multiple neurons. To do so, we had to overcome two challenges: 1. The excitation spectra of ChR2 and VChR1 overlap substantially with G-CaMP. Thus measurement of the G-CaMP signal could lead to spurious activation of channel-rhodopsin in others; 2. When the same promoter drives expression in multiple neurons, we need the ability to specifically excite the neuron of choice. Using a Digital Light Processing mirror array to spatially confine the excitation light and a different light path to measure G-CaMP activity without activating ChR2, we achieved the goal of interrogating and monitoring multiple neurons simultaneously.

We present our experimental results for the synapses between ASH, AVA and AVD, and the gap junction between RIM and AVA. The polymodal sensory neuron ASH has synaptic connections to the command interneurons AVA and AVD required for backward locomotion. In conjunction with behavioral experiments, we used the optical setup to find that specific stimulation of ASH only activated both AVA and AVD. This activation was destroyed in eat-4 mutant animals. Our results suggest that ASH using glutamate to activate the downstream backward command interneurons AVA and AVD. The inter-motor neuron RIM has several gap junctions with AVA. We expressed ChR2 in RIM and found that animals rapidly initiated reversals upon blue light illumination. Furthermore, specific stimulation of RIM produced calcium activity in AVA, suggesting that RIM depolarization activated AVA. The results show that exclusively optical methods can be used to map synaptic connections formed by chemical synapses or gap junctions.

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Neuropeptides are essential for the serotonergic stimulation of aversive responses mediated by the ASH sensory neurons. **Gareth P. Harris**, Rachel Wragg, Vera Hapiak, Amanda Korchnak, Philip Summers, Richard Komuniecki. Dept Biological Sci, Univ Toledo, Toledo, OH.

Serotonin modulates many key behaviors in *C. elegans*, including the stimulation of aversive responses to dilute octanol mediated by the ASH sensory neurons (Harris et al., 2009, J. Neuroscience 29, 1446-1456). The serotonergic stimulation of ASH-mediated aversive responses requires the expression of SER-5 in the ASHs, but the site of SER-5 action is unclear. For example, SER-5 signaling may increase the release of glutamate and/or neuropeptides from the the ASHs. Indeed, the ASHs express multiple peptide encoding genes, including *nlp-3* and *nlp-15*, and the peptides encoded by these genes have multiple effects on ASH signaling. For example, *nlp-3* null animals exhibit wild-type basal responses to dilute octanol, but do not increase aversive responses in the presence of food or 5-HT. In contrast, *nlp-15* null animals exhibit elevated basal responses to dilute octanol in the absence of either food or 5-HT and these elevated basal responses are not inhibited by octopamine. As predicted, animals overexpressing *nlp-15* respond only weakly to dilute octanol and exhibit dramatically reduced basal responses. Interestingly, in these *nlp-15* overexpressors, aversive responses can be stimulated to near wild-type levels by food or 5-HT. Together, these results suggest that *nlp-15* inhibits ASH signaling by a mechanism that may not directly involve NT release from the ASHs. These observations have been confirmed by the ASH rescue and RNAi knockdown of *nlp-15* and *nlp-3*. In addition, we have identified a number of other genes encoding peptides and peptide receptors that are also involved in modulating various aspects of ASH mediated signaling, highlighting the complexity of peptidergic modulation. These studies are continuing with the goal of identifying the receptors and downstream signaling pathways mediating the differential effects of *nlp-3* and *nlp-15* on signaling in the ASH sensory neurons.

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Serotonin enhances feeding only in response to familiar food. **B. Song**, L. Avery. Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX.

Serotonin has long been regarded as a food signal because serotonin mimics food in controlling behaviors, such as feeding, egg laying, and locomotion. However, serotonin depletion only moderately decreases feeding rate on food and does not noticeably affect development or brood size. This suggests that serotonin may not function as a major food signal, but function to enhance feeding under certain conditions. Here, we report that serotonin from ADF neurons enhances feeding in response to previously experienced food by activating serotonin receptor, SER-7, probably in MC and M4 pharyngeal motor neurons. Feeding rate of *tph-1* , which can't synthesize serotonin, decreased to 75% of *N2*. tph-1 expression in ADF neurons fully restored feeding rates of both *tph-1* and *mod-5; tph-1*, suggesting that ADF neurons release serotonin and activate feeding. Consistent with this, laser ablation of ADF neurons decreased feeding rate comparable to *tph-1*. We also found that serotonin from ADF neurons enhances feeding via SER-7. Like *tph-1*, feeding rates of both *ser-7* and *tph-1; ser-7* decreased to 75% of *N2*, which suggests that SER-7 is the major receptor through which serotonin enhances feeding. SER-7 probably in MC and M4 neurons activate pharyngeal pumping and isthmus peristalsis rate, respectively. Then, under what conditions does serotonin enhance feeding? Previous studies suggested that ADF neurons sense food. Furthermore, it was reported that serotonin from ADF neurons enables worms to remember previously experienced food. It was also shown that serotonin from ADF neurons suppresses DAF-16 mediated stress response, which is crucial for survival on pathogenic bacteria such as PA14. These studies, taken together with our observation that serotonin from ADF enhances feeding, suggest that serotonin enhances feeding only in response to previously experienced and safe food. In fact, our preliminary results strongly support this hypothesis. On previously experienced food, feeding rate of *ser-7* decreased to 75% of *N2*, suggesting that serotonin signal is on. On the contrary, on previously unexperienced food, feeding rates of *N2* and *ser-7* were only marginally different, suggesting that serotonin signal is off. Interestingly, feeding rate of *N2* was significantly higher on familiar food than on unfamiliar food, further supporting our hypothesis. From these results, we suggest that only in response to familiar food, serotonin is released from ADF neurons and enhances feeding. We speculate that serotonin serves as a safeguard in the natural habitat filled with possible pathogens by promptly activating feeding only in response to previously experienced- and safe food.

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Fat storage and metabolic genes regulate quiescence as a result of satiety. **Young-jai You**, Leon Avery. Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX.

Despite the current epidemic of obesity and metabolic syndrome, how metabolism regulates food intake is not clear. Rodents, after being fully fed, stop eating and often go to sleep. This is defined as the behavioral sequence of satiety. We found that *C. elegans* mimics the same behavioral sequence of satiety; after full feeding, worms stop eating and become quiescent as if sleeping. We found that insulin, TGF β and cGMP signals acting through PKG (cGMP-dependent protein kinase) are necessary to regulate quiescence induced by satiety [1]. During fasting, worms use their stored fat [2]. The fat lost in 12 hours of fasting is fully recovered after 3 hours of refeeding (personal observation, You YJ). To investigate if fat storage and metabolism regulate quiescence, we modified fat storage and examined changes in quiescence. To examine the effect of increased fat storage in quiescence, we fed worms oleic acid, a monounsaturated fatty acid [3]. To examine the effect of reduced fat storage, we tested several mutants that fail to store fat. Adding oleic acid enhances quiescence in wild type, suggesting that fat storage can change quiescence behavior. A *fat-6; fat-7* double mutant stores less triglyceride than wild type (personal communication, Watt J.) and show reduced quiescence. The defect of *fat-6; fat-7* in quiescence could be rescued by adding oleic acid during refeeding. Furthermore, RNAi of *sbp-1*, a worm homolog of SREBP-1c which is necessary for worms to store fat [4], also reduced quiescence. To further investigate the roles of metabolism in quiescence, we performed an RNAi screen with 163 metabolic genes identified by Wang et. al. [5]. So far we have found 11 candidate genes most of which are involved in fat metabolism, suggesting that proper fat storage is necessary for worms to show intact quiescence. This work was supported by research grant DK074065 from the National Institutes of Health. Reference 1.You, Y.J., et al., Insulin, cGMP, and TGF-beta signals regulate food intake and quiescence in C. elegans: a model for satiety. Cell Metab, 2008. 7(3): p. 249-57. 2.McKay, R.M., et al., C elegans: a model for exploring the genetics of fat storage. Dev Cell, 2003. 4(1): p. 131-42. 3.Brock, T.J., J. Browse, and J.L. Watts, Genetic regulation of unsaturated fatty acid composition in C. elegans. PLoS Genet, 2006. 2(7): p. e108. 4.Yang, F., et al., An ARC/Mediator subunit required for SREBP control of cholesterol and lipid homeostasis. Nature, 2006. 442(7103): p. 700-4. 5.Wang, M.C., E.J. O'Rourke, and G. Ruvkun, Fat metabolism links germline stem cells and longevity in C. elegans. Science, 2008. 322(5903): p. 957-60.

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Distributed neural sex differences modify *C. elegans* locomotory behaviors. William Mowrey, **Douglas Portman**. Interdepartmental Graduate Program in Neuroscience and Center for Neural Development and Disease, University of Rochester, Rochester, NY.

Naturally-occurring variation is a fundamental feature of innate behaviors, but its mechanistic basis is poorly understood. To better understand adaptive variation in behavior and how it is regulated, we are investigating the relationship between sex-dependent modification of neural circuits and sex differences in behavioral output in *C. elegans*.

The regulation of locomotion is central to the expression of goal-directed behaviors in the worm. Interestingly, the two sexes of *C. elegans* exhibit significant differences in the body waves used to mediate locomotion: males propagate these waves with markedly increased frequency and greater angles of bending than the hermaphrodite. To identify the circuitry controlling these behavioral differences, we are analyzing the behavior of animals in which specific subsets of the nervous system have been genetically sex-reversed. Surprisingly, we have found that multiple neural sex differences are involved the modification of this relatively simple behavior. In particular, we have identified the sexual modification of non-sex-specific sensory neurons as a major contributor to the regulation of body wave frequency. Genetic evidence indicates that this regulation of body wave frequency requires glutamate signaling. Additionally, we find that sex differences in *glr-1*-expressing neurons also have a role in regulating the frequency of body waves. Further elucidation of the mechanisms controlling sex differences in body waves may offer insights into how changes at the molecular level can adaptively alter the properties of a rhythmic pattern generating circuit and its behavioral output.

Sexual modification of the *C. elegans* nervous system also regulates the higher-order organization of locomotory behaviors. We have found that genetic masculinization of the nervous system of hermaphrodites can suppress the sex-typical expression of "dwelling" behavior (characterized by bouts of consecutive short, slow forward movements), whereas genetic feminization of the nervous system can confer this dwelling behavior to males. Preliminary evidence suggests that multiple neural subtypes are involved in regulating this qualitative difference in locomotory pattern, reinforcing the idea that diverse modifications of the worm nervous system are required to impart sex differences to locomotion. Identification of the specific circuit- and molecular-level modifications controlling these sex differences can offer important insight into how nervous systems are modified to produce novel adaptive behavioral phenotypes.

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An integrated model of *C. elegans* locomotion: from swimming to crawling. **Stefano Berri**[1], Jordan H. Boyle[1], Manlio Tassieri[2], Ian A. Hope[3], Netta Cohen[1,4]. 1) School of Computing, University of Leeds, Leeds LS2 9JT, United Kingdom; 2) School of Physics, University of Leeds, Leeds LS2 9JT, United Kingdom; 3) Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, United Kingdom; 4) Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, United Kingdom.

C. elegans is capable of coordinated locomotion both when swimming in water and when crawling on an agar surface, two behaviors with distinct kinematics. By analyzing the worm's locomotion in a range of fluids with increasing visco-elasticity, we were able to demonstrate that swimming and crawling are merely two snapshots out of a continuum of locomotory behaviors that are achieved by a modulation of a single gait[1]. This finding suggests that a single neural mechanism underlies this entire range of behaviors. We developed an integrated model of the worm's forward locomotion that consists of a ventral cord nervous system, muscles, a body and an environment. The neural model consists of excitatory B-like bistable neurons that receive sensory feedback (mediated, in the model, by posteriorly directed stretch receptors on B class motorneurons) as well as inhibition from D-type neurons. Muscles receive both excitatory and contralateral inhibitory inputs and control the shape of a worm, instantiated by a physical model of the *C. elegans* body, embedded in a model of the visco-elastic environment. The integrated model can generate and coordinate oscillations, and captures the entire swim-crawl transition as the properties of the environment are changed. The neuromuscular control of the locomotion is modulated solely by the stretch receptor input which in turn varies with the external physics. No parameters are changed in the neuromuscular control. The model does not require a central pattern generator (or distinct neural mechanism) in the head, and can start from arbitrary initial body shape. Tests of the model are presented in virtual knockout and laser ablation simulation experiments, as well as in complex environments such as microfluidic "artificial dirt" chips and irregular granular media, which the worm is likely to encounter in its natural habitat. We are now using the model to gain insight into various uncoordinated phenotypes, including those that have a stronger effect in liquid than on agar.

[1] Berri S, Boyle JH, Tassieri M, Hope IA and Cohen N. 2009. "Forward locomotion of the nematode *C. elegans* is achieved through modulation of a single gait." HFSP journal, In press.

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The worms crawl in, the worms crawl out: An analysis of C. elegans locomotory gaits. **Christopher Fang-Yen**[1], Risa Kawai[1], Sway Chen[1], Matthieu Wyart[2], Quan Wen[2], Dmitri Chklovskii[2], Aravinthan Samuel[1]. 1) Department of Physics, Harvard University, Cambridge, MA; 2) Janelia Farm Research Campus, Ashburn, VA.

C. elegans exhibits very different locomotory patterns when swimming in fluids and crawling on a solid substrate. Compared with crawling, swimming is characterized by a longer wavelength and higher frequency of undulations. We are interested in understanding how a single circuit composed of command neurons, motor neurons, and muscles is capable of supporting these two locomotory gaits. We estimate that when crawling on moist surfaces, worms are held by capillary forces up to 100,000 times greater than the viscous forces experienced during swimming. To investigate in more detail the effect of this mechanical loading on locomotion, we immerse worms in solutions of varying viscosity and measure their wavelength, frequency, and curvature by machine vision algorithms. We show that worm exhibit a continuous transition from swimming to crawling behavior as viscosity is increased from that of water to a value 5 orders of magnitude larger. Worms in fluids of intermediate viscosity exhibit locomotory patterns intermediate between swimming and crawling. Swimming and crawling can therefore be seen as low-load and high-load limits of a continuum of locomotory patterns. We show that our results can be understood in terms of a biomechanical model of C. elegans locomotion in which the swimming to crawling transition is associated with a transition between elastic-dominated and viscous-dominated dynamics. Next we analyze the behavior of worms undergoing spatial transitions between swimming and crawling behaviors, such that mechanical loading varies over the length of the worm. The analysis of such transitions gives insights into the generation and propagation of the sinusoidal bending wave, and the mechanisms of modulation of locomotory gaits. We show that the worm behavior follows neither strictly local nor strictly global modulation, but rather can be described by a set of rules governing the generation and propagation of undulatory waves.

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HPL-2 and MUT-7 in odor-adaptation of the AWC neuron. **Bi-Tzen Juang**, Noelle L'Etoile. Ctr Neuroscience, Univ California, Davis, Davis, CA.

A sensory neuron must be able to respond to signals from the environment and to either attenuate or amplify its responsiveness as a function of experience. *C. elegans* relies extensively upon its sense of smell to locate food, thus, survival requires that a worm is able to ignore (adapt to) odors that are not associated with food. We exploit the ability of the genetically tractable, anatomically simple nematode to adapt to persistent profitless odors in order to examine the molecular mechanisms underlying neuronal plasticity.

In previous work we found that long-term adaptation requires nuclear translocation of a cGMP-dependent protein kinase (EGL-4) in the AWC olfactory neuron (Lee et al., submitted). Here we show that proteins within the RNA-induced transcriptional silencing (RITS) pathway may be targets of EGL-4 in the nucleus. Though RNAi has been well characterized in *C. elegans* development, our studies may be the first to demonstrate a role for this process for the plasticity of the adult sensory neuron.

We screened through all known RNAi defective mutants that are also able to chemotax for those that also fail to adapt to AWC-sensed odors. We found that the Histone H3 K9 methyl binding protein HPL-2 and the RNAase III MUT-7 are both required within the AWC neuron at the time of odor exposure for proper odor-adaptation. From our genetic analysis, we found that HPL-2 and MUT-7 are likely to work in the same pathway to promote adaptation. We are testing the hypothesis that HPL-2 and/or MUT-7 are phosphorylated by EGL-4 in response to odor stimulation.

Histone modification has been shown to be important for memory formation in mice (Nature 447:178-183). To analyze the effect of histone modification in maintaining neuronal plasticity, we examined that the H3 K4 histone methyltransferase SET-2 and found that it antagonizes the functions of HPL-2 and MUT-7 in olfactory adaptation. Our work opens up the possibility that this olfactory memory formation is dependent on chromatin changes and is actually initiated or maintained by small non-coding RNAs.

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The Role of the Calcium/Calmodulin-dependent Protein Kinase Cascade in Mechanosensory Habituation. **Tiffany A Timbers**, Catharine H Rankin. Brain Research Centre and Psychology Department, University of British Columbia, Vancouver, British Columbia, Canada.

Studies of CaMKIV, CaMKII and CaMKK in knockout mice show that these genes are critical for learning and memory. Curiously, CaMKI, a widely expressed CaMK has received little attention on its role in learning and memory. Our objective was to test the hypothesis that cmk-1 (homologous to CaMKI) is necessary for learning and/or memory. Because cmk-1 is highly homologous (and perhaps ancestral) to CaMKIV, and is widely expressed in the nervous system we predicted that animals with mutations in cmk-1 would show deficiencies in learning and/or memory. Worms strains with mutations in cmk-1, and ckk-1 (homologous to CaMKK and a known activator of cmk-1; Kimura et al. 2001) were tested for deficits in learning, short-term and long-term memory. Worms were habituated to mechanical stimuli (taps to the side of the Petri dish) using different stimulation protocols known to induce short-term, and long-term habituation. Worms with mutations in cmk-1 habituate normally when stimuli are presented at a 10s interstimulus interval (ISI), but do not habituate as deeply as wild-type animals when stimuli are presented at a 60s ISI, and cannot form long-term memory. No deficits in either short or long-term habituation were found in animals with a mutation in ckk-1. These results are similar to those of Satterlee et al. (2004) who also found that cmk-1, but not ckk-1 regulated gene expression, morphology, and functions of the AFD thermosensory neurons. This evidence suggests that a novel kinase may play the role of activating cmk-1 in the context of short-term habituation and long-term memory of habituation. To investigate whether the long-term memory deficit of the cmk-1 mutants is a result of the animals short-term habituation deficit or whether it is independently involved in both processes we created a strain that expresses the cmk-1 gene without the nuclear localization signal (NLS), restricting its expression to the cytoplasm (CaMKIV, and perhaps cmk-1 in some animals, are thought to participate in the induction of long-term memory by phosphorylating the transcription factor CREB in the nucleus). We predict that cmk-1 without the NLS will rescue the short-term habituation deficit but will be incapable of rescuing the long-term memory deficit, demonstrating that cmk-1 acts in independently in these two processes. In these studies we show, for the first time, that cmk-1 is critical for learning and memory. We also present data that support the hypothesis that cmk-1 is ancestral to mammalian CaMKIV and suggest that in species where CaMKIV does not exist, cmk-1 may function in its place.

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A Genetic and Cellular Circuit for *C. elegans* Long-term Associative Memory. **Amanda L Kauffman**, Geneva M Stein, Coleen T Murphy. Lewis-Sigler Institute for Integrative Genomics and Department of Molecular Biology, Princeton University, Princeton, NJ.

In humans, aging often is associated with a decline in cognitive function. Progress toward an understanding of the molecular mechanisms underlying the initiation and progression of age-related neuronal decline could be hastened by the development of experimental systems that quickly test early and true symptoms (rather than the correlative downstream effects) of neuronal decline and disease. In contrast to muscle degradation, the nervous system of C. elegans is structurally remarkably well-preserved, leaving open the question of how to define age-related changes in neuronal function. To begin to address this question, we developed two positive associative olfactory memory assays, and found that long-term, but not short-term, associative memory requires the C. elegans CREB transcription factor, *crh-1*. Furthermore, transcriptional analysis identified CREB targets that are changed during long-term memory training, which include genes involved in vesicle trafficking (*tom-1*, *snt-1*, *sng-1*, *unc-18*, etc.), ion channels (*slo-1*, *lgc-27*, *lgc-33*, *acr-7*, etc.), synaptogenesis (*syd-1*, *nid-1*, *klc-2*), RNA binding (*puf-4*, *sym-2*), neural migration (*vab-8*, *frl-1*, *ptr-1*, etc.), and uncharacterized genes. Functional analyses revealed that many of these transcriptionally-identified candidate genes are required for long-term associative olfactory memory. Feed-forward activation of CREB activity through transcription of its upstream activators JNK and CaMKII may explain its amplified response with increased training. During long-term memory training, CREB is activated in the AIM and SIA interneurons, via the AIY/AIA interneurons, inducing gene expression in other neurons and in glial cells. Our results provide a new molecular and cellular framework for understanding long-term memory, and allow quick screening of mutations and conditions that improve age-related associative learning and memory decline.

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A cell-free C. elegans embryonic system reveals maternal translation control by the miR-35-42 microRNA family through deadenylation. Edlyn Wu[1], Mathieu Flamand[1], Caroline Thivierge[1], Marc Fabian[1], Geraldine Mathonnet[1], James Wohschlegel[2], Nahum Sonenberg[1], **Thomas F. Duchaine[1]**. 1) Goodman Cancer Centre & Dept. Biochemistry, McGill University, Montreal, Quebec, Canada; 2) Department of Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, CA 90095-1737.

Powerful genetic methods in C. elegans have unraveled numerous translational control phenomena, including microRNA-mediated silencing. Better resolution on the mechanisms of microRNA-mediated silencing requires in vitro translation systems that are currently missing from the C. elegans experimental paradigm. We report here the first C. elegans embryonic extract able to sustain potent 5'-Cap- and 3'-poly(A)-synergic translation initiation of exogenous transcripts. Using a reporter system based on the abundant and maternally loaded miR-35-42 family we recapitulated efficient microRNA-mediated silencing in vitro. We found mRNA reporters to be rapidly and completely deadenylated in a microRNA-dependent manner. Unexpectedly, complete deadenylation did not result in the destabilization of the targeted transcripts up until at least 2 hours post-deadenylation. The stability of the deadenylated intermediate prompted us to propose a model wherein miRNA-mediated silencing of maternally contributed transcripts is reversible via the regulated control of poly(A) tail length. Finally, functional proteomic analysis of the embryonic microRNA-mediated silencing machinery, and the systematic validation in this system is underway and progress will be presented. *This work is supported by the Canadian Institute of Health and Research and by the National Sciences and Engineering Research Council of Canada*..

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Multiple germline 22G-RNA pathways maintain genome integrity in *C. elegans*. **Weifeng Gu[1]**, Masaki Shirayama[1], Darryl Conte, Jr[1], Jessica Vasale[1], Pedro Batista[1], Julie Claycomb[1], Daniel Chaves[1], Jennifer Keys[1], Craig Mello[1,2]. 1) Program in Molecular Medicine, Univ Massachusetts Medical School, Worcester, MA 01605; 2) Howard Hughes Medical Institute.

While analyzing RNA prepared from various *C. elegans* strains, we noticed that a small RNA species visible in ethidium-bromide stained gels was absent in a number of mutant strains deficient in RNAi and transposon silencing. To explore the nature of this abundant RNA species, we prepared cDNA libraries and utilized deep-sequencing technology to obtain reads corresponding to millions of genome-matched small RNAs. We refer to these small RNAs as 22G-RNAs due to the predominance of a 22-nucleotide length and the presence of a 5' G. Surprisingly, roughly 50% of the 22G-RNAs are antisense to over 5000 germline-expressed mRNAs, while the remaining 50% match intergenic loci, pseudogenes and transposons. A breakthrough in our understanding of the functions of 22G-RNAs came with the analysis of small RNAs associated with two distinct, germline-expressed Argonaute proteins R06C7.1 (WAGO-1) and CSR-1. Both Argonautes localize prominently to P-granules (see also abstract by Claycomb et al.). Despite their overlapping expression pattern, WAGO-1 and CSR-1 interact with non-overlapping sets of the germline-expressed 22G-RNA species. CSR-1 interacts with the majority of the mRNA-targeted 22G-RNAs, while WAGO-1 interacts with 22G-RNAs that target transposons, pseudogenes, intergenic loci, and a minority of genes. Tiling array studies suggested that only a small number of mRNAs targeted by 22G-RNAs are down-regulated. These mRNAs were exclusively the targets of WAGO-1, consistent with the role of this pathway in transposon silencing. In contrast, CSR-1 appears to utilize its small RNA co-factors to promote the proper alignment of kinetochores, rather than to down-regulate its targets (see Claycomb et al.). Our genetic studies indicate that CSR-1 and WAGO-1 share several upstream factors required for 22G-RNA biogenesis, including the dicer-related helicase DRH-3, the tudor-domain protein EKL-1, and the RNA-dependent RNA polymerases (RdRPs) RRF-1 and EGO-1. How these upstream factors properly identify distinct targets and load the resulting 22G-RNA species onto the appropriate Argonautes remains a mystery. Insight into this question may come from analyzing pathway-specific factors such as the β-nucleotidyl transferase-related protein RDE-3 and the 3'-5' exonuclease-related protein, MUT-7, which are both required for the WAGO-1 pathway. Our findings suggest that distinct Argonaute/22G-RNA pathways exert genome-scale surveillance that is essential for germline maintenance as well as the faithful transmission of both genetic and epigenetic information. (Funded by NIH GM58800).

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New Roles for Mitotic Condensin Proteins in RNAi and Chromatin-Based Gene Silencing. **James F. Carey[1]**, James Thompson[2], John R. Yates III[2], Kirsten A. Hagstrom[1]. 1) Molecular Medicine, Univ Massachusetts Med School, Worcester, MA; 2) Department of Cell Biology, Scripps Research Institute, La Jolla, CA.

Genome integrity requires proper chromosome structure and faithful segregation of chromosomes during mitosis. Protein complexes called condensins are conserved key regulators of chromosome architecture, and are essential for chromosome segregation during cell division. Here we describe new roles for condensins in RNAi and chromatin-based gene regulation.

Immunoprecipitation of condensin II recovered proteins that influence nucleosome positioning or histone tail modification, as well as proteins in small RNA pathways. In several functional assays, we demonstrate that *C. elegans* condensin complexes are involved in chromatin-based gene silencing phenomena that utilize RNAi machinery. For example, condensins are required to silence transgenes that undergo chromatin- and RNAi-dependent transcriptional gene silencing. Notably, condensin II also appears to be required for RNAi. Condensin II mutants are less sensitive to dsRNA treatment, and fail to elicit an effective RNAi response. Moreover, this effect even was observed in post-mitotic cells, suggesting a novel non-mitotic role for condensins in RNAi mediated silencing.

Thus, we speculate that condensins influence a transcriptional silencing component of RNAi that has not been well characterized in worms. These findings reveal an unexpected link between condensins and gene silencing mediated through small RNA and chromatin modification, providing evidence for additional mechanisms by which condensins maintain genome integrity.

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RDE-8 encodes a novel protein with a conserved domain required for RNAi. **Hsin-Yue Tsai**[1], Chun-Chieh G. Chen[2], James J. Moresco[3], Weifeng Gu[1], John R. Yates III[3], Craig Mello[1]. 1) Program in Molecular Medicine, University of Massachusetts Medical school, Worcester, MA; 2) Whitehead Institute for Biomedical Research, Cambridge, MA; 3) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

Several classes of small RNAs have been identified in mammals, zebrafish, Drosophila, and the nematode C. elegans. These include Dicer-dependent products (primary siRNAs and miRNAs) as well as secondary siRNAs produced by RNA-dependent RNA polymerase (RdRP). We have recently identified a new RNAi pathway component, RDE-8, which is essential for the accumulation of RdRP-derived small RNAs in both the exo- and endo- RNAi pathways. RDE-8 contains a novel protein motif that is highly conserved throughout all three kingdoms of life. Interestingly, in other organisms the majority of proteins containing the RDE-8 motif also contain RNA binding domains, most often of the CCCH zinc finger or KH domain families. Although RDE-8 itself is not highly conserved, one C. elegans RDE-8 family member (C30F12.1) has a human homolog in which both the amino acid composition and organization of its CCCH and RDE-8 motifs are well conserved. A third C. elegans RDE-8-motif protein also contains a Cytidine deaminase motif and is required for male fertility (See abstract by Colin Conine). These findings suggest that proteins containing the RDE-8 motif function in RNA binding and modification. To explore the role of RDE-8 in small-RNA pathways, we have utilized mass-spectrometry-based proteomics to identify protein interactors, and small-RNA deep sequencing to identify endogenous small RNAs whose biogenesis/stability depend on RDE-8. Our data suggests that RDE-8 is required for several distinct small-RNA pathways and resides in at least two protein complexes. RDE-8 interacts with the sap-domain exonuclease ERI-1b, and is required for the accumulation of ERI-pathway small RNAs. Interestingly, RDE-8 not only co-purifies with ERI-1b, but is required for a ssRNA trimming activity associated with the native ERI-1b/RDE-8 complex. We speculate that this trimming activity is necessary for the processing of substrates (or products) of the RdRP RRF-3. RDE-8 appears to form a second complex with components of the RNAi and transposon silencing small RNA pathway, including RDE-3, MUT-15, and MUT-16. We have not yet identified specific biochemical activities associated with this complex. However, it may also be involved in recruiting RdRPs: in this case, RRF-1 and EGO-1, which function to amplify silencing signals in the exo- and endo-RNAi pathways. We are currently investigating the biochemical properties of the RDE-8 complexes, and are exploring the activity of the RDE-8 motif.

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26G RNAs regulate gene expression during spermatogenesis and larval development. **Ting Han**[1], Arun Prasad Manoharan[1], Colin Fitzpatrick[2], Diana Chu[2], John Kim[1]. 1) The Department of Human Genetics and the Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109; 2) Department of Biology, San Francisco State University, San Francisco, CA 94132.

Endogenous siRNAs (endo-siRNAs) fine tune gene expression in eukaryotes (1-4). Although endo-siRNAs were initially discovered in *C. elegans* (5), the biology of these small RNAs has not been studied in depth. Here we show that a class of endo-siRNAs is generated in the proliferating germline and regulates specific transcriptional programs during parental spermatogenesis and filial larval development. Using Solexa (Illumina) deep sequencing technology, we identified a unique class of endo-siRNAs that are 26nt in length and invariantly start with a G nucleotide (therefore, we name them 26G RNAs; 6). The 26G RNAs are highly enriched in purified germ cells (sperm and oocytes), depleted in the soma (*glp-4(bn2)* grown at 25°C), and antisense to mRNA transcripts. There are two distinct classes of 26G RNAs. Class I 26G RNAs peak in L4/young adult stages and tightly associate with the spermatogenesis transcriptional program. Class II 26G RNAs are maternally inherited, with highest expression in oocytes and embryos, and decline through the four larval stages. Importantly, by monitoring target transcript levels, we show that these two 26G RNA classes regulate non-overlapping sets of mRNA transcripts. Class I preferentially silences genes expressed during spermatogenesis and this target regulation is strongly coupled with 26G RNA levels. Class II 26G RNAs regulate maternal transcripts and the silencing effect perdures throughout larval development, indicating Class II 26G RNAs function as maternally deposited silencing factors for regulating zygotic gene expression. We further probed the genetic requirements for 26G RNA expression. In addition to several known eri pathway genes (*eri-1, 3, 5, 7*), an argonaute (*ergo-1*), and an RNA-dependent RNA polymerase (*rrf-3*) are required for 26G RNA expression. The fact that these mutants all exhibit enhanced exogenous RNAi suggests a competition between the 26G endo-siRNA pathway and exogenous RNAi for limiting factors. We are currently studying the biochemical mechanisms of 26G RNA biogenesis and target regulation. Take together, our findings indicate that 26G RNAs are generated by the endogenous RNAi machinery in the parental germline and regulate gene expression during paternal spermatogenesis and zygotic development.• 1. Ghildiyal, M. et al., Science 320, 1077 (2008). •2. Okamura, K. et al., Nature 453, 803 (2008). •3. Tam, O. H. et al., Nature 453, 534 (2008).• 4. Watanabe, T. et al., Nature 453, 539 (2008).• 5. Ambros, V. et al., Curr Biol 13, 807 (2003). •6. Ruby, J. G. et al., Cell 127, 1193 (2006).

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Small RNA Pathways Required to Produce Thermo-tolerant Sperm in C. elegans. **Colin Conine**[1], Pedro Batista[1], Hsin-yue Tsai[1], Craig Mello[1,2]. 1) Program in Molecular Medicine University of Massachusetts Medical School, Worcester, MA 01605, USA; 2) HHMI.

Argonaute-mediated small RNA pathways are involved in a diversity of cellular processes in numerous organisms. In C. elegans null mutations are available for the entire family of over 24 Argonaute genes, and at least 5 different combinations of these mutants result in lethal or sterile phenotypes. Here, we focus on the role of two members of the AGO clade of Argonautes, T22B3.2 and ZK757.3 that are essential for functional sperm at elevated temperatures. The predicted products of these two AGOs are 96% identical at the amino acid level, and while either single mutant is viable and fertile the double mutant (TZK) is temperature sensitive sterile. TZK worms have a reduced brood size at 20°C, and are completely sterile at 25°C. This sterility appears to reflect a defect in sperm function at high temperature, as, even males reared at 15°C fail to produce cross progeny when shifted to 25°C. Furthermore, wild-type males can rescue sterile TZK hermaphrodites suggesting that the defect is specific to the male germ line. Consistent with this phenotype we found that a rescuing GFP::T22B3.2 transgene was expressed exclusively in germ-cells undergoing spermatogenesis and in mature spermatozoa. The TZK sterile phenotype was very similar to male-specific infertility observed in a null mutant in the rde-8-related gene C29F5.3. In addition to the rde-8 motif, C29F5.3 contains a cytidine-deaminase domain. RDE-8 is a novel protein with defects in both the exo- and endo-RNAi pathways (see abstract by Tsai et al.). RDE-8 domains have no known function but are found in organisms ranging from bacteria to humans. Surprisingly, certain classes of small RNAs that are absent in rde-8 mutants appear to be restored in rde-8, C29F5.3 double mutant animals. These findings suggest that the loss of these small RNAs may result from an ectopic function of C29F5.3 that is acquired when RDE-8 protein is absent. Gametogenesis appears to be an inherently thermosensitive process both in C. elegans and in many other metazoa. In humans the core body temperature is lethal to sperm, and external male gametogenesis is likely to represent an adaptation that was basal to the evolution of endothermia in the vertebrate lineage. Understanding how TZK argonautes and the novel protein C29F5.3 function to facilitate sperm function may shed light on the inherently ts-process that underlies spermatogenesis. We hope to gain insight into these questions by sequencing the small RNAs that engage T22B3.2, and by systematically applying transcriptomics to identify cytidine-deaminated RNAs expressed in sperm.

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SID-1 expression in neurons enhances neuronal RNAi in *C. elegans*. **Andrea Calixto**[1,2], Chelur Dattananda[1], Topalidou Irini[1], Chalfie Martin[1]. 1) Dept Biol, Columbia Univ, New York, NY; 2) School of Biological Sciences, Center for Cell Regulation and Pathology, P. Universidad Catolica de Chile.

SID-1 is a multispan transmembrane protein required for systemic RNAi in *C. elegans*. The *sid-1* gene is expressed in all but neuronal tissue, where systemic RNAi works poorly. We expressed SID-1 in all neurons using the pan-neural promoter from the *unc-119* gene ($P_{unc-119}$*sid-1*) and found that this protein increased the response of neurons to dsRNA delivered by feeding. This effect was further increased by mutations in the *lin-15b* and *lin-35* genes. dsRNA that did not induce a phenotype in wild-type animals phenocopied known neuronal mutants in $P_{unc-119}$*sid-1* animals. Phenotypes were also discovered for several genes for which no previous information was known. A striking secondary effect produced by *sid-1* expression in the neurons was a decrease in the response in non-neuronal cells to RNAi. This effect can be used to uncover neuronal defects for genes that, when eliminated in other tissues, produce lethality. For example, in wild-type animals RNAi for *pat-4*, which encodes an integrin-linked kinase, results in embryonic and larval lethality. In contrast $P_{unc-119}$*sid-1* animals are selectively touch insensitive, suggesting a previously unidentified role for *pat-4* in mechanosensation .

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Distinct mechanisms of microRNA post-transcriptional regulation by LIN-28 and LIN-42. **Priscilla M. Van Wynsberghe**, Zoya Kai, Valentino M. Gantz, Amy E. Pasquinelli. Biology Dept, UCSD, La Jolla, CA, 92093-0349.

Mature microRNAs (miRNAs) are small, noncoding RNAs that post-transcriptionally regulate gene expression. Though miRNAs are essential for many fundamental cell processes, the mechanisms regulating miRNA biogenesis are poorly understood. miRNA biogenesis is a multi-step process in which long, genetically-encoded primary (pri-) miRNAs are cleaved into precursor (pre-) and then mature miRNAs. The *let-7* miRNA is conserved across phyla, an important tumorigenesis regulator in humans and crucial for proper development in *C. elegans*. *Pri-let-7* is transcribed from two transcriptional start sites and trans-splicing to the Spliced Leader 1 RNA produces a third isoform important for mature *let-7* production. Unexpectedly, by reporter and molecular analyses we found that pri- and mature *let-7* expression is uncoupled suggesting that *pri-let-7* is post-transcriptionally regulated. Since mutations in the RNA binding protein LIN-28 or the period protein homolog LIN-42 suppress strong loss-of-function *let-7* alleles, we investigated the mechanisms by which these heterochronic pathway members might regulate *let-7* biogenesis. Recently, distinct models by which LIN-28 controls mammalian *let-7* miRNA expression were proposed. To ascertain if *C. elegans* LIN-28 similarly regulates *let-7* expression, we studied *let-7* biogenesis in *lin-28(n719)* worms. Contrary to WT worms, we find that concurrent with initial *pri-let-7* expression, pre- and mature *let-7* accumulate prematurely during late L1 in *lin-28* mutants. Interestingly, *pri-let-7* isoform levels vary in *lin-28(n719)* worms pointing to a specific *pri-let-7* isoform as the target of LIN-28 repression. Surprisingly, *lin-28* also differentially regulates maturation of *let-7* sister miRNAs, suggesting that *lin-28* activity can positively and negatively affect miRNA biogenesis. Unlike LIN-28, which affects specific miRNAs, we found that LIN-42 globally regulates miRNA expression. Using a previously identified allele and a novel C terminal allele of *lin-42*, which was identified as a spontaneous *let-7* suppressor, we discovered that LIN-42 activity down-regulates mature *let-7* levels. Our results suggest that LIN-42 post-transcriptionally regulates *let-7* expression since *pri-let-7* levels are unchanged, *pre-let-7* levels decrease and mature *let-7* levels increase in *lin-42* mutant worms. We observed similar effects on pre- and mature miRNA levels for other embryonic and larval stage miRNAs in *lin-42* mutant worms. Thus, we hypothesize that LIN-42 inhibits pre to mature miRNA processing. Our results propose a new role for the period protein homolog in globally regulating miRNA processing in *C. elegans* and raise the possibility that period proteins in other organisms could play similar roles.

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A novel nuclear export route for miRNAs? **Ingo Buessing**, Monika Fasler, Helge Grosshans. Friedrich Miescher Institute for Biomedical Research (FMI), Basel, Switzerland.

MicroRNAs (miRNAs) are small, noncoding RNAs that regulate numerous target genes to control major developmental pathways. The biogenesis of miRNAs has been investigated extensively leading to the following model: MiRNAs are transcribed by the RNA polymerase II from dedicated genetic loci, yielding a primary transcript that is polyadenylated and capped. The nuclear RNase Drosha cleaves the pri-miRNA to generate the precursor miRNA (pre-miRNA) of about ~70 nucleotides. In flies and vertebrates, the pre-miRNA is then recognized and exported to the cytoplasm by the importin-β family member Exportin-5. Cytoplasmic processing into the mature miRNA of ~22 nucleotides is mediated by the Dicer RNase. However, *C. elegans* lacks an Exportin-5 orthologue, and we tested other members of the importin-β family for their function in miRNA biogenesis.

I will present an alternative nuclear export route that is used in *C. elegans*. This novel pathway involves the Crm1/Xpo1 orthologue and the cap-binding complex (CBC), composed of NCBP-1 (CBP-80) and NCBP-2 (CBP-20). Depletion of each of these factors results in alae defects and burst animals reminiscent of the *let-7* miRNA mutant phenotype. Consistent with the observed phenotypes, the levels of mature *let-7* are reduced upon *xpo-1*, *cbp-20* or *cbp-80* depletion by RNAi. Additionally, we observed a decrease in the abundance of different other mature miRNAs, indicating that these genes are essential for the biogenesis of many, if not all, miRNAs. Moreover, the abundance of pre-*let-7* is reduced to a similar extend as seen for the mature miRNA, whereas the pri-*let-7* and SL1-transspliced *let-7* intermediates accumulate.

These findings support the notion that *C. elegans* miRNA biogenesis substantially deviates from the canonical biogenesis pathway. Nuclear export of *C. elegans* miRNAs not only involves a different set of proteins, XPO-1 and the cap-binding proteins CBP-20 and CBP-80, but also uses capped pri-miRNAs as export substrates. The alternative biogenesis pathway might provide an explanation for the difference in the virtually complete lack of intronic miRNAs in *C. elegans*.

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Genes containing binding sites for multiple microRNAs. **Yoshiki Andachi**[1,2]. 1) Genome Biology Lab, National Inst Genetics, Mishima, Japan; 2) Dept Genetics, Graduate Univ Advanced Studies, Mishima, Japan.

MicroRNAs (miRNAs) are roughly 22-nucleotide regulatory RNAs. Animal miRNAs down-regulate target genes by forming imperfect base pairs with the 3' untranslated regions of their mRNAs. A common feature of miRNA-target combinations is almost perfect base pairing between the nucleotides 2-8 of the miRNA, referred to as the seed, and complementary nucleotides in the target. Since founding members of the miRNA family, *lin-4* and *let-7*, were discovered in *C. elegans*, much effort has been devoted to the extensive analyses of small RNAs in several organisms, and thousands of miRNAs have already been isolated. The next major challenges in miRNA research include the identification of target genes for each of the miRNAs and the elucidation of mechanisms for regulation of the targets.

We have recently developed a novel biochemical method to identify target genes of individual miRNAs. In this method, cDNAs are synthesized from mRNAs by reverse-transcription using endogenous miRNAs that binds to the mRNAs *in vivo*. Then, cDNAs containing the sequence of a miRNA of interest are selectively amplified by PCR, and products are cloned to construct a cDNA library. An alignment of the sequence of each cDNA clone with the genome sequence reveals the gene from which the cDNA was derived. To demonstrate the usefulness of the method, it was applied to *lin-4*, resulting in the isolation of many cDNA clones of the known target gene *lin-14*. Application of the method to *let-7* and subsequent experimental validation of genes corresponding to isolated clones identified a new target gene, K10C3.4. The method was also applied to other *C. elegans* miRNAs and yielded many candidate target genes. Base pairing in each miRNA-candidate hybrid can be deduced from complementarity between the miRNA sequence and the 3'-flanking sequence of the cloned sequence. The presence of a sequence complementary to the seed and the conservation of the sequence among orthologous genes in phylogenetically related nematodes strongly support the notion that the 3'-flanking sequence is a functional binding site for the miRNA. Interestingly, several genes contain binding sites for two or more miRNAs. For example, cDNA clones of *pqn-47* were isolated by application of the method to *lin-4*, *let-7*, *mir-35* and *mir-71*, and conserved sequences almost complementary to the miRNA seeds were detected in the 3'-flanking sequences of the cloned sequences. These results suggest that the genes are targets of the multiple miRNAs and that redundant and combinatorial regulations of the genes are possible in the tissues where at least two of the miRNAs are expressed along with the genes.

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Systematic analysis of dynamic miRNA-target interactions during C. elegans development. Liang Zhang[1,2], Molly Hammell[3], **Brian A Kudlow**[1], Victor Ambros[3], Min Han[1]. 1) Howard Hughes Medical Institute and Department of MCDB, University of Colorado at Boulder, Boulder, CO; 2) Current Address:Laboratory of Mammalian Cell Biology and Development, The Rockefeller University, New York, NY; 3) Program in Molecular Medicine, University of Massachusetts School of Medicine, Worcester, MA.

Although microRNA (miRNA)-mediated functions have been implicated in many aspects of animal development, the majority of miRNA::mRNA regulatory interactions remain to be characterized experimentally. We used an AIN/GW182 protein IP approach to systematically analyze miRNA::mRNA interactions during C. elegans development. We first characterized the composition of miRNAs in functional miRISCs at each developmental stage and classified them into three major clusters. We then identified thousands of miRNA targets in each developmental stage including a significant portion that were subject to differential miRNA regulation during development. Our experimental data support the ideas that miRNAs preferentially target genes involved in signaling processes and avoid genes with housekeeping functions, and that miRNAs orchestrate temporal developmental programs by coordinately targeting or avoiding genes involved in distinct biological functions at different stages. By matching miRNAs and mRNAs with temporally correlated AIN-2-association patterns, combined with computational analysis, we identified 1589 potential miRNA family::mRNA pairs during development. This pattern matched data provides valuable information about the principles of physiological miRNA::target recognition.

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Roles of Rb and its related genes in regulating RNA interference. **Mingxue Cui**, Min Han. HHMI and Dept. of MCDB, Univ. of Colorado.

Extensive genetic and biochemical analysis revealed the essential functions of many factors such as Dicer, RNA-dependent RNA polymerase, helicases, and dsRNA endonucleases in the double-stranded RNA-mediated interference (RNAi) process. These factors are important for either the generation of small interfering RNA (siRNA) or the reorganization/degradation of the target mRNA. Some of the SynMuv B class genes, including lin-35/Rb (C. elegans ortholog of the RB tumor suppressor) and its related genes, have been shown to play roles in RNA interference, but the mechanism of their functions remains unclear. Since these synMuv B genes encode proteins that are either transcription factors or chromatin regulators, we hypothesized that they regulate the expression of genes that play a critical role in RNAi. By analyzing available microarray data, we first noticed that the expressions of several genes with known functions in exogenous RNAi were significantly up-regulated in the lin-35/Rb and lin-15B mutants. Quantitative RT-PCR analysis confirmed that these genes were up-regulated in the SynMuv B mutants with enhanced RNAi sensitivity, but not in the SynMuv B mutants that display wild type RNAi efficiency. This result suggests a functional link between the expression of these candidate Rb target genes and RNAi functions. We then found that loss of function mutations in three of these genes suppressed the enhanced RNAi efficiency in the lin-15B mutant, consistent with the idea that these genes mediate the regulation of SynMuv B genes on RNAi. Enhanced sensitivity to exogenous RNAi could be due to a disruption of endogenous RNAi, such as in the rrf-3 mutant that is defective in endogenous siRNA generation. To exclude the possibility that the up-regulation of these candidate genes was the result of endogenous RNAi reduction, we determined that expression of these candidate genes did not increase in the mutants of rrf-3. To further investigate the possibility that Rb and its related genes are involved in the production of endogenous siRNAs, we analyzed the expression of nrde-3::GFP in the mutants of these genes. The argonaute protein NRDE-3 has been shown to translocate into the nucleus when endogenous siRNAs are present, and remains in the cytoplasm when endogenous siRNAs synthesis is blocked in eri-1 and rrf-3 mutants. Our experimental results showed that NRDE-3::GFP was normally distributed in the mutants of Rb and several other SynMuv B genes, suggesting that endogenous siRNAs were normally produced. These data suggest that Rb and related SynMuv B genes likely affect RNAi by regulating the transcription level of genes required for exogenous RNAi. Future work to test this model is being carried out.

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Rictor/TOR complex 2 regulates fat metabolism, feeding, growth, and lifespan in *C. elegans*. **Alexander Soukas**, Elizabeth Kane, Christopher Carr, Buck Samuel, Justine Melo, Gary Ruvkun. Dept. of Genetics, Harvard Medical School and Dept. of Molecular Biology, Massachusetts General Hosp, Boston, MA.

The target of rapamycin (TOR) protein exists in two structurally and functionally distinct multi-protein complexes, TOR complex 1 (TORC1) and TORC2. TORC2 is composed of TOR, Rictor, LST8, and Sin-1. TORC2 is the activating, hydrophobic motif (HM) kinase of AKT, but may also phosphorylate other AGC kinases such as PKC and serum- and glucocorticoid-induced kinase (SGK). In yeast and mammals, genetic inactivation of TORC2 is lethal. Thus, the physiologic significance of this kinase complex remains unknown in the context of the living animal. We identified mutations in the *C. elegans* homologue of the essential TORC2 component *rictor* in a forward genetic screen for increased body fat. *rictor* is critical for balancing energy utilization and storage, as mutants inappropriately store fat as triglyceride despite being growth delayed, small, short lived, and laying an attenuated brood. Although *rictor* is expressed in neurons, muscle, intestine, and pharynx, expression in the intestine is sufficient to rescue fat mass and whole animal growth. Out of a pool of growth-delayed mutants with high body fat, we also identified loss-of-function mutations in the sole *C. elegans* homologue of SGK, *sgk-1*, which we found to be in a genetic pathway with *rictor*. Growth, lifespan, and reproductive defects of *rictor* mutants can be entirely explained by *sgk-1*. Alternatively, the high fat phenotype of *rictor* is partially dependent upon *akt-1*, *akt-2*, and *sgk-1* as assessed by quantitative lipid biochemistry (solid phase chromatography and GC/MS). Surprisingly, insulin-like signaling via *insR/PI3K/FOXO* is not the input to or output of TORC2, since *daf-2* (*insR*), *daf-18* (*PTEN*), and *daf-16* (*FOXO*) regulate fat mass in parallel to *rictor*. Thus, through as yet unidentified inputs and outputs, TORC2 signals through SGK-1 to regulate lifespan, growth, and reproduction, and through AKT and SGK to regulate fat metabolism. Genetic and genomic approaches identified numerous candidate signaling molecules that may participate in a genetic pathway with *rictor* regulating metabolism. Further, to begin to determine which nutrients might provide input directly or indirectly to TORC2, we took advantage of our observation that *rictor* mutants avoid a lawn of HB101, displaying phenotypes consistent with caloric restriction: slower growth, a further reduced body size, decreased energy expenditure, and extended lifespan. We have identified candidate bacterial genes and metabolic characteristics which cause *rictor* mutants to avoid HB101, giving insight as to how nutrients regulate metabolism and feeding behavior through TORC2.

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Transketolase is a new target of SBP-1 and is required for lipid homeostasis in *C. elegans*. **Veerle Rottiers**[1,2], Amy K Walker[1,3], Jennifer L Watts[4], Anne C Hart[1,3], Anders M Näär[1,2]. 1) Cancer Center, Massachusetts General Hospital, MA; 2) Department of Cell Biology, Harvard Medical School, MA; 3) Department of Pathology, Harvard Medical School, MA; 4) School of Molecular Biosciences, Washington State University, WA.

Several prevalent diseases such as obesity and type II diabetes are linked to aberrant lipid homeostasis. The *C. elegans* SREBP/SBP-1 transcription factor is, like its mammalian homologs, critical for fatty acid and lipid homeostasis. Depletion of SBP-1 or its co-activator MDT-15 results in clear, sterile, lethal and slow growth phenotypes and a reduction in the fatty acid oleic acid, a precursor for triacylglycerides (TAGs) and poly-unsaturated fatty acids. Dietary supplementation with oleic acid markedly improved the phenotypes of *sbp-1* and *mdt-15* knockdown animals, suggesting a central role for oleic acid in SBP-1-function and lipid homeostasis. To identify novel conserved regulators of lipid homeostasis we performed an oleic acid auxotrophy screen. We screened the Ahringer RNAi library clones that cause clear, sterile, lethal, and slow growth phenotypes (≈1,300 genes) in search of clones that are, like *sbp-1* and *mdt-15*, rescued upon dietary addition of oleic acid. We identified 6 genes in the screen. Three genes are required for mitochondrial function: *sco-1* (C01F1.2) and mitochondrial S9 and S17 ((F09G8.3; C05D11.10) confirming the role of mitochondria in lipid homeostasis (McKay et al, 2003). We also found *cul-1, pyr-1* and *tkt-1*(F01F10.2) in our screen. We focused on *tkt-1*, the homolog of human transketolase (TKT) since TKT mouse knockouts are lean. *C. elegans tkt-1* is also necessary for normal lipid homeostasis since *tkt-1* depleted animals contain less TAGs by GC-MS and show reduced Sudan Black staining. TKT is an enzyme of the Pentose Phosphate Pathway and is necessary for the production of NADPH. A large number of SREBP/SBP-1 targets need NADPH as a cofactor and mammalian SREBP activates transcription of NADPH generating enzymes. This activation seems to be conserved in *C. elegans* since 3 NADPH producing enzymes (G6PDH, PGD and cytosolic IDH) were identified in our microarray expression studies of *sbp-1* depleted animals. *tkt-1* seems to be a new, direct target of SBP-1 since P*tkt-1::gfp* expression is dependent on SBP-1 and *tkt-1* was identified in our microarray analysis of *sbp-1*(RNAi) animals. We are currently testing if mammalian TKTs are also activated by SREBP in cultured cells. Our oleic acid auxotrophy screen has identified genes necessary for lipid homeostasis and found a new target of SBP-1. We suggest a molecular mechanism for lipid depletion in TKT depleted animals, possibly across species, through SBP-1 and the requirement for NADPH.

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Hormonal Control of the Dietary Restriction Response in C. elegans. **Bree Heestand**, Adam Antebi. Huffington Ctr on Aging, Baylor College Med, Houston, TX.

Environmental alterations can cause profound changes in organismal lifespan. One example is dietary restriction (DR), a reduction in caloric uptake without malnutrition, which can increase health and life span in different species across taxa, including worms, flies and rodents. Although a few studies have identified candidate genes required for DR-induced longevity, the molecular mechanisms remain largely elusive. In C. elegans, a widely used method of inducing DR involves the eat-2 mutant, which has reduced pharyngeal pumping and food intake. The C. elegans transcription factor, SKN-1/NF-E2, regulates DR-induced longevity from the pair of ASI neurons. This implies that downstream signals mediate the systemic physiological response to DR. Conceivably, this DR response could be communicated through hormonal signaling. If so, then specific hormones and their hormone receptors should be required for DR-induced longevity. To test this hypothesis, we are screening through candidate peptide hormones, G-protein coupled receptors, and nuclear receptors in an attempt to identify genes required for lifespan extension induced by DR. Currently, we have potentially identified a receptor necessary for DR-induced longevity and are testing for specificity using others forms of DR. Our studies will potentially unravel novel genes involved in a highly conserved biological process conferring increased lifespan.

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Intraflagellar transport/Hedgehog-related signaling components couple sensory cilium morphology and serotonin biosynthesis in *C. elegans*. **Mustapha Moussaif**, Ji Ying Sze. Dept Mol Pharm, Albert Einstein Col Med, Bronx, NY.

Intraflagellar transport in cilia has been proposed as a crucial mediator of Hedgehog signal transduction during embryonic pattern formation in both vertebrates and invertebrates. We found that the Hh receptor Patched-related factor DAF-6 and intraflagellar transport modulate serotonin production in *C. elegans* animals, by remodeling the architecture of dendritic cilia of a pair of ADF serotonergic chemosensory neurons. Wild-type animals under aversive environment drastically reduce DAF-6 expression in glia-like cells surrounding the cilia of chemosensory neurons, resulting in cilium structural remodeling and up-regulation of the serotonin-biosynthesis enzyme tryptophan hydroxylase *tph-1* in the ADF neurons. These cellular and molecular modifications are reversed when the environment improves. Mutants of *daf-6* or intraflagellar transport constitutively up-regulate *tph-1* expression. Epistasis analyses indicate that DAF-6/intraflagellar transport and the OCR-2/OSM-9 TRPV channel act in concert regulating two layers of activation of *tph-1* in the ADF neurons. The TRPV signaling turns on *tph-1* expression under favorable and aversive conditions, whereas inactivation of DAF-6 by stress results in further up-regulation of *tph-1* independently of OCR-2/OSM-9 activity. Behavioral analyses suggest that serotonin facilitates larval animals resuming development when the environment improves. Our study revealed the cilium structure of serotonergic neurons as a trigger of regulated serotonin production, and demonstrates that a Hedgehog-related signaling component is dynamically regulated by environment and underscores neuroplasticity of serotonergic neurons in *C. elegans* under stress and stress recovery. On going experiments are focusing on mapping and cloning signaling components acting between IFT/hedgehog-related signaling and *tph-1* expression in the ADF neurons.

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Neuronal Regulation of L1 Survival Under Starvation Condition. **Brian Han Lee**, Kaveh Ashrafi. Dept Physiology, Univ California, San Francisco, San Francisco, CA.

For most organisms, nutrient is a limiting resource; therefore, molecular mechanisms that coupled growth and development to food availability are critical for survival. One essential aspect of this regulation is the detection of food availability in the environment by the sensory nervous system and the relay of this information to other tissues in the organism. To address this question, we determined the starvation survival phenotype of mutants defective in sensory transduction and found that a TRPV (transient receptor potential vanilloid subfamily) calcium channel, OCR-2, negatively regulate starvation survival, while a cGMP gated calcium channel, TAX-2/TAX-4, positively promote survival. *ocr-2* mutants are defective in neuroendocrine secretion leading to a reduction in insulin signaling, which extends starvation survival in a *daf-16* dependent manner. *tax-2* and *tax-4* mutants, on the other hand, are sensitive to starvation and act in parallel to insulin signaling. Furthermore, *ocr-2* is expressed in a small subset of chemosensory neurons that is distinct from *tax-2/tax-4* expressing neurons. Taken together, our data suggests that competing signals from different part of the sensory nervous system regulate growth and development arrest via distinct mechanisms.

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Identification of a novel conserved master stress response pathway. **Natalia V. Kirienko**, David S. Fay. Dept of Molecular Biology, Universuty of Wyoming, Laramie, WY.

The maintenance of a homeostatic interaction with the environment is crucial for the growth and survival of all living organisms. This equilibrium is maintained by the activation of stress response networks, whose function is to reestablish homeostatic balance. Despite its importance, the mechanisms by which stress response pathways are triggered are still incompletely understood. Here we report that a previously described C. elegans zinc finger protein, SLR-2, coordinates the expression of a large and functionally diverse cohort of stress responsive genes. Through microarray studies and computational analysis, we independently identified a nucleotide motif, the ethanol stress response element (ESRE), that is present in genes differentially regulated by SLR-2 under multiple stress conditions. Moreover, the ESRE motif is highly conserved in orthologs of SLR-2 responsive genes in both Drosophila and mammals. We demonstrate that SLR-2 is required for normal responses to conditions including heat shock, hypertonic, ethanol, and oxidative stresses. We further show that T28F2.4, a gene conserved from C. elegans to mammals, is responsive to a wide array of stress conditions and, like SLR-2, is required for the normal induction of ESRE genes. In addition, expression of T38F2.4 following stress is dependent on the presence of SLR-2. Furthermore, similar to slr-2 mutants, loss of T28F2.4 activity in C. elegans leads to hypersensitivity to pleiotropic stress conditions. Finally, we report that the Drosophila homolog of T28F2.4, CG2982, is also required for survival under stress conditions and also regulates ESRE-containing genes in Drosophila. Taken together, we have uncovered a novel phylogenetically conserved stress response network including several previously unknown master regulators of the stress response.

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Pore-forming toxins, stress and the hypoxia pathway in *C. elegans*. **Audrey Bellier**, Chang-Shi Chen, Raffi Aroian. Dept Cell & Developmental Biol, Univ California, San Diego, San Diego, CA.

Pore-forming toxins (PFTs) represent the largest class of bacterial protein toxins and often play an important role in the bacterial pathogenesis. The pores formed by these proteins in the membrane of target cells lead to ion imbalances and signal transduction activation that represent a tremendous stress on cell. We are studying pore-forming Crystal (Cry) proteins made by the bacterium *Bacillus thuringiensis* (Bt) to understand how cellular factors and pathways respond to PFTs. In particular, our lab is interested in understanding what genes and pathways act in concert with the pore to undermine cellular health and integrity. The prediction is elimination of such gene and pathways will lead to animals resistant to the PFTs. A previous genetic screen in the laboratory for *C. elegans* resistance mutants to the PFT Cry5B led to the identification of glycolipid carbohydrates as key receptors for Cry5B toxin. These results, although important, did not actually point to broadly applicable mechanisms. Therefore, to try to get to these intoxication pathways, we screened for mutants resistant to a different PFT, Cry21A. These results will be presented here. Despite extensive screening, only one robust mutant resistant to Cry21A was found. Surprisingly this mutant presents an early stop codon in *egl-9*, which encodes for a key regulator of the hypoxia pathway. In an *egl-9* mutant, the hypoxia-inducible factor (HIF-1) is stable and activates transcription of genes involved in cell survival under hypoxia. We found that *egl-9* mutants are also resistant to various other Cry PFTs. These data suggest that repression of the hypoxia pathway by *egl-9* makes animals more susceptible to PFTs. Loss-of-function mutation in *egl-9* has already been reported to confer resistance to pathogenic attack (Darby et al., 1999), therefore we tested *egl-9* to different stresses. We found that *egl-9* mutants are also resistant to heat shock, oxidative stress, pathogenic bacteria and have an extended lifespan. However, the dependence of these stress responses on HIF-1 can be diametrically opposite, suggesting that both stabilization and loss of function of HIF-1 lead to stress resistance, depending on the stress. We believe we have also identified a factor downstream of HIF-1 required for a normal response to PFTs. Our data show that HIF-1 levels play an important role on cellular stress resistance and longevity and that maintaining a proper and dynamic balance of HIF-1 activity is crucial for proper survival of the animal under varying conditions. Lethal paralysis of *Caenorhabditis elegans by Pseudomonas aeruginosa*. 1999. Darby C, Cosma CL, Thomas JH, Manoil C. Proc Natl Acad Sci U S A. 96:15202-7.

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Unfolded protein response (UPR) protects C. elegans from hypoxic injury. **Xianrong Mao**[1], C. Michael Crowder[1,2]. 1) Anesthesiology, Washington Univ Sch o, Saint Louis, MO; 2) Developmental Biology, Washington Univ Sch o, Saint Louis, MO.

We have previously identified a missense mutation of arginyl tRNA synthethase (rrt-1) gene that strongly protects C. elegans from hypoxic insults. We also established a positive correlation between the protein translation rate and hypoxic death. Because most of protein maturation processes take place in endoplasmic reticulum (ER), we desire to investigate if the ER unfolded protein response (UPR) is involved in hypoxic death in C. elegans. We found that a short exposure to the UPR-inducing reagent tunicamycin could significantly protect worms from subsequent hypoxic insult. Canonical UPR pathway genes ire-1, xbp-1, and atf-6 but not pek-1 were required for tunicamycin-induced protection. We have previously showed that a brief exposure to hypoxia could induce a protective response to subsequent hypoxic insults. Interestingly, this hypoxic preconditioning (HP) could induce the UPR, as the expression of the hallmark of UPR hsp-4 was elevated shortly after HP period. This HP-induced protection was dependent on ire-1 but not xbp-1, pek-1, or atf-6. This suggests that the mechanism for HP-induced protection overlaps with but is not identical to that for tunicamycin-induced protection which utilizes the entire core UPR pathway. Hypoxia-induced protein unfolding may be an early result of hypoxia to which cells attempt to mount a protective adaptive response that includes the UPR. Modulation of the UPR is a novel target for the design of therapeutics against hypoxia related diseases.

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Ascaroside Signalling and Lifespan in C. elegans. **Andreas Ludewig**[1], Rabia Malik[1], Chirag Pungaliya[2], Bennett Fox[2], Frank Schroeder[1,2]. 1) Boyce Thompson Institute, Cornell University , Ithaca, NY; 2) Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY.

In the nematode Caenorhabditis elegans the endogenously produced ascarosides differentially regulate development and behaviour. At low concentrations, ascarosides act as mating signal (Srinivasan, 2008), whereas at higher concentrations, they induce developmental arrest at the dauer stage (Butcher, 2007, Butcher 2008). Here we report that the ascarosides ascr#2 and ascr#3 influence adult lifespan and stress tolerance of C. elegans. Combinations of ascr#2 and ascr#3 increased lifespan of wild type animals by 30 % and increased thermotolerance up to 70%. Thermotolerance and ageing assays in different mutant background revealed complex regulatory networks for the activity of ascr#2 and 3. Notably, only ascr#3-, but not ascr#2-, mediated heat stress and tolerance and longevity are abolished in reduced insulin signalling background indicating that ascr#2 and ascr#3 act through different pathways. Ascr#2 and ascr#3-mediated increases in ageing and thermotolerance are dependent on the histone deacetylases SIR2.1 and SIR2.3, which also mediate caloric restriction-dependent increases in lifespan. These studies provide the first examples for endogenous small molecules that strongly increase lifespan and thermotolerance.

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Live imaging and quantification of proteasome activity using C. elegans. **Geert Hamer**, Olli Matilainen, Carina Holmberg. Molecular and Cancer Biology Program, Institute for Biomedicine, Biomedicum Helsinki, University of Helsinki, Haartmaninkatu 8, FIN-00014 Helsinki, Finland.

In healthy cells, improperly folded or otherwise defective proteins are cleared by the proteasome, a large supra-molecular protein complex that degrades proteins that are targeted by the ubiquitin-proteasome system. In addition, the ubiquitin-proteasome system orchestrates many cellular processes by selectively degrading key regulatory proteins. As a consequence, impaired proteasomal activity is involved in several neurological diseases and certain types of cancer.

We have developed a novel *in vivo* method for live imaging and quantification of proteasome activity and protein turnover using *C. elegans*. We have generated strains that express photo-convertible fluorescent reporter proteins that are targeted for degradation by the ubiquitin-proteasome system. Quantification of the fluorescent signals therefore directly translates to protein turnover and proteasome activity *in vivo*. By using this method, we can visualize and quantify proteasome activity over many hours in selected cells in living animals. Not only have we been able to demonstrate impairment of the proteasome by using RNAi or mutant strains, but we have also shown significant differences in proteasome activity between cell types in our transgenic animals.

Our results reveal, for the first time, cell type-specific proteasome activity in a living multi-cellular organism. Because proteasome activity and its regulatory pathways are evolutionary conserved, *C. elegans* provides an excellent model system to increase our knowledge on regulation of the proteasome and thus on human proteasome-related diseases.

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p53/CEP-1 Increases or Decreases Lifespan, Depending on Level of Mitochondrial Bioenergetic Stress. **Natascia Ventura[1,2]**, Shane Rea[1,3], Alfonso Schiavi[2], Alessandro Torgovnick[2], Roberto Testi[2,4], Thomas Johnson[1]. 1) Institute for Behavioral Genetics, University of Colorado at Boulder, Box 447, Boulder, CO 80309, USA; 2) Department of Experimental Medicine and Biochemical Sciences, University of Rome "Tor Vergata", Rome, Italy; 3) Sam and Ann Barshop Institute for Longevity and Aging Studies and the Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA; 4) Fondazione Santa Lucia, Rome, Italy.

Mitochondrial pathologies underlie a number of life-shortening diseases in humans. In the nematode Caenorhabditis elegans, severely reduced expression of mitochondrial proteins involved in electron transport chain-mediated energy production also leads to pathological phenotypes, including arrested development and/or shorter life; in sharp contrast, mild suppression of these same proteins extends lifespan. Here we show that the C. elegans p53 ortholog cep-1 mediates these opposite effects. We find that cep-1 is required to extend longevity in response to mild suppression of several bioenergetically relevant mitochondrial proteins, including frataxin–the protein defective in patients with Friedreich's Ataxia. Importantly we show that cep-1 also mediates both the developmental arrest and life shortening induced by severe mitochondrial stress, and that this effects partially rely on the induction of the cep-1 transcriptional target egl-1. Our findings support an evolutionarily conserved function for p53 in modulating organismal responses to mitochondrial dysfunction and suggest that metabolic checkpoint responses may play a role in longevity control and in human mitochondrial-associated diseases.

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eIF-4G post-transcriptionally remodels stress response and cellular homeostasis gene expression in C. elegans. **Aric N. Rogers[1]**, Di Chen[1], Gregg Czerwieniec[1], Gawain McColl[2,3], Alan Hubbard[1], Brad Gibson[1], Simon Melov[1], Gordon Lithgow[1], Pankaj Kapahi[1]. 1) Buck Institute for Age Research, Novato, CA; 2) Mental Health Research Institute of Victoria, Parkville, VIC 3052, Australia; 3) Center for Neuroscience, University of Melbourne, Parkville, VIC 3052, Australia.

Attenuation of mRNA translation extends lifespan in metazoans by as yet unknown mechanisms. Eukaryotic translation initiation factor (eIF)-4G is an mRNA cap-binding complex member that positively regulates translation by acting as a scaffold between transcript 5' and 3' ends via eIF-4E and poly A binding protein, respectively. Here we show that reducing translation via suppression of eIF-4G (ifg-1 in C. elegans) also differentially regulates expression of genes involved in responding to stress. Furthermore, we observe a positive correlation between mRNA size and translation as measured by ribosome loading when ifg-1 is inhibited. This correlation extends to overall protein levels assayed using quantitative mass spectrometry. Genome-wide analysis shows that longer mRNAs are enriched with genes important for stress responses and that positively regulate lifespan and cellular homeostasis. Inhibition of some of these stress response genes substantially diminishes the increased lifespan under eIF-4G inhibition and include the FOXO transcription factor daf-16 and the energy sensor AMP kinase catalytic subunit aak-2. Expression of SCA-1, a Calcium ATPase involved in ER homeostasis, is essential for lifespan extension in an ifg-1 mutant. Our results indicate that ifg-1 controls a switch in mRNA translation between growth and development and somatic maintenance, which provides a novel mechanism for the tradeoffs between growth and longevity.

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The axial element protein HTP-3 promotes cohesin loading and assembly of the meiotic chromosomal axis. **Aaron Severson**, Barbara Meyer. HHMI and Department of Molecular & Cell Biology, Univ California, Berkeley, CA.

Faithful transmission of the genome through sexual reproduction requires precise reduction of genome copy number during gametogenesis to produce haploid sperm and eggs. Meiosis therefore entails unique steps, that are absent from mitosis, to tether homologous chromosomes together during prophase of meiosis I and to separate homologs and then sister chromatids during anaphase of meiosis I and II. We show that HTP-3, a known component of the axial element (AE) that assembles along meiotic chromosomes and promotes crossover recombination, molecularly links these meiotic innovations.

When meiosis begins, sister chromatids are held together by sister chromatid cohesion (SCC), mediated by a protein complex called cohesin. Homologs become linked during crossover recombination. Once recombination is complete, SCC around the crossover holds homologs and sisters together. Their successive separation requires the stepwise proteolysis of Rec8, a meiosis-specific cohesin subunit. During meiosis I, cohesin regulators protect Rec8 locally, at discrete domains of each homolog pair, to keep sisters together.

We have found that global regulation of cohesin loading by HTP-3 is also required to forestall sister separation in anaphase I, and that cohesin, in turn, is required for HTP-3 loading and AE assembly. Unexpectedly, REC-8, the known REC-8 paralog COH-3 and the previously unknown paralog COH-4 are together essential for AE assembly. In contrast, REC-8 alone can keep sisters together after anaphase I; consequently, sister chromatids segregate away from one another in meiosis I of *rec-8* mutants (premature equational division). In a genetic screen for additional factors required to maintain SCC until meiosis II, we identified HTP-3, already known to promote meiotic double strand DNA break formation, homolog pairing, synapsis and recombination. We show that HTP-3 recruits all known AE components to meiotic chromosomes. Additionally, HTP-3 promotes loading of REC-8 containing cohesin complexes, the first demonstrated requirement for an AE protein in cohesin axis assembly. In *htp-3* mutants, sister chromatids separate equationally in anaphase I. Thus, HTP-3 is required for multiple events that distinguish meiosis from mitosis. Moreover, our data suggest that interdependent loading of HTP-3 and cohesin is a principal step in assembly of the meiotic chromosomal axis.

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Dynamic features of chromosome organization during *C. elegans* meiosis revealed through chromosome painting in the context of intact 3-D nuclear architecture. **Kentaro Nabeshima**[1], Anne Villeneuve[2]. 1) University of Michigan, Ann Arbor, MI; 2) Stanford University, Stanford, CA.

In preparation for the meiotic divisions, homologous chromosomes must identify and align with their appropriate pairing partners to ensure their faithful segregation. We have investigated global features of chromosome organization during this process, using a chromosome painting method that enables visualization of whole chromosomes along their entire lengths in the context of preserved 3D nuclear architecture. Our study illuminates several unappreciated features of chromosome dynamics during homologous pairing and synapsis: 1) Individual chromosomes occupy separate compact territories before entering into meiosis, and no preferential association of homologous territories is observed. Following meiotic entry, chromosomes undergo a transient, dramatic elongation of their territories (pre-pachytene elongation; PPE). 2) After chromosomes establish full homologous synapsis, they continue to increase their length until SC disassembles at the end of the pachytene stage (pachytene elongation; PE). 3) For the X chromosomes, both pairing centers (PC) and PC binding protein HIM-8 are involved in these elongation processes. Deficiency of the PC eliminates PPE but not PE. Interestingly, the presence of single PC is not sufficient to support PPE, suggesting that communication between two PCs is required for PPE. A mutant defective for the PC binding protein, *him-8*(*e1489*), is impaired for both PPE and PE, suggesting a role for HIM-8 both at PCs for PPE and outside of PCs for PE. Interestingly, partial loss of function mutant *him-8*(*me4*) is fully proficient in PPE and partially proficient in PE. Since this mutant is not proficient for PC pairing but the mutant protein is localized at PCs, this result suggests that HIM-8 has a function in regulating dynamic changes of chromosome morphology during meiotic prophase independent of its function in PC pairing. 4) Synapsis-independent homologous associations can support full juxtaposition of homologous chromosomes. In the absence of SYP-1, an essential component of the synaptonemal complex central region, homologous chromosome pairs are most often associated either along their entire lengths or only at the end of the chromosomes harboring the PCs. There is a relatively low abundance of homolog pairs exhibiting a partial longitudinal association compared to the PC-only and fully-aligned configurations, suggesting a rapid transition between these two states. Our observations support a model in which PC association promotes rapid lengthwise juxtaposition of chromosomes, thereby facilitating assessment of the suitability of potential pairing partners.

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Mechanism and regulation of meiotic recombination. **Simona Rosu**, Anne Villeneuve. Dept Genetics, Stanford Univ, Stanford, CA.

Meiosis is a fundamental process by which diploid organisms generate haploid gametes. During meiosis, crossovers (COs) between the DNA molecules of homologous chromosomes provide physical links that hold the homologs together and ensure proper segregation at the meiosis I division. Most organisms make few COs per chromosome pair and space them far apart, indicating the process is tightly regulated. COs are generated by homologous recombination (HR) initiated by DNA double strand breaks (DSBs) formed by the meiotic SPO-11 protein. Only a subset of DSBs enter the CO pathway, while the rest are repaired to give noncrossover (NCO) products. Despite the importance of crossing over for ensuring chromosome inheritance, the mechanisms that convert DSBs into COs and that regulate crossing over remain poorly understood.

To better understand this process, we are employing an assay system that uses controlled excision of a Mos1 transposon to induce a DSB at a defined site and in a defined time frame. Our system builds on the finding of Robert et al. (2008) that DSBs caused by Mos1 excision can be repaired by HR to yield both NCO and CO meiotic products. Several conclusions have emerged from our analysis. First, by performing time course analyses in which we monitored the outcomes of recombination events initiated at different time points during meiotic prophase, we have determined that: 1) Germ cells shut down access to the homologous chromosome as a DSB repair template for both CO and NCO pathways simultaneously, and 2) DSBs introduced during the mid-pachytene stage (after completion of SC assembly) are competent to become COs. Further, we have shown that COs induced at the Mos1 site inhibit the formation of COs elsewhere on the chromosome. Together these results indicate that Mos1-induced DSBs are able to compete with endogenous DSBs to become the sole CO on a chromosome pair, implying that a CO/NCO decision has not been irrevocably made early in prophase as proposed in recent models. We have also found that whereas COs represent only 12% of recombination events detected at the assay locus in a genetic background that is WT for the meiotic machinery, nearly all interhomolog DSB repair events in the *spo-11* mutant background (which lacks endogenous DSBs) become COs. This result indicates that a single DSB can be converted into a CO with high efficiency. By using additional variations of this powerful assay system, we will elucidate both the roles of different components of the meiotic machinery in promoting COs and the cytological relationship between the CO site and the large-scale chromosomal changes that are triggered by COs.

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Condensin I promotes accurate mitotic and meiotic chromosome segregation. **Karishma Collette**, Uchita Patel, Gyorgyi Csankovszki. Department of Molecular Cellular and Developmental Biology, Univ of Michigan, Ann Arbor, MI.

Condensins are five subunit protein complexes comprised of two SMC ATPase proteins (structural maintenance of chromosome) and three regulatory CAP (chromosome associated polypeptide) subunits. C.elegans has three condensin complexes that function in the diverse processes of dosage compensation and chromosome segregation. Condensin IDC binds to both X chromosomes in hermaphrodites to down regulate gene expression by half, equalizing X-linked gene product in males (XO) and hermaphrodites (XX), a process referred to as 'dosage compensation'. Condensin II is essential for chromosome organization and segregation during mitosis and meiosis. We recently showed that CAP subunits from Condensin IDC and SMC subunits from Condensin II interact to form an additional mitotic/meiotic condensin called Condensin I. To better understand the contribution of the newly discovered Condensin I to chromosome organization and segregation, we are focusing on a subunit of this complex, CAPG-1. capg-1 null mutants undergo larval arrest with a complete lack of germline development. Depletion of capg-1 by RNAi reduces brood size about four-fold and leads to a slight him phenotype. capg-1 RNAi also results in chromatin bridges and aneuploidies in embryonic and adult somatic nuclei and endomitotic oocytes in the germline. During embryonic mitosis, CAPG-1 coats chromosomes in a discontinuous pattern and colocalizes with Histone 3 phosphorylated on serine 10 (an AIR-2 dependent modification). During meiosis, CAPG-1 localizes at the midbivalent of diakinesis chromosomes, between homologs in meiosis I and between sister chromatids in meiosis II. As in mitosis, the meiotic localization of CAPG-1 also corresponds to the chromosomal association pattern of AIR-2. AIR-2 phosphorylates a subunit of cohesin that holds homologs and sister chromatids together, marking it for degradation and thereby initiating segregation (Rogers et al, 2002). Strikingly, capg-1 depletion disrupts AIR-2 localization at the midbivalent, which may contribute to the meiotic defects observed in these animals. Our work focuses on further exploring how Condensin I and AIR-2 might cooperate to achieve accurate chromosome partitioning during mitosis and meiosis.

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Three-Dimensional Positioning of Dosage Compensation Complex Binding Sites. **Emily Crane**, Satoru Uzawa, David Mets, Deborah Thurtle, Barbara Meyer. HHMI/UC Berkeley, Berkeley, CA.

In many species, dosage compensation equalizes X-linked gene expression between males (1X) and females (2X). Typically, a dosage compensation complex (DCC) is targeted to the X chromosomes of only one sex to modulate transcript levels. Dosage compensation requires chromosome-wide regulation of gene expression, which could be achieved through *cis* elements that act locally on individual genes or elements that act over long distances on many genes.

The *C. elegans* DCC is a multi-subunit complex that binds to both X chromosomes of hermaphrodites to repress gene expression by half. The DCC includes 5 proteins with homology to condensin, a conserved complex essential for the compaction, resolution, and segregation of mitotic and meiotic chromosomes. Similarity between the DCC and condensin suggests the DCC may achieve chromosome-wide gene repression through changes in X-chromosome structure. The DCC binds X using two classes of sites. *rex* (recruitment elements on X) sites recruit the DCC in an autonomous, DNA sequence dependent manner using a conserved motif enriched on X. *dox* (dependent on X) sites are more prevalent than *rex* sites, but unlike *rex* sites, occur preferentially in expressed genes and cannot recruit the DCC when removed from X. To test the theory that changes in chromosome architecture facilitate DCC binding, dispersion, or activity, we visualized the location of *rex* sites in embryonic nuclei through fluorescent in situ hybridization (FISH). We found that pair-wise combinations of *rex* sites separated on X by 12.5, 9, or 9.5 Mb of DNA were co-localized in 3D at a frequency higher in XX than XO embyros, suggesting a specific role for the DCC in bringing distantly encoded *rex* sites together.

The *rex* sites colocalize at the nuclear periphery, coincident with nuclear pore complexes (NPCs), suggesting NPCs provide a scaffold for DCC binding and X-chromosome restructuring. Additional data support a direct interaction between the DCC and NPCs. In XX and XO embryos co-stained with DCC and NPC antibodies as well as FISH probes to X or chromosome III, the DCC co-localized with NPCs and brought X, but not III, to NPCs in XX but not XO animals. Mass spectrometric analysis of proteins immunoprecipitated from embryo extract with DCC antibodies revealed an association between the DCC and NPC subunit NPP-9. In fact, the DCC co-localizes with NPCs caused to aggregate by RNAi to *npp-9*. These data support a model in which the DCC associates with NPCs and tethers X to pores. Anchoring of *rex* sites to NPCs my play a role in DCC binding, dispersion, or activity.

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Differentiation associated spatial separation of active and silent loci. **Benjamin D. Towbin**, Peter Meister, Brietta L. Pike, Susan M. Gasser. Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, 4058 Basel, Switzerland.

Chromatin is non-randomly organized in the interphase nucleus. In particular, silent heterochromatin is found adjacent to the nucleolus or at the nuclear periphery, where it is bound to the nuclear lamina. In yeast and flies, certain active genes are bound to nuclear pores. It is, however, still unclear whether clustering of silent genes at the nuclear envelope confers a biological function. In order to study the subnuclear distribution of genes in vivo and throughout development we have used recognition of lacO repeats by GFP-lacI for visualization of genetic loci in *C. elegans*. To this end, we applied microparticle bombardment to generate chromosomally integrated low-copy transgenes that contain arrays of LacO sites, and a developmentally regulated promoter driving mCherry (*myo*-3::mCherry or *pha*-4::mCherry-H2B). By expression of GFP-lacI from a ubiquitously expressed promoter, these transgenes are detected as fluorescent foci. In early embryonic cells, transgenes, like many endogenous loci, are randomly distributed throughout nuclear space. Over the course of differentiation, we observe spatial segregation of the transgenes, depending on their transcriptional status. Silent transgenes show high enrichment at the nuclear periphery while active ones become sequestered internally. In contrast to these low-copy transgenes created by bombardment, integrated repetitive transgene arrays derived form gonadal plasmid injection accumulate high levels of heterochromatic marks (H3K9me3 and H3K27me3). These silent domains are attached to the nuclear periphery throughout development. However, similar to low-copy transgenes, repetitive arrays can overcome peripheral anchoring upon activation of a developmentally regulated promoter located on the array. In summary, we observe spatial compartmentalization of active and silent genes in the nucleus, driven by differentiation. Our results suggest that the trigger for localization to the nuclear rim may be structural components of heterochromatin and not simply the binding of sequence specific factors. Preliminary experiments indicate that gene attachment to the nuclear envelope may stabilize heterochromatic silencing.

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Lateral microtubule bundles promote chromosome alignment during acentrosomal oocyte meiosis. **Sarah Wignall**, Anne Villeneuve. Dept. of Developmental Biology, Stanford University, Stanford, CA.

In mitotically-dividing cells, duplicated centrosomes are used as structural cues to define and organize the spindle poles. These centrosomes nucleate microtubules that capture the sister kinetochores of each chromosome, facilitating chromosome biorientation, congression, and segregation. In contrast, during meiosis in female animals the centrosomes are degraded prior to the meiotic divisions, and therefore oocyte spindles form in their absence. How these spindles are organized and the mechanisms by which they promote proper chromosome partitioning are poorly understood. We are using *C. elegans* oocyte meiosis as an *in vivo* model for investigating these mechanisms. First, we use high resolution imaging of both bipolar spindles and experimentally-generated monopolar spindles to reveal a surprising organization of microtubules and chromosomes within acentrosomal structures. While bivalents orient axially in bipolar spindles, with the chromosome ends pointing towards the poles, we find that lateral (not end-on) microtubule associations predominate. Bivalents are ensheathed by microtubule bundles that run along their sides, whereas microtubule density is low at chromosome ends despite a concentration of kinetochore proteins on those regions. This organization is a special feature of acentrosomal spindles, since centrosome-containing male meiotic spindles appear to have end-on associations. Further, we find that the chromokinesin KLP-19 forms a ring around the center of each bivalent during prometaphase/metaphase and plays a key role in chromosome congression on acentrosomal spindles by providing a polar ejection force. The chromosome passenger complex (CPC) also localizes in mid-bivalent rings and is required for proper KLP-19 localization. KLP-19 and the CPC leave the chromosomes in anaphase, but surprisingly retain their ring configurations in the central spindle. Moreover, depletion of KLP-19 causes defects in microtubule organization during anaphase. Together, these observations create a new picture of chromosome/microtubule association in acentrosomal spindles and reveal mechanisms that promote accurate chromosome partitioning. Specifically, we propose a model in which: 1) Ensheathment by lateral microtubule bundles places spatial constraints on the chromosomes, promoting biorientation, 2) Chromokinesins localized in mid-bivalent rings mediate movement along these bundles, promoting alignment, and 3) Central spindle rings maintain spindle integrity during anaphase, promoting segregation.

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Female Meiotic Spindle Dynamics in *C.elegans*: Roles for ASPM-1 and BMK-1. **M.H. Price**, V. Davis Haug, D.W. Turnbull, M.L. Drumond, E.A. Johnson, S.R. Lockery, B.A. Bowerman. Univ Oregon, Eugene, OR.

Female meiotic cell divisions in *C.elegans* provide a model for acentrosomal spindle assembly and function during meiosis I and II (MI & MII). Although wild-type spindle dynamics in oocytes have been described[1], little is known about the genetic pathways that orchestrate these dynamic events. We performed forward genetic screens and isolated seven recessive, temperature-sensitive, embryonic-lethal mutants with multiple maternal pronuclei in one-cell embryos. Two, *or642*ts and *or646*ts, are *mei-1* alleles, affecting a katanin subunit; *or447*ts is an allele of the kinesin gene *klp-18*; *or645*ts is an allele of *aspm-1*, the *C.elegans* ortholog of human ASPM (abnormal spindles and primary microcephaly)[2]. Using a new approach[3] to positionally clone mutant loci, we have found that *or627*ts is an allele of *bmk-1*, the *C.elegans* ortholog of Eg5 kinesin, previously thought to have no essential requirements[4,5]. We are examining MI spindle assembly and dynamics in these mutants using spinning disk microscopy, with GFP::β-tubulin and mCherry::Histone H2B fusions and a new method for immobilizing adult hermaphrodites without anesthetics[3]. In *bmk-1*(*or627*ts) mutants, multiple unstable poles form but never coalesce into a stable bipolar spindle. Rather, the poles converge to generate a monopolar spindle that extrudes all chromosomes into one polar body. Three mutants (*or600*ts, *or643*ts, *or646*ts) form spindles without obvious polar organization. Unlike *mei-1*(*or646*ts) spindles that fail to translocate to the oocyte cortex and fail to extrude a polar body, *or600*ts and *or643*ts spindles do translocate to the cortex, but fail to accurately segregate chromosomes at anaphase. *aspm-1*(*or645*ts) mutants form bipolar spindles that are much larger than wild-type; these spindles also fail to rotate once they reach the cortex. Interestingly, chromosome masses that remain near the cortex induce the formation of two polar bodies, consistent with chromatin signals organizing actomyosin contractile forces at the plasma membrane to promote polar body extrusion. Three of these mutants have extended cell cycle times, while the meiotic cell cycle in *aspm-1*(*or645*ts) is shorter than in wild-type. Thus a spindle assembly checkpoint may influence meiotic cell cycle progression, and ASPM-1 may be required for its function. A GFP::ASPM-1 fusion localizes to meiotic spindle poles where MDF-2, a Mad2p ortholog and known SAC component, also is present[6]. [1]Yang et al. 2003. Dev Biol. [2]Van Der Voet et al. 2009. Nat Cell Biol. [3]Price et al. 2009. International Worm Meeting. [4]Bishop et al. 2005. Mol Biol Cell. [5]Genome-wide RNAi screens. Wormbase.org. [6]Kitagawa and Rose. 1999. Nat Cell Biol.

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Action of CED-3 in meiotic and mitotic chromosome segregation. **Ashish Kumar**, Joel H. Rothman. Molecular, Cellular and Developmental Biology, University of California, Santa Barbara, CA 93106.

Separation of sister chromatids at the metaphase-to-anaphase transition is crucial for accurate chromosome transmission during both meiosis and mitosis. Separase, a CD clan cysteine protease, mediates this separation. Separase cleaves SCC-1, a member of the cohesion complex that holds sister chromatids together, allowing chromosomes to separate and to be drawn towards the poles of the spindle. Caspases also belong to the CD clan of the cysteine protease family and have been shown to cleave SCC1/RAD21 during apoptosis in mammalian cells. We found that the *C. elegans* caspase CED-3, which has been known strictly from its role in activating programmed cell death, may perform a separase-like function during meiosis I in the hermaphrodite germline and in subsequent embryonic mitotic divisions.

To investigate the interplay between apoptosis and other essential cellular processes, we performed an RNAi screen for lethal mutants that are suppressed by a mutation in the pro-apoptotic regulator *ced-4(-)*. We found that debilitating the function of *C. elegans* separase *sep-1* by RNAi leads to diminished lethality in a *ced-4(-)* background compared to N2. In contrast, we were surprised to find that the lethality of *sep-1(RNAi)* is significantly enhanced by mutations in *ced-3*. In addition, *ced-3(n717)* enhances X chromosome non-disjunction of *sep-1(RNAi)* animals (evident by a Him phenotype), suggesting a role for CED-3 in meiotic chromosome segregation. We visualized chromosome segregation during meiosis and subsequent embryonic mitotic divisions with an H2B::GFP marker. Time-lapse revealed a more severe defect in chromosome segregation in *ced-3(n717)*; *sep-1(RNAi)* embryos compared to *sep-1(RNAi)* embryos. Further, our preliminary observations indicate that while maternal and paternal chromosomes congress to an apparently normal metaphase plate in *sep-1(RNAi)* embryos, no metaphase plate forms in *ced-3(n717); sep-1(RNAi)* embryos, revealing a further role for CED-3 in promoting metaphase plate formation. Analysis of several different *ced-3* alleles suggest that the pro-apoptotic function of CED-3 is separable from its action in chromosome segregation. We conclude that the CED-3 caspase may be capable of performing a separase-like role in chromosome segregation during meiosis I and is involved in metaphase plate formation in early embryos. Since both caspases and separases belong to the CD clan of the cysteine protease family, our findings further suggest that the components of chromosome segregation pathway may have been co-opted by the cell death machinery during the course of evolution.

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RACK-1 directs dynactin-dependent RAB-11 endosomal recycling during mitosis. **Erkang Ai**, Daniel Poole, Ahna Skop. Dept Genetics, Univ Wisconsin, Madison, Madison, WI.

Membrane trafficking pathways are necessary for the addition and removal of membrane during cytokinesis. In animal cells, recycling endosomes act as a major source of the additional membranes during furrow progression and abscission. However, the mechanisms and factors that regulate recycling endosomes during the cell cycle remain poorly understood. Here, we show that the *C. elegans* RACK-1 is required for cytokinesis, germline membrane organization, and the recruitment of RAB-11-labeled recycling endosomes to the peri-centrosomal region and spindle. RACK-1 is also required for proper chromosome separation and astral microtubule length. RACK-1 localizes to the centrosomes, kinetochores, the midbody, and nuclear envelopes during the cell cycle. We found that RACK-1 directly binds to DNC-2, the *C. elegans* p50/dynamitin subunit of the dynactin complex. Lastly, RACK-1 may facilitate the sequestration of recycling endosomes by targeting DNC-2 to centrosomes and the spindle. Our findings suggest a mechanism by which RACK-1 directs the dynactin-dependent redistribution of recycling endosomes during the cell cycle, thus ensuring proper membrane trafficking events during cytokinesis.

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Anoxia-Induced Suspended Animation Requires a Nucleoporin Protein for Prophase Arrest in Blastomeres and Oocytes in *Caenorhabditis elegans*. **Brent A. Little**, Vinita A. Hajeri, Mary L. Ladage, Pamela A. Padilla. Biological Sciences, University of North Texas, Denton, TX.

The use of environmental stress, such as microtubule-depolymerizing agents or DNA damage, was instrumental for understanding cell cycle arrest and checkpoints. Previously, we showed that severe oxygen deprivation (anoxia) induces a state of suspended animation in which cell cycle progression is reversibly arrested at interphase, late prophase, and metaphase in blastomeres. The spindle checkpoint genes are required for metaphase arrest; the mechanisms regulating anoxia-induced prophase arrest are not known. Live cell imaging of *tbg-1::GFP;pie-1::GFP::H2B* animals, to monitor cell cycle progression, shows that an immediate response to anoxia is chromosome association with the inner nuclear membrane (chromosome docking) in prophase blastomeres. In these blastomeres nuclear envelope breakdown and the transition from prophase to prometaphase is arrested. The phenomenon of chromosome docking is also observed in the oocytes of hermaphrodites exposed to anoxia, aged hermaphrodites and *fog-2* mutants, but not other stresses such as starvation and heat shock, suggesting that chromosome docking is a characteristic of quiescent cells. To determine if chromosome docking is a response to reduced ATP we exposed animals to sodium azide and find that chromosome docking is induced in blastomeres and oocytes; however, unlike anoxic animals this affects their viability which suggests anoxia-induced cell cycle arrest is not a function of mitochondrial dysfunction. To identify genetic mechanisms required for prophase arrest we used RNAi to screen a subset of nuclear membrane associated and kinetochore genes. This screen identified *npp-16*, a non-essential component of the nuclear pore complex, as being required for anoxia-induced prophase arrest. The *npp-16(ok1839)* embryos exposed to anoxia have a reduction in the number of prophase blastomeres, an increase in abnormal nuclei, and a decrease in embryonic viability relative to control. Live cell imaging indicates that blastomeres of *npp-16(ok1839);tbg-1::GFP;pie-1::GFP::H2B* embryos exposed to anoxia do not arrest at prophase resulting in abnormal nuclei. Experiments are underway to test the hypothesis that anoxia induces a prophase checkpoint involving *npp-16* and understand the role a nucleoporin may have in arrested cells. Since oxygen deprivation plays a central role in resistance of solid tumor cells to radiation and chemotherapy treatments, these studies could elucidate how oxygen deprivation influences cell division and reveal mechanisms involved with a less understood cell cycle arrest position during mitosis-prophase arrest.

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Global analysis of histone subtype composition in C. elegans sperm using MudPIT mass spectrometric analysis. **Tammy F. Wu**[1], Colin F. Fitzpatrick[1], Catherine Wong[2], Michael C. Yee[1], Kieran Hervold[1], Aiza C. Go[1], John R. Yates III[2], Diana S. Chu[1]. 1) Dept Biol, San Francisco State Univ, San Francisco, CA; 2) Scripps Research Institute, La Jolla CA.

Sperm are remarkable cells specialized for the delivery of the paternal genome to the oocyte, while embryos consist of rapidly dividing, largely undifferentiated cells. Unlike embryos, sperm have jettisoned most cellular components, have highly compacted chromatin, and are thought to be largely transcriptionally inactive. Thus, sperm rely on substitution histones and post-translational modification (PTM) of existing proteins for development and function. In other organisms, replacement of S-phase histones by histone variants and protamines fundamentally changes chromosome architecture at the nucleosome level. How chromatin differs between these two tissue types has been largely unexplored.

To investigate the nature of *C. elegans* sperm chromatin composition, we conducted a global analysis of sperm and embryo chromatin proteins using Multidimensional Protein Identification Technology (MudPIT) mass spectroscopy. One story that emerges from these data reveals that variant histone H2A proteins are incorporated to a higher degree in a terminally differentiated cell type compared to actively dividing cells. In embryos, spectral counts of peptides corresponding to the four histone H2A variants confirms S-phase histone H2A as the most abundant form, while HTZ-1 and HIS-35 (an alternative H2A that differs from S-phase H2A by one residue) are much less abundant. However, this profile differs in sperm. Here, we detect the sperm-specific incorporation of the HTAS-1 variant. HTAS-1 is required for optimal male fertility, is expressed only in sperm as detected by Western Blot and immunostaining, and intriguingly marks paternally-contributed chromatin after fertilization. Also, despite the high level of sequence identity, HIS-35 is employed in greater proportion in sperm. Interestingly, unlike S-phase H2A histone genes, *his-35* contains an intron. This feature may reflect a mechanism for regulating HIS-35 expression to fine-tune chromatin status during spermatogenesis.

Our novel approach to determine chromatin composition between disparate cell types has identified over 1000 proteins from embryos and sperm each, laying the groundwork for analysis of other histone subtypes as well as protamine orthologs. In addition, our ongoing large-scale identification of histone PTMs is a valuable resource to examine how paternal epigenetic marks contribute to embryonic development.

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The PMK-1 p38 MAPK pathway regulates *C. elegans* innate immunity through phosphorylation of the bZIP transcription factor ATF-7. **Robert P. Shivers**[1,3], Daniel J. Pagano[1], Tristan Kooistra[1], Naoki Hisamoto[2], Kunihiro Matsumoto[2], Dennis H. Kim[1]. 1) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA; 2) Department of Molecular Biology, Nagoya University, Nagoya, Japan; 3) Department of Biological Sciences, The Commonwealth Medical School, Scranton, PA.

Caenorhabditis elegans responds to pathogen infection with the activation of conserved innate immune signaling pathways and the transcriptional up-regulation of putative immune effectors including C-type lectin domain proteins and antimicrobial peptides such as lysozymes. A forward genetic screen for mutants for enhanced susceptibility to killing by *P. aeruginosa* PA14 previously defined a role for a conserved TIR-1-NSY-1-SEK-1-PMK-1 mitogen-activated protein kinase (MAPK) pathway comprised of *C. elegans* orthologs of the Toll-Interleukin-1 Receptor (TIR) domain protein SARM, ASK1 MAPKKK, MKK3 MAPKK, and p38 MAPK. Using our previously reported genome-wide microarray analysis of PMK-1-regulated genes, we developed a GFP reporter strain that serves as an indicator of PMK-1 signaling. We used this strain to carry out a large-scale COPAS® worm sorter-aided screen for mutants with diminished PMK-1 activation and enhanced susceptibility to killing by PA14. From this screen, we isolated a mutation in the bZIP transcription factor ATF-7. We found that ATF-7 regulates the expression of genes previously identified as transcriptional targets of the PMK-1 pathway. We observed that *atf-7* is expressed in the intestine, the predominant site of infection by PA14. Genetic interaction analysis with multiple alleles of *atf-7* and PMK-1 pathway components show that ATF-7 functions downstream of PMK-1 as a key regulator of the innate immune response. Furthermore, we determined that ATF-7 is phosphorylated by PMK-1 *in vitro* suggestive that PMK-1 directly regulates the activity of ATF-7. Taken together these data are suggestive of an ancient conserved role for ATF-7 in the transcriptional regulation of innate immunity mediated by the p38 MAPK pathway.

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Further dissection of the epidermal antifungal response. **Olivier Zugasti**, Jerome Belougne, Jonathan Ewbank. Centre d'Immunologie, CIML, Marseille, France.

Following sterile injury or infection by the fungal pathogen *Drechmeria coniospora*, *C. elegans* produces NLP antimicrobial peptides in the epidermis, as part of a PKC-delta/p38-MAPK-dependent response [1, 2]. A non-canonical TGF-beta signalling pathway regulates a second group of putative antimicrobial peptides (of the Caenacin/CNC family). It involves the TGF-β ligand DBL-1, its heterodimeric receptor SMA-6/DAF-4 and the SMAD protein SMA-3, but not SMA-2 or SMA-4, which are otherwise required for the known functions of DBL-1. Neuronal expression of *dbl-1* controls *cnc-2* expression in the epidermis in a dose-dependent paracrine fashion. But infection does not influence the level of *dbl-1* mRNA [3]. This suggests that an immature form of DBL-1 is activated upon infection, either by an endogenous protease, or by one from the pathogen. This contrasts with the apparent direct modulation of *ins-7* expression, and thereby the DAF-2/DAF-16 pathway, by *Pseudomonas aeruginosa* [4]. On the other hand, it would be analogous to the processing required to activate Toll during Drosophila's innate immune response to fungal infection. We are pursuing the characterization of DBL-1 maturation through biochemical approaches and combining this with a genome-wide RNAi screen for genes that regulate the expression of *nlp-30* and/or *cnc-2* after infection.

1. Pujol *et al*. Anti-fungal innate immunity in *C. elegans* is enhanced by evolutionary diversification of antimicrobial peptides. *PLoS Pathog*, 2008. 4(7): p. e1000105.

2. Ziegler *et al*. Antifungal innate immunity in *C. elegans*: PKCδ links G-protein signaling and a conserved p38 MAPK cascade. *Cell Host and Microbes*, in press.

3. Zugasti and Ewbank. Neuroimmune regulation of antimicrobial peptide expression by a noncanonical TGF-beta signaling pathway in *Caenorhabditis elegans* epidermis. *Nat Immunol*, 2009. 10(3): p. 249-256.

4. Kawli and Tan. Neuroendocrine signals modulate the innate immunity of *Caenorhabditis elegans* through insulin signaling. *Nat Immunol*, 2008. 9(12) 1415-24.

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Tissue- and pathogen-specific innate immune signalling. Sophie Cypowyj, Jonathan Ewbank, **Nathalie Pujol**. CIML, Marseille, France.

It is now clear that pathogens trigger conserved regulatory pathways in *C. elegans*. These lead to the production of antimicrobial peptides (AMP) and proteins in the intestine and epidermis, under the control of the p38 MAPK, PMK-1. The protein kinase C delta TPA-1 is required to activate PMK-1 in both tissues, thus defining a core signalling cascade, also involving TIR-1, NSY-1 and SEK-1 {Liberati, 2004; Pujol, 2008}. Outside this core cassette, there is divergence, as for example, the kinase DKF-2, required in the intestine, is not required in the epidermis {Ziegler et al, in press; Ren et al, in press}. In the epidermis, sterile injury also provokes the expression of *nlp*-family AMP genes. Several mutants with epidermal defects show a high constitutive level of AMP gene expression too. This might serve to prevent any opportunistic infection of the injured epidermis {Pujol et al, 2008b}. In a *dapk-1* (death associated protein kinase) mutant, however, this constitutive expression is not a consequence of the epidermal defects, as a suppressor of the morphological defects, *sydn-1*, does not abrogate the high level of AMP expression. Conversely, *pmk-1* abolishes AMP gene expression but has no effect on the morphological phenotype of *dapk-1* mutants. This suggests that *dapk-1* functions as a negative regulator of the epidermal response to damage {Tong et al, 2009}. As GPA-12, a G-alpha protein is required for the regulation of *nlp-29* in the epidermis, one or more GPCRs are likely to contribute to the innate immune response {Ziegler et al, in press}. But just as different signalling components are used in different tissues, so too is there divergence within the epidermis regarding the genes involved in the response to fungal infection and wounding. For example, the Tribbles like kinase NIPI-3 is only require for fungal infection and is therefore of particular interest. We have found that *nipi-3* acts cell autonomously in the epidermis {Pujol, 2008b}, downstream of dapk-1. We conducted a Y2H screen and identified CEPB-1 as an interactor of NIPI-3. Although alone, *cepb-1* loss of function mutants appear normal, *cepb-1* suppresses the *nipi-3* phenotype, such that a *cepb-1 nipi-3* double mutant is able to respond to infection. CEBP-1 is a homologue of NF-IL6, which functionally interacts with Tribbles 1 in vertebrates {Yamamoto et al, 2007}, further strengthening the notion of deep conservation of innate immune signalling. We are currently studying whether CEBP-1 acts as a repressor of the whole p38 pathway or is specific to NIPI-3, and whether the physical interaction reflects a translocation of NIPI-3 into the nucleus. Thanks to A. Chisholm, and C. Rubin for sharing unpublished results.

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HDA-4/MEF-2 Is a DKF-2A Target-Effector that Regulates Inducible Innate Immunity. **Y. Fu**, M. Ren, H. Feng, M. Zeng, C. S. Rubin. Dept. Mol. Pharmacol., Albert Einstein Col. Med., Bronx, NY.

When *C. elegans* intestinal epithelial cells encounter bacterial pathogens, host genes encoding microbicidal peptides and other defense-related proteins are switched on and robustly expressed. The response constitutes induced innate immunity. A signaling cascade that includes EGL-8, TPA-1 and DKF-2A contributes to the immune response. Activation of DKF-2A, a *C. elegans* protein kinase D (PKD), triggers induction of 84 mRNAs. The mRNAs encode immune effector proteins that protect intestinal epithelium against infection by Gram negative and Gram positive pathogens. Both DKF-2A and pathogens induced HDA-4, a class IIa histone deacetylase (HDAC), and MEF-2, a transcription factor which binds HDA-4. HDACs repress gene expression by (a) deacetylating histones (altering chromatin) and transcription factors and (b) directly binding and inhibiting transcriptional activators. We determined whether an HDA-4/MEF-2 complex is a critical DKF-2A target-effector in a signaling pathway that up-regulates innate immunity. Biochemical and cellular analysis yielded a model. The distribution of HDA-4 between nucleus and cytoplasm was regulated by DKF-2A catalyzed phosphorylation of 2 serines. Non-phosphorylated HDA-4 was concentrated in nuclei of transfected cells. After phosphorylation by DKF-2A, HDA-4 bound 14-3-3 protein and was exported to cytoplasm. Segregation of HDA-4/14-3-3 complexes in cytoplasm should activate transcription of MEF-2 controlled genes. The model was tested *in vivo*. WT and mutant (regulatory serines replaced with Ala) HDA-4-GFP proteins were expressed in transgenic animals. WT HDA-4-GFP accumulated in both cytoplasm and nuclei of various cells. However, depletion of DKF-2A, or mutation of DKF-2A phosphorylation sites, targeted HDA-4-GFP exclusively to PML-like bodies (sites of gene repression) in nuclei. Elimination of HDA-4 potently enhanced innate immunity by de-repressing MEF-2. A severe loss of function mutation in *hda-4* (*hda-4(oy57)*) produced animals that are highly resistant to infection by *P. aeruginosa* (PA14) pathogen. DKF-2A and PMK-1 (p38 MAP kinase), a DKF-2A effector, were indispensable for MEF-2 mediated pathogen resistance in HDA-4 deficient *C. elegans*. Animals lacking MEF-2 (WT or *hda-4(oy57)* background) were hyper-susceptible to PA14 infection/killing, indicating that MEF-2 and HDA-4 are in a common signaling pathway. Conclusions: DKF-2A phosphorylates and regulates the location and functions of HDA-4 *in vivo*. DKF-2A promotes MEF-2 induced pathogen resistance by HDA-4 dependent and independent mechanisms. A novel signaling module that includes DKF-2A, HDA-4/MEF-2, and PMK-1 (as well as EGL-8 and TPA-1) plays a key role in inducing *C. elegans* innate immunity.

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The small GTPase RHO-1 is required to alter cell morphology during the *C. elegans* innate immune response. **Rachel McMullan**, Jennifer Winter, Stephen Nurrish. MRC Cell Biology Unit, University College London, London, United Kingdom.

We demonstrate a novel requirement for the RHO-1 GTPase in innate immunity. Expression of constitutively active RHO-1(G14V) from a heatshock promoter causes tail swelling known as the deformed anal region (*dar*) phenotype. *Dar* is part of the innate immune response to the pathogen *Microbacterium nematophilum* and is associated with changes in the morphology of cells in the anal region, including the rectal epithelial cells. Expression of RHO-1(G14V) in the rectal epithelial cells causes *dar* suggesting that RHO-1 acts cell autonomously to control the morphology of these cells. We are currently using bus-1p::GFP to visualize the rectal epithelial cells in order to address the mechanism of cell swelling and RHO-1's role in this process. RHO-1 signaling is required for the innate immune response as mutations in two Rho guanine nucleotide exchange factors (GEFs) (*ect-2(ku427)* and *unc-73(ce362)*) block swelling following *M. nematophilum* infection. *M. nematophilum* still adheres to the rectal cuticle suggesting that RHO-1 is required for the cell size and shape changes induced by infection. Expression of UNC-73E from a heatshock promoter partially rescues the bacterially unswollen (*bus*) phenotype of *unc-73(ce362)* indicating that RHO-1 signaling is required, at least in part, in adult animals.

Loss of components of the ERK/MAPKinase pathway has been shown to suppress bacterially-induced swelling. Inhibition of the MAPKinase pathway using the MEK inhibitor U0126 also suppresses swelling caused by constitutively activate RHO-1(G14V). Furthermore RNAi of mpk-1 or expression of activated RHO-1(G14V) in *mek-2(n1989)* or *mpk-1(ku1)* mutants is sufficient to suppress RHO-1 induced swelling suggesting that RHO-1 acts upstream of, or in parallel to, the MAPKinase pathway to mediate the innate immune response. Cross talk between the Rho and ERK/MAPKinase signaling pathways have been reported in mammals and interactions between the two pathways may contribute to several diseases including cancer. Using this system we are currently investigating the relationship between these two pathways.

Our data identify a role for RHO-1 signaling in the control of cell morphology during the *C. elegans* innate immune response. Using a candidate approach we have identified several RHO-1 effectors and regulators that are required for this process and we aim to use forward genetics and RNAi screening to identify further components of the RHO-1 signaling pathways involved in this process in order to understand how Rho signaling is activated in response to infection to regulate cell morphology.

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Single gene immunological trade-offs in the nematode *Caenorhabditis elegans*. **Elizabeth Marsh**[1], Maiike van Den Berg[2], Robin May[1]. 1) School of Biosciences, University of Birmingham, United Kingdom; 2) University of Utrecht, The Netherlands.

Mounting evidence has firmly established *Caenorhabditis elegans* as a powerful model system for the study of host-pathogen interactions. *C. elegans* has no cell-mediated immunity; instead any response to pathogenic attack elicited by the animal is entirely dependent upon the action of antimicrobial molecules. We identified one such factor secreted by the system, *lys-7*, as a putative anti-cryptococcal molecule in two *C. elegans* strains which are intrinsically resistant to killing by the fatal fungal human pathogen *Cryptococcus neoformans*. We show that *lys-7* is indeed a major mediator of resistance to cryptococcosis but, surprisingly, that the expression of this defensive molecule results in increased sensitivity to another pathogen: the Gram negative bacterium *Salmonella enterica* serovar Typhimurium. Expression and survival analyses have revealed this "balanced immunity" phenotype to be mediated by a trade-off between the activity of LYS-7 and the tyrosine kinase ABL-1, which is dependent upon the major stress-response factor, DAF-16. This is the first report of an immunological trade-off in *C. elegans*, and as such, has significant implications for our understanding of the evolution of immune regulation.

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The IRE-1 Branch of the Unfolded Protein Response Functions in C. elegans Pathogen Resistance During Larval Development. **Claire E. Richardson**, Tristan Kooistra, Dennis H. Kim. Biology, MIT, Cambridge, MA.

The Unfolded Protein Response (UPR), a mechanism to sense and respond to the accumulation of unfolded proteins in the endoplasmic reticulum (ER), has an important role in development and stress resistance from C. elegans to mammals. We asked if the UPR functions in C. elegans immune defense against the bacterial pathogen Pseudomonas aeruginosa. We found that the conserved IRE-1-XBP-1 branch of the UPR is required specifically for immunity during larval development. IRE-1 is an endoribonuclease that activates the transcription factor XBP-1 by splicing a short exon from its mRNA. Using quantitative PCR, we show that the relative amount of IRE-1-spliced xbp-1 transcript increases after pathogen exposure. Furthermore, a fluorescent reporter for IRE-1 activation is induced after exposure to Pseudomonas specifically at the site of infection–the intestine. We analyzed the relationship between the IRE-1 branch of the UPR and the PMK-1 pathway, which is known to promote C. elegans immunity, and found that IRE-1 activation in response to Pseudomonas is downstream of the PMK-1 pathway. The IRE-1-XBP-1 branch of the UPR has been recently shown to be required for cellular defense against pore-forming toxins, also functioning downstream of PMK-1 (Bischoff et al, 2008). Our data point to a more general role for the UPR in innate immunity against infection. Since PMK-1 pathway activates transcription of putative pathogen resistance proteins, we suggest that the IRE-1 branch of the UPR promotes pathogen resistance by accommodating a PMK-1-driven influx of pathogen resistance proteins to the secretory pathway. Bischof, L.J., Kao, C.-Y., Los, F.C.O., Gonzalez, M.R., Shen, Z., Briggs, S.P., van der Goot, F.G., Aroian, R.V. (2008) Activation of the unfolded protein response is required for defenses against bacterial pore-forming toxin in vivo. PLOS pathogens, 4, 1-11.

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A multi-prong genome-wide approach to studying cellular defenses against the bacterial pore-forming toxin. **Cheng-Yuan Kao**[1], Ferdinand C.O. Los[1], Shinichiro Wachi[2], Youn Sagong[1], Christine Ha[1], Danielle L. Huffman[1], Larry J. Bischof[1], Raffi V. Aroian[1]. 1) Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA 92093; 2) Center for Eukaryotic Gene Regulation, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802.

Pore-forming toxins (PFTs), the largest single class of bacterial virulence factors, are made by many important pathogenic bacteria such as *Streptococcus pneumoniae* and *Staphylococcus aureus*. These toxins form holes in cell plasma membranes and disrupt normal cell functions, although the consequences are poorly understood. Our laboratory has developed a powerful *in vivo* system for studying the host defense against PFTs, the intoxication of *C. elegans* by the bacterial PFT Cry5B. For example, we have previously shown that the p38 MAPK pathway and the IRE-1/XBP-1 limb of endoplasmic reticulum unfolded protein response (UPR) are activated to defend *C. elegans* against Cry5B. Comparable results have been obtained with PFTs that target mammalian cells, indicating that results from *C. elegans* may have broad relevance. Given the importance of PFTs, we have decided to undertake a multi-prong genome-wide analysis of *C. elegans* response to PFTs. Specifically, we have performed: (1) genome-wide RNAi screening for genes involved in *C. elegans* defense against PFTs. Using the Ahringer library, approximately 200 defense genes have been identified; (2) a comprehensive set of expression profiling in wild-type animals using Affymetrix microarrays at 1, 2, 4, and 8 hr of exposure of *C. elegans* to the PFT with temporal changes of PFT-regulated genes further clustered based on the response kinetics; (3) a set of expression profiling experiments comparing the response of wild-type animals to animals lacking 2 different signal transduction pathways at a single time-point; and (4) a limited study using proteomics to identify proteins up and down-regulated by the PFT. These data present a unique and powerful opportunity to simultaneously compare at a genome-wide level these different approaches to studying pathogenesis, for example analyzing how functional data, transcriptome data in wild-type and in signal-transduction mutants, and proteomic data correlate with each other. In addition, these data are now being used to study how various genes are involved in specific pathways, for example asking which of the 200+ defense genes are involved in p38 and UPR pathways. Our eventual goal is to assemble networks of genes involved in defense against PFTs and to determine how these networks are interconnected in space and time.

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Intestinal epithelial cell destruction during *S. aureus* pathogenesis in *C. elegans*. **Javier E. Irazoqui**[1,2], Frederick M. Ausubel[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Staphylococcus aureus is a human pathogen with enormous impact on human health both in the hospital and the community. *S. aureus* is capable of causing a large number of diseases in humans and other animals, including skin infections and abscesses, pneumonia, endocarditis, and osteomyelitis to name a few. Due to increased community prevalence of antibiotic-resistant virulent strains, *S. aureus* has become a major public health concern. In fact, more deaths were reported in the US in 2005 from *S. aureus* infection than from AIDS. Therefore, it is critical that we understand the molecular mechanisms utilized by *S. aureus* to cause disease. Among the many known virulence factors utilized by *S. aureus*, secreted pore-forming toxins (PFTs) are most prominent. PFTs such as alpha-hemolysin cause cellular damage in mammalian hosts, and are required for bacterial escape from phagocytic vesicles once internalized by professional phagocytes of the mammalian immune system. We used *C. elegans* to understand the basic, most conserved, fundamental aspects of pathogenesis by *S. aureus*. Previous data from our lab showed that many clinical isolates of *S. aureus* infect and kill *C. elegans*, which required live bacteria. However, until recently the mechanism of killing remained mysterious. We undertook a detailed characterization of the early steps of *S. aureus* infection using light and transmission electron microscopy. This analysis showed intestinal luminal accumulation of *S. aureus* early during the infection process, with evidence of interaction between bacterial and intestinal epithelial cells. Our data show progressive and extensive damage to the apical surface of the intestinal epithelial cells, involving enterocyte effacement, membrane blebbing, and loss of cell volume, eventually resulting in cell lysis, bacterial dissemination, and complete digestion of nematode internal organs. Importantly, similar observations have been reported using other systems, and in human intestines colonized with *S. aureus*. This suggests that at least some of the mechanisms used to cause disease in humans may also be used to destroy the nematode, consistent with previous findings that virulence factors required for disease in mammals were also important for *C. elegans* killing. We propose that the extensive cellular damage we observed in *C. elegans* is caused by digestion of the host tissues by bacterially produced PFTs and secreted lytic enzymes to be identified. We also propose that *C. elegans* is an excellent model to study the interactions between host epithelial cells and *S. aureus* in vivo.

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Cell Vacuolation, a Manifestation of *Vibrio cholerae* Hemolysin in *Caenorhabditis elegans* Intestine. **Hediye N. Cinar**[1], Mahendra Kothary[1], Atin Datta[1], Ben D. Tall[1], Robert Sprando[3], Kivanc Bilecen[2], Fitnat Yildiz[2], Barbara McCardell[1]. 1) Division of Virulence Assessment, Food & Drug Administration, Laurel, MD; 2) Department of Environmental Toxicology, UCSC, Santa Cruz, CA; 3) Division of Toxicology, Food & Drug Administration, Laurel, MD.

Vibrio cholerae (VC), a natural dweller of aquatic ecosystem, causes acute gastroenteritis in humans, using virulence factors such as cholera toxin (CT) and toxin co-regulated pili (TCP). *Vibrio cholerae* O1 and O139 serogroups are associated with cholerae epidemics, while non-O1 non O-139 strains, which mostly lack CT and TCP, and vaccine strains with deleted CT and TCP loci are also capable of causing diseases such as diarrhea, soft tissue infections, sepsis, and inflammatory enterocolitis in humans in a sporadic fashion through mechanisms that are currently unclear. VC causes lethal infection in *Caenorhabditis elegans* via a CT / TCP independent process providing an excellent model to determine the roles of other virulence factors in pathogenesis (1, 2). We found that hemolysin (*hlyA*) deletion mutants showed attenuated killing in *C. elegans* which is an effect that can be complemented by the presence of an intact *hlyA* locus (3). Furthermore, O1 classical strains, which produce less hemolysin than O1 El tor strains, showed milder lethality and reduced developmental delay. It has been reported that VC hemolysin causes extensive vacuolization and lysis in cultured cells. We examined worms fed with either wild type or *hlyA* deletion mutants of VC for intestinal pathologies. Forty-six percent of the worms exposed to wild type VC exhibited intestinal vacuoles while none one of the worms fed an *hlyA* deletion mutant showed this defect after 48-hour exposure. Other intestinal pathologies such as distention of the intestinal lumen and intestinal cell shrinkage were observed in both hly+ and hly- conditions, although the defects were more severe in hly+ conditions. Intestinal lumen distention and cell shrinkage preceded vacuolization suggesting that intestinal colonization of the bacteria may be necessary for vacuole formation. Hemolysin A is a pore forming exotoxin whose role in VC pathogenesis is not fully understood. Further studies in *C. elegans* infection model will contribute to a greater understanding of the role of hemolysin virulence in the pathogenesis of cholera. (1) Vaitkevicius K. et al. PNAS, 103 (2006) 9280-9285 (2) Cinar HN. et al. 16 th International C. elegans Meeting, 2007 (3) Cinar HN. et al. Aging Stress and Pathogenesis Meeting, 2008.

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Using quantitative proteomics to identify proteins involved in innate immunity of *C. elegans*. **Karina T. Simonsen**[1], Jakob Møller-Jensen[1], Anders Riis Kristensen[1,2], Jens S. Andersen[1], Donald L. Riddle[3], Birgitte H. Kallipolitis[1]. 1) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark; 2) Centre for High-Throughput Biology, Department of Biochemistry & Molecular Biology, University of British Columbia, Vancouver , Canada; 3) Department of Medical Genetics and Michael Smith Laboratories, University of British Columbia, Vancouver, Canada.

Adherent-Invasive *Escherichia coli* (AIEC) plays an important role in inflammatory bowel diseases. The AIEC strain LF82 was originally isolated from a patient with Crohn's disease and here we show that *C. elegans* can be used as a model host for this *E. coli* strain. When *C. elegans* feeds on it the life span is shortened significantly compared to survival on the normal laboratory food, OP50. Death is associated with bacterial colonisation of the intestine and this infection is shown to be persistent.

To gain insight into the *C. elegans* host response during infection, a quantitative proteomic approach was used, in which samples of ^{15}N labeled *C. elegans* from two different times during infection were analyzed by mass spectrometry in comparison with unlabeled worms fed OP50. This comparison resulted in a list of several proteins upregulated in response to LF82. A majority of these proteins have also been identified in other studies, including Lysozyme-7 and three different C-type Lectins. Some proteins are novel with respect to their implied function in the innate immune response, including an uncharacterized member of the Transthyretin-like family and FTN-2; a *C. elegans* ferritin heavy chain homolog. We are currently examining the potential role of iron in pathogenesis.

So far, large-scale investigations of the *C. elegans* immune response have been performed using microarrays. This study is the first to make use of quantitative proteomics to directly follow the protein dynamics during the infection process. These data will provide new knowledge about the *C. elegans* host defense repertoire.

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Wnts regulate asymmetric spindle to generate asymmetry of β-catenin localization. **Kenji Sugioka**[1,2], Hitoshi Sawa[1,2]. 1) RIKEN CDB, Kobe, Japan; 2) Department of Biology, Graduate School of Science, Kobe University.

Asymmetric cell division is a fundamental process that produces cellular diversity during development. In *C. elegans*, many asymmetric divisions are regulated by the Wnt signaling pathway. After the cells are polarized by Wnt proteins secreted from their posteriorly located cells, WRM-1/β-catenin localizes asymmetrically to the posterior nucleus at telophase to regulate asymmetric fates of the daughter cells. However, the mechanism of asymmetric WRM-1 nuclear localization is largely unknown. We found by drug assays that WRM-1 nuclear asymmetry is generated in a microtubule dependent manner. We then analyzed GFP::β-tubulin signal during the division and found that the numbers of astral microtubules were asymmetric during telophase; higher at the anterior spindle pole than the posterior. And this spindle asymmetry was regulated by Wnt. To know the importance of spindle asymmetry in asymmetric division, we analyzed the correlation between asymmetry of spindle and that of cell fates using *mom-2/wnt* RNAi embryos and found that embryos with symmetric spindle had strong tendency to have cell fate defects. The correlation was further confirmed by experimentally disrupting the spindle asymmetry using laser irradiation of the microtubule organizing center (MTOC). When the posterior MTOC was irradiated, nuclear WRM-1 asymmetry was enhanced. In contrast, when the anterior MTOC was irradiated, nuclear WRM-1 asymmetry was disrupted. These results strongly suggest that WRM-1 asymmetry is controlled by microtubule number asymmetry and microtubules inhibit nuclear localization of WRM-1. Kinesin may also be involved in this system because kinesin inhibitor disrupts WRM-1 asymmetry and *zen-4/kinesin* mutants show symmetric WRM-1 localization at telophase. We propose a model that kinesin enhances nuclear export of WRM-1 differently between anterior and posterior nucleus by transporting WRM-1 from perinuclear region toward cell cortex along asymmetrically organized astral microtubules.

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Two ARF GTPase cycles and a Frizzled pathway collaborate to regulate the asymmetric cell division of the Q.p neuroblast. **Jerome Teuliere**[1], Aakanksha Singhvi[1], Shaun Cordes[1], Karla Talavera[1], Guangshuo Ou[2], Ron Vale[2], Gian Garriga[1]. 1) Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Department of Cellular and Molecular Pharmacology, University of California, San Francisco, CA.

Much of the cellular diversity in the metazoan nervous system is generated by asymmetric cell divisions (ACDs), in which a mother cell divides to produce daughters that adopt different fates. While the regulation of asymmetric neuroblast divisions has been well studied in the fruit fly Drosophila melanogaster, little is known about the regulation of these divisions in other organisms. Since all neurons in the nematode Caenorhabditis elegans are produced from asymmetric divisions, the worm is an attractive system to address this question. The Q.p neuroblast normally divides to generate a smaller cell that dies (Q.pp) and a larger precursor (Q.pa) that divides to produce the A/PVM and SDQ neurons. We have identified several genes required for the Q.p division. Mutations in these genes cause Q.p to divide symmetrically to produce two precursors that are more similar in size. Several of these genes encode components of the Arf GTPase cycle, which is involved in membrane trafficking. The Arf GEF GRP-1, the Arf GAP CNT-2 and the Arfs ARF-1 and ARF-6 are involved in the Q.p ACD. While these genes function cell autonomously in the Q.p lineage, genetic interactions indicate that CNT-2 is an ARF-1 GAP and GRP-1 is an ARF-6 GEF, suggesting that two separate Arf cycles regulate the Q.p ACD. We are currently testing whether a more centrally positioned mitotic spindle in these mutants can explain the equivalent sizes of the Q.p daughters. We have also implicated the LIN-17 and MOM-5 Frizzled receptors for Wnt secreted glycoproteins in the Q.p ACD: *lin-17 mom-5* double mutants but not *lin-17* or *mom-5* single mutants display Q.p lineage defects. Because loss of a single Frizzled can suppress the Q.p defects of either *cnt-2* and *grp-1* mutants, we propose that the function of the Arf cycles is to negatively regulate the Frizzled pathway. One surprising finding is that various Wnt mutant combinations or *mig-14* mutants, which are defective in Wnt secretion, do not display Q.p defects, suggesting that the role of Frizzleds in this division might be Wnt independent. We are now investigating the mechanistic relationships between the Arf and Wnt signaling pathways in the Q.p ACD.

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The Homeodomain protein CEH-51 and the T-box factor TBX-35 share function in specification of the MS blastomere. **Morris F. Maduro**, Gina Broitman-Maduro, Wendy Hung, Melissa Owraghi. Dept Biol, Univ California, Riverside, Riverside, CA.

The MS blastomere makes some 80 embryonic cells, including pharynx cells, body muscle cells and coelomocytes. The MED-1,2 divergent GATA factors are important for specification of MS, as loss of *med-1,2* results in a penetrant embryonic arrest in which embryos lack MS-derived cell types (and some of which also lack intestine, which is made by the E lineage). We previously reported the identification of the T-box factor gene *tbx-35*, a direct target of MED-1,2, as an important MS specification gene. However, while loss of *med-1,2* results in embryos that arrest before two-fold elongation, a null mutant of *tbx-35* has variable arrest phenotypes. The most severely affected *tbx-35* mutants arrest at 1-fold and resemble med-1,2(-) mutants, while ~15% elongate to greater than threefold and can hatch into (inviable) larvae. At 15°C, even more *tbx-35* mutants (up to 50%) elongate and hatch, and such embryos appear to have a partially-restored posterior pharynx, which normally contains many cells made by MS. The incomplete expressivity of the *tbx-35* defects strongly suggests the existence of another gene that acts in MS specification downstream of MED-1,2. We have identified the homeobox-containing gene *ceh-51* as a direct target of TBX-35. There are four binding sites for TBX-35 in the *ceh-51* promoter, which define a consensus sequence similar to that of Brachyury. Consistent with its being a direct target of TBX-35, *ceh-51* is expressed in the early MS lineage. However, *tbx-35* mutant embryos continue to express *ceh-51* at a reduced level. While *tbx-35* mutants have a relatively strong MS specification defect, *ceh-51* mutants have milder defects in differentiation. Mutants arrest as Unc larvae with pharynx morphology defects, and loss of *ceh-51* synergizes with loss of function in *hlh-1* or *unc-120* to produce paralyzed, arrested two-fold (Pat) embryos (see abstract by Kuntz *et al.*). CEH-51 appears to be a divergent NK-2 type homeoprotein, suggesting it is an activator of genes that specify tissues among MS descendants. To directly test whether CEH-51 and TBX-35 might have overlapping functions, we constructed a *tbx-35; ceh-51* strain. We found that double mutant embryos arrest with a phenotype that is very similar to *med-1,2(-)* embryos in elongation and development of MS-derived cell types, showing that TBX-35 and CEH-51 together account for most, if not all, of MS specification downstream of MED-1,2. Our results suggest that a gene cascade consisting of MED-1,2, TBX-35 and CEH-51 specifies the MS fate. As collaboration of function of T-box and NK-2 regulators in mesoderm development is also conserved in other animals, these results suggest that similar mechanisms specify mesoderm across multiple phyla.

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Analysis of the early steps of trans-differentiation *in vivo* in *C. elegans*. **Konstantinos Kagias**, Nadine Fischer, Sophie Jarriault. IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Department of Cell and Developmental Biology ; INSERM U964, CNRS UMR 7104, Université de Strasbourg, 67404 Illkirch-CU Strasbourg, France. kkagias@igbmc.fr, sophie @ igbmc.fr.

We study the potential implication of chromatin structure in one type of cell plasticity, namely trans-differentiation (TD), defined as the reprogramming of a differentiated cell. Understanding how a differentiated cell can change its identity is important to increase our knowledge of developmental processes and has important implications for cancer and regenerative medicine.

We have focused on one cell identity change that occurs during the development of the worm. The epithelial cell named “Y”, born in the embryo, forms initially part of the rectum–a tube made of 6 cells. During the second larvae stage Y detaches from the rectum, migrates anteriorly and becomes a motor-neuron named “PDA”, without cell division. We have established that this identity change has the hallmark of trans-differentiation. We have characterized a collection of transgenic strains expressing GFP in the various cells in the rectal area, including specific markers for Y and PDA. Our model, where a rectal epithelial cell changes its identity to become a motor-neuron, will allow us to address the underlying mechanisms *in vivo* in a physiological context.

Because chromatin remodeling processes have been involved in cell identity maintenance and cellular plasticity potential, we decided to examine the potential importance of chromatin structure during this trans-differentiation event. We have conducted an RNAi screen targeting factors potentially involved in chromatin remodelling and analyzed their effect on TD. We have identified 5 genes important for trans-differentiation. Here, we will report on one of these genes, a *NuRD* complex component that is found to be overexpressed in many tumors and is important for the maintenance of ES cells pluripotency. Our characterization of mutants suggest that it is a key player of the early steps of TD and we are addressing its spatial and temporal focus of action. We have identified genetic interactors and have hypothesized a genetic pathway for its action. We believe that our detailed analysis of the early steps of Y-to-PDA trans-differentiation will allow us to propose a model for how cellular plasticity proceeds.

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Mechanisms that exclude somatic differentiation in the *C. elegans* germline. **Nate R. Dudley**, Joel H. Rothman. UC Santa Barbara,CA Dept MCDB.

We are investigating the mechanisms that prevent somatic differentiation in the *C. elegans* germline. When ectopically expressed, the END-3 GATA transcription factor is sufficient to redirect early embryonic cells towards endoderm differentiation; however, cells at later stages of development, including the adult germline, become refractory to such reprogramming. To identify factors that prevent somatic differentiation in the germline, we performed screens to assay for genes that when inactivated by RNAi result in hs-END-3-induced expression of the END-3 target gene, *elt-2::gfp* in the germline. Studies by Ciosk et al (1) showed that animals simultaneously lacking both the GLD-1 and MEX-3 RNA binding proteins show ectopic somatic differentiation in the germline. We found that RNAi of either *gld-1* or *mex-3* alone is sufficient to result in substantial ectopic gut differentiation. Notably, this effect reflects not only *elt-2* expression, but activation of the endoderm differentiation pathway, as we observe distinctive birefringent /autofluorescent gut granules, a hallmark of endoderm differentiation, within the germline. These findings demonstrate that disruption of single genes can cause germline cells, which are normally prevented from undergoing somatic differentiation, to be susceptible to reprogramming into a somatic fate. To extend our studies, we performed additional analyses for genes required to prevent the germline from adopting endoderm fate by RNAi. This screen identified a number of factors that result in END-3-dependent expression of *elt-2::gfp* to the same degree or greater than that seen in either *gld-1* or *mex-3* (RNAi) animals. These genes fall into a number of functional classes, including those encoding chromatin modifiers, cell signaling factors, and PARs. *par-3* interacts with genes involved in IP3 signaling, and we found that many of the genes that function in IP3 signaling also result in ectopic endoderm expression in the germline in this assay, suggesting that Calcium signaling systems may inhibit activation of somatic differentiation in the germline. Further characterization of these genes is likely to provide additional insights into their functions in regulating the commitment of cells to a germline fate. (1) Ciosk et al, Science v311 p851(2006).

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The BTB/Zinc finger protein EOR-1 and its obligate binding partner EOR-2 may be direct targets of MPK-1 ERK. **Kelly L. Howell**[1], Swathi Arur[2], Tim Schedl[2], Meera Sundaram[1]. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Washington University School of Medicine, St. Louis, MO.

Ras signaling controls many aspects of normal animal development including the specification of several *C. elegans* cell fates. Although the core components of the pathway have been identified, much less is known about the targets of Ras signaling that function to mediate cell-specific responses.

Our lab previously identified EOR-1 and EOR-2 as potential targets of the *C. elegans* Ras pathway that act downstream of MPK-1 ERK to promote the excretory duct, P12, and vulva cell fates. EOR-1 is a BTB/Zinc finger protein most similar to the mammalian transcriptional repressor PLZF. EOR-2 is a novel but conserved protein. We have shown that the EOR-1 and EOR-2 proteins physically interact *in vitro* and *in vivo*, and that mutations that eliminate this physical interaction also eliminate biological function. We hypothesize that the EOR-1-EOR-2 complex mediates transcriptional responses to Ras/ERK signaling.

EOR-1 is likely a direct target of MPK-1 ERK. EOR-1 has two potential ERK docking sites and is robustly phosphorylated by mammalian ERK2 *in vitro*. A point mutation in one of the potential docking sites significantly reduces phosphorylation of EOR-1 *in vitro* and eliminates its function *in vivo*. Interestingly, in Ras(gf) animals, EOR-1 protein levels are increased compared to wild-type. We are currently testing the model that MPK-1 ERK-dependent phosphorylation of EOR-1 may affect its stability and function in Ras signaling.

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The LET-60/Ras effectors RGL-1 (RalGEF) and LIN-45 (Raf) play antagonizing roles during vulval fate patterning. **Tanya P. Zand**[1], Vanessa González-Pérez[2], Channing J. Der[1], David J. Reiner[1]. 1) Lineberger Comprehensive Cancer Center and Department of Pharmacology; 2) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC 27599.

Anchor cell-dependent LIN-3/EGF signaling induces vulval precursor cells (VPCs) to assume a 2°-1°-2° pattern, yet current models conflict on the mechanism underlying this patterning. The "Morphogen gradient" model posits that spatially graded LIN-3/EGF signal differentially induces both 1° and 2° cell fates, while the "Sequential Induction" model argues that LIN-3 only activates LET-23/EGFR→LET-60/Ras in P6.p, which in turn induces P5.p and P7.p via LIN-12/Notch to adopt the 2° cell fate, and thus 2° fate is not directly dependent on the LIN-3 signal. However, a recent study showed that the LET-60→LIN-45/Raf cascade is also transiently induced in P5.p and P7.p, demonstrating that presumptive 2° cells receive an EGF signal with unknown consequences. We provide evidence that reconciles these two models, and consequently propose the "Morphogen-Reinforced Sequential Induction" model. Recent mammalian studies established that a pivotal second Ras effector cascade, RalGEF activation of the Ral small GTPases, cooperates with Raf to promote oncogenesis and metastasis. How RalGEF→Ral transduces Ras activity remains poorly defined. Unexpectedly, we found that loss of RGL-1/RalGEF or RAL-1/Ral function enhanced rather than blocked the Muv phenotype of activated *let-60(*gf*)* animals. Conversely, in the *let-60(*gf*)* background we expressed in VPCs either activated RAL-1(75L) or an effector domain mutant of activated LET-60(12V,37G) that signals primarily through RGL-1 and not LIN-45/Raf. Both constructs suppressed the *let-60(*gf*)* Muv phenotype. Taken together, these results suggest that LET-60 signals through RGL-1→RAL-1 to antagonize the function of the LET-60→LIN-45/Raf cascade. In epistasis experiments, *rgl-1* or *ral-1* RNAi enhanced the Muv phenotype of all mutants analyzed, including the downstream *lin-31* transcription factor, suggesting that RGL-1→RAL-1 signaling acts parallel to or downstream of LIN-45/Raf→MPK-1. A *ral-1 promoter::gfp* fusion drives GFP specifically in 2° but not 1° lineages. Furthermore, activated RAL-1(75L) enhances EGF-independent LIN-12/Notch activity, suggesting that RGL-1→RAL-1 signaling is pro-2°. Data from other groups suggest that the LIN-45/Raf→MPK-1 effector pathway is quenched in presumptive 2° cells. We hypothesize that LET-60 signals through LIN-45/Raf in 1° cells, but that in 2° cells LET-60 effector usage is switched to RGL-1→RAL-1, and thus in presumptive 2° cells the LIN-3/EGF signal transduced by LET-60/Ras promotes a 2° fate instead of a 1° fate. Thus, the EGF morphogen gradient can reinforce the initial patterning established by sequential induction.

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A Tetraspanin Promotes Notch Signaling in *C. elegans*. **Cory David Dunn**, Iva Greenwald. HHMI and Dept Biochem/Molec Biophysics, Columbia Univ, New York, NY.

The Notch signaling pathway is critical for normal development of metazoans, and alterations in Notch signaling have been associated with human disease. Many core components and modulators of the Notch pathway have been identified, but there are potentially other proteins involved that remain to be discovered. We are approaching the identification of such factors through both unbiased RNAi screens and RNAi assessment of candidate genes.

Tetraspanins are a large family of polytopic membrane proteins identified in animals and fungi. Tetraspanins have four transmembrane domains and a characteristic topology with conserved and variable regions. Biological roles of tetraspanins and their mechanism of action are just beginning to be elucidated, but evidence points to their involvement in protein trafficking or in signaling microdomains at the cell surface. We hypothesized that one or more tetraspanin proteins might contribute to Notch signaling.

Using RNAi targeting 18 of 21 individual tetraspanin genes, we determined that depletion of *tsp-12* uniquely suppresses the sterility of *glp-1(ar202)*, a hyperactive glp-1/Notch mutant. A null allele of *tsp-12* also suppresses *glp-1(ar202)* sterility, confirming this interaction. Furthermore, *tsp-12(0)* suppresses or enhances different alleles of *glp-1*, *lin-12* and other genes in the Notch pathway, affecting several different cell fate decisions. The genetic analysis indicates that *tsp-12* has a general role in promoting Notch activity. We are currently determining whether *tsp-12* acts in the ligand producing cell, the receiving cell, or both cells during a productive Notch signaling interaction.

Because many tetraspanin null mutants, including *tsp-12(0)*, have no discernible phenotype on their own, we have investigated possible functional redundancy between *tsp-12* and other worm tetraspanins. We have observed that *tsp-12(0)* is synthetically lethal with *tsp-14(0)*, and that segregants from heterozygous parents display synthetic visible phenotypes, including egg laying defects. We are currently testing whether any of the observed synthetic phenotypes are consistent with an absolute requirement for tetraspanin function in Notch pathway activity.

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Computational Modeling of Vulval Development Reveals Cell-cycle Regulation of LIN-12 Notch Signaling. S. Nusser[1], I. Rimann[1], N. Piterman[2], A. Hajnal[1], **J. Fisher**[3]. 1) Institute of Zoology, Zurich University, Switzerland; 2) Dept of Computing, Imperial College London, UK; 3) Microsoft Research Cambridge, UK.

We have previously introduced computational models of the molecular events underlying cell fate decisions of the six vulval precursor cells (VPCs). Our models are based on multiple modes of crosstalk between the EGFR and the LIN-12 Notch signaling pathways, which together determine the fates of the six VPCs. The models pass intensive testing, and reproduce many experimental observations. A key notion of our models, called *bounded asynchrony*, allows each VPC to change its state independently of other VPCs. But, VPCs are not allowed to drift apart too far by forcing them to proceed 'more or less' at the same time through the use of a *scheduler* that synchronizes the progression of all VPCs within a certain boundary. Bounded asynchrony is an abstraction of the biological processes that allow equipotent cells to adopt different fates. While searching for a biological counterpart of a scheduler, we hypothesized that the progression of the VPCs through the cell-cycle likely corresponds to the "scheduler" in the computational model. We thus devised an experiment to specifically arrest cell-cycle progression in one VPC without preventing the progression of the neighboring VPCs and observed the effect of this intervention on the specification of VPC fates. The concept of bounded-asynchrony predicted that blocking the progression of one VPC should disrupt the temporal separation between activation of the EGFR-mediated inductive and the LIN-12 Notch-mediated lateral signaling and thus interfere with robust pattern formation. Indeed, arresting the central VPC (P6.p) in the G1 phase of the cell-cycle through expression of the Cdk inhibitor cki-1 prevented proper down-regulation of a translational LIN-12::GFP reporter in P6.p. While LIN-12::GFP in a non-arrested P6.p cell is initially localized to the apical plasma membrane and then degraded, LIN-12::GFP often remained diffusely localized in the cytoplasm and/or concentrated in the nucleus of arrested P6.p cells. This suggested that the intracellular cleavage product of LIN-12 failed to be degraded upon G1 cell-cycle arrest. Thus, down-regulation of LIN-12 in P6.p after activation of the lateral signal is tightly linked to cell-cycle progression. Moreover, we observed a disruption of the normal cell fate pattern, as G1-arrested P6.p cells often expressed high levels of a secondary cell fate marker (LIP-1::GFP). These findings indicate that the formation of a stable cell fate pattern during vulval development requires the coordinated progression of VPCs through the cell-cycle as postulated by the computational concept of bounded-asynchrony.

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Evolution of developmental mechanisms in vulva formation in nematodes C. elegans and C. briggsae. **B. P. Gupta**, N. Bojanala, P. Cumbo, S. Marri, A. Seetharaman, B. Thillainathan. Dept Biol, McMaster Univ, Hamilton, ON, Canada.

We are interested in understanding how genes and signaling pathways have evolved to specify distinct cell fates during animal development. Towards this, we are doing a comparative analysis of vulval development in *C. elegans* and its sister species *C. briggsae*. Studies in *C. elegans* have shown that the adult vulva is formed by the progeny of three of six multipotential vulval precursor cells (VPCs) that are induced by signal transduction pathways mediated by Wnt, Ras and Notch. This provides an excellent opportunity to study evolutionary changes in signaling pathway components and their effect on cell fate specification and organ formation.

To this end we have carried out large-scale genetic screens to isolate *C. briggsae* vulval mutants that display multivulva (Muv), Vulvaless (Vul), and protruding vulva (Pvul) phenotypes. Using a combination of phenotypic and single nucleotide polymorphism-based markers we have mapped 23 mutants leading to the identification of 17 different genes (4 Muv class, 8 Vul class, and 5 Pvul class). Current experiments focus on the genetic and molecular analysis of these loci. So far we have molecularly characterized three genes that represent orthologs of *pry-1* (Muv), *lin-39* (Vul), and *lin-11* (Pvul). The *pry-1* gene encodes an Axin-like protein that functions in the canonical Wnt signaling pathway to negatively regulate vulval cell proliferation. We have found interesting differences in the phenotype of *pry-1* mutants between *C. elegans* and *C. briggsae* suggesting a possible evolutionary change in *pry-1*-mediated Wnt signaling in vulval development. We are now focusing on the identification of Wnt target genes in *C. briggsae* to understand the mechanism of differences in Wnt signaling function between the two species.

Our analysis of *lin-11* function in vulval development has revealed its role in multiple processes. *lin-11* is a founding member of the LIM homeobox gene family and is necessary for the invagination and differentiation of vulval progeny. We have found that although *C. briggsae lin-11 (Cbr-lin-11)* is involved in vulval development similar to *Cel-lin-11*, there are subtle differences in the regulation and function of the two orthologs. We have dissected the *lin-11* enhancer region and identified several important regulatory sequences and transcription factor binding sites. Current experiments focus on the analysis of these sequences in conferring species-specific function on *lin-11* in vulval morphogenesis.

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ngn-1 and *hlh-2* Are Required for a Left-Right Asymmetric Neurogenesis Decision. **Shunji Nakano**[1], Ronald Ellis[2], Bob Horvitz[1]. 1) HHMI, Dept. Biology, MIT, Cambridge, MA; 2) Dept. Molecular Biology, UMDNJ, Stratford, NJ.

The body plan of *C. elegans* is mostly bilaterally symmetric. Much of this symmetry arises from analogous blastomeres, which through bilaterally symmetric cell lineages generate sets of left-right paired cells. To create either asymmetric cells or three-fold symmetry, this bilateral symmetry must be broken. The e3 pharyngeal epithelial cells form a three-fold symmetric structure that is composed of three cells, located on the ventral left (VL), ventral right (VR) and dorsal (D) region of the pharynx. e3VL and e3VR are generated as left-right lineal homologs, whereas e3D is generated by breaking left-right symmetry in a specific cell lineage. Specifically, the blastomeres ABaraapa and ABaraapp are sister cells that give rise to identical sets of left-right paired cells, except that the ABaraapaaaa cell becomes the e3D cell and its lineally homologous cell ABaraappaaa becomes the MI neuron. The differential determination of cell fate by these two cells breaks left-right symmetry in these cell lineages.

We sought mutations that transform MI into an e3-like cell or e3D into an MI-like cell, thereby regenerating symmetry in these cell lineages. From screens of 10,000 mutagenized haploid genomes, we identified two genes, *ngn-1* (*neurogenin-1*) and *hlh-2* (*helix-loop-helix-2*), that when mutated cause the absence of MI and the presence of an extra e3-like cell. Laser ablation of the grandmother of the presumptive MI neuron in the *ngn-1* mutant results in the absence of the extra e3-like cell. These results suggest that MI is transformed into an e3-like cell in the mutants.

ngn-1 encodes a basic helix-loop-helix (bHLH) protein. *hlh-2* is the *C. elegans* ortholog of *E2A/Daughterless*, a bHLH protein known to dimerize with other bHLH proteins, including those within the neurogenin subfamily. Mosaic analysis of *ngn-1* suggests that *ngn-1* acts cell-autonomously to specify the MI fate. We are currently examining the expression of *ngn-1* and *hlh-2* to test whether these genes are expressed asymmetrically to break the left-right symmetry in this cell lineage.

In short, we have identified two bHLH genes required for breaking symmetry in a left-right asymmetric cell lineage. We hypothesize that NGN-1 and HLH-2 form a transcriptional heterodimer that acts cell autonomously to induce left-right asymmetric neurogenesis. We hope that further analysis of these genes will uncover a mechanism by which *ngn-1* and *hlh-2* are differentially regulated to result in an invariant determination of this aspect of left-right asymmetry.

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Computational analysis of quantitative information of cell division dynamics of early embryos. **K. Kyoda**, E. Adachi, K. Shimada, J. Kuramochi, S. Onami. Developmental Systems Modeling Team, RIKEN ASI, Yokohama, Japan.

A collection of quantitative information of cell division dynamics under a wide variety of perturbations of gene expression is a rich resource for understanding four-dimensional spatiotemporal developmental process. The collection enables us to perform highly objective and quantitative analyses for elucidating developmental mechanisms of cell division dynamics. Our computer system obtains the outlines of three-dimensional nuclear regions and their transition over time as quantitative cell division dynamics information by combining 4D DIC microscope imaging and image-processing. Here we targeted all 97 embryonic essential genes on chromosome III, and measured and analyzed the cell division dynamics during the first three rounds of cell divisions after fertilization in 50 wild type and 136 RNAi embryos. To show the advantages of the quantitative cell division dynamics information, we first performed RNAi phenotype analysis. By mathematically defining 365 quantitative developmental characteristics of cell division dynamics, we objectively identified >7000 phenotypic alterations in these characteristics for the 136 RNAi embryos. Our analysis identified all 33 reproducible phenotypic alterations in the 16 defect categories for nuclear and spindle dynamics reported in previous large-scale manual analysis. We identified about 13 times more phenotypic alterations in the same defect categories that included not only subtle alterations but also obvious alterations likely to be overlooked in the previous manual analysis. Our analysis also identified alterations in four-dimensional spatial and temporal characteristics that are difficult to isolate in manual analysis. These alterations included those in cell division axes of the third round of cell divisions and those in cell division timing of each cell. We next performed developmental flowchart analysis, which predicts a model of developmental mechanisms of early embryo by using our cell division dynamics information. We predicted developmental flowchart that represents 1656 spatiotemporal associations between 329 developmental characteristics by finding the correlations between these characteristics in the wild type embryos. We also predicted total >8000 genes involved in these associations by finding the outliers on the correlations from the RNAi embryos. Our approach using the quantitative information provides useful information for understanding spatiotemporal developmental process.

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The monounsaturated fat vaccenic acid negatively regulates innate immunity through its effect on insulin signaling. **Madhumitha Nandakumar**[1], Man-Wah Tan[1,2,3]. 1) Prog in Immunology, Stanford Univ, Stanford, CA; 2) Dept of Genetics, Stanford Univ, Stanford, CA; 3) Dept of Microbiology Immunology, Stanford Univ, Stanford, CA.

Monounsaturated fatty acids (MUFAs) can have profound effects on human health. Diets rich in MUFAs, such as oleic acid, lower cholesterol levels and improve cardiovascular health. On the other hand, chronic exposure of islet cells to elevated levels of vaccenic acid, an omega-7 MUFA, causes increased insulin resistance and decreased glucose oxidation, two typical symptoms of diabetes. Diabetes is also thought to compromise the innate immune response, and diabetic patients show an increased frequency of infections. The potential effects of MUFAs on immunity, however, are not known. Using the *C. elegans–P. aeruginosa* infection model, we demonstrate that vaccenic acid has a detrimental effect on *C. elegans* immune function, but also plays a vital role in the virulence of *P. aeruginosa*. Specifically, we show that the *C. elegans fat-5* mutant, which has decreased levels of vaccenic acid, is resistant to *Pseudomonas* infection. This increased resistance is suppressed when vaccenic acid levels are restored through dietary supplementation. Transcriptional profiling of the basal expression of infection-response genes in the *fat-5* mutant revealed a significant up-regulation of genes required for survival against pathogen infection, including a number of known and putative antimicrobial peptides. Gene expression in the *fat -5* mutant is strongly correlated with the insulin-receptor mutant *daf-2*, but not mutants in other immune pathways, such as the Sma/TGF-beta or the p38 MAP kinase pathways. Thus, the detrimental effect of vaccenic acid on *C. elegans* immunity is likely through its effect on insulin signaling. Interestingly, a *fabF1* mutant of *P. aeruginosa* that has decreased levels of vaccenic acid is attenuated in its ability to infect and kill *C. elegans*. *fabF1* bacteria grown in the presence of vaccenic acid are no longer compromised in virulence, indicating a specific role for this fatty acid in virulence. The *fabF1* mutant is defective in its ability to both infect and persist in the worm, and appears to be more sensitive to the cationic antimicrobial Polymyxin B. We will further elaborate on the roles of vaccenic acid on *C. elegans* immunity and virulence of *P. aeruginosa*.

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Microsporidia are natural intracellular parasites of C. elegans. **Emily Ruth Troemel**[1], Marie-Anne Félix[2], Noah Whiteman[3], Antoine Barrière[2], Frederick Ausubel[4]. 1) Biology, UC-San Diego, La Jolla, CA; 2) Institut Jacques Monod, Paris, France; 3) Harvard University, Cambridge, MA; 4) Massachusetts General Hospital, Boston, MA.

The intestine is a common site for pathogens to invade the body, but little is known about how pathogens interact with fully differentiated intestinal cells. We have developed a new model for studying intracellular infection in the intestine by identification and characterization of a natural pathogen of the nematode C. elegans. We analyzed a strain of C. elegans isolated from a compost pit near Paris, France, which harbors intracellular microbes in its intestinal cells. These microbes are pathogenic, and are transferred from worm to worm. Using PCR with universal primers against ribosomal sequence, coupled with fluorescence in situ hybridization, we determined that these worms are infected with a species of microsporidia. Microsporidia comprise a phylum of eukaryotic intracellular parasites that are related to fungi. Microsporidia infect virtually all animal phyla, including humans. These pathogens have been added to the NIH list of priority pathogens, as well as the EPA list of waterborne microbial contaminants of concern. The microsporidian species infecting C. elegans defines a new genus and a new species, which we named Nematocida parisii, or nematode killer from Paris. We have also found similar infections in worms isolated from natural environments in other parts of France, in Portugal and in India, indicating that microsporidia may be a common cause of infection for C. elegans in the wild. By determining when worms are infectious to their neighbors, we identified a likely exit strategy of these pathogens, which involves restructuring a conserved cytoskeletal structure in the intestine called the terminal web. C. elegans defense against microsporidian infection does not require the p38 MAPK or insulin signaling pathways, which are involved in defense against bacterial pathogens. The C. elegans/microsporidia system provides a unique opportunity to identify the signaling pathways involved in interaction with microsporidia, a class of pathogens that are medically and agriculturally important, but poorly understood.

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Spatio-Temporal Regulation of the Dauer Decision. **Oren Schaedel**, Paul Sternberg. Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA.

The dauer decision integrates food abundance, population density and temperature. Dauer frequency in a population increases as conditions shift from favorable (e.g. high food concentration and low population density) to unfavorable (e.g. low food concentration and high population density). This decision is coordinated over the whole animal resulting in dauer or L3 fates, as wild-type animals do not display mosaicism of either fate. *daf-9* and *daf-12*, which encode a cytochrome P450 and a nuclear hormone receptor respectively, act downstream the insulin and TGFβ signaling pathways. Dafachronic acid, a lipophilic hormone product of DAF-9, works cell non-autonomously directing L3 programs. Dafachronic acid binds to DAF-12, which then upregulates hypodermal expression of *daf-9* via a positive feedback loop. We hypothesize that information from environmental sensing is integrated through cellular and molecular mechanisms and reduced to a single cell non-autonomous regulator, thereby explaining the tight binary nature of the decision. We propose a fate coordination mechanism in which secretion of a small amount of hormone is propagated across the anterior-posterior axis, locking in the L3 fate. Specifically, we show that Δ^7-dafachronic acid is necessary for the L2d/L3 fate transition and execution of L4 programs. Using a combination of laser ablations and time lapse image analysis, we demonstrate that upon L2d to L3 decision, XXXL/R cells act as a source releasing Δ^7-dafachronic acid. As a result, hypodermal *daf-9* expression propagates via the DAF-12 positive feedback loop from anterior to posterior, generating high amounts of dafachronic acid. Moreover, we show that high amounts of Δ^7-dafachronic acid are required for proper distal tip cell migration, an L4 program, suggesting that this is a fate locking mechanism. This mechanism of positive feedback acting on a small amount of signal has been shown in other cell fate decisions and can help explain how one fate is propagated in a coordinated manor across the whole organism.

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Caenorhabditis elegans chemical biology: lessons from small molecules. **Frank C. Schroeder**[1], Chirag Pungaliya[1], Jagan Srinivasan[2], Fatma Kaplan[3], Arthur Edison[4], Paul Sternberg[2]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell Univ, Ithaca, NY 14853; 2) 156-29 Biology Division, California Institute of Technology, 1200 E. California Blvd, Pasadena, CA-91125; 3) USDA-ARS, 1700/1600 S.W. 23rd Drive, Gainesville, FL 32608; 4) Department of Biochemistry and Molecular Biology, University of Florida, PO Box 100245, Gainesville, FL 32610-0245.

How can we complement *C. elegans* genomics and proteomics with a comprehensive structural and functional annotation of its metabolome? Several lines of evidence indicate that small molecules of largely undetermined structure play important roles in *C. elegans* biology, including key pathways regulating lifespan, development, and metabolism. We have developed NMR spectroscopic methodology that enables linking small molecule metabolites directly with corresponding mutant phenotypes and probable biological functions. Application of this approach to identifying the C. elegans mating and dauer pheromones revealed a complex signaling system based on a bi-functional group of signaling molecules, the ascarosides ascr#1-ascr#8. Low concentrations of ascarosides attract males and thus appear to be part of the C. elegans sex pheromone, whereas higher concentrations induce developmental arrest at the dauer stage. Intriguingly, only mixtures of several ascarosides produce strong phenotypes at physiologically relevant concentrations, and individual ascarosides exhibit different though overlapping activity profiles. For example, the ascaroside ascr#2 is a stronger dauer inducer than ascaroside ascr#3, whereas ascr#3 is a stronger male attractant than ascr#2. However, mixtures of ascr#2 and ascr#3 are much more active than either compound alone, and addition of a third component, ascr#8, further increases activity, indicating synergistic modes of action. Cellular and genetic analyses of ascr#3 suggest the role of both core and sex-specific sensory neurons in regulating the response to this metabolite. Additional studies indicate that different ascarosides act through different neuronal and genetic pathways. These findings present a significant departure from the one-compound one-phenotype paradigm and emphasize the need to develop systemic approaches for correlating genome, phenotype, and metabolome. 1. J. Srinivasan et al. (2008). A blend of small molecules regulates both mating and development in *C. elegans*. Nature 454:1115-1118. 2. C. Pungaliya, J. Srinivasan, B. W. Fox, R. U. Malik, H. A. Ludewig, P. W. Sternberg, F. C. Schroeder (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *C. elegans*. PNAS, in press.

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The C. elegans Gen-1 Holliday Junction Resolvase links DNA double strand break repair and DNA damage signalling. Aymeric Bailly[1], Anne-Cécile Déclais[2], Julie Hall[3,5], Alasdair Freeman[2], Arno Alpi[4], David Lilley[2], Shawn Ahmed[3], **Anton Gartner**[1]. 1) Gene Regulation/Expression, Sch of Life Sciences, Dundee, UK; 2) Cancer Research UK Nucleic Acid Structure Research Group, University of Dundee, UK; 3) Department of Genetics, Lineberger Cancer Center, and Department of Biology, University of North Carolina, Chapel Hill, NC 27599-3280, USA; 4) Current address: LMB, Cambridge, UK; 5) 4Current address: Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, Durham, NC, USA.

Coordination of DNA repair with cell cycle progression and apoptosis is a central task of the DNA damage response machinery. A key intermediate of recombinational repair and meiotic recombination, first proposed by Robin Holliday in 1964, are four-way DNA intermediates. These intermediates have to be resolved to allow for proper chromosome segregation. Enzymes resolving Holliday junctions by symmetric endonucleolytic cleavage have been isolated from bacteriophages, bacteria, and were found in yeast mitochondria. Although nuclear Holliday junction resolvase activities had been measured for many years, the corresponding Holliday Junction resolving enzyme(s) have remained elusive. The purification of a Holliday Junction resolvase activity was recently reported, and resolvase activity was linked to GEN1, but the in vivo functions of GEN1 remained unclear 1). Using unbiased genetic screening, followed by a positional mapping we identified several alleles of C. elegans gen-1. We find that GEN-1 is required for recombinational repair in response to DNA double strand breaks, but is not necessary for processing other forms of DNA damage. In addition, GEN-1 is not required for meiotic recombination. Importantly, the C-terminus of GEN-1 plays a role in DNA damage signalling to affect germ cell cycle arrest and germ cell apoptosis that is separable from its role in DNA repair. Our biochemical analysis suggests that GEN-1 Holiday Junction resolvase activity may be required for DNA repair but may be dispensable for DNA damage signalling. The DNA damage signalling function of GEN-1 defines a pathway that acts in parallel to the canonical DNA damage response pathway mediated by RPA loading, ATM/ATR and cep-1/p53 activation. Furthermore, we show that GEN-1 is needed in conjunction with the 9-1-1 DNA repair complex to ensure survival even in the absence of DNA damaging agents. Our results point towards the intriguing possibility that a dual function Holliday junction resolvase may coordinate DNA damage signalling with a terminal step in DNA double strand break repair. 1) Ip et al., Nature, 2008, Nov20th, 456, p357-361.

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CPS-5, a substrate of the CED-3 cell death protease, promotes cell death in *C. elegans* upon cleavage by CED-3. **Akihisa Nakagawa**, Yong Shi, Ding Xue. MCD Biology, University of Colorado, Boulder, CO.

Programmed cell death (apoptosis) is a complex and tightly controlled process that is vital for the proper development of an organism as well as for maintaining its homeostasis. *ced-3*, a key player in programmed cell death in *C. elegans*, encodes a member of the caspase family of cysteine proteases. One particularly important area that has not been studied is the *in vivo* targets of the death caspases. In order to reveal downstream targets of CED-3, we have developed a sensitized genetic screen to isolate mutations that partially suppress or delay cell death caused by constitutively activated CED-3 death protease. From this screen, we have identified at least fourteen new genes (*cps-1* to *cps-14*; CED-3 protease suppressors). Here we report the genetic, molecular, and biochemical characterization of the *cps-5* gene, which is directly activated by CED-3 to promote cell death.

cps-5 is identified by a single mutation, *sm55*, which causes a delay of cell death defect and can block cell death in sensitized genetic backgrounds. We mapped *cps-5* to the left arm of LG III by single nucleotide polymorphism mapping and cloned the gene by transformation rescue. *cps-5* encodes a novel protein that interestingly turns out to be a substrate of CED-3 *in vitro*. We identified the single CED-3 cleavage site in CPS-5 by N-terminus sequencing of the CPS-5 cleavage products. An Aspartate to Glutamate substitution in CPS-5 that blocks cleavage of CPS-5 by CED-3 *in vitro* abolishes CPS-5 pro-apoptotic activity *in vivo*. Moreover, expression of the N-terminal cleavage product of CPS-5 but not the C-terminal cleavage product induces ectopic apoptosis in *C. elegans*. Therefore, cleavage of CPS-5 by CED-3 is required to activate CPS-5's proapoptotic activity and CPS-5 is a bona fide CED-3 *in vivo* target. Interestingly, the N-terminus of CPS-5 contains a putative transmembrane domain that is required for its function. We are now looking for factors that interact with CPS-5 and investigating how CPS-5 promotes cell killing. The identification of CPS-5 as an *in vivo* CED-3 substrate suggests that our sensitized CED-3 suppressor screen is effective in identifying *in vivo* CED-3 caspase targets.

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Fat metabolism: a missing link between reproduction and longevity. **Meng Wang**[1,2], Eyleen O'Rourke[1,2], Gary Ruvkun[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

A precise balance of fat mobilization and storage is a basic feature of animal physiology. The endocrine role of adipose tissue in regulation of animal lifespan has been implied by genetic studies in both invertebrate and vertebrate organisms. However it is unclear how fat metabolism is related to lifespan control. As an energy-intensive process, reproduction has a significant impact on fat metabolism. In C. elegans and Drosophila, signals from the reproductive system regulate organism lifespan. Thus, understanding the mechanisms by which fat metabolism is coupled to reproductive cues may shed light on the systemic regulation of fat metabolism and provide insights on the control of aging. In our studies, we have identified a specific fat lipase (lipl-4) that connects lipid hydrolysis, germline stem cell proliferation and longevity in C. elegans. We found that germline stem cell arrest promotes systemic lipolysis by inducing the expression of the lipase. This lipase is also necessary upon germline stem cell arrest to prolong lifespan. Strikingly, constitutive expression of the lipase in fat storage tissue induces lipid hydrolysis and leads to longevity. The lifespan extension is independent of the Forkhead transcription factor/Daf-16. We also found that the transcriptional regulation of this lipase is complex. Its basal expression and induction by germline stem cell arrest are modulated by multiple forkhead transcription factors and nuclear hormone receptors. These results suggest the existence of an endocrine axis from germline stem cells to fat storage tissue, which plays a vital role in regulation of somatic maintenance and longevity. This work is supported by Life Sciences Research Foundation and Ellison Medical Foundation fellowships.

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A Transcription Elongation Factor that Links Signals from the Reproductive System to Lifespan Extension. **A. Ghazi**, S. Henis-Korenblit, C. Kenyon. Department of Biochemistry & Biophysics, University of California, San Francisco (UCSF), San Francisco, CA.

Aging and reproduction are central aspects of an animal's life history, so the discovery that in worms and flies, germline-stem cells regulate the aging of the entire animal is particularly fascinating. In both species, loss of these cells extends lifespan dramatically. The mechanism by which germline signals affect aging of somatic tissues is not well understood. We have found that a predicted transcription elongation factor, TCER-1, plays a key role in this process. TCER-1 is required for loss of the germ cells to increase *C. elegans'* lifespan, and it acts as a regulatory switch in the pathway. When the germ cells are removed, the levels of TCER-1 rise in somatic tissues. This increase is sufficient to trigger key downstream events, as overexpression of *tcer-1* extends the lifespan of normal animals that have an intact reproductive system. Our findings suggest that TCER-1 extends lifespan by promoting the expression of a set of genes regulated by the conserved, life-extending transcription factor DAF-16/FOXO. Interestingly, TCER-1 is not required for DAF-16/FOXO to extend lifespan in animals with reduced insulin/IGF-1 signaling. Thus, TCER-1 specifically links the activity of a broadly deployed transcription factor, DAF-16/FOXO, to longevity signals from reproductive tissues.

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Systemic regulation of starvation response in *Caenorhabditis elegans*. **Chanhee Kang**, Leon Avery. Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX.

When the supply of environmental nutrients is limited, multicellular animals can make both physiological and behavioral changes so as to cope with nutrient starvation. Although physiological and behavioral effects of starvation are well known, the mechanisms by which animals sense starvation systemically remain elusive. Furthermore, what constituent of food is sensed and how it modulates starvation response is still poorly understood. In this study, we use a starvation-hypersensitive mutant to identify molecules and mechanisms that modulate starvation signaling. We found that specific amino acids could suppress the starvation-induced death and excessive autophagy of *gpb-2* mutants, and that MGL-1 and MGL-2, *C. elegans* homologs of metabotropic glutamate receptors, were involved. MGL-1 and MGL-2 acted in AIY and AIB neurons respectively. Treatment with leucine suppressed starvation-induced stress resistance and life span extension in wild-type worms, and mutation of *mgl-1* and *mgl-2* abolished these effects of leucine. Taken together, our results suggest that metabotropic glutamate receptor homologs in AIY and AIB neuron may modulate a systemic starvation response, and that *C. elegans* senses specific amino acids as an anti-hunger signal. Acknowledgments: This work was supported by research grant HL46154 from the U.S. Public Health Service.

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MOLECULAR AGING DRIVEN BY THE ELT-3/ELT-5/ELT-6 GATA TRANSCRIPTION CIRCUIT IN C. ELEGANS. **Yelena V. Budovskaya**, Stuart K. Kim. Dept Dev Biol, Stanford Univ, Stanford, CA.

To define the C. elegans aging process at the molecular level, we used DNA microarray experiments to identify a set of 1254 age-regulated genes, and found that the GATA transcription factors ELT-3, ELT-5 and ELT-6 are responsible for age-regulation of a large fraction of these genes. Expression of elt-5 and elt-6 increases during normal aging and both of these GATA factors repress expression of elt-3, which shows a corresponding decrease in expression in old worms. elt-3 regulates a large number of downstream genes that change expression in old age including ugt-9, col-144 and sod-3. elt-5(RNAi) and elt-6(RNAi) worms have extended longevity indicating that elt-3, elt-5 and elt-6 play an important functional role in the aging process. These results identify a novel transcriptional circuit that guides the rapid aging process in C. elegans, and indicates that this circuit is driven by drift of developmental pathways rather than accumulation of damage.

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Serine/Threonine Phosphatases in the regulation of Insulin/IGF-1 signaling. **S.D. Narasimhan**[1], S. Padmanabhan[1], A. Mukhopadhyay[2], H.A. Tissenbaum[1]. 1) Program in Gene Function & Expression, University of Massachusetts Medical School, 364 Plantation St., Worcester, MA 01605; 2) National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110006, India.

The *C.elegans* insulin/IGF-1 signaling (IIS) pathway regulates longevity, stress resistance, lipid metabolism and dauer diapause. Activation of the DAF-2 insulin/IGF-1 receptor initiates a AGE-1/PI3-kinase signaling cascade that activates the downstream serine threonine kinases PDK-1, AKT-1/2 and SGK-1. These kinases negatively regulate the FOXO transcription factor DAF-16 by phosphorylation. While many of the kinases in the IIS pathway have been well-studied, little is known about the phosphatases that counterbalance their activity. We were interested in identifying phosphatases that were master regulators of IIS such as the PTEN homolog DAF-18, which antagonizes the pathway at the level of AGE-1. Identification of additional phosphatases would provide a more detailed understanding of IIS as well as DAF-16 regulation. A directed RNAi screen for serine/threonine phosphatases in C.elegans revealed several interesting candidates. In this screen, we identified *pptr-1*, a B56 regulatory subunit of the PP2A holoenzyme. Knockdown of *pptr-1* by RNAi affects dauer formation, fat storage, thermotolerance as well as longevity–all of which are IIS-mediated processes. Genetic epistasis analyses placed *pptr-1* at the level of *akt-1*, and this interaction was biochemically verified using immunoprecipitation experiments. Further, confocal microscopy using strains bearing fluorescent tags of both proteins showed a remarkable overlap. Phosphorylation of AKT at two critical residues is essential for its full activity. Remarkably, PPTR-1 modulates AKT-1 phosphorylation at the Thr 350 residue and to a lesser extent, Ser517. Ultimately, this modulation results in increased DAF-16 nuclear localization as well as activity and increased lifespan. Our results identify a conserved role for PPTR-1/B56 as a major regulator of IIS. Preliminary analyses have shown that similar to PPTR-1, other top candidates from the screen are also affect of IIS-mediated processes. Further characterization of these novel regulators would help to determine how they regulate IIS, thereby having important implications for cancer and diabetes research. Padmanabhan et al. *Cell.* 2009. 136(5),939-51.

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RNAi screening reveals SKN-1 involvement in lifespan extension deriving from inhibition of translation. Jinling Wang, Stacey Robida, **T. Keith Blackwell**. Department of Pathology, Harvard Medical School Harvard Stem Cell Institute Joslin Diabetes Center, One Joslin Place Boston MA 02215, USA.

Various processes have been shown to influence longevity in C. elegans, including levels of insulin/IGF-1-like signaling (IIS), caloric intake, and expression of translation factor genes during adulthood. The first two of these involve the transcription factor SKN-1, which acts in the ASI neurons to influence respiration during calorie restriction, promotes longevity by acting in the intestine, and orchestrates a conserved defense against oxidative damage by inducing protective genes in the intestine in response to stress. Many of these SKN-1 targets are classified as "Phase 2" detoxification genes, which metabolize reactive species and facilitate repair of damaged cellular structures. In the intestine SKN-1 is regulated in part through phosphorylation: its localization to nuclei requires p38 signaling, and its constitutive activation is inhibited by GSK-3 and IIS. To identify additional processes that regulate SKN-1 and its target genes, here we have used RNAi screening to detect genes that prevent constitutive activation of a well-characterized SKN-1 Phase 2 target gene (*gcs-1*). This screen has identified new cellular pathways and biochemical mechanisms that affect SKN-1 activity and stress resistance, and uncovered SKN-1- and p38-independent pathways of Phase 2 gene regulation. Interestingly, some genes that we identified in this screen encode general mRNA translation factors. It has seemed likely that reductions in translation factor levels increase lifespan through decreases in protein synthesis. However, here we show that SKN-1 nuclear occupancy, Phase 2 gene activation, and stress resistance are increased dramatically by RNAi of some but not all translation factors. Importantly, *skn-1* is required for lifespan to be extended by knockdown of some but not all of the translation factors we have analyzed. We propose that perturbations in mRNA translation send stress signals through SKN-1 that increase both stress resistance and longevity.

259A

A genetic screen for regulatory partners and effectors of DAF-16. **Daniel Ackerman**[1], David Weinkove[1,2], David Gems[1]. 1) Institute of Healthy Ageing and G.E.E., University College London, Gower Street, London WC1E 6BT, UK; 2) School of Biological and Biomedical Sciences, Durham University, UK.

The longevity of *daf-2* mutants requires the FoxO forkhead transcription factor DAF-16, and many studies have explored the biological processes that DAF-16 regulates. However, the architecture of the regulatory network crowned by DAF-16 remains poorly understood. To identify new regulatory partners and effectors of DAF-16, we undertook a novel screen. A gene that is regulated by insulin/IGF-1 signaling (IIS) in a DAF-16-dependent fashion was tagged with GFP, and then RNAi used to identify transcription factors influencing GFP expression.

We prepared promoter::gfp reporter lines for a number of genes previously identified as IIS-regulated using microarray analysis (1). One reporter, *pftn-1::gfp* was highly expressed and showed strong IIS regulation that was easily detectable using a microplate reader. Levels of *ftn-1* mRNA, encoding the iron storage protein ferritin (2), were ~50-fold higher in *daf-2* than *daf-16; daf-2* strains (1).

We then performed a screen using a *C. elegans* transcription factor RNAi library, using the microplate reader to detect changes in GFP levels. The test strain contained an integrated *pftn-1::gfp* reporter in a *daf-2; rrf-3* background. This identified dozens of genes where RNAi significantly decreased or increased GFP levels. We are now characterizing selected genes further, and have identified several where RNAi reduces *daf-2* longevity. We will present an update of our findings at the meeting.

1. McElwee et al. J. Biol. Chem. 279, 44533 (2004).
2. Gourley et al. J Biol Chem 278, 3227 (2003).

260B

Caenorhabditis elegans him-6 protein exhibits RecQ helicase activity. Hana Jung, Jina Lee, Moonjung Hyun, **Byungchan Ahn**. Dept Life Sci, Univ Ulsan, Ulsan, Korea.

Mutations in human RecQ helicases result in rare diseases of aging and cancer characterized by genome instability. Bloom syndrome is known as segmental progerias. A BLM helicase ortholog, him-6, has been identified in Caenorhabditis elegans. Defects in the him-6 mutant show a shortened lifespan and phenotypic signs of genomic instability. However, little is known about their enzymatic activities. Recombinant CeBLM proteins were purified from E. coli. We found that Ce BLM is a 3'-5' helicase and characterized its substrate specificities in vitro in order to better understand their function in vivo. The abilities of CeBLM to unwind these DNA structures may increase access for DNA repair and replication proteins that are important for preventing the accumulation of abnormal structures, contributing to genome stability.

261C

Osmotic stress extends C. elegans lifespan. Mark Corkins, **Edward N. Anderson**, Gerard Somers, Anne C. Hart. Mass. General Hosp Center for Cancer Research & Dept of Pathology, Harvard Med School, Charlestown, MA 02129.

Moderate levels of environmental stress, such as oxidative stress, dietary restriction, or heat shock, can cause organisms to live longer. In S. cerevisiae, osmotic stress has been shown to cause lifespan extension, and this extension is regulated by sirtuins via the NAD salvage pathway. (Kaeberlein et al. 2002). We find that moderate osmotic stress can increase the lifespan of C. elegans.

The major osmolyte in NGM media is NaCl. Wild type animals reared on NGM containing increased NaCl live longer than those reared on standard 51 mM NaCl. This increased lifespan extension is dose-dependant, and maximal lifespan is observed at roughly 300 mM NaCl with approximately 30% lifespan extension. Increased longevity is likely not due to a developmental change, animals shifted at the L4 stage to 300 mM NaCl have a lifespan similar to those reared from birth on 300 mM media.

Currently, we are assessing the contribution of genes known to extend lifespan in this paradigm. Osmotic stress induced lifespan extension is prevented by loss of the FOXO transcription factor daf-16, but is unaffected by loss of the AMP kinase aak-2. Osmotic lifespan extension is not further extended by the eat-2 model of dietary restriction. Overall, our results suggest that osmotic stress can increase longevity and that a subset of conserved pathways are critical for this lifespan extension.

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Prohibitin couples diapause signaling to mitochondrial metabolism during ageing in *C. elegans*. **Marta Artal-Sanz**[1], Nektarios Tavernarakis[2]. 1) Instituto de Biomedicina de Valencia, CSIC. Valencia, Spain; 2) Institute of Molecular Biology and Biotechnology, FORTH. Heraklion, Crete, Greece.

Mitochondrial biogenesis and function are considered primary longevity determinants in eukaryotes. However, the molecular mechanisms regulating mitochondrial energy metabolism during ageing are poorly understood. Prohibitins are ubiquitous and evolutionarily conserved proteins, which form a ring-like, high molecular weight complex at the inner membrane of mitochondria. Prohibitin function has been implicated in carcinogenesis and replicative senescence. We are investigating the role of prohibitin in mitochondrial metabolism regulation during ageing in *C. elegans*. We find that the mitochondrial prohibitin complex promotes longevity by moderating fat metabolism and energy production in the nematode. While, prohibitin deficiency shortens the lifespan of otherwise wild type animals, remarkably, knockdown of prohibitin promotes longevity in diapause mutants or under dietary restriction. In addition, prohibitin deficiency extends the lifespan of animals with compromised mitochondrial function or fat metabolism and restores normal lifespan in mutants with lethal germline tumours. Depletion of prohibitin influences ATP levels, adipose tissue fat content and mitochondrial proliferation in a genetic background- and age-specific manner. Together, these findings reveal an intricate mechanism of regulating mitochondrial energy metabolism and fat utilization during diapause and ageing in *C. elegans*.

263B

Molecular mechanisms leading to extreme longevity in C. elegans. **S Ayyadevara**[1,3], P Bharill[2], R Shmookler Reis[1,2,3]. 1) Dept Geriatrics, Univ Arkansas Medical Sci, Little Rock, AR; 2) Dept Biochemistry & Molecular Biology, Univ Arkansas Medical Sci, Little Rock, AR; 3) Central Arkansas Veterans Health System, Little Rock, Arkansas.

We reported that two nonsense mutants of age-1 confer exceptional longevity and stress resistance to C. elegans. These traits, observed in second-generation (F2) homozygotes of both mutants, are blunted at the first generation (F1). In these mutants, the class-I phosphatidyl¬¬inositol 3-kinase catalytic subunit (PI3KCS) is truncated upstream of its kinase domain. Both longevity and stress-resistance phenotypes require, at least in part, the DAF-16/FOXO transcription factor. F2 homozygous adults have transcriptional and protein profiles that differ from the genetically identical F1 generation, and from other mutants. The extremely long-lived worms show transcriptional feedback attenuation, silencing many components of insulin/IGF-1 signaling (IIS), as well as other signaling pathways that cross-talk with IIS. We have demonstrated that their kinase activities are silenced, and phosphoprotein levels are reduced, as are their transcript levels. Mutational disruption of PI3KCS activity results in depletion of the phospholipid PIP3. The F1 generation of this mutant, however, is maternally protected and presents relatively moderate survival phenotypes. PIP3 is a key signaling molecule, required for membrane tethering of many signaling molecules, and for phosphorylation of the AKT kinase and possibly others. Its disruption may account for post-translational suppression of multiple signaling pathways, but the mechanism of transcriptional inhibition remains to be elucidated.

264C

Transcriptional- and kinase-attenuation mechanisms associated with extreme longevity. **P. Bharill**[1], S. Ayyadevara[2,3], R. Shmookler Reis[1,2,3]. 1) Dept Biochem & Molec Biol, Univ Arkansas, Little Rock, AR; 2) Department of Geriatrics, Univ Arkansas, Little Rock, AR; 3) Central Arkansas Veterans Health System, Little rock,Arkansas.

A temperature-sensitive mutant of the age-1 gene increases life span and stress resistance of C. elegans. We recently reported that two nonsense alleles of age-1 confer a greater than 10-fold increase in life-span and 3- to 8-fold increases in resistance to several stresses. These effects are seen only in second-generation (F2) homozygotes, in which extreme survival benefits are observed in the absence of wild-type age-1 mRNA, full-length AGE-1/PI3Kcs protein, and/or the PIP3 phospholipid that AGE-1 generates. We also assessed the effects on H2O2 resistance of treating worms with phosphatidylinositide analogs (PIAs), and found that PIA6 conferred marked protection against hydrogen peroxide. We compared transcriptional and kinase-activity profiles of homozygous age-1 nonsense mutations at the F2 and F1 homozygous generations. Transcript steady-state levels were assessed on microarrays for several independent cohorts per group. Kinase activities were compared in vitro, for lysates of worms showing mild vs. strong age-1 phenotypes, by assessing their activities toward synthetic peptide substrates arrayed on glass slides. Gene expression changes and kinase activity profiles especially affected by age-1-null mutants at the very long-lived F2 generation may thus provide insights into mechanisms not previously implicated in life extension.

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The microRNA *mir-71* Is Involved in the Regulation of Longevity and Stress Responses in *C. elegans* . **Konstantinos Boulias**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA.

MicroRNAs (miRNAs) constitute a class of small (20-24 nt) non-coding RNAs found in *C. elegans*, *Drosophila*, plants, mammals and other organisms. Studies over the past few years indicate that miRNAs are critical regulators of gene expression in diverse biological processes, including developmental timing, cell-fate specification, cell proliferation and differentiation. The first miRNAs discovered were *lin-4* and *let-7*, which control the timing of developmental processes in *C. elegans*. Since aging can be regarded as a temporally-regulated developmental process, it is plausible that miRNAs also control aging. The genetic basis of *C. elegans* aging has been studied extensively, and a number of genes that define conserved regulatory pathways that affect lifespan have been characterized.

To identify miRNAs that might function in the regulation of the aging process, we screened our collection of miRNA mutants for those abnormal in aging. We analyzed deletion alleles of 95 miRNA genes for abnormalities in lifespan and the response to heat stress. We identified *mir-71* as a miRNA gene required for normal lifespan and stress responses, since worms lacking *mir-71* are short-lived and hypersensitive to heat shock and oxidative stress. *mir-71* is likely involved in the control of *C. elegans* aging, since *mir-71* adults undergo an early decline of locomotion and pharyngeal pumping, two physiological behaviors that normally decline with age. We are currently performing site-of-action studies to identify the tissues in which *mir-71* functions to regulate lifespan and stress responses, and we are investigating the possible role of *mir-71* in the pathways that are known to control *C. elegans* aging.

266B

Characterization of hydroxyurea resistance and Aging in C. elegans. **Jeanette Brejning**, Lone Schøler, Helle Jakobsen, Anders Olsen. The Interdisciplinary Research Consortium on Geroscience. Department of Molecular Biology, Aarhus University, Gustav Wieds Vej 10, 8000 Aarhus C, Denmark. JB and LS contributed equally. Corresponding author ano@mb.au.dk.

Checkpoints are evolutionary conserved surveillance mechanisms activated upon DNA damage to halt the progression of the cell cycle. Appropriate checkpoint function is required to preserve the genomic content of descendant cells and aberrant checkpoint function is connected to development of cancer. We have found that inactivation of certain checkpoint proteins, including p53, cause resistance to the chemotherapeutic drug hydroxyurea (HU) that stalls replication. HU is an inhibitor of ribonucleotide reductase (RNR) in many organisms, but the target in C. elegans is less clear. RNR is involved in synthesis of deoxyribonucleotide (dNTP) precursors for DNA replication and repair and consists of two homodimeric subunits RNR-1 and RNR-2. We report that in C. elegans, HU induces transcription of both RNR subunits, indicating that HU targets RNR in C. elegans as well. Inactivation of some S-M checkpoint proteins can increase stress resistance and lifespan of C. elegans1. Interestingly, several genes that influence HU resistance also influence lifespan and stress resistance. We find that at least one of these genes appears to function in the S-M checkpoint pathway. 1.A. Olsen, M. C. Vantipalli, G. J. Lithgow, Science 312, 1381 (2006).

267C

Do methionine sulfoxide reductases protect against aging in *C. elegans*? **Filipe Cabreiro**, Ryan Doonan, David Gems. Institute of healthy ageing and G.E.E, University College London, London, United Kingdom.

Oxidation of proteins by reactive oxygen species (ROS) seems likely to contribute to the accumulation of oxidized proteins that is a correlate of aging. One possibility is that this accumulation reflects insufficiency or age-decline in the enzymatic systems that reduce or eliminate oxidized proteins. Methionine residues are particularly susceptible to oxidation, but this can be reversed within proteins by methionine sulfoxide reductases A (MsrA) and B (MsrB). As one of the few repair systems for oxidized proteins, MsrA and MsrB represent an important line of defence against oxidative stress. If ROS contributes significantly to aging, as the oxidative damage theory proposes, then MsrA and/or MrsB might play a role in longevity assurance.

C. elegans MsrA, encoded by msra-1, catalyzes the thioredoxin-dependent reduction of the S-isomer of methionine sulfoxide to methionine1. The gene F44E2.6 encodes a protein containing the highly conserved MsrB signature domain, which may therefore be an MsrB capable of reducing the R-isomer of methionine sulfoxide. We are currently using *C. elegans* to test whether these two enzymes contribute to longevity assurance, and to protection against age-related diseases associated with age-related loss of protein folding homeostasis.

Lee et al. (2005) Arch Biochem Biophys 434: 275.

268A

The *C. elegans* PNC-1 nicotinamidase increases stress resistance and mediates caloric restriction-induced longevity. **Juan J. Carmona**[1,4], Shaday Michan[1], Tracy Vrablik[2], James N. Sleigh[3], Wendy Hanna-Rose[2], Anne C. Hart[4,5], David A. Sinclair[1]. 1) Paul F. Glenn Laboratories for the Biological Mechanisms of Aging, Department of Pathology, Harvard Medical School, Boston, MA 02115; 2) Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802; 3) Department of Biology and Biochemistry, University of Bath, Claverton Down, Bath UK BA2 7AY; 4) Massachusetts General Hospital Cancer Center, Charlestown, MA, 02129; 5) Department of Pathology, Harvard Medical School, Boston, MA 02115.

A common and powerful non-genetic method used to extend an organism's lifespan is caloric restriction, yet the underlying molecular mechanisms of this increased longevity remain unclear. In yeast, Pnc1 (nicotinamidase), an enzyme in the NAD+ salvage pathway that converts nicotinamide to nicotinic acid to assist in regenerating NAD+, can mediate lifespan extension under caloric restriction and mild stresses. We have previously shown that Pnc1 is nuclear, cytoplasmic, and peroxisomal in yeast and that caloric restriction and stress, like salt, causes a significant up-regulation in expression (Anderson, 2003). To understand the impact of this nicotinamidase on longevity and stress resistance in multi-cellular organisms, we are using *C. elegans* as a model. We show that the worm ortholog PNC-1 also has robust nicotinamidase activity *in vitro* and that increased gene dosage in *C. elegans* protects against salt stress and extends lifespan. Interestingly, this longevity effect is sirtuin dependent. In addition, lifespan extension mediated by PNC-1 over-expression is not additive with caloric restriction-induced longevity, suggesting that PNC-1 is an important component of this pathway. Accordingly, inactivation of the *pnc-1* gene leads to a defect in caloric restriction-induced longevity in *C. elegans*. Using a GFP reporter, we found that *pnc-1* is expressed in the pharynx and nervous system, including the chemosensory ASK neurons. We are currently using cell-specific promoters to dissect the biological relevance of these sites of expression. Interestingly, invertebrate PNC-1 and the mammalian PBEF/Visfatin catalyze analogous biochemical steps; we are expressing the human PBEF/Visfatin in *pnc-1* loss-of-function animals to test the functional conservation of these enzymes across species. Collectively, this study elucidates a functional role for PNC-1 in metazoans and expands our understanding of conserved pathways involved in longevity and stress resistance across organisms.

269B

HIF-1 modulates dietary restriction-mediated lifespan extension via the IRE-1 ER stress pathway. **Di Chen**, Emma Thomas, Pankaj Kapahi. Buck Institute for Age Research, 8001 Redwood Blvd, Novato, CA 94945, USA.

Dietary restriction (DR) extends lifespan in various species and also slows the onset of age-related diseases. Previous studies have demonstrated that the target of rapamycin (TOR) pathway, which regulates growth and metabolism in response to nutrients, is essential for DR-mediated longevity phenotypes in flies and yeast. The hypoxia inducible factor-1 (HIF-1) is one of the targets of the TOR pathway in mammalian cells. HIF-1 is a transcription factor complex that plays key roles in oxygen homeostasis, tumor formation, glucose metabolism, cell survival and inflammatory response. Here we describe a novel role for HIF-1 in modulating lifespan extension by DR in *C. elegans*. We found that under rich nutrients, a *hif-1* mutation extends lifespan, which overlaps with that by inhibition of the RSKS-1/S6 kinase, a key component of the TOR pathway. Under DR, the *hif-1* mutant fails to show further lifespan extension. Conversely, a mutation in *egl-9*, which increases HIF-1 activity, diminishes the lifespan extension under DR. This deficiency is rescued by tissue-specific expression of *egl-9* in specific neurons and muscles. Increased lifespan by *hif-1* or DR is dependent on the ER stress regulator IRE-1 and is associated with lower levels of ER stress. Therefore, our results demonstrate a tissue-specific role for HIF-1 in the lifespan extension by DR involving the IRE-1 ER stress pathway.

270C

Regulation of longevity by two novel heat-shock factor-1 regulators, *ddl-1* and *ddl-2*. **Wei-Chung Chiang**[1], Hee-chul Lee[2], Tsui-ting Ching[2], 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, Unversity of Michigan, Ann Arbor, MI.

Heat-shock transcription factor-1 (HSF-1) acts as a cellular survival factor against various stresses, including but not limited to the heat stress. The activation of HSF-1 increases thermotolerance and longevity in *C. elegans*. However, how HSF-1 response to stress and influence longevity at molecular level remains largely unclear.

Recently, two genes, *ddl-1* and *ddl-2* (ddl, *daf-16* dependent longevity), were shown to influence lifespan and thermotolerance in *C. elegans* when inactivated by RNAi. To confirm this observation, we analyzed the lifespan of animals overexpressing *ddl-1* and *ddl-2*. Consistent with previous findings, overexpression of *ddl-1* or *ddl-2* shortens lifespan. It has been previously suggested by yeast-two hybrid results that DDL-1 may physically interacts with DDL-2 and heat-shock factor binding protein-1 (HSB-1), a known negative regulator of HSF-1. Therefore we asked whether *ddl-1* and *ddl-2* regulate longevity by modulating *hsf-1* activity. Our result showed that *ddl-1* and *ddl-2* RNAi fails to increase lifespan in *hsf-1* mutant (*sy441*), suggesting that *hsf-1* functions downstream of *ddl-1* and *ddl-2*. Our biochemical evidences also showed that knock-down of *ddl-1* and *ddl-2* by RNAi increases HSF-1 activity. Taken together, our data suggest that DDL-1 and DDL-2 might influence longevity by regulating HSF-1 activity.

We hypothesize that DDL-1and DDL-2 regulate HSF-1 activity by forming a complex with HSF-1, and this complex is important for keeping HSF-1 in an inactive form. Our preliminary results have confirmed the interaction between DDL-1 and HSF-1; DDL-1 and HSB-1; DDL-1 and DDL-2 by co-immunoprecipitation. By understanding the role of DDL-1 and DDL-2 in regulating HSF-1, we will be able to reveal the underlying mechanism by which HSF-1 regulates heat-shock response and promotes longevity in *C. elegans*.

271A

An RNAi screen for regulators of phase II detoxification genes. **Helen Marie Crook**[1], Monika Oláhová[1], T. Keith Blackwell[2], Elizabeth A. Veal[1]. 1) Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, United Kingdom; 2) Joslin Diabetes Center, Boston MA 02215.

The phase II detoxification system is an important cellular defence against the toxic effects of free radicals and xenobiotics which cause oxidative tissue damage that has been implicated in many diseases and in ageing. Thus, in response to stress, the expression of many phase II detoxification enzyme genes is co-ordinately induced. The antioxidant glutathione is utilized as an important component of many phase II detoxification reactions.γ-glutamine cysteine synthetase, encoded by *gcs-1*, catalyses the rate-limiting step in glutathione biosynthesis. Thus *gcs-1* is amongst the phase II detoxification genes that are transcriptionally activated in response to stress. Conserved bZIP transcription factors, including Nrf-2 in mammals and SKN-1 in *C. elegans*, are required for regulation of phase II detoxification genes, including *gcs-1*. Several signaling pathways including the PMK-1/p38 MAPK signaling pathway, act to regulate levels of active SKN-1 which is important for both stress resistance and ageing [1, 2]. However, in the absence of the conserved 2-Cys Peroxiredoxin PRDX-2, expression of phase II detoxification genes such as *gcs-1* is also activated by alternative SKN-1-independent mechanisms [3]. Moreover, activation by these SKN-1-independent mechanism/s is apparently sufficient to increase resistance to arsenite stress. To determine the mechanism/s by which PRDX-2 inhibits phase II detoxification gene expression, we are undertaking RNAi screens to identify genes required for expression of a stress-inducible P*gcs-1*::GFP reporter in *prdx-2* mutant worms. Phosphatases play key roles in the regulation of signal transduction and are intrinsically sensitive to oxidative stress. Hence, we have begun by conducting a screen of RNAi targeting *C. elegans* phosphatase-encoding genes. Here we will present the data obtained in this screen. It is expected that characterisation of genes identified by these screens will uncover new mechanisms for regulating phase II detoxification gene expression and provide insight into the important contributions made by PRDX-2 and phase II detoxification systems to stress resistance and longevity [2, 3].

272B

An extensive role for microRNAs in aging. **Alexandre de Lencastre**[1], Sylvia Lee[2], Frank Slack[1]. 1) Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT; 2) Dept. of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

MicroRNAs (miRNAs) constitute a novel class of regulatory elements with important roles in the control of gene expression and development in higher eukaryotes. Although highly abundant and predicted to target a wide proportion of the genome, miRNAs have so far only been implicated in a handful of biological roles. Recent work has demonstrated that mutations to *lin-4* and its target *lin-14* significantly affect the lifespan of *C. elegans* [1]. In addition, microarray analysis in *C. elegans* has revealed dynamic miRNA expression changes during aging [2]. These observations suggest that miRNAs may function in pathways that impact life span.

In order to identify new roles of miRNAs in lifespan, we have undertaken a deep sequencing survey of miRNAs from aged tissue in *C. elegans*. We sequenced nearly 1 million small RNAs from *C. elegans* at different stages of adulthood and in mutants with aberrant lifespan. We observe significant expression changes of multiple miRNAs during aging, including several miRNAs that were not previously identified by microarray analysis. In order to test the functional role of these miRNAs, we tested the lifespan of strains of *C. elegans* containing mutations in miRNAs that exhibit the most significant changes of expression during aging. Our results demonstrate that three of the miRNAs that are most over-expressed in aged animals are necessary for a normal lifespan in *C. elegans*, while a fourth miRNA causes extension of lifespan when its expression is abrogated. In order to understand the pathways that mediate the function of these miRNAs, we are currently searching for predicted targets of the miRNAs in the sequences of the 3'UTRs of *C. elegans* genes, particularly those that have already been implicated in aging.

Finally, our deep sequencing survey identified multiple candidate novel miRNAs in aged *C. elegans*. We are currently validating and characterizing the putative role of these candidate miRNAs on aging. Given the high conservation of miRNAs across species, it is likely that insights uncovered by this research will have high relevance towards our understanding of aging in higher organisms and humans.

References:

1. Boehm, M. and F. Slack, Science, 2005. 310(5756). 2. Ibanez-Ventoso, C., et al., Aging Cell, 2006. 5(3).

273C

LET-418/Mi2 a novel determinant of ageing in *C. elegans*. **Véronique de Vaux**, Catherine Pfefferli, Marlène Nebiker, Diego Freti, Fritz Müller, Chantal Wicky. Department of Biology, University of Fribourg, Ch. du Musée 10, 1700 Fribourg, Switzerland.

The structure of chromatin changes dynamically during development and reproduction. Remodeling of chromatin involves highly specialized enzymes. One of them is the evolutionarily conserved chromatin remodeling factor Mi-2, the core subunit of the human NuRD (Nucleosome Remodeling and histone Deacetylase) complex. The gene *let-418* encodes one of the two *C. elegans* Mi-2 orthologues. Strong loss-of-function alleles of *let-418* show a highly pleiotropic phenotype that includes sterility, vulva defects and a L1 larval arrest associated with ectopic expression of germline specific genes (von Zelewsky et al., 2000; Unhavaithaya et al., 2002). Altogether, these phenotypes suggest an important developmental role of *let-418*.

Here we report on an additional aspect of the *let-418* phenotype. We observed that loss of *let-418* induces a significant lifespan extension, increases dauer formation and strongly enhances resistance to oxidative and heat stress. Thus, LET-418 may be part of a mechanism that is required to maintain a chromatin state allowing normal growth and reproduction. Reduction or depletion of *let-418* activity results in a shift towards somatic endurance, longevity and sterility.

For extension of lifespan and stress resistance *let-418* acts synthetically with *daf-2*, suggesting that *let-418* functions in parallel of the insulin pathway. Moreover, *let-418* partly requires the activity of daf-16. To see, whether let-418 acts through *daf-16*, we tested if depletion of *let-418* influences the localization and/or the expression level of *daf-16* and found that neither of them was changed. To test whether LET-418 may affect the transcriptional activity of DAF-16, we analyzed the expression patterns of the heat shock protein gene *hsp-16.2* and the superoxide dismutase gene *sod-3*, which are targets of *daf-16*. We found that both genes show elevated mRNA levels in a *let-418* mutant background. The over-expression of *sod-3* mRNA was dependent on DAF-16, whereas that of *hsp-16.2* was totally independent. Currently, we are further investigating the molecular mode of action of LET-418 on the expression of *sod-3* and *hsp-16.2*.

274A

Protein turnover in long-lived Insulin/IGF-1-mutant and dietary restricted *Caenorhabditis elegans*. **Geert Depuydt**, Jacques R. Vanfleteren, Bart P. Braeckman. Biology Department, Ghent University, K.L. Ledeganckstraat 35, B-9000 Ghent, Belgium.

In recent years, the importance of protein metabolism in the aging process has become more eminent [1-4]. According to the protein turnover hypothesis, high protein turnover rates (i.e. high synthesis and degradation rates) are beneficial to the animal because this process removes and replaces damaged protein molecules, thereby delaying the progressive accumulation of proteins damage–a hallmark of aging. A decline in these turnover rates is thus expected to contribute to the aging process. In order to put this hypothesis to the test, we conducted a series of pulse-chase experiments to measure the bulk protein degradation as a function of age in long-lived *daf-2* (Insulin/IGF-like receptor) mutant- and dietary restricted (DR) *C. elegans*, as both are expected to show enhanced protein turnover [1-3]. Monitoring ^{35}S in the TCA-insoluble worm fraction, we found that protein degradation rates declined drastically with age in both the control strain and DR animals, whereas in *daf-2* mutants, degradation rates seemed to be low over their whole life span, even tending to rise slightly at advanced age. Surprisingly, we found equal levels of ^{35}S in the TCA-soluble worm fraction (also a measure for protein degradation) of *daf-2* mutants and DR animals that were much higher than their respective control, irrespective of age. Furthermore, *daf-2* subjected to DR had ^{35}S levels in the TCA-soluble fraction higher than *daf-2* or DR alone. Based on these results, we hypothesize that decreased Insulin/IGF-1 signaling (IIS) and DR contribute to longevity at least in part by upregulating protein degradation in an additive manner. However, discrepancy between TCA-soluble and TCA-insoluble worm fractions indicate IIS and DR have only partially overlapping effects on protein metabolism. One possibility is that intracellular recycling of aminoacids (e.g. by autophagy) may be strongly upregulated in *daf-2* mutants, resulting in high protein turnover rates that may not be readily detected by classical pulse-chase experiments.[1] Melendez, A., et al., Autophagy genes are essential for dauer development and life-span extension in *C. elegans*.Science, 2003. 301(5638): p. 1387-91. [2] Jia, K.L. and B. Levine, Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. Autophagy, 2007. 3(6): p. 597-599. [3] Hansen, M., et al., A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet, 2008. 4(2): p. e24. [4] Syntichaki, P., K. Troulinaki, and N. Tavernarakis, Protein synthesis is a novel determinant of aging in *Caenorhabditis elegans*. Ann N Y Acad Sci, 2007. 1119: p. 289-95.

275B

Do Sfrp proteins have a role in *C. elegans*? **SIMON DESCAMPS**[1,2], Abdelhalim LOUKIL[1,2], Caroline ARAIZ[1,2], Marie-Thérèse Château[1], Simon GALAS[1]. 1) CRBM, CNRS UMR 5237, MONTPELLIER, France; 2) Université Montpellier 2, MONTPELLIER, France.

Wnt proteins are known to be involved in embryonic and post-natal development, in tissues homeostasis and in cancers. More recently they have been shown to participate in ageing processing specially for body wall muscle (1). In mammals, Wnt activity is modulated by a few proteins among which Secreted Frizzled Related Proteins (Sfrp, 5 in mammals) are secreted proteins capable to interact with Wnt proteins and their receptors and to modulate muscle differentiation in mammals (2). *C. elegans* possess only one Sfrp like protein (Q9GUF5). Therefore, we chose this simplified model to study the role of this Sfrp protein in the regulation of Wnt signaling during ageing and stress response with particular interest for muscle. RNAi feeding experiments inhibiting the expression of the *C. elegans* Sfrp protein lead to size modifications, muscle defects, increase of lipid storage, expanded longevity and modulation of stress resistance. Thus Sfrp protein seems to have important roles in *C. elegans*, not only in muscle homeostasis but also in the regulation of stress and longevity. (1) Maiese et al. (2008) The Wnt signaling pathway: Aging gracefully as a protectionist? *Pharmacology & Therapeutics* 118, 58-81. (2) Descamps et al. (2008) Inhibition of myoblast differentiation by Sfrp1 and Sfrp2. *Cell Tissue Res.*332, 299-306.

276C

Does organismal overexpression of superoxide dismutase directly test the oxidative damage theory of aging? **Ryan Doonan**, Filipe Cabreiro, Daniel Ackerman, Nicolaos Mathoudakis, Cassandra Coburn, David Gems. Institute of Healthy Aging and G.E.E., University College London, London, WC1E 6BT, United Kingdom.

The oxidative damage theory of aging proposes that accumulation of molecular damage caused by reactive oxygen species (ROS), particularly superoxide (O_2-), contributes significantly to aging. Accordingly, the enzyme superoxide dismutase (SOD), which catalytically eliminates O_2-, should contribute to longevity assurance. Thus, a prediction of the theory is that experimentally induced increases in SOD activity should lead to retardation of aging. This prediction has been tested in several model organisms, with mixed results.

A common weakness of such studies is a lack of direct measurements of ROS levels or distribution *in vivo*, or measurements of oxidative damage and/or effects on cell physiology, such as redox state. This is important, since redox state can influence multiple signal transduction pathways, and mild oxidative insults can trigger protective hormetic effects. Moreover, elevation of SOD can markedly increase net ROS production[1]. In short, it is naive to think that effects of SOD overexpression on lifespan simply reflect protection from ROS-mediated damage. Given these concerns, we ask whether SOD overexpression can deliver what it promises as a means of testing the oxidative damage theory of aging in *C. elegans*.

Ubiquitous overexpression of SOD-1, the major cytosolic Cu/ZnSOD, results in a small, but significant increase in lifespan[2]. We are now characterizing further the biochemical and cellular consequences of SOD overexpression in *C. elegans*. We find that it increases H_2O_2 production as predicted[1]. However, simultaneous overexpression of catalase does not suppress the extended lifespan, implying that H_2O_2 over-production is not a driver of longevity here.

Strikingly, we find that the increase in lifespan is dependent on the FoxO transcription factor DAF-16. This suggests that elevated SOD results in a hormetic effect, mediated by activation of stress-sensitive signalling pathways. We also find that SOD overexpression lowers protein oxidation, and are now testing whether this effect is DAF-16 dependent too. We are also testing whether the increased lifespan is *aak-2*, *hsf-1*, and/or *skn-1* dependent, and will present our findings at the meeting.

1. Buettner et al. Free Radic Biol Med 41, 1338 (2006). 2. Doonan et al. Genes Dev 22, 3236 (2008).

277A

A lifespan-based assay to assess the long-term toxicity of prolonged exposure to ecotoxic agents using *C. elegans*. **Toshihiko Eki**, Hisashi Morise, Masaru Kurauchi, Hiroaki Harada. Dept. Ecological Engineering, Toyohashi University of Technology, Toyohashi, Aichi, Japan.

Nematodes are highly abundant organisms found in soil or sedimentary habitats and the free-living nematode *Caenorhabditis elegans* (*C. elegans*) has been used as an excellent model for monitoring ecotoxicity in soil. The acute toxicities of toxic agents have been well examined with respect to several endpoints, such as mortality, for application to toxicity tests for environmental assessments. However, chronic influences of these agents on multicellular organisms still need to be determined. Here we studied long-term effects on the lifespan of a free-living nematode *C. elegans* resulting from prolonged exposure to heavy metals metals ($CuSO_4$ and $CdCl_2$), detergents (sodium dodecyl sulfate and a commercially available household detergent), a perfluoro organic compound (pentadecafluorooctanoic acid) and an organophosphate insecticide (dichlorvos) as well as short-term inhibitory effects on egg production and growth. These agents except for dichlorvos inhibited growth of hatched larvae and reproductive capacity in a concentration-dependent manner. They also effectively shortened the lifespan of the adult nematode over the same concentration range. Since toxic effects on both the growth and the lifespan were observed over similar concentration ranges, where acute toxicities in various endpoints are detected, the shortening of the lifespan can be used as a new endpoint for the assessment of various ecotoxic agents. We have also used a *daf-16(mu86)* mutant CF1038 strain, which has a deficient transcription factor DAF-16 regulating a variety of the genes involved in longevity and stress response, for ecotoxicity assays, however, both strains unexpectedly exhibited comparable reductions in these endpoints including lifespan by exposure to these ecotoxicants, indicating that DAF-16 does not largely contribute to tolerance to these agents. By virtue of a shorter assay period, the lifespan-based assay using the *daf-16* mutant can be useful for assessing the ecotoxicity of ecotoxic chemicals.

Ref. Harada *et al., Ecotoxicol. Environ. Saf.*, **66**, 378-383 (2007).

278B

The longevity gene *misc-1* modulates apoptosis in *C. elegans* and human cell lines. **M. Gallo**[1], D. Riddle[1,2]. 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.

We identified and characterized the *C. elegans* orthologue of human 2-oxoglutarate carrier (*OGC*) and called it *misc-1* (MItochondrial Solute Carrier). The human orthologue is responsible for importing α-ketoglutarate, an intermediate of the Krebs cycle, and glutathione (GSH), a detoxifying molecule, into mitochondria. Because of its role in metabolism and detoxification, we thought *misc-1* was a candidate longevity gene. *misc-1* RNAi increased wild type and *daf-2* mean adult lifespan by 20% and 42%, respectively, but it failed to induce significant longevity in *eat-2* mutants, suggesting that *misc-1* may be in the dietary restriction pathway. Unlike Mit mutants, *misc-1* knock-down and knock-out allow a normal rate of pharyngeal pumping, normal body size and developmental timing. We hypothesized the absence of Mit phenotypes was due to the activation of compensatory detoxifying pathways in *misc-1* mutants. We assessed gene expression levels for the *sod* genes. Only the Cu/ZnSOD *sod-5* was upregulated (by ~40%) in *misc-1*. *sod-5* is a known target of DAF-16 and strongly upregulated in *daf-2* and dauer larvae. It is known that H_2O_2–the end product of the dismutase reaction catalyzed by SOD-5–inhibits most steps in the insulin signalling pathway. We believe *misc-1* may modulate life-span by increasing *sod-5* expression, reducing insulin signaling and causing a metabolic shift from glycolysis/mitochondrial respiration to other mitochondria-independent pathways, such as the glyoxylate cycle. *misc-1* RNAi results in mitochondrial fragmentation. siRNA targeting *OGC* in HEK293 (Human Embryonic Kidney) cells also resulted in mitochondrial fragmentation. We showed that germline apoptosis in *misc-1* knock-out animals is increased two-fold compared to wild-type N2, but that mitochondrial fragmentation alone is not sufficient to increase the apoptotic rate. It is known that *OGC* over-expression in human cells protects against chemically induced apoptosis. Germline apoptosis in *C. elegans* can be triggered by three different pathways, namely the DNA damage, physiological and stress-induced pathways. All pathways ultimately converge on the core apoptotic machinery composed of CED-9/Bcl-2, CED-4/Apaf1 and CED-3/Caspase-9. Epistasis experiments showed that *misc-1* acts through the physiological apoptotic pathway and is dependent on LET-60/KRas. We propose a conserved, mitochondria-driven apoptosis mechanism dependent on control of mitochondrial Ca^{2+} stores by MISC-1 and CED-9/Bcl-2 and impinging on Ca^{2+} signalling mediated by LET-60/KRas.

279C

Divergence in DAF-16 dependent phenotypic coupling within the *Caenorhabditid* nematodes. **Francis Amrit Raj Gandhi**, Claudia Boehnisch, Robin May. School of Biosciences, University of Birmingham, Birmingham, West Midlands, United Kingdom.

In animals, ageing, immunity and stress tolerance are co-ordinately regulated by the forkhead transcription factors in response to upstream signals from Insulin-like growth factor-like signalling pathway. Previous work has emphasized that the transcription factor DAF-16, a key evolutionarily conserved gene regulator, functions as a "molecular link" that governs these processes. Although *daf-16* is highly conserved in a broad range of organisms, it is unclear how the downstream co-regulation of immunity, lifespan and stress resistance changes during evolution. In nematodes, these phenotypes have been shown to have both a gender specific and species-specific variability. Here for the first time we investigate this phenotypic coupling by comparing ageing, stress-resistance and immunity in four species of *Caenorhabditid* nematode. We show that among these species there is a significant difference in expression levels between the *daf-16* homologues. This expression level, correlates directly with the observed phenotypes of lifespan, stress resistance and immunity. Using constitutively active *daf-16* mutants, backed by bioinformatic studies looking at *daf-16* target regions, we propose that the divergence of DAF-16 responses, depending upon the niche and life history traits of the species concerned, could explain differences in stress response, immunity and aging among species. Finally, we suggest that the evolutionary conundrum of post-reproductive aging could be explained since enhanced immunity would also increase lifespan even after the animal has reproduced.

280A

Role of autophagy in *C. elegans* longevity pathways. **Sara Gelino**, Louis Lapierre, Binnan Ong, Philip McQuary, and Malene Hansen. Burnham Institute for Medical Research, 10901 North Torrey Pines Road, La Jolla, CA.

C. elegans lifespan is modulated by multiple genetic pathways and processes. These include the DAF-2/insulin/IGF-1-like and TOR pathways, dietary restriction, protein translation and mitochondrial activity (reviewed in 1). Whereas our molecular understanding of these pathways comprises intricate roles for multiple transcription factors, including the transcription factors DAF-16/FOXO, PHA-4/FOXA, SKN-1 and HSF-1 (2, 3), much less is known about the downstream cellular events.

The cellular process of autophagy has recently been linked to aging in *C. elegans.* During autophagy, cytoplasm and organelles in the cell is degraded and recycled (4). We and others have shown that autophagy is required for at least some of the known *C. elegans* longevity pathways (see references in 1). For example, autophagy genes are essential for the long life of dietary-restricted animals and these animals have increased levels of autophagy. In contrast, autophagy does not appear to play a critical role in the extended lifespan observed in animals with mutations in genes involved in protein translation (5).

We have continued to investigate the role of autophagy in the known longevity pathways in *C. elegans.* For example, we find that the autophagy gene *bec-1*, the worm ortholog of the yeast and mammalian autophagy gene ATG6/VPS30/beclin 1, affects specific stress responses frequently observed in long-lived animals, such as thermotolerance. These results, along with our efforts to better characterize autophagy in *C. elegans*, will be discussed.

1. Melendez, Hall and Hansen, Methods Enzymol, 2008
2. Kenyon, Cell, 2005
3. Mair and Dillin, Annu Rev Biochem, 2008
4. Levine and Kroemer, Cell, 2008
5. Hansen *et al.*, PLoS Genetics, 2008.

281B

RNAi screen for genes that regulate stress resistance and lifespan. **B. Gerisch**[1], H. Lehrach[2], A. Antebi[3]. 1) MPI for Biology of Ageing, Cologne, Germany; 2) MPI for Molecular Biology, Berlin, Germany; 3) Huffington Center on Aging, Houston, TX.

Management of stress appears to be intimately related to the regulation of animal lifespan. Notably, most known long-lived mutants tested so far are resistant to various forms of environmental stress. In particular, long-lived mutants of the *daf-2*/insulin-like receptor (insulin/IGF-1 signaling) show increased resistance to heat and oxidative stress. We are using the correlation between longevity and stress resistance to identify genes that are involved in the process of lifespan regulation.

We screened over 600 RNAis corresponding to kinases with wild type for enhanced survival under stress, and obtained 115 strong to weak candidates involved in heat and/ or oxidative stress resistance. These candidate RNAis are currently being tested in aging experiments. Up to now we identified eight kinases that enhance stress resistance and result in longevity when down regulated. For example, the down regulation of a glycogen synthase kinase, implicated to be involved in the hormonal control of several regulatory proteins, results in strong heat stress resistance and longevity. Other candidate RNAis have to be re-tested in lifespan experiments.

In a similar approach we also screened the kinase RNAis for suppression of *daf-2*. Using a long-lived and stress resistant *daf-2* mutant, we screened for reduced survival under heat and/or oxidative stress. 32 candidates were identified in the stress screens and analyzed in aging experiments. So far we identified 12 candidates that lengthen or reduce the lifespan of *daf-2*. Four of these kinases specifically regulate *daf-2* and show no or minor effects on wild-type controls. Further characterization and assignment of the identified genes to pathways should yield insights into cellular processes involved in stress resistance and longevity.

282C

Characterizing Two *coq-3* Mutants in *Caenorhabditis elegans*. **F. Gomez**[1,3], R. Saiki[1], R. Chin[1], C. Srinivasan[2], C.F. Clarke[1,3]. 1) Dept Molecular Biol, Univ California, Los Angeles, Los Angeles, CA; 2) Department of Chemistry and Biochemistry, California State University Fullerton, Fullerton, CA; 3) Molecular Biology Interdepartmental Program, University of California, Los Angeles, Los Angeles, CA.

Coenzyme Q (referred to simply a Q) is a lipophilic component of the electron transport chain in mitochondria. The biosynthesis of Q is known to include several steps, including two *O*-methyltransferase reactions catalyzed by COQ-3. The *coq-3* gene is well-conserved among various organisms, from *Homo sapiens* to *E. coli*, where the gene is termed *ubiG*. Expression of *C. elegans* COQ-3 restores respiratory growth and Q biosynthesis in an *E. coli ubiG* mutant, and in a *S. cerevisiae coq-3* mutant. In *C. elegans, coq-3* mutants demonstrate decreased fertility, activity, survival and behavior. One mutant, *coq-3(qm188)*, possesses a deletion of the third and fourth exons. Sequencing of the *coq-3(qm188)* mRNA transcript shows a spliced product containing exons 1, 2 and 5 in a complete reading frame. Another mutant, *coq-3(ok506)*, is missing exon 3 only; however, the spliced mRNA is frameshifted and is predicted to generate an early stop codon. Interestingly, *coq-3(qm188)* mutants are sterile on a Q-replete bacterial diet of OP50, whereas *coq-3(ok506)* mutants generate approximately 100 larvae that slowly develop into adults with limited fertility. Brood size analysis on heterozygotes show that the *coq-3(qm188)* allele is not dominant negative. Both mutant strains are sterile when fed the Q-deficient bacteria strain GD-1. Neither the *coq-3(qm188)*, nor the *coq-3(ok506)* mutant is rescued, however, when grown on media containing NovaSol Q_{10}, a water soluble formulation containing coenzyme Q_{10}. Western blot using COQ-3 protein antibody indicates that both mutants also lack the wild-type protein. RT-PCR conducted on these strains shows mRNA product of the predicted sizes for both strains. Sequencing of these transcripts reveals no cryptic splice sites. The *coq-3* gene is situated in a three-gene operon. Transcripts of the flanking genes in the operon from both *coq-3* mutants are present; however, qT-PCR analysis shows that transcript levels of the upstream gene, *nuo-3*, in *coq-3(qm188)* mutants are widely variant compared to wild-type and *coq-3(ok506)* mutants. This erratic expression pattern indicates that *coq-3(qm188)* is not representative of a *coq-3* mutant, and thus further studies are to be conducted with the *coq-3(ok506)* mutant exclusively.

283A

A Molecular Mechanism for cGMP-Mediated Differential Production of Neuronal Insulin, Leading to Changes in Adult Longevity of *Caenorhabditis elegans*. **Jeong-Hoon Hahm**, Sunhee Kim, Young-Ki Paik. Department of Biochemistry, College of Life Science and Biotechnology and Yonsei Proteome Research Center, Yonsei University, Seoul, Korea.

G-proteins, including GPA-3, play an important role in regulating physiological responses in *Caenorhabditis elegans*. When confronted with an environmental stimulus such as dauer pheromone, or poor nutrients, *C. elegans* receives and integrates external signals through its nervous system (i.e., amphid neurons), which interprets and translates them into biological action. Here we show that a suppressed neuronal cGMP level caused by GPA-3 activation leads to a significant increase (47.3%) in the mean lifespan (MLS) of adult *C. elegans* through forkhead transcription factor family O (FOXO)-mediated signal. A reduced neuronal cGMP level was found to be caused by an increased cGMP-specific phosphodiesterase activity at the transcriptional level. Our results using *C. elegans* mutants with specific deficits in TGF-β and FOXO RNAi system suggest a mechanism in that cGMP, TGF-β and FOXO signaling interact to differentially produce the insulin-like molecules, ins-7 and daf-28, causing suppression of the insulin/IGF-1 pathway and promoting lifespan extension. Our findings provide not only a new mechanism of cGMP-mediated induction of longevity in adult *C. elegans* but also a possible therapeutic strategy for neuronal disease, which has been likened to brain diabetes.

284B

Identification of QTL involved in the *C. elegans* response to soil bacteria. **Michael A. Herman**[1], Basten Snoek[2], Ziyi Wang[1], Jan E. Kammenga[2]. 1) Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, KS; 2) Laboratory of Nematology, Wageningen University, Wageningen, NL.

We are investigating the genetic basis of nematode interactions with soil bacteria. To this end, we have isolated soil bacteria in association with native soil nematodes from grassland soils. We are using *C. elegans* to model the interactions of native soil nematodes with soil bacteria and have identified *C. elegans* genes induced when worms are grown on various soil bacteria. We have used available mutants to demonstrate that the functions of many of these genes are important for life history traits, including fitness and lifespan (Coolon et al., submitted). However, these experiments were not designed to identify natural variation in genes that may be able to respond to selection pressures presented by soil bacteria. To discover such genes we have taken a quantitative genetic approach to identify quantitative trait loci (QTL) that underlie *C. elegans* responses to soil bacteria. For this experiment we chose to investigate *C. elegans* response to *Stenotrophmonas maltophila*, a ubiquitous bacterium that we isolated in association with an *Oscheius sp.* from grassland soils. *S. maltophila* can cause nosocommial infections in immuno-compromised individuals, thus is also of interest to understanding conserved innate immune responses that could affect human health. We have observed that exposure to *S. maltophila* shortens lifespan in both wild-type and *daf-2* animals (see abstract by Vinod et al.), suggesting the involvement of other, perhaps uncharacterized, genes and innate immunity pathways. We used a set of recombinant inbred lines (RILs) generated by crosses between N2 and CB4856 (Li et al., 2006) to map QTL associated with lifespan in the presence of both *E. coli* and *S. maltophila*. We scored survivorship in the RILs and performed three replications of 10 animals each. Heritability of average lifespan was high on both *E. coli* and *S. maltophila*; 75 and 73 percent, respectively, indicating a large genetic component in the response. We were able to map QTL on LG I, II, IV and X and are now using near isogenic lines (NILs) to refine the positions of these QTL to identify the genes responsible. We will report our progress on these experiments.

Reference:

Li et al. 2006, PLoS Gen 2, e222.

285C

Trehalose extends longevity in the nematode *Caenorhabditis elegans*. **Yoko Honda**, Masashi Tanaka, Shuji Honda. Genomics Longevity & Health, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

Trehalose is a disaccharide widely used as a cryo- or anhydro-protective agent of cells and biomolecules. Here, we demonstrate that this simple sugar extends mean lifespan by over 30% without obvious side effects in the nematode *Caenorhabditis elegans*. It also increased reproductive span and retarded aging, as well as enhanced thermotolerance and suppressed polyglutamine aggregation. These results suggest that trehalose enhances longevity by suppressing internal and external stresses that disrupt protein homeostasis. Moreover, we show that RNAi inactivation of the trehalose biosynthesis genes trehalose-6-phosphate synthase-1 (*tps-1*) and *tps-2* decreases the lifespan of a long-lived insulin-like receptor (*daf-2*) mutant, indicating that the organism adopts trehalose in an aging-prevention system.

286A

Effects of Naphthoquinone Derivatives on Gene Expression and Aging in C. elegans. **Piper R. Hunt**, Mark A. Wilson, Quian-sheng Yu, Nigel H. Grieg, Catherine A. Wolkow. Laboratory of Neurosciences, National Institute on Aging, Baltimore, MD.

Macromolecular damage caused by the accumulation of reactive oxygen species (ROS) is thought to underlie many of the deleterious changes associated with aging. In numerous studies in *C. elegans*, resistance to oxidative stress has been linked to extended lifespan. Therefore, treatments that reduce cellular levels of ROS may also extend lifespan. The term hormesis refers to the beneficial effects on health of low doses of compounds, often plant derived, that are toxic at higher doses. ROS may be the modulators of hormesis; if concentrations of ROS are high enough to stimulate an adaptive response at the cellular level, yet low enough not to cause irreparable damage, increased health and longevity at the organismal level may result. Fourteen commercially available phytocompounds were screened for hormetic effects in *C. elegans*. In this initial screen, three compounds had beneficial effects at low doses. For further study, we chose to focus on plumbagin, a naphthoquinone present in the *Plumbago* and *Juglans* plant genera. Species from these plant families are used in the traditional medicines of various cultures around the world to treat ailments ranging from infections and ringworm to acne and inflammatory diseases. Plumbagin treatment induces the generation of ROS and is toxic at high doses. However, low doses of plumbagin have been shown to have anti-microbial, anti-malarial, anti-atherosclerotic, and anticarcinogenic effects. We generated a panel of plumbagin (5-hydroxy-2-methyl-1,4-napthoquinone) analogs with the goal of potentiating plumbagin's beneficial effects on morbidity while minimizing its toxicity. Here we show that, in *C. elegans*, expression of the Phase II reporter gene *pgst4::gfp* responds in a dose dependent manner to plumbagin. Toxic concentrations of menadione (2-methyl-1,4-napthoquinone) and 5,8-dihydroxy-1,4-napthoquinone also induce increases in *pgst4::gfp* expression of 15% or more over background. For the non-toxic compounds in our panel, excessive environmental concentrations of 500uM and above were required to consistently see even a 10% increase in *pgst4::gfp* expression, indicating that *pgst4::gfp* expression levels of 15% or more over background may act as a biosensor for potentially toxic compounds.

287B

A mutagenesis screen for genes involved in the regulation of longevity response to temperature by thermosensory neurons. **Ara Hwang**, Dahye Jeong, Seung-Jae Lee. Department of Life Science/I-BIO/WCU ITCE, POSTECH, Pohang, Korea.

Recently, we found that multiple thermosensory mutants, including *tax-2* mutants, which lack a subunit of the neuronal cyclic-nucleotide gated channel, lived even shorter than wild type at 25°C (the warm temperature) *(1)*. The short lifespan of *tax-2* mutants at 25°C was completely suppressed by a null mutation in *daf-12*/NHR (nuclear hormone receptor) gene *(1)*. From these and other molecular genetics data, we proposed that thermosensory AFD neurons moderate the effect that increased temperature otherwise have on the lifespan of *C. elegans* by changing the activity of the transcription factor DAF-12 *(1)*. In addition to the effects on aging, the thermosensory *tax-2* mutations cause a constitutive dauer phenotype at 27ºC *(2)*. Interestingly, *daf-12* mutation suppresses this dauer phenotype *(2)* as well as the short 25°C-lifespan phenotype caused by *tax-2* mutations *(1)*. Thus, we hypothesized that other mutations that suppress the 27°C dauer phenotype of *tax-2* mutations may also suppress the lifespan phenotype as well. We first tested whether known dauer suppressor mutations suppressed the 27°C dauer phenotype of the *tax-2* mutants. We confirmed that the dauer phenotype of the *tax-2* mutants was significantly suppressed by *daf-12* mutation. However, *daf-3*, *daf-5*, *scd-1*, *scd-2* or *scd-3* mutations, which have been shown to suppress dauer formation of various dauer-constitutive mutants, did not affect the *tax-2* mutants' dauer phenotype. Since only a few known dauer suppressor mutants suppressed the *tax-2* mutants' dauer phenotype, these data suggest that a dauer-suppressor mutagenesis screening may produce novel mutants rather than already identified mutants. We then carried out EMS mutagenesis screening for mutants that suppressed constitutive dauer phenotype of *tax-2* mutants at 27°C. We found and confirmed 7 *tds* (*tax-2* dauer suppressor) mutants from the screening. All of them were recessive mutants. We will carry out complementation and mapping analyses. We will also determine whether any of the *tds* mutants suppress lifespan phenotypes of the *tax-2* mutants. Our goal is to identify new components involved in the longevity response to temperature and to understand how the AFD neurons affect DAF-12 activity to regulate lifespan at high temperature. *(1) Lee and Kenyon, 2009, Curr Biol (in press) (2) Ailion and Thomas, 2000, Genetics*.

288C

CeWRN-1 RecQ protein is responsible for processing DSB with CeRPA. **Moonjung Hyun**, Hana Jung, Jina Lee, Byungchan Ahn. Dept Life Sci, Univ Ulsan, Ulsan, Korea.

RecQ helicases play essential roles in maintenance of genomic stability from E. coli to humans. The RecQ proteins interact with proteins involved in DNA metabolic pathways such DNA repair, recombination, and replication. Our previous studies showed that C. elegans WRN-1 RecQ protein (human WRN ortholog) has ATP-dependent 3'-5' helicase activity and that C. elegans replication protein A stimulates WRN-1 helicase activity on a long DNA duplex substrate. However, the mechanism for CeRPA stimulation of DNA unwinding is not characterized yet. We found that CeWRN-1 physically interacted with both subunits of CeRPA. However, a CeRPA73 subunit only stimulated CeWRN-1 helicase activity on a long DNA duplex, indicating a functional interaction of CeWRN-1 with the CeRPA73 subunit. When treated with CPT, replication-associated DSBs inducer, C. elegans wrn-1(gk99) is more sensitive than N2 and it was detected by COMET assay that DNA strand breaks more accumulated in wrn-1(gk99), suggesting that WRN-1 is required for responding to DSBs. Furthermore, we observed nuclear colocalization of CeWRN-1 and CeRPA in germ line cells after CPT. These findings propose that the WRN-1 is involved in processing DSB during replication with CeRPA protein.

289A

Analysis of EGF receptor-related proteins reveals an unexpected but potent role of EGF signaling in promoting healthy aging. **H. Iwasa**, Y. Simon, J. Xu, M. Driscoll. Dept Molec Biol & Biochemistry, Rutgers Univ, Piscataway, NJ.

Conserved pathways such as insulin/IGF signaling (IIS) and dietary restriction (DR) promote healthy aging and extend longevity across species. Genetic screens focused primarily on extending the period of healthy aging rather than lengthening lifespan have not yet been a focus in aging biology, and thus the genetics of healthspan remain poorly understood.

Previous bioinformatic analysis identified 54 *C. elegans* proteins related to the insulin receptor by homology to the extracellular ligand-binding domains. Importantly, these proteins appear more similar to secreted potential ligand-binding proteins than to true receptor kinases. As part of a large screen for genes that impact age-associated locomotory decline, we systematically targeted their knockdown via RNAi, assaying swimming rates of treated animals during mid/late life. We identified two candidates (*hpa-1* and *hpa-2*) for which RNAi confers **h**igh **p**erformance in **a**dvanced age (*hpa*) phenotype.

To examine how *hpa-1* and *hpa-2* modulate aging, we tested for genetic interactions with IIS or DR mutants. Unexpectedly, our data indicated that healthspan-promoting effects of *hpa-1* and *hpa-2* are exerted independently of IIS as well as DR pathways. We revisited sequence comparisons of *hpa-1* and *hpa-2*, and noted that these two genes actually resemble EGF receptor more closely than insulin receptor. We therefore probed EGF pathway mutants for aging phenotypes. We found that a gain-of-function mutation in EGF receptor confers healthspan benefits; reduction-of-function in the EGF receptor has the opposite effect. We conclude that the EGF pathway impacts healthy aging.

EGF signaling promotes activates multiple downstream pathways, including a RAS/MAP kinase pathway, a diacylyglycerol pathway, and an IP3 receptor/PLC-γ receptor pathway. We show that it is the IP3 receptor pathway that is involved in heathspan-promoting effects of EGF signaling. We also showed that this pathway, as well as EGF itself, is required for HPA-1 and HPA-2 modulation of healthspan. Pathway activation in the adult appears sufficient to extend healthspan. In sum, we show that the EGF pathway and two novel regulators that are candidate EGF-binding proteins have a strong impact on successful aging in a simple animal model. HPA-1 and HPA-2 are related to human proteins that bind EGF, and thus we suggest the regulatory mechanism we describe, and its impact on aging, may be conserved across species.

290B

Characterization of novel genes found to accelerate amyloid-beta toxicity in a *C. elegans* model for Alzheimer's disease. **Louise Jensen**[1], Helle Jakobsen[1], Andrzej Swistowski[2], Dale Bredesen[2], Anders Olsen[1]. 1) Department of Molecular Biology, Aarhus University, Aarhus C, Denmark; 2) Buck Institute for Age Research, Stem Cells/Neuroscience, Novato, CA, USA.

Alzheimer's disease (AD) is the most common type of dementia among elderly, but the mechanisms causing AD are still largely unknown. Cerebrovascular deposits of amyloid-beta (Aβ) plaques and tau protein tangles have long been considered hallmarks of AD. However, although they are present in the brains of the diseased, it is becoming clear that the deposits themselves may not be toxic. On the contrary, these aggregates may provide a protective mechanism whereby the cell immobilizes toxic species of soluble Aβ-oligomers. Aβ is generated from cleavage of Aβ precursor protein (APP). To understand the biology of APP, binding partners of APP binding protein 1 X11a (APPB1) was identified (Bredesen Lab, unpubl.), 22 of these with good homology to *C. elegans* proteins. In order to test these APPB1 binding proteins in an *in vivo* model, we RNAi inactivated these genes in a *C. elegans* model expressing human Aβ42 in muscle, showing an age-related increase in Aβ-aggregation and paralysis[1]. We found that inactivation of three of these genes dramatically alters toxicity in Aβ-worms, but not in controls. Paralysis over time was greatly accelerated when these genes were inactivated and for one of the genes this was extremely severe. Interestingly, this gene also seems to play a role in the toxicity of protein aggregation in general, as is the case for several Aβ-toxicity modulating genes, e.g. *hsf-1* and *hsp-70*. When we inactivated the gene in a model for Parkinsons disease overexpressing α-synuclein in muscle cells, this lead to an increased formation of vesicles of α-synuclein, as previously reported in this model[2]. Complementing the RNAi analysis in the Aβ-model, Western blots probed with anti-Aβ42-antibody revealed large differences in the distribution of Aβ-oligomers in RNAi treated animals compared to RNAi controls. We are currently investigating the Aβ-distribution further using WB and immunostaining as well as investigating genetic pathways these candidate genes for Aβ toxicity may operate in. 1: Link, C.D. 1995. PNAS 92: 9368-9372 2: van Ham, T.J. et al. 2008. PLoS Genet 4: 1-11.

291C

Identification of genes involved in the longevity response to temperature using microarray analysis. **Dahye Jeong**[1], Ara Hwang[1], Cynthia Kenyon[2], Seung-Jae Lee[1,2]. 1) Department of Life Science/I-BIO/WCU ITCE, POSTECH, Pohang, Korea; 2) Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA.

Recently, we showed that thermosensory AFD neurons play a regulatory role in the temperature dependence of lifespan *(1)*. We found that the AFD neurons counteract the life-shortening effect that high temperature would otherwise have on lifespan through changes in the expression of daf-9, which in turn alters the activity of the transcription factor DAF-12/NHR (nuclear hormone receptor) *(1)*. Thus it is possible that the thermosensory neurons influence expression of longevity genes at different temperatures differently. For example, high temperature sensed by the AFD neurons may alter the expression of DAF-12 targets and possibly additional genes to moderate the lifespan-shortening effect of high temperature. To test this idea, we compared the expression profile of animals grown at 25°C (high temperature) with that of animals grown at 15°C (high temperature) by performing DNA microarray analysis. We found that 144 transcripts were differentially expressed at different temperatures (1% false positive gene discovery rate as an arbitrary cutoff with the Significance Analysis of Microarray). We then tested whether any of these genes were involved in lifespan regulation at different temperatures, by performing RNAi for each of the top 90 differentially expressed genes and examining the effect on lifespan at different temperatures. Several of them displayed a temperature-dependent lifespan phenotype differently at different temperatures. These data suggest that animals may regulate the expression of these genes at high temperature to reduce the lifespan-shortening effect of high temperature. Further characterization of these genes at a molecular level is ongoing. *(1) Lee and Kenyon, 2009, Curr Biol (in press)* .

292A

Autophagy Genes Protect Against *Salmonella typhimuruim* Infection and Mediate Insulin Signaling-Regulated Pathogen Resistance. **Kailiang Jia**[1], Collin Thomas[2], Muhammad Ahkbar[1], Qihua Sun[1], Beverley Huet[3], Christopher Gilpin[4], Beth Levine[1,5,6]. 1) Dept of Internal Medicine, UT Southwestern Medical Center, Dallas, TX; 2) Center for Advanced Studies in Math and Natural Sciences, Collin College, Plano, TX; 3) Department of Biostatistics and Clinical Sciences, UT Southwestern Medical Center, Dallas, TX; 4) Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX; 5) Department of Microbiology, UT Southwestern Medical Center, Dallas, TX; 6) Howard Hughes Medical Institute.

A conserved insulin-like pathway modulates both aging and pathogen resistance in *Caenorhabditis elegans*. However, the specific innate effector functions that mediate this pathogen resistance are largely unknown. Autophagy, a lysosomal degradation pathway, plays a role in controlling intracellular bacterial pathogen infections in cultured cells but less is known about its role at the organismal level. Here we report the effects of autophagy gene inactivation on *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) infection in two model organisms, *Caenorhabditis elegans* and *Dictyostelium* discoideum. In both organisms, RNAi inactivation of autophagy genes increases bacterial intracellular replication, decreases animal lifespan, and results in apoptotic-independent death of infected cells. In *C. elegans*, genetic knockdown of autophagy genes abrogates pathogen resistance conferred by a loss-of-function mutation, *daf-2(e1370)*, in the insulin-like signaling pathway. Moreover, overexpression of the DAF-16 FOXO transcription factor, a major target of the insulin pathway, induces autophagy and confers autophagy gene-dependent resistance to *Salmonella* infection. Thus, autophagy genes play an important role in host defense *in vivo* against an intracellular bacterial pathogen and mediate pathogen resistance in long-lived mutant nematodes. These findings raise the novel hypothesis that age-related declines in autophagy function may contribute to immunosenescence and increased susceptibility to infectious diseases in the elderly.

293B

Role of *daf-2*/insulin signaling pathway genes on preservation of mobility and muscle function in *Caenorhabditis elegans* . **Luv Kashyap**, Alfred Fisher. Medicine, Division of Geriatric Medicine, University of Pittsburgh, Pittsburgh, PA.

Sarcopenia is noted in a wide range of species, from nematodes, flies, rodents, and non-human primates to humans. While sarcopenia is considered to be a normal aspect of aging, the loss of strength can have significant effects on the health and functioning of elderly individuals. A recent study in *Caenorhaleditis elegans* showed gradual, progressive deterioration of muscle in this short-lived nematode that resembled human sarcopenia from midlife, resulting in decline in mobility during aging. A mutation in *daf-2*/insulin signaling pathway is known to affect a wide range of physiological processes ranging from longevity to metabolism, development, stress resistance, thermotolerance, resistance to hypoxia, and resistance to bacterial pathogens. One of the remarkable features of the *daf-2* mutants is the delay in the development of sarcopenia. The mechanisms by which changes in *daf-2* signaling protect muscle by delaying the development of sarcopenia are completely unknown. Since, multiple genes regulated by *daf-2* signaling have been identified, and these genes may represent possible candidate effectors for the delaying in the onset of sarcopenia. We investigated the effect of knockdown of a total of 41 genes required for the effects shown by *daf-2* mutations on longevity. To assess muscle function, we used a chemotaxis assay which assesses the ability of worms to move in response to attractant chemicals. Our experiments demonstrate that *daf-16* is necessary for preservation of mobility in worms since we see significant differences between *daf-2* mutants treated with *daf-16* RNAi and control as early as day #4 of adulthood and the differences are magnified with increasing age. In contrast, *daf-2* mutant worms on *skn-1* RNAi did not show any significant decline with progressing age. Moreover, we were also successful in identifying several novel genes which might have a possible role on preservation of mobility in *Caenorhabditis elegans*. These findings in worms may offer significant insight towards the identification of genes involved in sarcopenia in higher organism including humans.

294C

Genetic analysis of *sub-1* and *sub-2*, two suppressors of *bec-1* dependent lethality in *C. elegans* development. **Sana Khan**[1,2,3], John Attonito[2,3], Zahava Rubel[2], Lana Tolen[2], Alicia Meléndez[1,2]. 1) The Graduate Center, CUNY, New York, NY; 2) Biology, Queens College-CUNY, Flushing, NY; 3) have contributed equally.

Autophagy is a process by which a cell degrades cytoplasmic components such as organelles. It may be induced by stress, over-crowding, or starvation conditions in addition to its role in homeostasis. *C. elegans bec-1* is the ortholog of *beclin1/Atg6/Vps30* in mammals and yeast, an important regulator of autophagy. Yeast Atg6/Vps30p was identified in a genetic screen for mutants that are starvation sensitive or defective in vacuolar protein sorting. Human Beclin 1 was identified as a protein interacting with the anti-apoptotic protein Bcl-2 in a yeast two-hybrid assay and is monoallelically deleted in up to 75% of various human cancers. Therefore, any insights on the function of *bec-1* and autophagy in *C. elegans* will likely shed light on the role of autophagy in tumorigenesis in humans.

We have established *C. elegans* as a multicellular genetic model system to study the role of BEC-1 (the ortholog of Atg6/Vps30/Beclin 1) in development. We and others have found that *bec-1* functions in various fundamental biological processes, including survival, longevity, fat accumulation, dauer and reproductive development. A complete loss of function mutation of *C. elegans bec-1(ok691)* is lethal. We have isolated two mutants that suppress the *bec-1* dependent lethality and named these mutants *sub-1* and *sub-2* (suppressor of *bec-1* lethality). Interestingly, these two mutations also suppress the decrease in fat accumulation observed in *bec-1* homozygous mutant animals, as well as the shortening of lifespan associated with heterozygous *bec-1* mutants. Using single nucleotide polymorphism (SNP) mapping, we have mapped *sub-1* to a small interval on chromosome X, and *sub-2* to a small interval on chromosome IV. We will report on the molecular and genetic characterization of the *sub-1* and *sub-2* suppressor mutations.

295A

Defective responses to oxidative stress in protein L-isoaspartyl repair-deficient *Caenorhabditis elegans*. **Shilpi Khare**[1,2], Tara Gomez[1,2], Steven Clarke[1,2]. 1) Dept. of Biochemistry & Molecular Biology, University of California, Los Angeles, Los Angeles, CA; 2) The Molecular Biology Institute, University of California, Los Angeles, Los Angeles, CA.

We have shown that *Caenorhabditis elegans* lacking the PCM-1 protein repair L-isoaspartyl methyltransferase are more sensitive to oxidative stress than wild-type nematodes. Exposure to the redox-cycling quinone juglone upon exit from dauer diapause results in defective egg laying (Egl phenotype) in the *pcm-1* mutants only. Treatment with paraquat, a redox-cycling dipyridyl, causes a more severe developmental delay at the second larval stage in *pcm-1* mutants than in wild-type nematodes. Finally, exposure to homocysteine and homocysteine thiolactone, molecules that can induce oxidative stress via distinct mechanisms, results in a more pronounced delay in development at the first larval stage in *pcm-1* mutants than in wild-type animals. Homocysteine treatment also induced the Egl phenotype in mutant but not wild-type nematodes. All of the effects of these agents were reversed upon addition of vitamin C, indicating that the developmental delay and egg-laying defects result from oxidative stress. Furthermore, we have demonstrated that a mutation in the gene encoding the insulin-like receptor DAF-2 suppresses the Egl phenotype in *pcm-1* mutants treated with juglone. Our results support a role of PCM-1 in the cellular responses mediated by the DAF-2 insulin-like signaling pathway in *C. elegans* for optimal protection against oxidative stress.

296B

Dissecting Alternative Lengthening of Telomeres in *Caenorhabditis elegans* . **Chun-A Kim**, Beomseok Seo, Junho Lee. Research Center for Functional Cellulomics, Institute of Molecular Biology and Genetics, Department of Biological Sciences, Seoul National University, Seoul, Korea.

Eukaryotic cells have linear chromosomes which is protected by telomeres. Telomeres are distinguished from the double strand breaks (DSBs) which is a kind of DNA damage. With telomeres, the chromosomal ends can be protected from degradation or fusion with another chromosome. Telomeres of somatic cells are gradually shortened with repeated replication. However, telomeres of cancer cells and germ cells are not shortened because they can maintain telomere length by the telomerase, a special kind of reverse transcriptase. In *Caenorhabditis elegans* it was known that *trt-1(ok410)* mutant that lacks telomerase catalytic subunit has shortened telomeres and fused chromosomes. These data correspond with the study of mammals and it is known that telomere maintenance mechanisms are evolutionally conserved. However, in other model organisms like yeast and mouse, telomerase independent telomere maintenance mechanism has been reported; This is called Alternative Lengthening of Telomeres (ALT). ALT mechanism is used in about 15% of cancer cells. The telomere repeat numbers of ALT cells are heterogeneous. Some ALT cells show non-traditional telomere repeats with other sequence inserted. Loss-of-function experiments suggested that ALT mechanism is based on homologous recombination-mediated replication. So far, the existence of ALT mechanism has not been confirmed at the organismal level. Recently, we have found novel suppressor mutants of *trt-1(ok410)* mutant by EMS mutagenesis screening. Currently we are mapping the suppressor mutation by Single Nucleotide Polymorphisms(SNPs) mapping method that uses SNPs of restriction endonuclease recognition site as a canonical mapping marker. By mapping the putative ALT genes using this approach, we expect to elucidate the molecular mechanisms of ALT.

297C

Genomic Approach to Identify Novel Genes to Regulate Lifespan and ROS Resistance. **Yongsoon Kim**, Hong Sun. Cancer Genomics, Nevada Cancer Inst, Las Vegas, NV.

DAF-2/Insulin receptor-like signaling pathway in C. elegans is a highly conserved pathway and is involved in regulation of longevity, development and metabolism. DAF-16, a member of forkhead transcription factor, is a major downstream target for DAF-2 pathway. To identify new genes involved in regulation of longevity, we have been carrying out genome-wide RNAi screens. In the primary screen, we screened for RNAi clones which can give rise to resistance towards reactive oxygen species (ROS) phenotype In the secondary screen, we identified RNAi clones that produce lifespan extension phenotype. To this date, we have completed screens of one third of the genome. We have identified 84 genes, most of them novel, that regulate both ROS resistance and lifespan (Kim and Sun 2007). We have found that 29 genes act to daf-16 dependent manner. Interestingly, several of these genes appear to act in a pathway parallel to AKT to regulate DAF-16 activity. We are in the process to determine the mechanism. In addition, we have identified a group of genes that act daf-16 independent manner to regulate lifespan. Our screens have uncovered new genes that participate in important cellular processes such as signal transduction, cell-cell interaction, gene expression, protein degradation and energy metabolism. Through characterization of these newly identified genes, we hope to better understand the molecular mechanism underlying ROS resistance and animal aging process. Kim Y and Sun H (2007) Functional genomic approach to identify novel genes involved in the regulation of oxidative stress resistance and animal lifespan. Aging Cell 6, 489-503.

298A

USING RECOMBINANT INBRED LINES AND A HIGH THROUGHPUT ASSAY TO ASSESS THE BASIS OF DIFFERENCES IN LONGEVITY BETWEEN A LAB-ADAPTED (N2) AND A WILD STRAIN (CB4856) OF *C. ELEGANS*.. **Gunnar A. Kleemann**[1,2], Matthew Rockman[4,5], Alina Garbuzov[1,2], Leonid Kruglyak[1,3], Coleen T. Murphy[1,2]. 1) Lewis Sigler Institute of Integrative Genomics, Princeton University, Princeton, NJ; 2) Department of Molecular Biology, Princeton University; 3) Department of Ecology and Evolutionary Biology, Princeton University; 4) Department of Biology, New York University; 5) Center for Genomics and Systems Biology at New York University.

Advances in genetic research have often been gleaned from genes with large effects. While the single-gene, large-effect model has been extremely useful for the for the rapid identification of the major biological players influencing processes such as aging, our understanding of the mechanisms controlling processes in the natural world would be enhanced by studying the genetics of traits in wild strains with naturally occurring mutations. In order to better understand the type of alleles that influence longevity in a wild isolate strain, we are analyzing the longevity of recombinant inbred lines (RILs) arising from crosses between the wild strain CB4856 and N2. Since there are a large number of RILS to characterize, we have facilitated our analysis by developing a high-throughput longevity assay pipeline (CHRONOS). Plates are photographed at two time points, then our program “Chronos” (matlab) is used to align, filter, subtract images, count worms and estimate longevity curves. We have estimated the longevity of 173 of the 239 existing CB4856 X N2 RILs and have identified long-lived candidates. We are currently analyzing one long-lived RIL,105b and its sib strain 105. Although these RILs share ~ 70% of their genotype,105 is not long-lived. Comparison of the 105 and 105b genotypes reveal a polymorphism in the FOXO transcription factor *daf-16*. 105 has the N2 allele and 105b has the CB4856 allele. Additionally, microarray data for 105 and 105b reveals expression level differences in *daf-16*, in known *daf-16* target genes as well as in genes are not *daf-16* targets. Furthermore, the extended longevity of 105b is at least partially dependent on *daf-16*, and 105b has elevated dauer production (10%) when reared at 25°C. While these data suggest partial involvement of a FOXO/*daf-16* activity in the extended longevity of 105b, the genetic polymorphism in *daf-16* alone cannot cause the enhanced longevity of 105b since 105b is longer-lived than either parent line. These additional unknown loci may be in a *daf-16* independent pathway since non-*daf-16* targets were differentially regulated between 105 and 105b. We are currently working to determine the degree to which *daf-16*/FOXO activity affects longevity in 105b, and to identify other, perhaps *daf-16* independent, loci responsible for the enhanced longevity of 105b.

299B

Analysis of brap-2 and oxidative stress response in C.elegans. **Janet Koon**, Terry Kubiseski, Jennifer Alberts. Biology, York University, Toronto, ON, Canada.

In mammals, IMP/Brap2 (BRCA1 associated protein 2) is associated with the Ras signaling pathway and cytoplasmic retention of BRCA-1 and the cell cycle inhibitor p21 (1,2). The EEED8.16 protein in C.elegans has been identified as a homolog of the Brap2 gene in C.elegans and has been designated brap-2. It consists of a Brap2 domain, a RING-H2 domain, a ubiquitin-protease-like zinc-finger domain (ZnF-UBP) and leucine heptad repeats. A brap-2 deletion mutant was obtained from the Caenorhabditis Genetics Center (CGC) which contains a 1540bp deletion that removes the ZnF-UBP and leucine heptad repeats. Here we show that the brap-2 gene is associated with oxidative stress response in C.elegans. Exposure of the brap-2 mutant to either paraquat or peroxide resulted in developmental arrest at the L1 stage at significantly lower concentration compared to N2 worms. Nematodes exposed to oxidative stress have been shown to increase its expression of the phase II detoxification enzyme, gst-4. The gst-4::gfp reporter construct was expressed in the brap-2 deletion mutant and showed elevated levels of expression in the intestine compared to expression in the body wall muscles in the gst-4::gfp strain alone. Since Brap2 has shown to influence p21 activity, we also focused on the interaction between brap-2 and the p21 homologue in C.elegans, cki-1 as the cause for L1 arrest under oxidative stress conditions. Incorporation of the transcriptional reporter cki-1::gfp in the brap-2 mutant showed increase expression in many cells, most notably in the blast cells, upon exposure to paraquat. Co-transfection of cki-1 and brap-2 in HEK293T cells showed that brap-2 modified cki-1 localization by retaining it in the cytoplasm. We are currently reintroducing the wild-type brap-2 gene back into the brap-2 deletion strain to rescue its oxidative stress sensitivity phenotype and determine the stage of the cell cycle which arrest is occurring. References: 1.Asada M., et al. (2004). Molecular and Cellular Biology, 24(18): 8236-8243. 2.Li S, et al. (1998). Journal of Biological Chemistry, 273(11): 6183-6189. 3.An J.H, et al. (2003). Genes and Development. 17(15): 1882-1993.

300C

Genetic characterization of the *dhs-21* gene in *C. elegans*. **Son T. Le**[1], Tae-Woo Choi[1], Jeong-Min Kim[1], Kyungmin Ko[2], Jeong Hoon Cho[3], Joohong Ahnn[1]. 1) Life Science, College of Natural Sciences, Hanyang University, 17 Haengdang-dong, Seongdong-gu, Seoul, 133-791, Korea; 2) Gwangju Institute of Science and Technology, 261 Cheomdan-gwagiro, Buk-gu, Gwangju 500-721 Republic of Korea; 3) Division of Biology Education, College of Education, Chosun University, Gwangju 501-759, Republic of Korea.

The L-xylulose dehydrogenase enzyme interconverts L-xylulose into xylitol in the pentose phosphate pathway in both eukaryotes and procaryotes. Previous studies reported that xylitol serves as an osmolyte contributing osmotic control in cells. However, the fuction of L-xylulose dehydrogenase gene has not been elucidated. In *C. elegans*, the *dhs-21* is predicted to encode a short chain dehydrogenase reductase (SDR/XDR). It has well-conserved functional domains of mammalian L-xylulose dehydrogenases. *C. elegans* can thus be readily used for the relevant genetic studies of *dhs-21*. We have isolated a deletion mutant of *dhs-21*in *C. elegans*. The expression of transcriptional GFP fusion showed that the DHS-21 protein is expressed in hypodermis, uterus and spermatheca. The *dhs-21(jh129)* mutant exhibited various defects in phenotypes including small brood size, short life span, and hypertonic resistance. These results showed that *dhs-21*gene can be involved in many different pathways through the catalytic product xylitol. We are currently studying the genetic pathways that may be regulated by *dhs-21*as well as enzymatic function by the DHS-21 protein in order to clear understanding of the fuctions *in vivo*.

301A

Thermosensory neurons regulate the longevity response to temperature in *C. elegans*. **Seung-Jae Lee**[1,2], Cynthia Kenyon[1]. 1) Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA; 2) Present address: Department of Life Science/I-BIO/WCU ITCE, POSTECH, Pohang, Korea.

Many ectotherms, including *C. elegans*, have shorter lifespans at high temperature than at low temperature. High temperature is generally thought to decrease the lifespan of ectotherms simply through its effects on metabolic rates. We questioned this view and asked whether the temperature-dependence of lifespan is subject to active regulation. *C. elegans* has a pair of thermosensory neurons called AFD neurons, which allow the animal to sense and respond to temperature. We asked whether the AFD neurons have regulatory roles in the temperature dependence of lifespan. Surprisingly, we found that genetic or laser ablation of the AFD neurons led to even shorter lifespan at high temperature, suggesting that the thermosensory AFD neurons have a homeostatic role that counteracts the effect that increased temperature otherwise have on lifespan. The AFD neurons influenced lifespan through the steroid signaling pathway comprising DAF-9 (cytochrome P450) and DAF-12 (nuclear hormone receptor). The AFD neurons were required for normal expression of *daf-9* at high temperature, and genetic epistasis analysis indicated that DAF-9 affected lifespan by altering the activity of DAF-12. Together, our findings suggest that *C. elegans* actively transmits signals from thermosensory neurons to this steroid pathway to regulate lifespan at high temperature. We propose that this thermosensory system allows *C. elegans* (and possibly other cold-blooded animals) to reduce the effect that warm temperature would otherwise have on processes that affect aging, something that warm-blooded animals do by controlling temperature itself.

302B

Glucose shortens the lifespan of *Caenorhabditis elegans* by down-regulating aquaporin gene expression. **Seung-Jae Lee**[1,2], Cynthia Kenyon[1]. 1) Department of Biochemistry and Biophysics, University of California, San Francisco, CA, USA; 2) Present address: Department of Life Science/I-BIO/WCU ITCE, POSTECH, Pohang, Korea.

Many studies have addressed the effect of dietary glycemic index on obesity and diabetes, but little is known about its effect on lifespan itself. As recently shown in an independent study *(1)*, we found that adding a small amount of glucose to the medium (0.1-2%) shortened the lifespan of *C. elegans*. We found that glucose shortened lifespan by inhibiting the activities of lifespan-extending transcription factors that are also inhibited by insulin signaling: the FOXO family member DAF-16 and the heat shock factor HSF-1. We then found that most or all of this effect could be attributed to the down-regulation of an aquaporin glycerol channel, *aqp-1*. Our findings suggested that changes in glycerol metabolism underlie the lifespan-shortening effect of glucose, and that *aqp-1* acts cell-nonautonomously as a feedback regulator in the insulin/IGF-1 signaling pathway. Insulin downregulates similar glycerol channels in mammals *(2, 3)*, suggesting that this glucose-responsive pathway might be conserved evolutionarily. Together these findings raise the possibility that a diet with a low glycemic index might have beneficial effects on lifespan in higher organisms. *(1) Schulz et al., 2007, Cell Metab (2) Kishida et al., 2001, J Biol Chem (3) Kuriyama et al., 2002, Diabetes*.

303C

Functional Studies on Thioredoxin Reductases in *C. elegans*. **Weixun Li**[1], Yun-Ki Lim[1], Cha-Sun Cho[1], Yon Ju Ji[2], Jeong Hoon Cho[3], Jaya Bandyopadhyay[4]. 1) Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, 133-791, Korea; 2) Laboratory of Cell and Developmental Signaling, National Cancer Institute-Frederick, Frederick, Maryland 21702, USA; 3) Division of Biology Education, College of Education, Chosun University, Gwangju 501-759, Republic of Korea; 4) Department of Biotechnology, West Bengal University of Technology, BF142, Salt Lake City, Sector I, Kolkata, India.

Thioredoxins have been described as small (~12kDa), and ubiquitous disulfide reductases. Thioredoxins are multifunctional proteins; the anti-oxidative activity is one of the most well studied functions. Previously one of the thioredoxin (*trx-1*) in *C. elegans* has been characterized in our laboratory. The reducing activity of thioredoxin has been demonstrated *in vitro*. The *trx-1* has been found to express in head neurons and intestine. Null mutant of *trx-1*, has been found to show shorter life span than that of wild type, and showed less viability under stress condition. Further, these defects of mutant can be completely rescued by transforming full length construct of *trx-1*into mutants. These results showed that thioredoxin as an anti-oxidative protein, is important for maintaining the normal life span and surviving under stress condition. Thioredoxin reductases are enzymes belonging to the flavoprotein family of pyridine nucleotide-disulfide oxidoreductases. These enzymes use NADPH to reduce thioredoxin. There are two thioredoxin reductases in *C. elegans*, *trxr-1* and *trxr-2*. The *trxr-1* is predicted to localize in cytoplasm, whereas *trxr-2* is predicted to localized mitochondria.GFP reporter system revealed that both *trxr-1* and *trxr-2* are expressed in intestine the same as *trx-1*. Consistent with prediction and biochemical analysis, we found that *trxr-2* showed mitochondrial GFP expression pattern. Further, ectopic expression of *trxr-2* in muscle confirmed the mitochondrial localization of *trxr-2*. Interestingly, *trxr-2* seems to be induced by stress and knockout mutants of *trxr-2* were less viable under stress condition. These results suggest that *trxr-2* is mainly involved in stress response whereas *trxr-1* may be response for other biological phenomena. Further studies of these two genes are under investigation.

304A

Snip-SNP mapping a gene implicated to be a component of the insulin signaling pathway. **Anne C. Logie**, Catherine A. Wolkow. Laboratory of Neurosciences, National Institute on Aging, Baltimore, MD.

The insulin-like signaling pathway regulates larval development, stress resistance and adult lifespan in *Caenorhabditis elegans*. Mutations in *age-1*, which encodes a PI3K p110 catalytic subunit, cause constitutive developmental arrest as dauer larvae and extend adult lifespan. Genetic screens have isolated several mutations that suppress these phenotypes in *age-1(mg109)* mutants, including gain-of-function mutations in *akt-1* and *pdk-1* (Gami et al, 2006). This screen also recovered a mutation, *mg227*, which suppresses *age-1(mg109)* dauer arrest, but, surprisingly, appears to extend adult lifespan of *age-1(mg109)* animals. Here, we describe progress towards cloning the *mg227* allele. Snip-SNP mapping placed this mutation on the X chromosome, within a region devoid of other genes implicated as *age-1* pathway modifiers. The map position is being refined using Snip-SNP mapping.

305B

A neuromedin U receptor homolog modulates *C. elegans* lifespan in a food source-dependent manner. **Wolfgang Maier**, Bakhtiyor Adilov, Martin Regenass, Joy Alcedo. Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.

Subsets of sensory neurons, which can detect specific food-derived gustatory and olfactory cues (1), have been shown to have distinct effects on the lifespan of *C. elegans*. This is consistent with the observation that different bacterial food sources have also been shown to modulate *C. elegans* lifespan (2). Since the effects on lifespan of these food sources can be mediated by different types of cells (1,3-5), it is possible that their diverse effects are also promoted by different genes that act in a food source-dependent manner. These food source-dependent genes would presumably be unlike the many longevity genes, such as the insulin/IGF-1 receptor *daf-2*, that have been shown to affect lifespan consistently on different food sources, and thus act in a food source-independent manner.

We reasoned that some of the food source-dependent lifespan-influencing genes presumably encode signaling molecules, like neuropeptides, which are secreted from neuronal and/or non-neuronal cells in response to food-derived cues, and the receptors for such peptide signals. Among several neuropeptide and neuropeptide receptor genes that we tested for food source-dependent lifespan phenotypes, we identified *nmur-1*, a homolog of the mammalian neuromedin U receptors, which shortens *C. elegans* lifespan only on some *E. coli* food sources but not on others. Notably, the lifespan-influencing effect of *nmur-1*, which is expressed in the somatic gonad, sensory neurons and interneurons, requires the lipopolysaccharide (LPS) structure of its live *E. coli* food source. Furthermore, we found that the *nmur-1* food source-dependent regulation of lifespan can be uncoupled from its food source-dependent regulation of feeding rate, development and reproduction. Together our data suggest that *nmur-1* plays a role in processing information from different food sources to influence *C. elegans* lifespan and other aspects of its physiology.

References: (1) Alcedo and Kenyon, 2004, Neuron 41, 45-55. (2) Garsin et al., 2003, Science 300, 1921. (3) Apfeld and Kenyon, 1998, Cell 95, 199-210. (4) Wolkow et al., 2000, Science 290, 147-150. (5) Libina et al., 2003, Cell 115, 489-502.

306C

Expression mechanism of INS-18, one of the insulin-like peptides, in *C. elegans*. **Yohei Matsunaga**[1], Keiko Gengyo-Ando[2,3], Shohei Mitani[2,3], Tsuyoshi Kawano[4]. 1) Lab Bioorganic Chemistry, Tottori Univ, the United Graduate School of Agricultural Sciences, Tottori, Japan; 2) Dept Physiology, Tokyo Women's Medical Univ, Graduate School of Medicine, Tokyo, Japan; 3) CREST, JST, Saitama, Japan; 4) Dept Bioresources Science, Tottori Univ, Tottori, Japan.

The insulin/IGF-I signaling (IIS) exists in diverse species of animals. In the nematode *Caenorhabiditis elegans*, IIS regulates larval diapause and adult lifespan via a unique insulin receptor-like protein, DAF-2 followed by a transcription factor DAF-16. *C. elegans* has putative 40 insulin-like molecules, and one of them INS-18 functions as an antagonist as reported previously. Under a diapause-inducing condition, disruption of *ins-18* reduces larval diapause. In addition, under a lifespan-extending condition, the disruption cancels lifespan extension. Our interest is when and whether the gene functions in induction of diapause and extension of lifespan. To address this question, we constructed reporter gene in which *gfp* is connected to *ins-18* in a translational fusion manner, and then introduced into *C. elegans*, yielding a transgenic animal. We observed GFP luminescence under a diapause-inducing or a lifespan-extending condition. Moreover, we found DAF-16 recognition sequence in an upstream region of *ins-18*, suggesting that the gene expression is regulated by DAF-16 downstream of DAF-2. Therefore, we investigated the expression of *ins-18* under *daf-16*(-) conditions. In this meeting, we will show the results and propose a feedback model of regulation of *ins-18* expression.

307A

The physiological effects of polyphenol induced longevity. Kerstin Pietsch[1], Stephen Stürzenbaum[2], Nadine Saul[1], **Ralph Menzel[1]**, Christian Steinberg[1]. 1) Humboldt-Universität zu Berlin, Department of Biology, Laboratory of Freshwater & Stress Ecology, Späthstr. 80/81, 12437 Berlin, Germany; 2) School of Biomedical & Health Sciences, Pharmaceutical Science Division, King's College London, 150 Stamford Street, London SE19NH, United Kingdom.

The positive effects of polyphenols are well described. They have been shown to possess antioxidant activities, as well as anti-inflammatory and cancer-preventive properties. In *C. elegans*, exposure to the selected polyphenols was shown to prolong nematode's lifespan. Here we demonstrate that Quercetin could extend mean lifespan in a significant manner up to 10%, Rosmarinic acid up to 6%, and Caffeic acid up to even 13%. A variety of approaches was chosen to identify and differentiate underlying physiological mechanisms. To investigate whether food uptake is increased or diminished due to polyphenol attraction, the pharyngeal pumping rate was measured in control and polyphenol treated wild type worms at three different time points (day 3, 6 and 10 of adulthood). Moreover, to identify alterations in growth caused by polyphenol uptake, the growth rate was recorded for 8 days from L1. In addition, worms were monitored from L1 to the onset of egg laying to reveal possible developmental decelerations. To detect differences in behaviour and movement, the body waves were counted in young adults in the presence or absence of polyphenols. Nile Red staining was performed to analyze, whether the ingestion of polyphenols alters the fat storage during the adult lifespan. Finally, oxidative stress resistance tests were completed with control and treated *C. elegans*. All three compounds significantly increase thermotolerance in wild type nematodes. Additionally it could be shown that reproduction is not negatively influenced, although Rosmarinic acid and Caffeic acid affect the length of six days old adults significantly negative. Overall, tantalizing new insights were observed regarding general and compound specific modes of action of polyphenol induced longevity, findings that will be discussed in detail.

308B

C. elegans and the Disposable Soma Theory: Extended lifespan and reduced body size due to exposure to tannic acid and catechin. Nadine Saul, Kerstin Pietsch, **Ralph Menzel**, Christian Steinberg. Humboldt-Universität zu Berlin, Department of Biology, Laboratory of Freshwater & Stress Ecology, Späthstr. 80/81, 12437 Berlin, Germany.

Natural polyphenols occur, amongst others, in wine, fruits and vegetables; and several health benefits have previously been reported, especially for resveratrol, quercetin and the gallated forms of catechin. We focused our studies on the so far less observed non-gallated form of catechin and on tannic acid, which structure is based on glucose esters of gallic acid. Both polyphenols (or more precisely tannins) were found to extend the mean and median lifespan of the nematode *C. elegans*. Moreover, improved resistance to oxidative and thermal stress was monitored. Results of several further biotests suggested that hormesis, calorie restriction and simple antimicrobial or antioxidative action can be excluded as the main responsible factors for resulting longevity. On the contrary, the observed reduction in body size is in line with the Disposable Soma Theory. This theory implies that the amount of energy available to an organism is distributed to three sectors, namely maintenance, growth and reproduction. Additional energy consumption that is necessary to facilitate an increased lifespan should therefore cause an energy imbalance resulting in negative effects like reduction in body size or offspring quantity. In spite of these consistent results, there are several indices for variant background mechanisms for tannic acid and catechin action: i) life extending and growth reducing properties are much stronger under tannic acid exposure, ii) lifespan assays with various mutant strains revealed that the stress related genes, which are required for the life prolonging effect, differ between the substances, and iii) tannic acid showed higher resistance to thermal stress whereas catechin is more effective against oxidative stress. In conclusion, it is proposed that catechin and tannic acid modulate different energy-intensive stress response and repair systems, resulting in enhanced lifespan and reduced body size.

309C

The role of tissue and stress-specific activities of PRDX-2 in promoting longevity. **Monika Oláhová**, Helen M. Crook, Elizabeth A. Veal. Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, United Kingdom.

Oxidative stress-induced cellular damage is widely believed to play a fundamental role in ageing and in many diseases. However, certain reactive oxygen species, such as hydrogen peroxide, are also utilized as signaling molecules in control of diverse biological responses. As ubiquitous, abundant thioredoxin peroxidase enzymes, 2-Cys Peroxiredoxins (Prx) play a central role in responses to hydrogen peroxide. In addition to preventing oxidative damage by detoxifying peroxides, Prx have also been attributed activities as redox sensors and molecular chaperones. For instance, data suggest that thioredoxin peroxidase, chaperone and signaling activities all contribute to the important roles of PRDX-2, the *C. elegans* ortholog of the Prx1 tumor suppressor, in stress resistance [1]. Moreover, we have identified tissue-specific activities for PRDX-2 in stress resistance and longevity that provide new insight into the fundamental roles of these multifunctional antioxidants in the context of a whole animal. Although present in many tissues, like other stress-protective enzymes, PRDX-2 is abundant in the intestine, a primary site of exposure to exogenous stress. Indeed, intestinal expression of PRDX-2 completely rescues the hydrogen-peroxide sensitivity of *prdx-2* mutant worms and also increases their resistance to the oxidative stress-causing agent arsenite. In contrast, PRDX-2 in other tissues acts to inhibit phase II detoxification gene expression and *reduce* arsenite resistance. Paradoxically, the failure of intestinal PRDX-2 to increase the longevity of short-lived *prdx-2* mutant animals suggests that their accelerated ageing is also due to loss of non-intestinal PRDX-2. Together these data suggest that PRDX-2 is required for intrinsic mechanisms that promote longevity rather than through protection against environmental stress. Indeed, we find that environmental factors that influence ageing, such as bacterial growth and temperature, have minimal effects on the lifespan of *prdx-2* mutant worms. We have found that PRDX-2 is also important for the increased longevity associated with inhibition of insulin-like signaling. We will present the data from these and other studies addressing the underlying mechanisms by which PRDX-2 influences ageing. [1] Oláhová M, Taylor SR, Khazaipoul S, Wang J, Morgan BA, Matsumoto K, Blackwell TK, Veal EA. (2008) A redox-sensitive peroxiredoxin that is important for longevity has tissue- and stress-specific roles in stress resistance. Proc Natl Acad Sci U S A. 105 19839-44.

310A

A whole genome screen for checkpoint functions that determine lifespan in C. elegans. **Anders Olsen**[1], Maithili Vantipalli[2], Karla Mark[2], Dipa Bhaumik[2], Michael Benedetti[2], Xianmin Zeng[2], Adam L. Knight[3], Mike Zhang[3], Shusei Hamamichi[3], Kim A. Caldwell[3], Guy A. Caldwell[3], Gordon J. Lithgow[2]. 1) Interdisciplinary Research Consortium on GeroscienceMolec Biol, Univ Aarhus, Aarhus, Denmark; 2) Interdisciplinary Research Consortium on Geroscience, The Buck Institute, 8001 Redwood Blvd, Novato CA 94945; 3) Department of Biological Sciences, The University of Alabama Box 870344, Tuscaloosa, AL 35487-0344 USA.

Based on our previous observation that gene encoding cell cycle checkpoint functions determined lifespan in *C. elegans*, we aimed to define the genetic pathways at play. Three observations prompt this study. First we isolated a mutation in the gene checkpoint gene cid-1 which causes thermotolerance, increased lifespan and resistance to hydroxyurea (HU). Second we also found that mutations in other checkpoint genes and the tumor suppressor p53 confer resistance to HU. Third, since the nematode is post-mitotic, it is possible to study the effect of knocking down these genes in a whole organism without occurrence of lethal cancers. When wild-type eggs are placed on NGM plates spotted with HU the worms arrest development in a dose dependent manner. We screened for RNAi clones that suppressed this arrested development. All hits were re-tested at multiple doses of HU and resistance quantified as body size at three days of age. ~ 50 clones reproducibly result in HU resistance (hur genes) We are currently examining the hur genes for abnormal germline development, sterility and embryonic lethality. Many known cell cycle and checkpoint mutants show sensitivity to DNA damage. Therefore, to further test for cell cycle / checkpoint phenotypes we are exposing young worms to ionizing radiation and studying three different markers of DNA damage/checkpoint inefficiency, embryonic lethality, cell cycle arrest and apoptosis in the germline. We observed thermotolerance for 21 of the hits but not for all of them. Thus, HU resistance does not stem from a generalized increase in stress resistance but from a more specific mechanism. One of the clones increases thermotolerance of daf-2, daf-16 and eat-2 mutants and seems to function independently from insulin signaling and caloric restriction. Aging is one of the biggest risk factors for cancer. Our ongoing analysis suggests that the hur gene list is highly enriched for increased lifespan although some result in shortened lifespan. We are investigating these and similar genes in a number of neuronal cell death models including human cells.

311B

Metformin Induces a Dietary Restriction-Like State and the Oxidative Stress Response to Extend C. elegans Healthspan via an AMPK, LKB1, and SKN-1-Dependent Mechanism. **Brian D. Onken**, Monica Driscoll. Dept Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ.

Metformin, a biguanide drug commonly used to treat type-2 diabetes, has been noted to extend healthspan of non-diabetic mice, but this outcome, and the molecular mechanisms that underlie it, have received relatively little experimental attention. To develop a genetic model for study of biguanide effects on healthspan, we investigated metformin impact on aging C. elegans. We find that metformin increases nematode healthspan, slowing lipofuscin accumulation and extending median lifespan and youthful locomotory ability in a dose-dependent manner. Genetic data suggest that metformin acts through a mechanism similar to that operative in eating-impaired dietary restriction mutants, but independent of the insulin signaling pathway. Energy sensor AMPK and AMPK-activating kinase LKB1, which are activated in mammals by metformin treatment, are essential for health benefits in C. elegans, suggesting that metformin engages a metabolic loop conserved across phyla. We also show that the conserved oxidative stress-responsive transcription factor SKN-1/Nrf2 is essential for metformin healthspan benefits, a mechanistic requirement not previously described in mammals. skn-1, which functions in nematode sensory neurons to promote DR longevity benefits and in intestine for oxidative stress resistance lifespan benefit, must be present in both neurons and intestine for metformin-promoted healthspan extension, indicating that metformin improves healthy middle-aging by activating both DR and anti-oxidant defense longevity pathways. In addition to defining molecular players operative in metformin healthspan benefits, our data support that metformin may be a plausible pharmacological intervention to promote healthy human aging. Metformin has been proposed to promote DR metabolism by controlling flux through the glycolytic pathway. In keeping with this, we find a striking concentration of candidate DR inductions consequent to manipulations of the glycolytic/gluconeogenic components of metabolism. We show that disruption of glycolytic and gluconeogenic genes impacts lipofuscin accumulation, and our results suggest differential regulation of healthspan/lifespan by glycolytic and gluconeogenic pathway components. Overall, our findings identify new potential points for pharmacological induction of DR via these two metabolic processes.

312C

Novel Pathways Mediating Dietary-Restriction-Induced Longevity in C. elegans: NLP-7 Signaling and Endocytosis by Coelomocytes. **Sang-Kyu Park**, Thomas Johnson. IBG, University of Colorado at Boulder, Boulder, CO.

Dietary restriction (DR) is the most widely used intervention to promote longevity in a diverse range of organisms. Despite this, the mechanisms underlying efficacy of dietary restriction still remain elusive. The SKN-1 transcription factor mediates life extension under DR in Caenorhabditis elegans. We identified downstream targets of SKN-1 using genomic transcriptional profiling (Park *et al.,* in press). Two SKN-1-dependent genes, *nlp-7* and *cup-4*, were required for both resistance to oxidative stress and normal longevity. *nlp-7* encodes a neuropeptide-like protein and *cup-4* encodes a coelomocyte-specific ion-channel. Here, we report that *nlp-7* and *cup-4* are specifically required for DR-induced longevity in C. elegans. RNAi of *nlp-7* or *cup-4* significantly reduces the lifespan of a genetic model of DR, the *eat-2* mutant, but has no effect on the lifespan of long-lived mutants having reduced insulin/IGF-1 signaling or dysfunctional mitochondrial electron transport chain. There are several methods for imposing DR in the worm and we found that bacterial dilution also fails to increase the lifespan of *nlp-7* or *cup-4* mutants. RNAi of genes encoding candidate receptors of NLP-7 and genes involved in coelomocyte endocytosis also specifically shortens the extended lifespan of *eat-2* mutant. Based on these results, we conclude that two novel pathways, NLP-7 signaling and endocytosis in coelomocytes, are required for life extension under dietary restriction in C. elegans (supported by grants from the NIA).

313A

Increasing wild type hermaphrodite brood size does not affect life span. **Christopher L. Pickett**, Sara Collier, Brinda L. Armstead, Kerry Kornfeld. Developmental Biology, Washington University, Saint Louis, MO.

Aging is characterized by a progressive decline of tissue organization and function that correlates with chronological age. The link between aging, progeny production, and reproductive decline, however, is controversial. Antagonistic pleiotropy was proposed in the 1950s and theorized that extending life span required a reduction in progeny production, and vice versa. Consistent with this theory, loss- or reduction-of-function mutations in insulin signaling, mitochondrial electron transport, or caloric intake extend life span and often reduce progeny production in a number of organisms. However, the effects of increasing progeny production on life span have not been adequately investigated. To address this, we conducted longitudinal studies using *C. elegans* N2 hermaphrodites mated to N2 males, and we analyzed brood size, reproductive span, fast body movement span, pharyngeal pumping span, and life span. In addition, hermaphrodites were unmated or mated to sterile *fer-6(hc6)* males—these animals mate but do not transfer viable sperm. The brood sizes and reproductive spans of mated hermaphrodites were nearly double those of unmated or *fer-6(nc6)* mated hermaphrodites. We also observed that the fast body movement, pharyngeal pumping, and life spans did not significantly differ among unmated hermaphrodites or those mated to N2 males or *fer-6(hc6)* males. These data suggest that doubling brood size and extending the reproductive period does not decrease life span, contrary to the predictions of antagonistic pleiotropy. Additionally, our preliminary data suggest that different *E. coli* strains can increase cross brood size. Animals grown on HT115 have larger cross brood sizes than those grown on OP50. We draw two conclusions from these data. First, animals grown on OP50 are not maximizing their reproductive potential. Second, brood size and life span are not involved in a trade-off in hermaphrodites grown on food that does not maximize their reproductive potential. We are currently testing other bacterial strains and species to determine the maximal level of cross progeny production and the effect on life span. Furthermore, in order to analyze reproduction and life span in *C. elegans* populations, we are validating a liquid culture system where we control food, predation, and the environment.

314B

Total Worm Awareness: Longitudinal Studies of Aging Nematode Populations. **Zachary Pincus**, Frank Slack. Molecular, Cellular, and Developmental Biology, Yale University, New Haven, CT.

Within a given species, life span is surprisingly variable: for many metazoans, even genetically identical siblings reared in identical laboratory conditions can nevertheless experience dramatically different life-spans. In the case of *Caenorhabditis elegans*, identical wild-type animals can live from less than ten to more than thirty days. Understanding the sources of this variability, which may be stochastic, epigenetic, or environmental, is necessary for generating a fuller picture of the basic mechanisms of aging; for finding "biomarkers" that can predict the remaining lifespan of an organism; and, eventually, for developing anti-aging interventions and targeting them at appropriate sub-populations.

In order to understand the sources of inter-individual variability in longevity, measurements of relevant biological parameters must be made on individual, identifiable animals, so that these measures can be correlated with each individual's eventual life- and health-span (the time before senescent decline). Toward these goals, we have developed single-animal vermiculture techniques that allow life-long observation of multiple, physically separated nematodes with fluorescence microscopy.

We accomplish this by confining each animal to the upper surface of a flat and optically clear hydrogel membrane, 6mm in diameter, that is covalently attached to a glass support (itself containing many such "worm corrals"). A single egg and an OP50 food source is placed in each corral, after which the apparatus is sealed against desiccation and contamination with a clear and gas-permeable silicone elastomer. (A temperature-sensitive fertilization defect prevents further reproduction.)

We then use this system to measure space–time profiles of fluorescent reporters, with near-cell-level resolution, for multiple individuals. Image-processing tools locate the animals in each image and map fluorescence intensity onto a "standardized worm", so that tissue- and stage-specific patterns of gene expression can be compared against life-span and other quantitative image-based measures of health such as movement, pharyngeal pumping, gut autofluorescence accumulation, and maintenance of shape and turgor pressure. Further, by measuring several different parameters per individual, upstream and downstream factors can be distinguished by examining the correlation of one biomarker with life-span while controlling for the levels of the other biomarkers: in essence, performing statistical "epistasis" experiments. We have begun to examine genes in longevity pathways to determine whether variability therein underlies variability in longevity, and will present our preliminary findings.

315C

Elevated O-GlcNAc modification can extend Caenorhabditis elegans lifespan. **Mohammad M. Rahman**[1], Olga Stuchlik[3], Enas Karim[3], Lance Wells[3], Edward Kipreos[1,2]. 1) Genetics, University of Georgia, Athens, GA; 2) Cellular Biology, University of Georgia, Athens, GA; 3) Biochemistry & Molecular Biology, University of Georgia, Athens, GA.

O-linked beta-N-acetylglucosamine (O-GlcNAc) modification is an abundant nucleo-cytoplasmic post-translational glycosylation of proteins associated with age-related diseases like Alzheimer's, Parkinson's, and type II diabetes. However a link between O-GlcNAc modification of proteins and organismal aging has not been demonstrated. This work uses the nematode *C. elegans* to establish a link between nutrient availability, O-GlcNAc cycling, and longevity. We found that O-GlcNAc modification of protein(s) is critical for normal lifespan in adult animals, while unchecked O-GlcNAc modification of protein(s) increases adult lifespan in *C. elegans*. We demonstrate that the adult lifespan extension arising from an elevated level of O-GlcNAc modification is dependent on the DAF-16/FoxO transcription factor. DAF-16 is a key factor in insulin-like signal transduction, which regulates reproductive development, lifespan, and dauer formation in response to nutrient availability. Our current data indicates that O-GlcNAc cycling influences a subset of insulin-like signaling-mediated functions that regulate adult lifespan, without affecting other downstream aspects of the insulin-like signaling pathway.

316A

A variety of age-dependant aggregating proteins determine lifespan. **Pedro Reis Rodrigues**, Gregg Czerwieniec, Silvestre Alavez, Theodore Peters, Brad Gibson, Robert Hughes, Gordon Lithgow. Interdisciplinary research consortium on geroscience, The Buck Institute, Novato, CA.

Protein aggregation has for long been hypothesised as a determinant of lifespan. Briefly, normal cellular activity may give rise to damaged proteins causing them to become insoluble, missfold and aggregate. To test this hypothesis we adapted a protocol in order to extract insoluble proteins from synchronously aging populations of C. elegans. Proteins were separated based on their aqueous and detergent solubility and the insoluble fraction was resolubilized in 70%; formic acid. Insoluble proteins were chemically labelled, identified and quantified by liquid chromatography coupled with mass spectrometry (LC- ESI-MS/MS). We identified a range of proteins with roles in various cellular processes and possibly from a range of cellular compartments. 27%; of the proteins identified as forming aggregates have previously been shown to be important in keeping low levels of polyglutamine aggregation[1]. This suggests that reduced soluble levels of these proteins caused by age-related aggregation may cause increased risk of polyglutamine aggregation. We then considered whether proteins that appear to form aggregates during normal aging influenced lifespan. To test this notion we, reduced their expression in adult animals (from 4 days old) by RNA interference (RNAi). 34%; of the RNAi treatments were found to significantly extend mean lifespan in C. elegans suggesting that a variety of age-dependant aggregating proteins determine lifespan. Among the insoluble proteins, DAF-21, an ortholog of the mammalian HSP-90, showed age-dependant aggregation and is being used as a marker to study the role of several molecular pathways in protein aggregation. Taken together our results suggest that protein aggregation may play a common and key role in aging and age-related disease.

1–Nollen, E., Garcia, S., Haaften, G., Kim, S., Chavez, A., Morimoto, R., Plasterk, R., Genome-wide RNA interference screen identified previously undescribed regulators of polyglutamine aggregation. Proc Natl. Acad. Sci., 101, 6403-6408, 2004.

317B

In vivo determination of mitochondrial state during the *C. elegans* aging process. **Catalina Romero**, Javier Apfeld, Walter Fontana. Department of Systems Biology, Harvard University, Boston, MA.

Several mutations and RNAi knockdowns of mitochondrial genes affect the lifespan of *C. elegans*. Furthermore, environmental and genetic perturbations that increase lifespan, such as caloric restriction and reduction in insulin signaling, increase mitochondrial activity (Bishop & Guarente, Nature 447, 545-9, 2007; Houthoofd et al., Aging Cell 4, 87-95, 2005). We are interested in studying how alterations in mitochondrial activity lead to metabolic and physiological changes that result in an extended lifespan. We are quantifying mitochondrial matrix pH *in vivo* by targeting a pH-sensitive GFP variant to the mitochondrial matrix of muscle cells. This allows us to follow changes in mitochondrial function and morphology in single cells over time. We are currently investigating how (i) differences between individuals and (ii) differences between cells within each individual correlate with the large variation on lifespan that is observed in isogenic populations of worms. We hope that these studies will allow us to quantify the dependencies between changes in mitochondrial activity at the cellular level and the aging phenotype at the organismic level, both in wild type animals and in mutants with extended lifespans.

318C

An engineering approach to aging. **Dror Sagi**, Stuart Kim. Dept Developmental Biol, Stanford Univ, Stanford, CA.

We are taking a synthetic biology approach to aging by bio-engineering worms with increased lifespans. Our approach is to improve aging by over-expressing genes that strengthen various pathways such as stress resistance and damage repair. Worms are particularly well-suited for this project because they have the shortest life span among animal models, and thus their stress and damage repair pathways may be relatively inefficient compared to those from longer lived animals such as flies or mice. Furthermore, events that occur in old age are outside the force of natural selection, as animals in the wild die from predation and disease well before old age. Lack of natural selection in old worms may permit cellular and biochemical pathways to become dysfunctional or inefficient, and thus it seems reasonable that one could improve physiology in old worms using a directed engineering approach. To make worms live longer, we are expressing genes from the zebrafish, which lives up to 5 years (about 100 times more than C. elegans) and lives at a similar temperature. Our assumption is that stress and damage repair proteins from zebrafish need to preserve cell function for a longer time than proteins from worms, and thus zebrafish proteins may be more efficient or have higher function than their worm orthologs. We picked five pathways believed to play key roles in aging: Oxidative damage repair, mitochondria activity, protein turnover, the immune system and gene regulation. We then chose 1-4 genes known to work in each pathway, and expressed the zebrafish gene using the orthologous worm promoter. Overall, we have found six genes belonging to four pathways that increase lifespan when overexpressed. In two cases, we could extend worm lifespan by expressing proteins that are present in zebrafish, but are absent in worms. The first example is zebrafish UCP2, which is a mitochondrial uncoupling protein. Vertebrates have UCP2, whereas worms use another uncoupling protein called UCP4. We found that overexpression of zebrafish UCP2 extended lifespan, but worm UCP4 did not. One possibility is that vertebrate UCP2 extends worm lifespan because the vertebrate protein makes the mitochondria more efficient or less toxic. The second example is zebrafish lyzozyme. Vertebrates and worms use different types of lysozyme to combat bacterial pathogens. We found that zebrafish lysozyme but not worm lysozyme could extened lifespan, suggesting that the vertebrate protein may be more efficient in combating pathogenesis. We are currently trying to upgrade two (or more) pathways at the time to look for synergistic effects in extending lifespan. Our work shows that it is possible to rebuild weak links in an animal by adding parts from a longer-lived life form in order to extend lifespan.

319A

Insulin signaling pathway genes facilitating the maintenance of thermotolerance and protein homeostasis. **Andrew Samuelson**[1,2], Gary Ruvkun[1,2], Christopher Carr[1,3]. 1) Dept Molecular Biol, Massachusetts General Hosp, Boston, MA; 2) Department of Genetics, Harvard Medical School; 3) Department of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology.

The *daf-2* insulin-like signaling pathway is the most potent pathway for lifespan extension in *C. elegans*, converging on the DAF-16 transcription factor to regulate a large number of genes including free radical detoxifying genes and stress resistance genes. During aging, specific protein damage by reactive oxygen species increases exponentially to challenge protein homeostasis. Processes impacting protein homeostasis include protein synthesis, folding, assembly, repair, translocation and degradation. Activation of stress response pathways, with induction of heat shock proteins, is central to the maintenance of protein homeostasis after stress. The ability to maintain protein homeostasis depends on both chaperone-mediated protein folding (i.e. chronic maintenance) and induction of heat shock proteins (i.e. survive acute stress). Additionally, chronic growth at higher temperature shortens lifespan and accelerates aging. Protein homeostasis, as measured through protein aggregation, is controlled by insulin signaling, dependent on HSF-1 and DAF-16.

From a comprehensive functional genomic screen we have previously identified 103 genes that are necessary for decreased insulin signaling to extend lifespan. Animals are progeric after these gene inactivations by several independent measures including: premature age pigment accumulation and increased rate of aging, without drastically altering progeny production or an established biomarker for *C. elegans* aging; the activity ratio, or proportion of life an animal actively responds to stimuli. We sought to identify the progeric gene inactivations that impair the heat shock response or challenge protein homeostasis. We identified the progeric gene inactivations that are necessary for the increased thermotolerance conferred by decreased insulin signaling. Additionally, we tested the progeric gene inactivations for differential lifespan at varying temperature, premature protein aggregation (polyQ), and induction of heat shock proteins. This comprehensive functional analysis identifies the subset of progeric gene inactivations that negatively impact protein homeostasis after acute or chronic stress. Results from this analysis will be presented.

320B

Gene expression noise and aging stochasticity in C.elegans. **Adolfo Sánchez-Blanco**, Stuart K. Kim. Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

Aging stochasticity is a common property of aging in all organisms. In the case of C.elegans, a population kept under identical conditions will lead to some individuals living as much as three fold longer than others. We are trying to understand the molecular reasons for chronologically and genetically identical animals to age at different rates. In order to address this fundamental aging question we have developed a molecular odometer for aging in C.elegans. To create such an odometer, we have generated transgenic lines harboring fluorescent reporter constructs of C.elegans genes whose expression is age down-regulated. Using this molecular odometer for aging, we have been able to predict the remaining life span of same age individuals in a population while they are still young. Genetically-identical individual animals that have high expression of the aging reporter tend to live longer than siblings that have lower expression. Additionally, we have been able to identify a common source for both aging stochasticity and gene expression noise. We found that pathogenesis from E. coli affects individual worms to variable degrees, giving rise to variable levels of activation of the insulin-signaling pathway. We showed that worms grown on non-pathogenic bacteria or worms that are defective for daf-16 FOXO do not show a link between gene expression noise and aging stochasticity. In summary, we have developed a molecular odometer for aging that can predict remaining life span of individuals, and used this system to identify a major source of aging stochasticity.

321C

Interactions between insulin/IGF signaling and AMP-activated protein kinase in *C. elegans* ageing. **Matthew J. Sanders**[1], Eugene Schuster[1], Josh McElwee[1], David Carling[2], David Gems[1]. 1) Institute of Healthy Ageing, and G.E.E., University College London, Gower Street, London WC1E 6BT, UK; 2) MRC Clinical Sciences Centre, Imperial College, Hammersmith Hospital Campus, DuCane Road, London W12 0NN, UK.

Reduced insulin/IGF signaling (IIS) increases lifespan and this effect is dependent upon the DAF-16 FoxO transcription factor. However, the processes regulated by IIS/FoxO that directly control aging remain poorly understood. Previous studies have shown that genes involved in metabolism, stress resistance, cell cycle arrest and apoptosis are directly regulated by FoxO. The longevity of *daf-2* insulin/IGF receptor mutants is also dependent upon AMP-activated protein kinase (AMPK)[1,2]. This enzyme acts as a fuel gauge of the cell[3]: When the AMP:ATP ratio increases, AMPK is activated and phosphorylates key metabolic enzymes, resulting in increased catabolism and reduced biosynthesis. AMPK is a heterotrimer with a catalytic α-subunit, and regulatory β- and γ-subunits[3]. Binding of AMP to the γ-subunit activates AMPK. There is evidence that DAF-16 acts downstream of AMPK, e.g. the longevity resulting from an AMPK activation mutation is suppressed by mutation of *daf-16*, and AMPK phosphorylates DAF-16 (ref. 4).

In an attempt to identify direct targets of DAF-16, DamID (DNA adenine methyltransferase identification) was used in combination with microarray analysis (see abstract by E. Schuster et al.). Results using this approach imply that two of the five predicted AMPK γ subunits are up-regulated by DAF-16. Moreover, microarray data[5,6] reveals up-regulation of an α-subunit gene, *aak-2* and a β-subunit gene, *aakb-1*, in *daf-2* relative to *daf-16*; *daf-2*. Thus, DAF-16 is predicted to increase expression of the entire AMPK heterotrimer. This suggests action of AMPK downstream as well as upstream of DAF-16, and the presence of a positive feedback loop. We are now investigating further the relationship between IIS and AMPK in the control of aging in *C. elegans*.

1. Apfeld et al. Genes Dev. 18, 3004-9 (2004). 2. Curtis et al. Aging Cell 5, 119-26 (2006). 3. Hardie & Carling. Eur. J. Biochem. 246, 259-273 (1997). 4. Greer et al. Curr. Biol. 17, 1646-56 (2007). 5. McElwee et al. Genome Biol. 8, R132 (2007). 6. McElwee et al. J. Biol. Chem. 279, 44533-43 (2004)..

322A

A role of PKC-1 in regulation of aging in *C.elegans*. **Yutaro Sassa**[1], Yoshiyasu Ohara[1], Satoshi Itakura[1], Kazunori Kume[1], Masaki Mizunuma[1], Kohji Miyahara[2], Dai Hirata[1]. 1) Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Japan; 2) Department of applied Life Science, Sojo University.

We have found that GEI-1-RHO-1 signal-transduction pathway regulates the olfactory adaptation in AWC sensory neuron, and that PKC-1, which has been shown to affect chemotaxis and thermotaxis, acts in the downstream of the GEI-1-RHO-1 pathway. On the other hand, it has been shown that insulin-like signaling pathway plays an important role for not only adaptation in the ASER salt-sensing neuron but also regulation of aging. Therefore, we focused on the functional relationship between olfactory adaptation and aging. To investigate whether or not the gene(s) concerned with the olfactory adaptation, which performs in sensory neurons, plays an important role in regulation of aging, we measured the life-span of the mutants related to GEI-1-RHO-1 signaling pathway. We found that the life-span in *pkc-1* mutant is extended compared with wild-type strain. This extended life-span was dependent on DAF-16/FOXO. Consistent with the life-span, *pkc-1* mutant was resistant to oxidative stress. Further, the expression of *pkc-1wt* in AWC neuron in *pkc-1* mutant rescued the chemotaxis defects but not the extended life-span. These results suggest that PKC-1 plays an important role on regulation of aging, and that the regulation requires the expression of PKC-1 in the other cells except AWC neuron, or in both AWC and the others.

323B

Increased fitness in a long-lived mutant exposed to environmental stress. **Fiona. R. Savory**, Ian. A. Hope, Tim. G. Benton, Steve. M. Sait. Institute of Integrative and Comparative Biology, University of Leeds, Leeds UK.

In *C. elegans*, the conserved insulin / IGF-1 signalling pathway alters life-history, in response to environmental stress, by regulating the cellular localisation of the transcription factor DAF-16. When nuclear localised, DAF-16 diverts resources from development and reproduction to somatic maintenance and repair by activating the expression of stress response proteins. Mutants of the pathway are long-lived, have enhanced resistance to environmental stress, and display trade-offs, consistent with the evolutionary theories of senescence, which ultimately reduce lifetime fitness. Long-lived *age-1* mutants are unique because they can compete with wild type for multiple generations if food is not limiting[1]. However, fitness costs arise in these mutants during exposure to repeated cycles of starvation[1], indicating that the mutant allele disrupts the ability to adapt to fluctuations in resource availability. It remains unclear if fitness is altered in *age-1* mutants in response to additional forms of stress. To determine if trade-offs in *age-1* mutants are specific to nutrient limited conditions, we compared fitness in direct competition with wild type, by monitoring changes in genotype frequency over time, when populations of different density were exposed to various forms of environmental stress. Remarkably, *age-1* mutants displayed higher fitness than wild type following periodic exposure to either high temperature (30ºC) or oxidative stress (juglone). This was most apparent in low-density populations, where food was not limiting. The molecular basis of these population level responses was examined by comparing spatial and temporal distributions of DAF-16. Though nuclear localisation was observed in both genotypes in response to different forms of stress, this was typically more intense in *age-1* mutants. Furthermore, during prolonged exposure to stress, and after removal from stress, DAF-16 remained nuclear localised for considerably more time in *age-1* mutants than in wild type. Increased investment in somatic maintenance and repair thus appears to provide *age-1* mutants with a selective advantage over wild type, during and after exposure to thermal and oxidative stress. Though nutrient limitation is likely to be the most frequent stress encountered by *C. elegans* in nature, these results indicate that the *age-1* mutation could potentially increase toward fixation if it arose in populations which experience periodic fluctuations in temperature or oxidative stress. This is the first demonstration of a long-lived mutant having higher fitness than wild type and exemplifies that, in certain contexts, the evolutionary theories of senescence need not necessarily apply. [1] Walker *et al*. (2000). Nature 405: 296-297.

324C

How is the onset of biodemographic aging determined? **Y. Shimizu**, T. Shoyama, M. Hyodo, H. Suda. Department of Biological Science and Technology, School of High-technology for Human Welfare, Tokai University, Numazu, Shizuoka, Japan.

We have derived the equation of lifespan including the regulation system of aging such as a switching, timing, and memory. Indeed, in our model, two important parameters (t_0 and z) are contained (Shoyama et al., *Mech. Ageing Dev.* **128** (2007) 529-537). The one represents the onset of demographic aging. We have proved that another is proportional to the reciprocal of physiological decline rate. However, the biological meaning of the former parameter remains unclear. Thus, to address this issue, we approach from understanding the regulation mechanism at the molecular level. In this work, we used the long-lived *daf-2* mutant strain. Interestingly, when we quantitatively analyzed the survival curve of this mutant using the equation of lifespan, it was composed of two distinct components. The first component was close to that of *daf-16* or wild-type. This specific feature has to become a crucial key to perform our aim. On the other hand, we observed a periodic fluctuation in metabolism energy and body size after maturation. The heterogeneity revealed in biodemographic data seems to be concerned with this finding. Thus, to investigate how the onset of biodemographic aging is determined, we quantitatively analyze the survival curves obtained by varing the timing of *daf-16*-RNAi feeding, using the equation of lifespan.

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Quantitatively analyzing the respiratory activity with age in a *C. elegans* cohort. **T. Shouyama**, T. Horikoshi, H. Suda. Department of Biological Science and Technology, School of High-technology for Human Welfare, Tokai University,Numaazu Shizuoka, Japan.

Metabolic energy in humans is thought to linearly decline with age and to become zero at maximum lifespan. However, in *C. elegans*, it has been reported that it exponentially decays (B. P. Braeckman et al., 2002; T. Shoyama et al., 2007). To clarify a relationship between lifespan and aging from an energetic view, we measure oxygen consumption rates, or metabolic energy, as a function of age by using an optical oxygen-concentration-measurement apparatus that was originally developed in our laboratory. In this work, furthermore, we demonstrate whether this conclusion is established against various *C. elegans* mutant strains. As the present experimental condition, worms were grown on a UV-killed-bacterial lawn from hatching. We carefully improved the experimental condition to remove live bacteria as thoroughly as possible. As a result, we find that oxygen consumption rates exponentially decays after maturity, and those are closely related to mean and maximum lifespans. Here, we defined the physiological decline rate,λ. We also confirm that the respiratory activity is maintained in a finite value even at older age. We quantitatively analyze using the equation of lifespan and discuss in more details about this difference between humans and *C. elegans*.

References

B. P. Braeckman et al., 2002. Assaying metabolic activity in ageing *Caenorhabditis elegans*. Mech. Ageing Dev. **123**, 105-119. T. Shoyama et al., 2007. Basic principle of the lifespan in the nematode *C. elegans*. Mech. Ageing Dev. **128**, 529-537.

326B

Analysis of *C. elegans* telomere replication mutants. **Ludmila Shtessel**, Shawn Ahmed, Yan Liu. Genetics, UNC Chapel Hill, Chapel Hill, NC.

The ends of linear chromosomes, termed telomeres, are comprised of canonical repeat tracts that function to promote chromosome stability. RNA primers used by canonical DNA polymerases prevent complete replication of chromosome termini (Watson 1972). Due to this “end replication problem”, telomeres ought to progressively shorten with every round of DNA replication. However, the ribonucleoprotein telomerase is an enzyme that combats telomere erosion by adding telomeric repeats onto 3’ telomeric ends via reverse transcription of the telomeric template sequence encoded by the telomerase RNA subunit.

In order to elucidate how telomerase functions in multicellular organisms, forward genetic mutagenesis screens to identify mutations that confer progressive telomere shortening in the roundworm C. elegans were performed. Propagation of such mutants for multiple generations results in sterility and in formation of end-to-end chromosome fusions. Large-scale screens have yielded 6 mortal germline mutants with telomere replication defects, one of which corresponds to trt-1, the catalytic subunit of telomerase, as well as others that correspond to DNA damage response proteins. Mapping and characterization of several remaining telomere replication mutants will be presented.

327C

Life span of *C. elegans* is inversely proportional to the tissue level of the lipid peroxidation product 4-hydroxynonenal (4-HNE). **Sharda P. Singh**[1], Srinivas Ayyadevara[1,2], Ludwika Zimniak[1], Piotr Zimniak[1]. 1) Dept. Pharm. Tox., Univ. Arkansas for Medical Sci., Little Rock, AR; 2) Dept. Geriatrics, Univ. Arkansas for Medical Sci., Little Rock, AR.

We have previously shown that transgenic expression of 4-HNE-metabolizing glutathione transferases (GSTs) in *C. elegans* extends life span (*Aging Cell* 4: 257-271, 2005), whereas RNAi-mediated silencing of several such GSTs shortens life (*Aging Cell* 4: 299-307, 2005; *Mech. Ageing Dev.* 128: 196-205, 2007). To demonstrate that longevity is indeed affected by 4-HNE, rather than by another substrate (or function) of the GSTs whose expression was altered, we have now modulated 4-HNE levels by means distinct from manipulations of GSTs. It is unlikely that multiple interventions that utilize dissimilar reactions would all affect a substrate other than 4-HNE or its congeners. Therefore, a consistent effect on life span of several such treatments would indicate an involvement of 4-HNE.

In addition to the previously employed glutathione conjugation, two new approaches were used: lowering of 4-HNE levels by chemical low-molecular weight 4-HNE scavengers, and increasing 4-HNE levels through RNAi-mediated silencing of enzymes that metabolize 4-HNE via a reaction distinct from glutathione conjugation. In the first approach, hydralazine and carnosine were used. Both compounds were previously reported to act as 4-HNE scavengers in mammalian systems (S. Galvani *et al.*, *Free Radic. Biol. Med.* 45: 1457-1467, 2008; Y. Liu *et al.*, *Chem. Res. Toxicol.* 16: 1589-1597, 2003). We now demonstrate that treatment with both compounds extends the life span of *C. elegans*. The extension was concentration-dependent. In the second approach, *C. elegans* aldehyde dehydrogenase (Alh) isoforms were individually silenced. This resulted in a shortening of life span that correlated well with the contributions of the Alh isoforms to the whole-body capacity to oxidize 4-HNE.

Together, our results support the hypothesis that decreasing the tissue level of 4-HNE extends *C. elegans* life span, whereas elevated 4-HNE concentrations have the opposite effect. Although the mechanism of longevity modulation by 4-HNE remains unknown, our preliminary data indicate that it is at least in part mediated by the expression level of the insulin-like peptide Ins-7. *Supported by NIH grant AG028088.*.

328A

Natural variation reveals the autophagy gene unc-51 as a key connection between sex and death in C. elegans. **L. Basten Snoek**, Ana Viñuela, Evert W. Gutteling, Agnieszka Doroszuk, Joost A.G. Riksen, Jan E. Kammenga. Nematology, Wageningen University, Wageningen, Netherlands.

The cost of reproduction, i.e. a decreased lifespan due to reproduction, is a crucial trade-off which drives life-history evolution in nearly all species, including humans. A key question in evolutionary and aging research is how this trade-off is regulated in natural populations. Although some induced mutations affecting lifespan also alter the reproductive state, detailed knowledge about the genetic control of the trade-off between lifespan and offspring in natural populations is scant. We combined life-history analysis and mapping of global gene transcription profiles in a C. elegans population of recombinant inbred lines (RILs) obtained from a cross between wildtypes N2 and CB4856. We found a trade-off between lifespan and offspring across the RILs and detected 27 gene transcripts that were highly correlated to both traits in an opposite way. These genes had 20% more eQTLs, i.e. were more regulated, than the less correlated genes. Using a candidate gene approach we show that knocking-down one of these genes, the autophagy gene unc-51, with RNAi increased offspring with 30% while decreasing lifespan with 15%. By introgressing a trans-acting CB4856 locus into N2 we show that the RNAi effect on the trade-off was modulated. Together our results demonstrate that the trade-off between lifespan and offspring can be highly regulated early in life and suggest that natural variation can tune C. elegans life-history by regulating unc-51 transcription during the L3 stage. Interestingly, the results also indicate that RNAi phenotypes depend strongly on the genetic background due to epistatic interactions and trans-acting loci.

329B

Monitoring oxidative stress in aging *C.elegans*. **Maike Thamsen**, Daniela Knoefler, Ursula Jakob. MCDB, Univ Michigan, Ann Arbor, MI.

Aging is a complex physiological process and numerous aging theories have been proposed. One of the leading models is the free radical theory of aging, which suggests that the accumulation of reactive oxygen species (ROS), like superoxide (O_2^-) and hydrogen peroxide (H_2O_2), causes protein, lipid and DNA damage and leads to the observed age-related decline of cells and tissues.

To directly monitor the onset and extent of oxidative stress during the lifespan of *C. elegans*, we utilize two complementary approaches. In the first approach, we use the fluorescent H_2O_2-sensor protein HyPer (Belousov, 2006) to evaluate the accumulation of endogenous H_2O_2 in the muscle cells of *C. elegans*. This ratiometric sensor protein has two excitation maxima, which substantially change in the presence of H_2O_2. With this tool, we are now able to determine and monitor endogenous H_2O_2 levels over the whole life span of a worm population. In the second approach, we use a highly quantitative mass spectrometry based thiol trapping technique termed OxICAT to identify the protein targets of oxidative stress in aging animals. This technique allows us not only to detect and quantify oxidative thiol modifications in hundreds of different proteins in a single experiment but enables us to identify the affected proteins and to define their redox-sensitive cysteine(s).

Using this technique we were able to determine the redox status of numerous *C. elegans* proteins and monitor their age-related changes. The combination of these techniques provides us now with valuable insights into the underlying mechanism of aging and into the role that oxidative stress plays in this process.

Reference: Belousov, V.V., et al., Nat Methods, 2006. 3(4).

Maike Thamsen and Daniela Knoefler contributed equally to the work for this abstract.

330C

Requirement for the stress-responsive MAP kinase KGB-1 pathway in intermittent fasting-induced longevity in *C. elegans*. **M Uno**, S Honjoh, E Nishida. Department of Cell and Developmental Biology, Graduate School of Biostudies, Kyoto University.

Dietary restriction increases lifespan in many species and delays the onset of multiple age-related diseases. We have recently established an intermittent fasting (IF) regimen that effectively extends the lifespan of *C. elegans*, and shown that the insulin/IGF signalling effector DAF-16 plays an important role in the IF-induced longevity. In this study, we show that the *C. elegans* stress-responsive MAP kinase (MAPK) KGB-1 signalling pathway plays an essential role in the regulation of IF-induced longevity. A loss-of-function mutation in *kgb-1*, the gene encoding a JNK-type MAPK, results in marked suppression of IF-induced longevity. Loss-of-function mutations in *mek-1*, *mlk-1*, and *nsy-1*, the genes encoding an MKK7-type MAPKK, an MLK-type MAPKKK and a *C. elegans* ASK1 MAPKKK, respectively, also suppress IF-induced longevity. Although loss-of-function mutations in the components of the KGB-1 pathway apparently cause DAF-16 nuclear translocation to some extent under fed conditions, fasting-induced enhancement of its nuclear translocation is significantly suppressed in these worms. In addition, fasting-induced changes in expression of DAF-16 target genes are suppressed in *kgb-1* knockout worms. Collectively, these results demonstrate that the KGB-1 signalling pathway mediates IF-induced longevity, at least partly, through regulating DAF-16 responsiveness to fasting.

331A

Little influence of iron homeostasis on aging in *C. elegans*. **Sara Valentini**, Dan Ackerman, Filipe Cabreiro, David Gems. University College of London, London, UK., United Kingdom.

During normal metabolism, O2 is converted into reactive oxygen species (ROS) such as O2•, H_2O_2 and the particularly reactive •OH. According to the oxidative damage theory, ROS-induced damage to biomolecules is a major cause of ageing1,2. The cellular free iron pool can contribute to ROS by catalyzing the Fenton reaction, where iron (II) is oxidized by H_2O_2 to iron (III), generating •OH. Taken together, this suggests that iron homeostasis might protect against aging. Ferritins regulate the cytosolic concentration of iron by storing excess iron, and can provide protection against oxidative stress in some contexts 3,4. *C. elegans* has two ferritin genes, *ftn-1* and *ftn-2* (ref. 5). Long-lived *daf-2* mutants show a ~50-fold increase in *ftn-1* mRNA levels, raising the possibility that *ftn-1* might contribute longevity assurance. We have therefore tested the role of *ftn-1* in longevity assurance. We find that RNAi of *ftn-1*, alone or with *ftn-2*, does not affect *daf-2* mutant longevity, nor does over-expression of *ftn-1* increase lifespan. We are currently testing the effects of manipulation of ferritin levels on resistance to stress from iron (III) and H_2O_2. Overall, our results show that ferritin does not contribute to longevity assurance, and imply that iron homeostasis is not a critical determinant of aging in *C. elegans*. 1. Harman, J. Gerontol. 11, 298 (1956). 2. Beckman & Ames, Physiol. Rev. 78, 547 (1998). 3. Cozzi et al. J Biol Chem 275, 25122 (2000). 4. Balla et al. J Biol Chem 267, 18148 (1992). 5. Gourley et al. J Biol Chem 278, 3227 (2003).

332B

Elucidating aggregation pathways in a *C. elegans* model for α-synuclein inclusion formation. **Annemieke T van der Goot**[1], Tjakko J van Ham[1], Carlos W Bertoncini[2], Janet Kumita[2], Christopher M Dobson[2], Ellen A A Nollen[1]. 1) Department of Genetics, University Medical Center Groningen, the Netherlands; 2) Department of Chemistry, University of Cambridge, United Kingdom.

Fibrillar protein aggregates of misfolded proteins are the pathological hallmark of many ageing-associated neurodegenerative diseases, including Parkinson's disease (PD). The main component of these inclusions is fibrillar alpha-synuclein (AS). How these inclusions are formed and how this links to disease is poorly understood. Although these inclusions were initially thought to be pathogenic, more recent evidence suggests that they have a protective role and that it is the microscopically invisible precursors to these aggregates that are toxic to cells. We have previously established a *C. elegans* model expressing an AS-YFP fusion protein in body wall muscle cells, which allows us to monitor AS accumulation in living and ageing animals. By fluorescence recovery after photobleaching (FRAP) we have shown that, at old age, these inclusions contain immobile AS, resembling a key pathological feature in PD patients. By performing a genome-wide RNAi screen, we identified 80 genetic modifiers whose knockdown resulted in a premature increase in the number of microscopically visible inclusions (van Ham et al. 2008). We currently attempt to elucidate the effects that these genetic modifiers have on steps preceding inclusion formation. Studying the intermediates of aggregation *in vivo* has proven difficult due to the lack of methods to detect their presence and due to their transient and heterogeneous nature. Here, we show by several biophysical methods, including standard aggregation assays and transmission electron microscopy, that the AS-YFP fusion protein, *in vitro*, has similar amyloid forming properties as non-fused wild-type AS. We will report on our progress in elucidating AS aggregation pathways.

333C

Transcriptional cascades regulating *C. elegans* aging. **Eric L Van Nostrand**[1,2], Yelena Budovskaya[2], Stuart K Kim[1,2]. 1) Department of Genetics, Stanford University School of Medicine, Stanford, CA; 2) Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

Recent work in the Kim lab has identified the GATA transcription factors elt-3, elt-5 & elt-6 as a developmental circuit that becomes mis-regulated during aging, regulating lifespan. Microarray analysis of gene expression in young and old C. elegans populations led to the identification of a highly significant enrichment for a GATA sequence motif in promoters of genes with differential expression during aging. Further work determined that a developmental circuit, in which elt-5 and elt-6 act to repress elt-3 during normal development, inappropriately continues to drive elt-3 expression down with age, causing elt-3 regulatory targets to further decrease and negatively regulating lifespan. Using ChIP-Seq technology, we will identify the regulatory targets of elt-3 in order to determine whether elt-3 is chiefly responsible for age-regulated expression changes, or if the expression changes are due to the combination of a number of other regulators. In addition, a comparison of ChIP-Seq targets in young and old worms will determine whether decreased expression of elt-3 during aging affects specific targets or causes a general loss of regulation across all targets. In addition, microarray profiling identified an additional 30 age-regulated transcription factors, with a variety of functions and expression patterns. In an approach parallel to that described for elt-3, the roles of these additional regulators during aging will be determined. In particular, we will determine whether these regulators function as part of known aging regulatory circuits, or if they present additional examples of circuits that become mis-regulated during aging. Identification of regulatory targets for these additional age-regulated transcription factors by ChIP-Seq will enable the construction of a full aging expression regulatory network, allowing us to better understand the key regulatory factors controlling aging and age-dependent gene expression in C. elegans.

334A

Genetic analysis of the relationship between reproduction and longevity. **Meng Wang[1,2]**, Gary Ruvkun[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Reproduction and aging are intertwined phenomena throughout metazoan phylogeny. During aging, reproductive abilities decline and many organisms including C. elegans display a sharp decrease in progeny production, which refers to reproductive senescence. In human, the transition to reproductive senescence is associated with age-related diseases. Genetic studies have suggested that signals from the reproductive system influence organism lifespan in worms and fruit flies. In our studies, we have investigated the mechanistic interrelationship between reproduction and longevity. To investigate the molecular mechanisms underlying reproductive senescence, we have conducted a genome-wide RNA interference (RNAi) screen searching for genes whose inactivation postpones reproductive senescence. From the screen, we identified 94 genes when inactivated result in delayed reproductive senescence. Animals with reduced insulin/IGF-1 or serotonin signaling have delayed reproductive senescence, which is suppressed by inactivation of the Forkhead transcription factor. We have analyzed the genetic interactions of the newly identified genes with insulin receptor/daf-2, serotonin biosynthesis enzyme/tph-1 and Forkhead transcription factor/daf-16. These analyses have identified new factors in insulin and serotonin signaling, and also novel regulators of reproductive senescence. Further characterization has revealed the roles of these genes in regulation of stress resistance, fat metabolism and organism lifespan. These studies provide insights to the interaction between reproductive senescence and somatic aging and the mechanisms by which reproductive senescence is regulated. This work is supported by Life Sciences Research Foundation and Ellison Medical Foundation Fellowships.

335B

The Histone 3 Lysine 9 and 36 Tri-demethylase JMJD-2 Impacts *C. elegans* Longevity Through Genomic Integrity. Emily Forbes[1], James Sleigh[1], Juan Carmona[1,3], Anne Hart[1,3], **Johnathan Whetstine[1,2]**. 1) Massachusetts General Hospital Cancer Center; 2) Department of Medicine, Harvard Medical School; 3) Department of Pathology, Harvard Medical School.

Genomic stability is a major factor that influences aging and aging-related diseases. The integrity of the genome is influenced by histones and other chromosomal proteins that package and stabilize the genome as well as regulate gene expression. The posttranslational modifications of histones, especially lysine methylation, play an important role in these processes. The site and degree of methylation have specific affects on nuclear processes. For example, histone lysine 9 tri-methylation (H3K9me3) is a hallmark of chromosomal structures such as heterochromatin and telomeres and has distinct roles in gene repression. Consistent with H3K9me3 function in chromosomal stability, alterations in H3K9me3 levels have been shown to result in aging-related diseases (*i.e.*, progeria and cancer). We have uncovered a new class of enzymes that are responsible for demethylating H3K9me3. The JMJD2 protein family is conserved from worm to human and plays an important role in stabilizing the genome. Using *C. elegans*, we have established an important role for this protein in maintaining the genomic stability of the germline and in lifespan. Our initial data suggest that DNA damage response within the germline plays a fundamental role in the reduced lifespan of the jmjd-2 loss of function allele. In addition, we have observed an increase in generational lifespan that may be associated with structural changes within the chromatin and/or changes in DNA damage response. Our data with the histone demethylase begins addressing the fundamental relationship between chromatin change, tissue homeostasis and lifespan.

336C

EAK-7 and AKT-1 act through distinct and complementary mechanisms to control lifespan and dauer arrest via DAF-16/FoxO. Hena Alam[1], **Travis Williams[1]**, Sawako Yoshina[2], Shohei Mitani[2], Patrick J. Hu[1,3,4]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Physiology, Tokyo Women's Medical University School of Medicine, Shinjuku-ku, Tokyo, Japan; 3) Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI; 4) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI.

The *C. elegans* insulin receptor ortholog *daf-2* promotes reproductive development and controls lifespan by regulating nuclear translocation of the FoxO transcription factor DAF-16 via a conserved PI 3-kinase/AKT pathway. Nuclear localization of DAF-16/FoxO is necessary but not sufficient for full DAF-16/FoxO activity, suggesting that other inputs regulate the activity of nuclear DAF-16/FoxO.

We recently discovered the EAK (enhancer-of *akt-1*) pathway, which acts in parallel to AKT-1 to inhibit nuclear DAF-16/FoxO activity. Similar to mutation of other *eak* genes, mutations in the novel conserved gene *eak-7* enhance the dauer arrest phenotype of *akt-1* null mutants and increase mRNA levels of direct DAF-16/FoxO target genes such as *sod-3* in *akt-1* null mutants. However, in contrast to other EAK pathway components, which do not promote longevity when mutated, *eak-7* mutation extends lifespan by ~25% and promotes stress resistance in a DAF-16/FoxO-dependent manner. Whereas *daf-16/FoxO* mRNA levels and DAF-16::GFP subcellular localization are unaffected by *eak-7* mutation, DAF-16/FoxO protein levels are elevated in *eak-7;akt-1* double mutants compared to *akt-1* single mutants, suggesting that EAK-7 inhibits DAF-16/FoxO activity at least in part by reducing steady-state DAF-16/FoxO protein levels.

A functional EAK-7::GFP transgene is expressed in multiple tissues, including vulva, intestine, neurons, and the endocrine XXX cells. Tissue-specific expression experiments demonstrate that EAK-7 acts nonautonomously to control both lifespan and dauer arrest. Our results are consistent with a model whereby EAK-7 and AKT-1 act via distinct and complementary mechanisms to inhibit DAF-16/FoxO activity. EAK-7 and Akt/PKB may act similarly to regulate FoxO transcription factor activity in mammals.

337A

Developmental drift of GATA transcription factors during *C. elegans* aging. **Xiao Xu**[1,2], Stuart K. Kim[1]. 1) Department of Developmental Biology, Stanford University Medical Center, Stanford, California; 2) Cancer Biology Program, Stanford University Medical Center, Stanford, California.

To unveil the mechanisms underlying aging in *C. elegans*, we have carried out microarray analysis to profile changes in gene expression between young and old worms. Bioinformatics analysis revealed that the promoters of the 1254 age-regulated genes are highly enriched for the GATA binding motif. We previously found that age-related changes in expression of these genes is caused by three GATA transcription factors (*elt-3*, *elt-5* and *elt-6*)(Budovskaya *et al.*, *Cell* 2008). Here, we identify two additional GATA transcription factors (*egr-1* and *egl-27*) that may also play important roles during the aging process. We found that RNAi knockdowns of *egr-1* and *egl-27* specifically decrease *daf-2* lifespan without affecting wild type lifespan. Additionally, overexpression of *egl-27* extends lifespan, suggesting that *egl-27* normally functions to promote longevity. Expression of both *egr-1* and *egl-27* is age-regulated. *egr-1* expression decreases specifically in the head with age, while *egl-27* expression increases as worms age. In addition to containing GATA DNA binding domain, EGL-27 and EGR-1 are homologous to mammalian metastasis tumor antigen 1 (MTA1) family, which functions as part of the nucleosome remodeling and decacetylase (NuRD) complex. This raises the possibility that changes in these proteins in old worms affect not only the GATA transcriptional network but also chromatin structure in general.

338B

Inhibition of eIF2Bδ/F11A3.2 during adulthood extends lifespan in C. elegans. **Atsushi Yamaguchi**, Daisuke Tohyama. Dept. of Neurobiology, Graduate School of Med, Chiba University, Chiba, Chiba, Japan.

The critical role of protein synthesis in regulating lifespan has been evidenced. This study shows that adult-onset RNAi inactivation of eukaryotic initiation factor 2Bδ (eIF2Bδ/F11A3.2), a subunit of eIF2B, extends the mean lifespan of Caenorhabditis elegans. eIF2B is a GDP-GTP exchange factor for eIF2—a rate-limiting factor for protein synthesis initiation. 35S-Methionine incorporation assay showed that global protein synthesis is reduced by eIF2Bδ/F11A3.2 RNAi. Inhibition of eIF2Bδ/F11A3.2 during adulthood conferred thermal and oxidative stress resistance, and reduced the fecundity and fat storage. Lifespan extension by adult-onset eIF2Bδ/F11A3.2 RNAi is suppressed in FOXO transcription factor daf-16 deletion mutants. Adult-onset eIF2Bδ/F11A3.2 RNAi enhances the nuclear accumulation of DAF-16, and increases the expression of stress-resistant genes including hsp-16.2, hsp-70, hsp90, and sod-3, some of which are reported to be targets of DAF-16. Adult-onset eIF2Bδ/F11A3.2 RNAi in daf-16 mutants reduced fecundity, but did not extend lifespan. Furthermore, adult-onset eIF2Bδ/F11A3.2 RNAi did not extend the lifespan of germline-defective glp-4 organisms. Thus, it is possible that eIF2Bδ/F11A3.2 RNAi during adulthood prolongs lifespan via daf-16, which induces stress resistance in organisms.

339C

Regulation of steroid hormone signaling by the somatic reproductive tissues of *C. elegans*. **Tracy M. Yamawaki**, Mark McCormick, Cynthia Kenyon. Dept Biochemistry, Univ California, San Francisco, San Francisco, CA.

The reproductive system of *C. elegans* influences the lifespan of the rest of the animal. Removal of the germ cells extends lifespan by approximately 60%. This increase requires the presence of the somatic gonad, since removal of the whole gonad, both the somatic gonad and germ cells, does not extend lifespan. How the somatic gonad promotes an increase in longevity of other tissues remains an intriguing question.

Here, we present data suggesting that the somatic gonad promotes longevity through the activity of the DAF-9/DAF-12 steriod signaling pathway. It has been demonstrated that lifespan extension in animals that lack the germ cells requires the activity of both DAF-12, a nuclear hormone receptor, and DAF-9, a cytochrome P450 involved in the synthesis of the DAF-12 ligand. We find that the presence of the somatic gonad is required for the transcription of potential DAF-12 target genes when there are no germ cells. Furthermore, an increase in the amount of the DAF-12 ligand, dafachronic acid, extends the lifespan of animals that lack both the germ cells and the somatic gonad, but does not extend the longevity of animals with an intact gonad. Intriguingly, these results suggest that in the absence of the germline, the DAF-12/dafachronic acid complex promotes longevity whereas in the presence of the germline, the DAF-12/dafachronic acid complex suppresses a long lifespan.

340A

SOD-1 Deletions in *Caenorhabditis elegans* Alter the Localization of Intracellular ROS and Show Molecular Compensation. **Sumino Yanase**[1,2], Akira Onodera[2], Patricia Tedesco[3], Thomas E. Johnson[3], Naoaki Ishii[2]. 1) Dept Health Sci, Daito Bunka Univ, Saitama, Japan; 2) Dept Mol Life Sci, Tokai Univ Sch Med, Kanagawa, Japan; 3) Inst Behav Genet, Univ Colorado, Boulder, USA.

Superoxide dismutase (SOD) is an enzyme that catalytically removes the superoxide radical ($\cdot O_2^-$) and protects organisms from oxidative damage during normal aging. We demonstrate that not only the cytosolic $\cdot O_2^-$ level but also the mitochondrial $\cdot O_2^-$ level increased in the deletion mutants of *sod-1* gene encoding Cu/Zn SOD in *Caenorhabditis elegans* (*C. elegans*). Interestingly, this suggests that the activity of SOD-1, which so far has been thought to act mainly in cytoplasm, helps to control the detoxification of $\cdot O_2^-$ also in the mitochondria. We also found functional compensation by other SODs, especially the *sod-5* gene, which was induced several fold in the mutants. Therefore, the possibility exists that the compensative expression of *sod-5* gene in the *sod-1* deficit is associated with the insulin/insulin-like growth factor-1 (Ins/IGF-1) signaling pathway, which regulates longevity and stress resistance of *C. elegans*, because the *sod-5* gene may be a target of the pathway (1).

Reference (1) Yanase, S. and Ishii, N. (2008) Hyperoxia exposure induced hormesis decreases mitochondrial superoxide radical levels via Ins/IGF-1 signaling pathway in a long-lived *age-1* mutant of *Caenorhabditis elegans*. Journal of Radiation Research **49**(3): 211-218.

341B

Real-time in vivo proteomics: elucidating the contribution of translational dynamics in the maintenance of protein homeostasis at single-cell resolution. **Weiqun Yu**, Todd Lamitina. Physiology, University of Pennsylvania, Philadelphia, PA.

The accumulation of damaged or denatured proteins is a major consequence of diverse environmental stressors, normal physiological ageing, and diseases of protein aggregation. Protein homeostasis is the process by which cells prevent the accumulation of damaged or denatured proteins. Regulation of protein homeostasis involves the clearance of damaged proteins, as well as the synthesis of new proteins. Although the synthesis of new proteins by the ribosome makes a major contribution to the protein homeostasis equation, little is known about how the properties of the ribosome, such as overall ribosome number, spatial localization, and translational rates and accuracy, are influenced by disruptions in protein homeostasis. To investigate the role of the ribosome in the maintenance of protein homeostasis, we generated a GFP-tagged large subunit ribosomal protein (rpl-12p::RPL-12-GFP). Genetic evidence suggests that the RPL-12-GFP fusion protein can rescue rpl-12(lf) phenotypes. Biochemical analysis of RPL-12-GFP transgenic animals shows that all GFP signal is present in actively translating ribosomes and that none of the protein is detectable in a free form. Therefore, in this strain, GFP fluorescence reports the localization and number of functional ribosomes in vivo. Analysis of RPL-12-GFP expression shows that ribosome number is strongly regulated through development. In adults, ribosomes are most enriched in the intestine and spermatheca. Preliminary analyses suggest that ribosome number, as well as the relative proportion of translating polysomes, are strongly downregulated during normal physiological ageing. Currently, we are investigating whether the decrease in ribosome number is a stochastic effect of ageing or is regulated by ageing signaling pathways. Our studies provide the first cell biological analysis of the ribosome in C. elegans. These and other methods that we are developing for the in vivo analysis of real-time ribosome function at single-cell and subcellular resolution will help to elucidate the role of translational regulation in the complex process of protein homeostasis.

342C

Quinic Acid Extends Lifespan and Improves Stress Resistance of C. elegans by Scavenging Free Radicals and Up-regulating sir-2.1 and daf-16 Expression. **Longze Zhang**[1], Junjing Zhang[1], Xi Zhao-Wilson[2], Baolu Zhao[1]. 1) Institute of Biophysics, Chinese Academy of Sciences, Beijing, Beijing, China; 2) BioMarker Pharmaceuticals, Inc. 5941 Optical Court, San Jose, CA 95138, USA.

Quinic acid (QA) is an active ingredient of Cat's Claw (Uncaria tomentosa) which is used in Traditional Chinese Medicine and found to be active in enhancing DNA repair and immunity in model systems. However, it is not known whether QA could play a role in anti-aging. Here we report that QA could extend C. elegans lifespan by 11.4% under normal culture conditions, 17.8% under thermal stress, and 29.7% under oxidative stress. Both sir-2.1 and daf-16 are required for QA to extend the worm lifespan, which suggests that QA causes worms to live longer in a signaling pathway involved of sir-2.1 and daf-16. In the QA-treated worms, the downstream DAF-16-targeted genes, sod-3 and hsp-16.2, were consequently up-regulated. However, hsp-16.2 was not indispensable for the lifespan-extending effect of QA suggesting that HSP-16.2 was only one of the effectors for QA. Meanwhile, QA exhibited an ability to keep the Reactive Oxygen Species (ROS) at a lower level in worms by directly and indirectly scavenging free radicals. The ability of QA to extend worm lifespan combined with the association of QA with sir-2.1, daf-16, sod-3, hsp-16.2 and ROS demonstrates that QA possesses great potential in anti-aging.

343A

Understanding tissue-specificity of DAF-16/FOXO activity in gene regulation and lifespan control. **Peichuan Zhang**, Cynthia Kenyon. Dept Biochem & Biophysics, Univ California, San Francisco, San Francisco, CA.

In long-lived mutant animals with decreased insulin/IGF-1 signaling activity, expression of many longevity-associated genes requires the forkhead transcription factor DAF-16/FOXO [1]. In order to understand the regulatory mechanisms for longevity control by DAF-16/FOXO, we have analyzed how DAF-16/FOXO functions in animal's tissues to control the expression of its downstream target genes. We have found that DAF-16/FOXO acts in a cell-autonomous fashion to up- or down-regulate many of its target genes in long-lived daf-2 mutant animals. In our analysis of dod-8, a target gene that is regulated cell autonomously by DAF-16/FOXO, we find that DAF-16/FOXO can bind to both DAF-16/FOXO-associated element (DAE) [1] and other non-canonical sub-optimal sites that are located within the dod-8 promoter. Moreover, the DAE site is essential for in vivo expression of dod-8 in daf-2 mutant animals. Interestingly, we have also found that nematode GATA factors also bind to the DAE site. We are currently addressing potential co-regulation of dod genes by DAF-16/FOXO and GATA factors. Long-lived animals with activation of DAF-16/FOXO only in the intestine display enhanced locomotion behavior at mid-age and increased lifespan [2]. In accordance with such benefits, we have found that intestinal DAF-16/FOXO activity acts cell non-autonomously to protect muscles that lack daf-16/foxo from the deterioration that accompanies normal aging. Thus, intestinal DAF-16 must regulate certain signaling pathway(s) that influence the rate of aging of other tissues. Using RFP fusions, we showed that intestinal DAF-16/FOXO can turn on expression of certain DAF-16-regulated genes in daf-16(-) tissues. Such cell non-autonomous regulation likely underlies the ability of intestinal DAF-16/FOXO to promote the survival of daf-16(-) tissues. We are currently analyzing candidate RNAi clones that perturb the cell non-autonomous gene regulation by DAF-16/FOXO to identify the downstream signaling pathway(s) that mediate tissue crosstalk and longevity control. Reference: 1)Murphy, C. et al. (2003) Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 427:277-83. 2) Libina, N., Berman, J., and Kenyon, C. (2003) Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. Cell 115:489-502.

344B

Distinct control of survival, and somatic and germline development during L1 diapause by the insulin/IGF signaling and AMPK pathways. **Masamitsu Fukuyama**[1], Kensuke Sakuma[1], Riyong Park[1], Yuriko Atsumi[1], Yumi Shimomura[1], Hidefumi Kasuga[1], Ann Rougvie[2], Toshiaki Katada[1]. 1) Dept of Pharmaceut Sci, Univ Tokyo, Tokyo, Japan; 2) Dept of GCD, Univ of Minnesota, MN, USA.

Environmental cues have great impact on developmental progression and physiology in many species of animals. Under nutrient-poor conditions, newly hatched *C. elegans* larvae can suspend larval development (L1 diapause) and sustain viability until food becomes available. Although previous studies have shown that the insulin/IGF signaling (IIS) and AMPK pathways are involved in these developmental and physiological responses (1-4), the precise roles of these pathways remain to be delineated.

We found that activation of the IIS signaling pathway only in the hypodermis can trigger multiple events of L1 development in somatic tissues even under the starvation conditions. In contrast, experiments using the *rrf-1* mutant background suggest that germline development is cell-autonomously regulated by the IIS and AMPK pathways during L1 diapause. Previous studies suggested that the IIS pathway controls germline proliferation in a *daf-16/foxo*-independent manner (2). In addition to *daf-16/foxo*, TORC1 (TOR complex 1) is another downstream effector of the IIS pathway, and also acts downstream of AMPK in mammalian cultured cells. Consistent with this, we observed that interference of genes encoding TORC1 as well as its activators significantly suppresses abnormal germline proliferation in both *daf-18/pten*, a negative regulator of the IIS pathway, and *aak-1,2/ampk* mutants during L1 diapause. In addition to the IIS and AMPK pathways, amino acids have been shown to regulate activity of TORC1 in mammalian cultured cells. Reminiscent of this, we previously reported that essential amino acids, accompanied with energy sources and salts, could activate L1 development like *E. coli* (4).

On the other hand, rescue experiments suggest that *daf-18/pten* in the hypodermis and intestine, and *aak-1,2/ampk* in sensory neurons and intestine play an important role in regulating survival during L1 diapause. Collectively, these findings illustrate that distinct sets of tissues control survival and, germline and somatic development during L1 diapause through the IIS and AMPK pathways. Further identification and characterization of the components of these pathways will shed the light on the molecular mechanism underlying the developmental and physiological responses of L1 larvae to nutrients and starvation. 1: *Genetics* 163, 171. 2: *Curr Biol* 16, 773. 3: *Curr Biol* 16, 780. 4: Fukuyama et al. 16th International *C. elegans* Meeting.

345C

The O-GlcNAc Modification Modulates Nutrient Stress and Differentiates DAF-2/DAF-16 Phenotypes in *C. elegans*. **Michelle A. Mondoux**[1], John A. Hanover[2], Michael W. Krause[1]. 1) Laboratory of Molecular Biology, NIDDK/NIH, Bethesda, MD; 2) Laboratory of Cell Biochemistry & Biology, NIDDK/NIH, Bethesda, MD.

O-linked-N-acetyl glucosamine (O-GlcNAc) is a post-translational modification implicated in cellular processes including transcription, translation, proteasome activity, and the stress response. Over 500 proteins are O-GlcNAc modified, including components of the insulin signaling pathway. Over expression of the O-GlcNAc transferase OGT-1 or inhibition of the O-GlcNAcase OGA-1 causes insulin resistance in mammalian cells, and OGA-1 is a human type II diabetes susceptibility locus. Glucose levels influence O-GlcNAc modification levels, suggesting that this pathway acts as a nutrient sensor. Although these enzymes are essential in mammals and highly conserved in *C. elegans*, knockouts of *oga-1* and *ogt-1* are viable in the worm, making it a good model system for genetic studies of the O-GlcNAc modification and its effect on nutrient and insulin signaling. Previous work in our laboratory demonstrated that loss of OGT-1 in a *daf-2* mutant partially suppresses dauer formation. We find that loss of OGA-1 results in a similar decrease in dauer formation in *daf-2* mutants, suggesting that O-GlcNAc cycling is important in insulin signaling. We also find that dauer formation in *daf-2 ogt-1* and *daf-2;oga-1* double mutants is DAF-16 dependent. In order to define the role of O-GlcNAc modification in insulin signaling, we tested how *ogt-1* and *oga-1* mutations affect other DAF-16-dependent *daf-2* phenotypes. We find that *oga-1* and *ogt-1* have different effects on different *daf-2* phenotypes: although both mutations suppress dauer formation, they have no effect on *daf-2* fertility and have opposing effects on *daf-2* lifespan (collaboration with CA Wolkow). Since the mammalian DAF-16 homolog FOXO1 is O-GlcNAc modified, we are testing whether DAF-16 is O-GlcNAc modified and how the modification could affect its localization, activity, and transcriptional program. Since O-GlcNAc levels respond to nutrient levels, we also tested whether this pathway is involved in the response to excess nutrition. We find that *ogt-1* mutants, but not *oga-1* or *daf-2* mutants, show decreased fertility on high glucose (3-5X reduced vs. N2). These data suggest that O-GlcNAc modification, but not insulin signaling, is necessary for the response to nutrient stress. However, high glucose suppresses dauer formation in *daf-2, daf-2 ogt-1* and *daf-2;oga-1* mutants, but not in the TGF-β mutants *daf-1* and *daf-7*, indicating that this effect is specific to insulin signaling but independent of O-GlcNAc. We plan to exploit this phenotype in a genetic screen for novel factors that connect nutrient stress and insulin signaling.

346A

C. elegans as a model system to understand environmental influence on germline health. **Patrick Allard**, Monica Colaiácovo. Genetics, Harvard Medical School, Boston, MA.

Although it is clear that environmental toxicants can alter reproductive ability, the dissection of the affected molecular pathways has been particularly challenging. We propose to use *C. elegans* to investigate the genetic mechanisms of meiotic disruption following environmental exposure. As a proof of principle, we concentrated our research on characterizing the biological effects of Bisphenol-A (BPA), a compound commonly used for the production of polycarbonate-containing plastics. Previous work in mice has shown that exposure to BPA *in utero* leads to aberrations during prophase of meiosis I including incomplete synapsis, end-to-end chromosome fusions, and an increased number of recombination foci corresponding to elevated recombination frequencies and altered exchange distribution. These defects likely result in increased chromosome nondisjunction as highlighted by the greater number of aneuploid eggs and embryos observed. Assessment of the fertility of adult hermaphrodites in *C. elegans* following exposure to BPA revealed that BPA causes a dramatic increase in embryonic and larval lethality as well as a decrease in the total number of eggs laid compared to control (exposed to vehicle). A two-fold increase in germ cell apoptosis is detected in BPA-exposed worms suggesting the activation of a DNA damage checkpoint that may stem from a defect in DNA double-strand break repair. Analysis of chromosome morphogenesis in control gonads revealed the presence of 6 DAPI-stained bodies, representing the six pairs of attached homologs in oocytes at diakinesis. In contrast, an aberrant DNA morphology is observed at this stage in BPA-exposed worms. Specifically, bivalents were decondensed and chromatin bridges were apparent. Furthermore, disassembly of the synaptonemal complex (SC) is impaired during late prophase as indicated by an incomplete unloading of SYP-1, a structural component of the SC, from chromosomes in late diakinesis. Taken together, these data demonstrate that exposure to BPA disrupts the meiotic machinery in *C. elegans*. We are currently further characterizing these meiotic defects by examining recombination levels and the expression of other synapsis components in BPA-exposed animals.

347B

Targeting DNA Repair to Enhance Radio-sensitivity in Tumor Stem Cells of *C. elegans*. **Xinzhu Deng**[1], Diana Rothenstein[1], Zvi Fuks[1,2], Richard Kolesnick[1]. 1) Dept Molecular Pharmacology, Sloan-Kettering Inst, New York, NY. 10021; 2) Dept radiation oncology, Sloan-Kettering Inst, New York, NY. 10021.

The *C. elegans* germ line presents several biological features similar to those of human cancer stem cells (CSCs). First, the germ line contains a small population of self-renewing cells represented as germ stem cells. Second, the distal tip cell (DTC) provides a niche that expresses the LAG-2 proliferative signal to the germ stem cell GLP-1/Notch receptor ortholog. Further, the *C. elegans glp-1* mutant *glp-1(ar202)* exhibits constitutive activity of GLP-1/Notch signaling, generating a germline tumor with a phenotype highly reminiscent of the behavior of human cancers. This germ stem cell population is resistant to apoptotic stimuli and has a high DNA repair capacity, resulting in resistance to the lethal effects of ionizing radiation (IR). The goal of this study is to identify and target genes that are responsible for the radiation resistance in the *glp-1(ar202)* CSC tumor model. In preliminary studies, we have developed an optimized readout system to assess IR-induced *glp-1(ar202)* tumor response, as a baseline for mechanistic studies to assess the role of DNA damage repair enzymes in the radioresistance phenotype. We also confirmed that *glp-1(ar202)* CSC death by IR is not through apoptosis, but rather appears akin to the mitotic death conferred by radiation treatment of most mammalian solid tumors. Further, we targeted *rad-51* and *mre-11*, genes that regulate homologous recombination (HR) and found that their silencing by RNAi significantly increased sensitivity of the germline tumor to IR, reducing the IR dose required to eradicate *glp-1(ar202)* tumors by 50%. We are also using immunostaining of DNA repair foci to quantitatively study the role of HR and other DNA repair pathways in the *glp-1(ar202)*tumor model response to IR. We anticipate that some gene products on these pathways will have a major impact on radiation sensitivity, and may lead to discovery of new targets for enhancing radiotherapy sensitivity in human cancer.

348C

PNC-1 Modulation of Nicotinamide Levels and NAD+ Biosynthesis Separately Impact Distinct Aspects of *C. elegans* Reproductive Development. **Tracy Vrablik**[1], Li Huang[2], Wendy Hanna-Rose[1]. 1) Dept Biochemistry & Molecular Biology, Pennsylvania State Univ, University Park, PA; 2) Institute for Genetic Medicine, Univ of Southern California, Los Angeles, CA.

NAD^+ is vital to cellular metabolism and is an obligate co-substrate for NAD^+ consumers, which affect processes ranging from lifespan and stress response to circadian rhythms. Cells regenerate the NAD^+ cleaved by NAD^+ consumers via the NAD^+ salvage pathway. We have uncovered surprisingly specific roles for the salvage pathway in *C. elegans* reproductive development. Hermaphrodites with mutant PNC-1, the first enzyme in the NAD^+ salvage pathway, have an egg-laying defect, temporally delayed gonadogenesis and abnormal death of uv1 cells. The nicotinamidase activity of PNC-1 is essential for these processes as ubiquitous expression of two different nicotinamidases robustly rescues the defects, while a catalytically dead version does not. In addition to probing the novel roles for NAD^+ regulation of development, these phenotypes serve as models to elucidate the mechanisms of how NAD^+ salvage influences organismal physiology.

PNC-1 hydrolyzes nicotinamide (NAM) to nicotinic acid (NA) in the NAD^+ salvage pathway, thereby reducing NAM levels and increasing NA and subsequently increasing NAD^+. NAM is a potent inhibitor of NAD^+ consumers, and studies in yeast demonstrate that Pnc1p promotes Sir2-mediated longevity. However, the relative importance of decreasing NAM versus increasing NAD^+ on Sir2p regulation has been unclear. We performed pharmacological analysis to determine whether the developmental defects of *pnc-1* mutants were due to increased NAM or decreased NA. Supplementing wild-type worms with NAM recapitulates the uv1 and egg-laying defects, but not the gonad timing defect. Interestingly, supplementing *pnc-1* mutants with NA restores only the gonad timing. Injecting worms with a different NAD^+ precursor, nicotinamide mononucleotide (NMN), also robustly rescued only the gonad timing defect, indicating that NA acts by increasing NAD^+. We conclude that PNC-1 modulates both NAM and NAD^+ levels in reproductive organ development, with each having distinct, tissue-specific roles. Interestingly, vertebrates employ a different salvage pathway, using NAMPT to convert NAM to NMN as the first step. PNC-1 and NAMPT are functionally equivalent since ubiquitous expression of human NAMPT rescues *pnc-1* reproductive defects. Mammalian NAMPT is secreted, and we discovered an isoform of *C. elegans pnc-1* with a predicted secretion signal. We confirmed secretion of this isoform by its ability to rescue from a distance. Therefore we postulate an evolutionarily conserved extracellular role for NAD^+ biosynthetic enzymes.

349A

Bacteria not normally pathogenic to humans evaluated in a *C. elegans* pathogen challenge model. **John Joseph Peloquin**, Diamond V Technical Center staff. DiamondV/Embria Technical Ctr, Cedar Rapids, IA. 52404.

Diamond V Mills produces food and feed supplements produced by our proprietary fermentation processes. The universe of products and potential new product prototypes that could be derived from our processes and available raw materials is vast. It is thus impractical to screen our many product prototypes in vertebrates to distinguish the most promising for further evaluation from prototypes of less potential. We have developed a number of initial *in-vivo* and *in-vitro* technologies for this purpose. In one of these techniques, we look for *C elegans* survivorship changes in the presence of our prototypes. If we were to challenge the worms with pathogen stress that we expect to significantly decrease worm survivorship, we could terminate our screening assays shortly after the deaths of the pathogen challenged controls and thus decrease the time needed to get a useful answer. We are always concerned about safety of the pathogens we use to humans and animals, so we are evaluating for use in this assay species and strains of bacteria known to infect and kill invertebrates but that are not expected to be pathogens to humans and other mammals under our experimental conditions.

350B

osm-8 encodes a mucin-like protein that negatively regulates osmotic stress responses. **Anne-Katrin Rohlfing**, Yana Miteva, Todd Lamitina. Department of Physiology, University of Pennsylvania, Philadelphia, PA.

Osmoregulation is involved in many physiological and pathophysiological processes, including cell growth, apoptosis, immunity, and renal function. While it is well-established that animals cells can transduce osmosensory signals, the molecular identification of animals cell osmosensors has remained elusive. We are using genetic approaches in the nematode *C. elegans* to identify genes that may function as osmosensors, as well as components of osmosensory signal transduction pathways. Following exposure to hypertonic stress, *C. elegans* synthesizes the organic osmolyte glycerol to balance intracellular and extracellular osmolarity without altering cytoplasmic ionic strength. Glycerol accumulation is preceded by the rapid transcriptional activation of the glycerol biosynthetic enzyme gpdh-1 in the intestine and hypodermis, which can be visualized in live animals with a *gpdh-1p::GFP* reporter. Using this reporter, we screened for existing and new mutants that either positively or negatively regulate *gpdh-1p::GFP* expression. One mutant identified in these studies, *osm-8(n1518),* is resistant to acute and chronic osmotic stress (*osr* phenotype), exhibits strong constitutive expression of the *gpdh-1p::GFP* reporter, and contains high glycerol levels under isotonic conditions. We positionally cloned osm-8 and found that it encodes a secreted mucin-like protein. *osm-8p::GFP* reporters, as well as cell-type specific rescue experiments all suggest that the OSM-8 protein is secreted from the apical membrane of the hypodermis. Using a heat shock inducible *osm-8* transgene, we found that expression of OSM-8 in L4 animals, but not adults, is sufficient to rescue *osm-8* mutants. During this period, the adults cuticle is synthesized and secreted by the hypodermis. To determine if *osm-8* mutant phenotypes were the result of cuticular disruption, we stained *osm-8* mutants with a DPY-7 antibody, which marks cuticular furrows. No striking defects in the organization of furrows were noted in *osm-8* mutants as compared to wild type animals. Based on these data, we hypothesize that OSM-8 plays a role in the establishment of an extracellular osmosensory complex, but not in the acute transduction of osmosensory signals. Currrently, we are using genetic suppressor screening to identify genes that function downstream from *osm-8* to transduce osmosensory signals, as well as to identify other genes that may link *osm-8* sensory complexes to these signal transduction pathways.

351C

Genetic and physiological activation of osmosensitive gene expression mimics transcriptional signatures of pathogen infection in *C. elegans*. **Anne-Katrin Rohlfing**[1], Yana Miteva[1], Sridhar Hannenhalli[2], Todd Lamitina[1]. 1) Department of Physiology, University of Pennsylvania, Philadelphia, PA; 2) Department of Genetics, University of Pennsylvania, Philadelphia, PA.

The soil-dwelling nematode *C. elegans* is a powerful model system for molecular analyses of innate immunity, which is the first line of cellular defense against invading pathogens. Exposure of worms to diverse types of bacterial and fungal pathogens causes the activation of well-characterized innate immune transcriptional programs in pathogen-exposed hypodermal and intestinal tissues. Here, we show that infection-activated transcriptional responses are, in large part, recapitulated by either physiological or genetic activation of the osmotic stress response. Microarray profiling of worms exposed to non-lethal levels of hypertonicity identified a set of genes that were also regulated by bacterial and fungal pathogens, including anti-microbial peptides (AMPs), caenicins, and C-type lectins. Expression profiles of five different osmotic stress resistant (*osr*) mutants, which cause the constitutive activation of osmotic stress responses, reiterated the wild type response to osmotic stress and also showed substantial similarity to infection-induced gene expression, suggesting that the activation of immunity genes by osmotic stress is a genetically regulated response. Like infection regulated genes, the promoters of osmotically regulated genes are enriched for GATA transcription factor binding sites. Both physiological and genetic activation of an osmotic stress specific reporter (*gpdh-1p::GFP*) requires the presence of consensus GATA binding sites in its promoter, suggesting that regulation by GATA factors is direct. Finally, we show that two GATA transcription factors previously implicated in pathogen-induced transcriptional responses, *elt-2* and *elt-3*, are also essential for coordinated tissue-specific activation of osmosensitive gene expression in the intestine (*elt-2*) and hypodermis (*elt-3*). *elt-2(RNAi); elt-3(gk121)* double mutant animals survive normally in isotonic environments but exhibit reduced survival in hyperosmotic environments as compared to either single mutant alone, suggesting that these GATA factors play functionally redundant roles in osmotic adaptation, in addition to their previously described roles in immunity. Together, our data suggest infection and osmotic stress elicit similar transcriptional responses that might be regulated via overlapping signaling mechanisms targeting GATA-type transcription factors.

352A

Systematic studies of ABC transporters function in heavy metal detoxification. Andy Chen, **Olena K. Vatamaniuk**. Dept Crop & Soil Sci, Cornell Univ, Ithaca, NY.

Understanding the cellular mechanisms of heavy metal detoxification is critical for the cure and prevention of heavy metal-caused diseases, such as neurodegenerative conditions, dysfunction of vital organs and cancer. In this regard, members of one of the largest and structurally-conserved family of integral membrane proteins, ATP-binding cassette (ABC) transporters have been shown to be involved in metal detoxification. Over 60 family members are known in *C. elegans*, 48 in humans, 57 in *Drosophila*, 103 in *Arabidopsis*, 30 in *Saccharomyces cerevisiae,* and 11 in *Schizosaccharomyces pombe*. However, how each one contributes to metal detoxification and the relationship among pathways are not completely understood. To fill this void, we initiated systematic screens of the *C. elegans* ABC transporters function in heavy metal detoxification. Fifty two mutant alleles with lesions in 44 ABC transporters genes are available at the *C. elegans* Genetics Center. We analyzed deletion alleles corresponding to lesions in 43 ABC transporter genes. In our screens, adult worms were placed individually on OP50-seeded NGM plates with or without $CdCl_2$ (50 or 75 μM) and allowed to lay eggs for 8 h at 20°C, after which adult worms were removed. After 4 days of culturing, larval arrest or developmental defects of the progeny were used as criteria of Cd^{2+} sensitivity for selecting ABC transporters with putative roles in metal detoxification. In doing so, we identified that nine ABC transporters, in addition to reported previously, HMT-1, MRP-1, PGP-1 and PGP-3 (Broeks et al 1996, Vatamaniuk et al 2005), function in detoxification of Cd^{2+}. Phylogenetic analyses of family members revealed that ABC transporters functioning in metal detoxification cluster together, allowing to identify "heavy metal tolerance sub-clusters" among the subfamily members. In addition, relating data from the mutants screens with data from phylogenetic analyses identified several yet unscreened ABC transporters that deserve closer scrutiny. Currently, we are performing targeted RNAi screens to determine the role in metal detoxification of the remaining ABC transporters for which mutant alleles are not available. In parallel, we study the relationship among the identified in our screens ABC transporters using epistasis analyses. Our data show that our screens have a potential to identify new pathways and ascribe functions to known pathways that have not previously been determined to be involved in metal detoxification, and thus to contribute to understanding of cellular resources for metal detoxification.

353B

The requirement of stress response and autophagy to cope with abnormal sensory ray morphogenesis. **Agnes K. Y. Hui**, King L. Chow. Biology, HKUST, Hong Kong, Hong Kong.

ER stress is generated by accumulation of misfolded proteins, deprivation of calcium ion or change in redox environment. Unfolded protein response (UPR) pathways are selectively activated by the ER stress to maintain the ER homeostasis. Autophagy/lysosomal degradation pathway and cell death may be triggered in severely stressed cells. We have been using *ram-2(bx32)* mutant as a model to study the impact of misfolded proteins in ray development. Previous studies have documented ER expansion, autophagy activation and signs of cell death in male tail of *ram-2(bx32)* mutant. In this report, we addressed the following issues: (1) which UPR pathway is required to handle the stress? (2) what is the role of autophagy? And (3), is cell death pathway activated in *ram-2(bx32)* mutant?

Transcriptional activation of *hsp-4* and enhancement of Ram phenotype in *ram-2(bx32);xbp-1(zc12)* and *ram-2(bx32);crt-1(bz50)* double mutants suggest that *ire-1/xbp-1* signaling pathway is required for handling the stress generated by *ram-2(bx32)* products. A potential downstream target of *pek-1*, *atf-5*, was shown to be activated in *ram-2(bx32)* mutant. Attenuation of autophagy activity can enhance the severity of Ram phenotype of *ram-2(bx32)* mutant, while elevation of autophagy by resveratrol treatment showed partial suppression of its Ram phenotype. Hence, autophagy is likely required for removal of aberrant organelle. As for the punctate nuclear membrane and positive acridine orange staining in the *ram-2(bx32)* mutant male tail, both TUNEL and annexin V staining showed negative results and ruled out the occurrence of cell death in *ram-2(bx32)*, a notion supported by the absence of specific effect of *egl-1(n487)* and *ced-4(n1162)* on *ram-2(bx32)* in double mutants. Absence of genetic interaction in *ram-2(bx32); unc-68(e540)* and *ram-2(bx32); itr-1(sa73)* double mutants also eliminate the possibility of having necrosis occurring in *ram-2(bx32)*. Interestingly, altered nuclear architecture in *ram-2(bx32)* mutant resembling that of aging nuclei can be revealed by confocal microscopy. The finding suggests the leakiness of nuclei may account for the acridine orange staining and implicates a potential link between aberrant collagen production with cellular senescence. (The research is funded by Research Grants Council, Hong Kong.).

354C

Proteasome subunit expression in vivo under normal and stress condictions. **CongYu Jin**, Carina I. Holmberg. Molecular Cancer Biology Program, and Institute of Biomedicine, University of Helsinki, Helsinki, Finland.

The well-conserved ubiquitin-proteasome system is responsible for degrading most of the intracellular proteins including key regulatory proteins and misfolded proteins. Thus, it is essential for cell function and survival. So far, very little is known about the expression of proteasome in various tissues in animals and changes in expression of proteasome in response to various stresses. In this study, we used transcriptional and translational fluorescent fusions to visualize expression patterns of proteasome core and regulatory subunits in vivo in C. elegans, both under normal and stress conditions. The subunits are widely expressed in various tissues, but the expression intensities are different. Additionally, the expression patterns of core and regulatory subunits are not identical. An increase in fluorescence after heat stress was observed, whereas a decrease in fluorescence was detected after exposure to oxidants. Our results indicate that the expression of proteasome core and regulatory subunits are controlled in a tissue-specific manner and are altered in response to different stresses.

355A

A Genetic Screen for mutations that Cause Reduced Fat Accumulation in *C. elegans*. **Rahul Gaur**, Bhaskar Reddy Gavinolla, Ava Hossein Zadeh, Simon Tuck. Umeå Center for Molecular Medicine, Umeå University, Umeå, Sweden.

To identify new genes involved in the regulation of fat metabolism in *C. elegans*, we have carried out a genetic screen for mutations causing reduced fat accumulation. From a screen of 50,000 haploid genomes, we isolated 30 mutations, 10 of which cause highly penetrant defects without causing embryonic or larval lethality. To date we have concentrated our efforts on five of these, *sv74*, *sv75*, *sv76*, *sv77* and *sv78*, which all map to *LG I* and fall into three complementation groups. These mutants have a pale appearance, are smaller than wild type, and have much reduced staining with the lipophilic dye, Sudan Black B. The *sv74* and *sv78* mutants show the most severe phenotypes. Interestingly, they are able to accumulate fat during dauer formation indicating that the fat synthesis pathways are intact and that fat accumulation is improperly regulated. The thin and pale phenotype of *sv74* mutation is not suppressed by *tph-1* or *daf-2* at semi-permissive temperatures implying that the defect is either downstream of the insulin pathway or in a parallel pathway. *sv74* worms also display behavioural defects including decreased performance in salt induced learning assay, clumping around the bacterial lawn, and increased roaming. They have an improved mean but not maximum life span compared to wild type, N2.

356B

Interactions between the Insulin/IGF-1 Signaling Pathway and Stearoyl-CoA Desaturase in the Regulation of Fat Storage, Growth, Stress Responses, and Lifespan. **Bin Liang**, Kyle Ann Brooks, Jennifer Watts. School of Molecular Biosciences, Washington State University, Pullman, WA.

Obesity is becoming a global pandemic. It is associated with diabetes, cardiovascular disease, hypertension, and many forms of cancer. Obesity develops when energy intake exceeds energy expenditure, leading to the net accumulation of triglycerides. New breakthroughs have revealed that stearoyl-CoA desaturase (SCD) plays a significant role in energy balance and obesity. In the biosynthesis of fat, SCD is a key enzyme that converts saturated fatty acids to monounsaturated fatty acids, which are the most abundant fatty acids of membrane phospholipids, triglycerides, wax, and cholesterol esters. The C. elegans fat-6;fat-7 SCD double mutants, similar to mammalian SCD1 mutants, have reduced fat stores and developmental defects. Likewise, the insulin/IGF-1 signaling pathway is remarkably conserved in worms, flies, and mammals. In C. elegans, the insulin/IGF-1 signaling pathway regulates life span, fat storage, dauer diapause, reproduction, and stress responses. Searches for downstream targets of this pathway identified genes involved in lipid and carbohydrate metabolism. The expression of fat-7 is upregulated in daf-2 mutants. These studies raise the questions of whether the regulation of fat storage by the insulin/IGF-1 pathway depends on SCD, and how insulin/IGF-1 signaling pathway and stearoyl-CoA desaturase coordinately or independently regulate growth, stress responses, and even lifespan. To address theses questions, we generated daf-2;fat-6;fat-7 triple mutants. We found that daf-2;fat-6;fat-7 triple mutants accumulate same amount of fat as daf-2 single mutant by GC/TLC and fixed Nile Red staining. The daf-2 mutation also suppresses the susceptibility of fat-6;fat-7 double mutants to heat and pathogen stress. However, the insulin/IGF-1 signaling pathway and SCD overlap to regulate growth rate and brood size. We are currently investigating target genes that regulate fat stores of daf-2;fat-6;fat-7 triple mutants by quantitative RT-PCR and RNAi. Taken together, these studies will provide insights into the mechanisms of fat accumulation and the development of diabetes and obesity in humans.

357C

Cytochrome P450-dependent metabolism of poly-unsaturated fatty acids (PUFA) in the nematode *Caenorhabditis elegans*. Jana Kulas[1], Mandy Kosel[2], Cosima Schmidt[2], Michael Rothe[3], Christian Steinberg[4], **Ralph Menzel[4]**. 1) Max Planck Institute for Molecular Genetics, Development & Disease, Ihnestr. 63-73, 14195 Berlin, Germany; 2) Max Delbrück Center for Molecular Medicine, Robert-Rössle-Str. 10, 13125 Berlin, Germany; 3) Lipidomix GmbH, Berliner Allee 261-269, 13088 Berlin, Germany; 4) Humboldt-Universität zu Berlin, Department of Biology, Laboratory of Freshwater & Stress Ecology, Späthstr. 80/81, 12437 Berlin, Germany.

We tested here the hypothesis that some of *Caenorhabditis elegans* cytochrome P450 (CYP) forms may be involved in the metabolism of polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA), the predominant PUFA of this nematode. Microsomes isolated from adult worms contained spectrally active CYP proteins and showed NADPH-CYP reductase (CPR) activities. They metabolized EPA and, with lower activity, also arachidonic acid (AA) to specific sets of regioisomeric epoxy- and ω-/(ω-1)-hydroxy-derivatives. 17(R),18(S)-epoxyeicosatetraenoic acid was produced as the main EPA metabolite with an enantiomeric purity of 72 %. The epoxygenase and hydroxylase reactions were NADPH-dependent, required the functional expression of the CPR-encoding *emb-8* gene, and were inhibited by 17-ODYA and PPOH, two compounds known to inactivate mammalian AA-metabolizing CYP isoforms. Multiple followed by single RNAi gene silencing experiments identified CYP-29A3 and CYP-33E2 as the major isoforms contributing to EPA metabolism in *C. elegans*. Liquid chromatography/mass spectrometry revealed that regioisomeric epoxy- and hydroxy-derivatives of EPA and AA are endogenous constituents of *C. elegans*. The endogenous EPA metabolite levels were increased by treating the worms with fenofibrate, which also induced the microsomal epoxygenase and hydroxylase activities. Heterologous expression of *cyp-29A3* and *cyp-33E2* in SF9 insect cells is in progress. These results demonstrate for the first time that *C. elegans* shares with mammals the capacity to produce CYP-dependent eicosanoids and may thus facilitate future studies on the mechanisms of action of this important class of signaling molecules.

358A

Gene silencing based functional analysis of *C. elegans*' cytochromes P450: PUFA metabolism, biotransformation and malfunction in fat storage. **Ralph Menzel**. Humboldt-Universität zu Berlin, Department of Biology, Laboratory of Freshwater & Stress Ecology, Späthstr. 80/81, 12437 Berlin, Germany.

Cytochromes P450 (CYP) are heme-thiolate monooxygenases and form one of the largest superfamilies of enzyme proteins. They are found in virtually all organisms and contribute to diverse vital processes, such as carbon source assimilation, biosynthesis of hormones and structural components; other forms can degrade xenobiotics and interact with carcinogens. Although 75 full length CYP genes have been identified in the genome of *C. elegans*, the individual biological function of the vast majority is mostly unknown yet. Our investigations started with the validation that *C. elegans* possesses spectrally active CYP proteins, as demonstrated by CO-difference spectrum from microsomal fractions. To complete the monooxygenase system CYP enzymes need a carrier protein used for conveying reducing equivalents coming typically from NADPH. A genomic screen suggested *emb-8* as the only *C. elegans* gene in question to encode a protein that resembles mammalian NADPH-CYP reductases (CPR). Indeed, the CPR component was easily detectable by measuring the microsomal NADPH-cytochrome *c* reductase activity and it abolished upon prevention of functional *emb-8* expression. Moreover, CPR expression was found essential for all CYP activities measured in this study. To identify CYP components of specific monooxygenase systems, a systematic gene silencing by RNAi as well as by using *cyp*-mutant strains was performed. Several pre-selected enzymatic reactions, proved as CYP dependent in *C. elegans*, were used as screening systems. By doing this we identified CYP-29A3 and CYP-33E2 as the major isoforms contributing to the metabolism of eicosapentaenoic acid (EPA) and arachidonic acid (AA) to specific sets of regioisomeric epoxy- and ω-/(ω-1)-hydroxy-derivatives. Heterologous expression of both CYP-forms in SF9 insect cells is in progress. Moreover, CYP34A6 and CYP14A isoforms were found to be required for hydroxylation of an *ortho*-substituted, non-coplanar tetrachlorbiphenyl (PCB52) to C3-, C4- and/or C6-hydroxy-PCB52. This finding shows for the first time the so far only predicted involvement of *C. elegans*' CYP forms in biotransformation. Further CYP-forms of subfamily 35A, which were under suspicion to be part of biotransformation, too, could be assigned to the fat storage pathway. Both individual gene silencing of *cyp-35A2*, *3*, *4*, and *5* resulted in a dramatic decrease of intestinal fat content of well fed young adults, measured by Nile red vital staining. Our investigation may facilitate future studies on *C. elegans*' CYP enzymes, which are much less investigated than its status as model organism lets expect.

359B

Cytochrome P450-dependent metabolism of PCB52 in the nematode *Caenorhabditis elegans*. Martin Müller[1], Patrick Schäfer[2], Angela Krüger[3], Christian Steinberg[2], **Ralph Menzel[2]**. 1) Universität Duisburg-Essen, Zentrum für Medizinische Biotechnologie, Genetik, Universitätsstr. 5, 45117 Essen, Germany; 2) Humboldt-Universität zu Berlin, Department of Biology, Freshwater and Stress Ecology, Späthstr. 80/81, 12437 Berlin, Germany; 3) Leibniz Institute of Freshwater Biology and Inland Fisheries, Central Chemical Laboratory, Müggelseedamm 310, 12587 Berlin, Germany.

There are 75 full length cytochrome P450 (CYP) genes known in the genome of the nematode *Caenorhabditis elegans*, the individual biological functions of the vast majority are mostly unknown yet. Here we investigated the impact of cytochrome P450 isoforms on the metabolism of PCB52, an *ortho*-substituted, non-coplanar 2,2',5,5'-tetrachlorbiphenyl, as model PCB of these world wide distributed pollutants. The first finding was that hydrophobic extracts, isolated from treated worms and analyzed by GC/MS, contained two obvious PCB52 derived product peaks which have been indentified as C3-, C4- and/or C6-hydroxy-PCB52. Moreover, these hydroxylase reactions strictly required the functional expression of the NADPH dependent cytochrome P450 reductase (CPR) encoding *emb-8* gene, which was recently shown to be essential for several other cytochrome P450 dependent enzymatic reactions, too. Multiple followed by single RNAi gene silencing experiments as well as the use of *cyp*-mutant strains identified members of the CYP-14A subfamily and CYP-34A6 as the major isoforms contributing to PCB52 metabolism in *C. elegans*. The decrease in generation of hydroxylated products ranged from 56 to 31% compared to the wild type. These results demonstrate for the first time that *C. elegans* shares with mammals the capacity to produce CYP-dependent PCB metabolites and may thus facilitate future studies on nematode's biotransformation, which is much less investigated than its status as model organism lets expect.

360C

Novel fasting-induced lipases regulate energy homeostasis in *C. elegans*. **Hyungmin Moon**, Jiwon Shim, Junho Lee. School of biological science, Seoul National University, Seoul, Korea.

Our recent study revealed that IRE-1, an ER protein, known to be involved in the unfolded protein response, and HSP-4, an ER chaperone, regulate the expression of the novel fasting-induced lipases which induce fat granule hydrolysis upon fasting in *C. elegans*. Physiologically, broken fat granules are used as an energy source to meet the cellular energy levels during fasting. However, precise regulatory mechanisms of lipase induction upon fasting have not been identified. First, to identify upstream regulators of the lipases, we carry out EMS mutagenesis and RNA*i* screening, on the assumption that there must be presence of activators or inhibitors of the lipases in both normal and fasting status. Secondly, to study the effect of the lipases on lifespan, we measure lifespan in the lipases knockdown animals using RNA*i*. Many lipid metabolic genes are known to affect lifespan via insulin mediated mechanisms in *C. elegans*, and we plan to verify the physiological significance of the lipases through this experiment. By various genetic approaches, our goal is to clarify the specific pathways between ER resident molecules and transcriptional regulation of the lipase genes in the nucleus, which can be applied to obesity and diabetic pathways in humans.

361A

A fasting-responsive transcriptional switch promotes fat accumulation and shortens lifespan in times of food abundance. **Eyleen J. O'Rourke**, Gary Ruvkun. Department of Molecular Biology, Massachusetts General Hospital, and Department of Genetics, Harvard Medical School, Boston, MA.

Fasting is the most usual nutritional state of animals in the wild. As such, selective pressure has acted over millions of years to maximize nutrient uptake when food is available and efficient utilization when is scarce. The "thrifty genotype" hypothesis gives a controversial explanation to the current epidemics of obesity. Although popular, the hypothesis that a thrifty genotype has adaptive value in natural environments but leads to excessive fat accumulation and shorter life span in conditions of excessive food availability lacks direct experimental evidence. Here we present Max-like 3 (Mxl-3), a conserved transcriptional regulator required for optimal adaptation of *C. elegans* to cycles of food and fasting that promotes fat accumulation and shortens lifespan when food is abundant. In conditions of food abundance Mxl-3 represses the lipases *lipl-1* to *lipl-3*. Upon fasting *mxl-3* transcription is downregulated leading to an upregulation of *lipl-1* to *lipl-3*. Activation of the lipases increases fat mobilization. Mxl-3-deficiency is sufficient to constitutively activate the lipl genes. Consequently, *mxl-3* mutant animals accumulate 30% less fat. In addition, Mxl-3 deficiency leads to a 35% increase in lifespan. Both, the lean and longevity phenotypes of *mxl-3* are rescued by lipls inactivation and LIPLs overexpression increases *C. elegans* lifespan. However, in cycles of food and fasting *mxl-3* worms are outcompeted in 8 generations by wild-type animals suggesting that constitutive lipolytic activation impairs nutrient uptake upon refeeding at a profound fitness cost. Our work shows a unique example of direct evidence of the adaptive value of a gene required for optimal response to fasting-food cycles that in conditions of food abundance allows to store extra fat and reduces lifespan.

362B

MicroRNAs–Aging, Stress and Longevity. **M. Vora**, C. Ibáñez-Ventoso, J. Xue, M. Shah, M. Driscoll. Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ.

MicroRNAs (miRNAs) are short, conserved, non-coding RNAs that repress gene expression by complementary binding within the target mRNA. miRNAs impact a range of biological processes including muscle development, developmental timing, and oncogenesis. In a notable study of deletions of 87 of 154 C. elegans miRNA genes, a striking observation that emerged was that most individual miRNAs are dispensable for development and basic function of the animal. miRNAs have been proposed to be mediators of biological robustness, conferring stability of cellular phenotypes and ability to withstand stresses or perturbations. Given that aging has been described as a decline in robustness (with enhanced susceptibility to stresses), the question arises as to how individual miRNAs might influence the quality of aging. We hypothesize that miRNAs may play a vital role in the overall maintenance of the organism through reproductive life, such that their absence will hold consequences for the health- and lifespan of the organism. Indeed, we have shown that miRNA expression in C. elegans is dynamic throughout adulthood and have compiled a list of 50 "age-regulated" miRNAs that change in abundance during adult life. Can we find node miRNAs that control gene networks critical to adult maintenance and healthy aging? Do miRNAs influence tissue-specific decline? Are transcripts of gerontogenes targeted by miRNAs? In an effort to address whether miRNAs modulate aging, we are currently screening conserved age-regulated miRNAs to identify those that impact the quality of aging. Although we are still at the beginning of our effort, we have already found that 8 mutant strains accumulate significantly higher age pigments (lipofuscin and autofluorescent gut granules) compared to wt animals in early adulthood, whereas 3 strains have significantly lowered levels. Since age pigments are thought to reflect the physiological age rather than the chronological age, these miRNAs may modulate physiological "cellular order" through life. We are also conducting swimming assays on young and old worms in order to identify atypical locomotory decline for select age-regulated miRNAs–mir-1, mir-256, mir-34, mir-238 and mir-71. 4/5 (mir-1, 34, 71, 238) exhibit a significant decline in swimming prowess compared to wt animals at late age. Significantly, these mutants also accrue higher age pigments early on in life, perhaps indicating that a "poor" start leads to a more rapid decline in integrity later in life. At the meeting we will present the current status of our screen. At the moment, our impression is that a high proportion of microRNAs may play an important role in maintaining homeostasis and general integrity of the aging animal.

363C

The Lifespan Machine: Automated *C. elegans* lifespan acquisition on agar plates. **Nicholas Stroustrup**, Javier Apfeld, Walter Fontana. Department of Systems Biology, Harvard Medical School, Boston, MA.

The current method for identifying individual death times within *C. elegans* populations is both labor-intensive and repetitive, limiting the quality and scope of demographic aging data available to the *C. elegans* research community. In response, we have developed a scalable and low-cost imaging platform to allow fully-automated acquisition of nematode mortality data. Our system captures month-long, time-lapse videos of entire *C. elegans* populations, with images taken once every two hours at 8 μm resolution. Animals are maintained on temperature-controlled NGM agar plates seeded with *E. coli*, allowing straightforward integration of machine-acquired results with mortality and behavioral data collected via traditional manual techniques. Through utilization of consumer-grade optical equipment, we have produced an inexpensive system suitable for both small, single-researcher installations and large, high-throughput clusters capable of monitoring tens of thousands of worms simultaneously. To complement the new hardware, we have developed a software package that analyzes time-lapse videos to extract survival curves in a fully-automated fashion.

We present survival curves acquired with our technique of large populations (>500 animals each) at high temporal resolution (observations made every two hours). We use this high-resolution mortality data as a basis for the quantitative comparison of aging demographics between various long-lived mutant strains. We also demonstrate the suitability of our method for performing RNAi screens. We hope to share this technology as a platform for the collection and analysis of more and higher quality demographic aging data in the *C. elegans* community.

364A

Functional characterization of the *C. elegans* mitochondrial thioredoxin system. **Briseida B. Cacho-Valadez**[1], Peter Swoboda[2], Simon Tuck[3], Antonio Miranda-Vizuete[1]. 1) Andalusian Center for Developmental Biology, Universidad Pablo de Olavide, Seville, Spain; 2) Karolinska Institute, Department of Biosciences and Nutrition, Huddinge, Sweden; 3) Molecular Medicine Center, Umeå University, Umeå, Sweden.

The thioredoxin system, formed by thioredoxin reductase (TrxR) and its substrate thioredoxin (Trx) catalyzes thiol-disulphide reactions to maintain the cellular redox homeostasis. Two main thioredoxin systems have been described in mammals, one localized in the cytoplasm (Trx-1 and TrxR-1) and the other one found in mitochondria (Trx-2 and TrxR-2). Different functions have been attributed to both thioredoxin systems including protection against oxidative stress, regulation of cellular differentiation and apoptosis. In C. elegans the mitochondrial thioredoxin system is composed of the ZK637.10/*trxr-2* and B0024.9/*trx-2* genes. The *trx-2* and *trxr-2* genes encode mitochondrial proteins as transgenic strains expressing translational GFP fusions display a general punctuated fluorescence in most worm tissues. Co-localization with the mitochondrial marker Mitotracker® confirmed the mitochondrial localization of TRX-2 and TRXR-2. As *trxr-2* is the third gene of an operon, transgenic strains expressing translational GFP fusions under the control of the putative internal promoter also resulted in fluorescence with an expression pattern different from that of the first gene of the operon. By RT-PCR and western blot, we showed that two different *trxr-2* alleles (*tm2047*) and (*ok2267*) are null mutants as they do not express *trxr-2* mRNA or protein. In contrast, the *trx-2* (*tm2720*) allele expresses an abnormal mRNA, which encodes an anomalous TRX-2 with an aberrant C-terminus. Double mutants *trxr-2*(*tm2047*) III; *trx-2*(*tm2720*) V and *trxr-2*(*ok2267*) III; *trx-2*(*tm2720*) V are viable, suggesting that another redox system acts redundantly to the mitochondrial thioredoxin system. Double mutants of the mitochondrial genes *trx-2* and *trxr-2* with the cytoplasmic thioredoxin reductase *trxr-1* are also viable. We studied whether embryonic development or fertility was compromised in *trx-2* and *trxr-2* mutants, as seen in some other organisms, and observed only a mild decrease of fertility, but no embryonic lethality in the *trxr-2* (*ok2267*) mutant. As thioredoxin systems are among the most important defence mechanisms against oxidative stress, we evaluated the role of *trx-2* and *trxr-2* in coping with paraquat-induced oxidative stress. Single and double mutants of the mitochondrial thioredoxin system have an increased resistance when compared to wild type, suggesting that the lack of the mitochondrial thioredoxin system might induce other antioxidant defences.

365B

In vivo function of presequence peptidase in *C.elegans*. **Min Ren**, Wei-Jen Tang. Ben-May Department of Cancer Research, The University of Chicago, Chicago, IL.

Mitochondria, essential organelles of all eukaryotic cells, serve critical function to many cellular processes. They are the main power house of cells and cell survival depends on their integrity and dynamics. The majority of mitochondrial proteins are synthesized in the cytosol in the form of preproteins and transported into the organelle with the help of targeting presequence peptides. After import into the mitochondria, the presequence peptides are cleaved off from the protein and subsequently degraded. Failed degradation and accumulation of these peptides may cause mitochondria toxicity since they are generally rich in charged and hydrophobic residues. One member of the M16 family metalloprotease, PreP (presequence peptidase), was postulated to degrade mitochondria presequence peptides. However, the exact location of PreP in mitochondria is unclear. Whether PreP can degrade presequence peptides and other physiological substrates *in vivo* remain to be elucidated.

For better understanding of PreP's function and signaling regulation *in vivo*, we used *C. elegans* as a genetic model system to approach these questions. Sequence analysis shows PreP proteins are highly evolutionarily conserved. Based on a structure homology modeling search, we identified one PreP-like protein (Gene C05D11.1) in the entire C. elegans genome. An allele of gene knockout animal, tm989, which has a ~1.8 kb deletion, was obtained from National Bioresource Project (Tokyo, Japan). The deletion in gene C05D11.1 does not affect viability, thus the PreP-like gene product is not essential for *C. elegans* survival. Consistent with the reported data, out crossed knockout animals have normal reproduction ability and no obvious developmental defects under routine culture condition. However, we found two interesting phenotypes. PreP-like knockout strain shows increased sensitivity to heat stress. Upon heat shock at 33°C, TM989 has 50% survival time at 25 hours in comparison with 40 hours for wildtype animals. Interestingly, this mutant strain of *C. elegans* is also more sensitive to alcohol intoxication. On alcohol saturated plates, PreP knockout animals were instantly intoxicated with complete motility loss, while wild type worms remain only partial paralyzed. This establishes a clear association of the PreP-like gene with hyper-sensitivity to heat and alcohol, both are associated with mitochondrial dysfunction. Thus, our studies suggest that PreP plays an important role in stress response and detoxification processes by modulating mitochondria function.

366C

Investigating the function of *pnc-1* in muscle development and function. **Wenqing Wang**, Tracy Vrablik, Wendy Hanna-Rose. Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA.

NAD^+ is a vital molecule in cellular redox reactions and acts as a cosubstrate for NAD^+ consuming enzymes, which is critical to a variety of biological processes. Our studies have revealed a novel developmental role of NAD^+ metabolism; compromised NAD^+ biosynthesis affects muscle development and function in *C. elegans*. PNC-1 is a key enzyme in the NAD^+ salvage pathway, acting as a nicotinamidase that catalyzes the deamination of nicotinamide (NAM) to nicotinic acid (NA), which is eventually processed to nicotinamide adenine dinucleotide (NAD^+). We discovered that *pnc-1* mutants exhibit male mating defects. Male mating behavior is a coordinated multi-step process including finding a mate, locating the vulva, insertion of the copulatory spicules and ejaculation. *pnc-1* males are capable of locating the hermaphrodite vulva, but are severely defective in spicule insertion. A portion of mating defective *pnc-1* males also show crumpled spicules. The spicule morphology and insertion defects are likely due to compromised nicotinamidase activity of *pnc-1*, as supplementation of *pnc-1* males with NA (product of nicotinamidase) during development can rescue both the spicule morphology and the spicule insertion defects, while supplementation of NAM (substrate of nicotinamidase) to wild-type males during development causes a mild mating defect. Since both spicule formation and insertion require proper function of the spicule muscles, these phenotypes suggest a role for *pnc-1* in muscle development and/or function. We have used pharyngeal pumping as an assay to study muscle function of pnc-1 more generally. Our data show that *pnc-1* mutants have a lower pharyngeal pumping rate than N2. Additionally, Hart and Sinclair labs indicate that overexpression of *pnc-1* increases the pumping rate[1]. These results are consistent with our hypothesis that impaired NAD^+ metabolism impacts muscle function.

Reference

[1] Personal communication with Anne C. Hart and David A. Sinclair labs, Harvard University.

367A

Genetic Factors Associated with Aging that Influence α-Synuclein Toxicity. **A. L. Knight**, S. Hamamichi, M. Zhang, S. M. DeLeon, S. K. Lee, K. A. Caldwell, G. A. Caldwell. Dept Biological Sci, Univ Alabama, Tuscaloosa, AL.

Aging is an underlying susceptibility factor of neurodegenerative diseases including Parkinson's disease (PD), wherein pathological features include progressive loss of dopamine (DA) neurons and misfolding of α-synuclein (α-syn) into proteinaceous inclusion bodies. In *C. elegans*, lifespan extension and increased stress resistance have been extensively studied and demonstrated to involve modification in the DAF-2/insulin-like signaling pathway. We previously reported *C. elegans* PD models that mimic pathological features of the disease including α-syn-induced neurodegeneration in DA neurons and α-syn misfolding/aggregation in body wall muscles (Cao *et al*., 2005, *J. Neurosci*.; Hamamichi *et al*., 2008, *PNAS*). Given the role of the DAF-2 pathway in maintaining a cytoprotective environment that allows the organism to double its lifespan, we postulated that *daf-2* mutation provides a fruitful platform to decipher the genetic link between aging and α-syn toxicity. To determine the genetic components downstream of DAF-2 that modify α-syn induced neurodegeneration, we generated *daf-2(e1370)* and *daf-16(m26)* worm strains that overexpress α-*syn* and *gfp* under the control of *dat-1* promoter. While *daf-2* strikingly suppressed neurodegeneration at day 7, *daf-16* enhanced it. Interestingly, the analysis of DA neurons at the mean lifespan (N2: day 20; *daf-2*: day 40) resulted in no *daf-2* mediated neuroprotection, suggesting that differential gene expression in *daf-2* background may reveal critical factors for DA neuron survival. To identify these genetic components, we performed a systematic RNAi screen using a *daf-2* strain that overexpresses α-*syn::gfp* in body wall muscles. Approximately 700 RNAi candidates were compiled based on the following criteria: 1) genes/proteins that are up-regulated in *daf-2* mutants identified via genome-wide (microarray, RNAi, SAGE, MS) analyses, 2) genes that are up-regulated upon pan-neuronal α-syn overexpression (Vartiainen *et al*., 2006, *Neurobiol. Dis*.), and 3) modifiers of α-syn toxicity (Hamamichi *et al*., 2008, *PNAS*; Kuwahara *et al*., 2008, *Hum. Mol. Genet*.; van Ham *et al*., 2008, *PLOS Genet*.). While α-syn::GFP was readily degraded in *daf-2* background, RNAi knock-down of ~50 genes enhanced α-syn misfolding/aggregation *in vivo*. Among the positives were genes involved in trafficking, signaling pathways, and metabolism. Taken together, as we discern the functional significance of these positives as putative cytoprotective gene products, this study is poised to further reveal a network of genetic factors that better defines the role of the DAF-2 pathway and its influence on aging and neurodegeneration.

368B

Mitochondrial genotoxicity during development leads to dopaminergic neurodegeneration in adult *Caenorhabditis elegans*. **Maxwell C.K. Leung**, Andrew E. Arrant, Amanda M. Smith, Madeline G. McKeever, Tracey L. Crocker, Joel N. Meyer. Nicholas School of the Environment, Duke University, Durham, NC.

Mitochondrial DNA damage and mutation have been correlated with neurodegeneration, but causation is unclear. Epidemiological studies have identified an association between neurological disorders and exposure to environmental chemicals such as pesticides and heavy metals. The current study investigates the effect of environmental exposures on mitochondrial DNA damage as well as dopaminergic neurons in *Caenorhabditis elegans*. In the first experiment, adult germ-line deficient *C. elegans* (*glp-1* strain) was exposed to aflatoxin B_1, paraquat, cumene hydroperoxide, and manganese. Mitochondrial and nuclear DNA damage was quantified based on the amount of PCR product amplified from a specific nuclear or mitochondrial DNA segment. Exposure to aflatoxin B_1 (100 uM), manganese (2.5 mM), and paraquat (20 mM) resulted in significant dose-dependent mitochondrial DNA damage (0.27, 0.13, and 0.82 lesion per 10^5 base pairs, respectively, $p < 0.05$ in all cases). Nuclear DNA damage, on the other hand, was detected only with aflatoxin B1 (0.08 lesion per 10^5 base pairs), but not with paraquat and manganese ($p > 0.05$). Exposure to cumene hydroperoxide resulted in no significant nuclear or mitochondrial DNA damage ($p > 0.05$) but a 30% decrease in the mitochondrial:nuclear DNA copy number ratio as compared to controls ($p < 0.05$). In the second experiment, the effect of larval exposure to aflatoxin B_1, paraquat, and cumene hydroperoxide on the development of dopaminergic neurons was examined. Transgenic *C. elegans* expressing GFP in dopaminergic neurons (*dat-1* strain) were treated with the three chemicals at the L1 stage. The initial observation revealed that exposure to any of the three chemicals can result in dose-dependent dendritic and cell body degeneration in later life stages. Furthermore, similar dopaminergic neurodegeneration could be induced using repeated low-dose ultraviolet C treatment, which resulted in persistent DNA damage in the mitochondria but not in the nucleus. The current findings suggest that (1) mitochondrial DNA is a potential target of environmental exposure; and (2) mitochondrial DNA damage leads to dopaminergic neurodegeneration in adult *Caenorhabditis elegans*.

369C

The E3 ligase RNF-121 is required for ER homeostasis and regulation of PAT-3/β-integrin expression. Amir Darom, Ulrike Bening-Abu-Shach, **Limor Broday**. Dept Cell & Dev Biol, Tel Aviv Univ, Tel Aviv, Israel.

RNF-121 is a newly identified, evolutionarily conserved E3 ligase RING finger protein that is highly expressed in the endoplasmic reticulum in various cells and tissues. Inactivation of RNF-121 induces elevation in BiP expression and causes increased sensitivity to ER stress that is reversed by co-depletion of the ER-associated degradation (ERAD)-related E3 ligase, RNF-5. Genetic analysis places RNF-121 in the same pathway with the UPR regulator PERK. Overexpression of RNF-121 during gonad development causes aberrations in oocyte development and maturation, defects in gonad migration and decrease in PAT-3::GFP levels in the gonad distal tip cell, while reducing RNF-121 activity is associated with accumulation of cytoplasmic PAT-3::GFP inclusions. Our results indicate that RNF-121 is a novel ER-anchored ubiquitin ligase and suggest that it regulates the degradation of unassembled β-integrin monomers. RNF-121 may therefore play a specific role in the ERAD pathway in multicellular organisms.

370A

THE EGF/RAS/MAPK PATHWAY AND THE UFD COMPLEX UPREGULATE THE UBIQUITIN-PROTEASOME SYSTEM AS ADULT NEMATODES MATURE Gang Liu and Christopher Rongo, The Waksman Institute, Department of Genetics, Rutgers University, Piscataway, NJ 08854. **Gang Liu**, Christopher Rongo. Waksman Institute, Piscataway, NJ.

The Ubiquitin-Proteasome System (UPS) is an important regulatory mechanism used to control acute protein turnover and scavenge misfolded proteins in numerous cellular processes. Indeed, the failure of the UPS to remove misfolded proteins is believed to contribute to aging as well as several neurodegenerative disorders. Although recent studies have explored the biochemical function and protein-protein interactions of the UPS, regulated changes in UPS activity have been difficult to observe in vivo in specific tissues as animals develop and age. Here we have employed the GFP reporter UbG76V-GFP, which was developed for monitoring UPS activity, to study the tissue- and age-specific regulation of proteolysis in C. elegans. The UbG76V-GFP reporter contains a mutated ubiquitin fused to GFP, and is a substrate for polyubiquitination and degradation by the 26S proteasome (1). Using tissue specific promoters, we have expressed UbG76V-GFP in C. elegans, where it is a substrate for the UPS. Mutations in the ubiquitin moiety that prevent K48 and K63 polyubiquitin chain addition block turnover of the reporter. Mutations and RNAi treatment that reduce 26S Proteasome activity also block reporter turnover, confirming the in vivo functionality of this UPS reporter. Interestingly, the UbG76V-GFP reporter is stable in the hypodermal tissue of larvae, indicating that growing animals maintain low levels of proteasome activity. Upon maturation, adult animals displayed an age-specific decrease in reporter stability (with no change in the levels of an mRFP internal control expressed from the same promoter), indicating an upregulation of UPS activity. The UFD complex, including the E3 ligase CHN-1 and the E4 enzyme UFD-2 (2), are required for this increase in UPS activity in maturing adults. Mutations that reduce UFD gene activity do not appear to grossly affect larval health, but greatly shorten the lifespan of adult nematodes, suggesting that the upregulation of UPS activity is critical for long-term viability. We find that EGF signaling through the Ras-MAPK pathway and the transcription factors EOR-1 and EOR-2 (3) are required for this change in UPS activity. Our results suggest that mature animals use the EGF/Ras/MAPK signaling pathway to upregulate UPS activity in anticipation of an increased burden of misfolded proteins during aging. References Dantuma et al., (2000) Nature Biotechnology 18:538-43. Hoppe et al., (2004) Cell 118:337-49. Howard et al., (2002) Genes & Development 16:1815-27.

371B

Role of *C. elegans* JUN-1 in UV-induced DNA damage. **Holli Marie Duren**, Susan Marie Hiatt, Y. John Shyu, Chang-Deng Hu. Dept. of Medicinal Chemistry and Molecular Pharmacology, Purdue University, West Lafayette, IN.

Genome integrity is critically important for organisms to survive and flourish. Cells have protective measures, such as cell cycle arrest and DNA repair, to combat the effects of DNA damage. If a particular signaling pathway such as cell cycle arrest, DNA repair, or apoptosis, is not functional properly, the possibility of mutations occurring and incorporating into the genome increases, thus leading to increased cancer risk. Skin cancer is the most common type of cancer in the United States with 90% of the non-melonoma skin cancers associated with UV exposure. Activator protein 1 (AP-1), a family of dimeric transcription factors comprised of Jun, Fos, and ATF subfamilies, has been shown to be an immediate early response gene upon UV exposure and overexpression of AP-1 has been shown to play a role in skin cancer development *in vitro* and *in vivo* suggesting a possible role of AP-1 in DNA damage-induced skin cancer. To determine the role of AP-1 genes in UV response in intact animals, we have identified the *C. elegans* Fos, Jun, and ATF2 homologous genes (*fos-1*, *jun-1*, and *atf-1*) and investigated how they are involved in UV response. Using RNA interference methods, we have shown that knockdown of these individual AP-1 genes sensitized the worms to UV-induced worm death. Because UV affects cell cycle arrest, cell death, and DNA repair; we proceeded to expose worms to UV and study the effect on cell cycle arrest. RNAi knockdown of the each AP-1 gene showed no significant effect; however, the *jun-1* deletion mutant demonstrated an increase in arrest at the higher dose of 120 Jm^{-2}, 12 hours after exposure. The *atf-1* deletion mutant showed no change in arrest as compared to vector. Further, germline apoptosis assays showed that RNAi knock-down of *jun-1*, but not *fos-1* or *atf-1*, sensitized the worms to UV-induced cell death. Similar results were obtained when a *jun-1* deletion mutant was used. Further genetic analysis suggested that JUN-1 acts upstream of the canonical programmed cell death pathway in C. elegans and may regulate the transcription *xpf-1*, an XP gene involved in nucleotide excision repair (NER). The results presented here strongly suggest that *C. elegans jun-1* is involved in the UV-mediated cell death pathway. Current effort is directed to determine what AP-1 dimers are involved in UV-induced cell death and DNA repair.

372C

How is RNA Pol I involved in DNA damage responses in *C. elegans*? **Ralf Eberhard**, Lilli Stergiou, Randal Hofmann, Michael Hengartner. Institute of Molecular Biology, University of Zurich, Switzerland.

Eukaryotic organisms preserve the integrity of their genome by exhibiting a properly regulated response when DNA is damaged. Failure of DNA lesions to be restored can lead to mutations or large-scale genomic instability, which may implicate tumorigenic potential. The germline tissue of *C. elegans* is a versatile model to dissect the signaling network that links damage inflicted by chemical or physical agents to cellular key responses; DNA repair, cell cycle arrest, cell death, and–as an integrated output–embryonic survival, can be studied in the context of a living organism. We performed a forward genetic screen for new mutant worm strains that are defective for cellular responses to treatment with UV-light or ionizing radiation. Based on the findings in one mutant, RNA polymerase I appears to be involved in the regulation of DNA-damage induced germ cell apoptosis. It is the topic of our current research to elucidate whether the defective response we observe in this mutant is due to a specific role of the affected RNA Pol I subunit in apoptosis, or whether it results from a more general implication on transcription of ribosomal RNA and on the synthesis of ribosomes. Positioning this novel aspect in relation to known signaling factors, in particular to *cep-1*, the *C. elegans* homolog of p53, might extend our genetic model of DNA damage-induced apoptosis by another branch. The elemental transcription apparatus as well as most damage response mechanisms are highly conserved in evolution, which should allow to extrapolate many of our observations and working models to higher organisms including humans.

373A

C. elegans MLK-1 MAPKKK is regulated by double phosphorylation in JNK-mediated stress response pathway. **Kota Fujiki**, Tomoaki Mizuno, Naoki Hisamoto, Kunihiro Matsumoto. Div Biological Sci, Graduate school of Science ,Nagoya Univ, Nagoya, Japan.

Mitogen-activated protein kinases (MAPKs) are integral to the mechanisms by which cells respond to physiological stimuli and a wide variety of environmental stresses. In *C. elegans*, the stress response is controlled by a JNK-like MAPK signaling pathway composed of MLK-1 MAPKKK, MEK-1 MAPKK and KGB-1 JNK-like MAPK. As components functioning in the KGB-1 pathway, we identified the max-2 and shc-1 genes, which encode *C. elegans* homologs of PAK-like kinase and Shc, respectively. Biochemical analysis revealed that MAX-2 phosphorylates MLK-1 at Ser-355 in the T-loop of the MLK-1 kinase domain, resulting in its activation. We also found that phosphorylation of MLK-1 at Tyr-940 creates a binding site for SHC-1 and results in the recruitment of the SHC-1-MEK-1 complex to MLK-1. These results suggest that MLK-1 is regulated by double phosphorylation: 1) phosphorylation of Ser-355 regulates its kinase activity; and 2) phosphorylation of Tyr-940 regulates the association with SHC-1, consequently interacting with its substrate, MEK-1. Thus, regulation of MLK-1 by the double phosphorylation in the KGB-1 pathway can modulate the amplitude and duration of the signal in the stress response.

374B

Intercellular Transmission of Genotoxic Damage in the Nematode Intestine. Tamako Jones, Celso Perez, Leticia Ortloff, **Gregory A Nelson**. Dept. Radiation Medicine, Loma Linda Univ, Loma Linda, CA.

We measure genotoxic damage in the *C. elegans* intestine by irradiating L1 larvae and quantifying anaphase bridges in DAPI-stained young adults. The method is reliable for all types of radiation tested, shows a robust dose-response and distinguishes between different radiation types. Analysis of dose-responses for cells in different intestinal rings shows that they have unique radiosensitivities not correlated with their lineages. We present evidence that germline cells may modulate individual E cell radiosensitivity leading to the observed patterns. Thus, mutations in *dig-1*, *mes-1*, and *glp-4* which alter P4 fates or shift gonad position change the E cell radiosensitivity patterns. A statistical analysis of responses for pairs of E cells shows that neighboring cells also influence each other such that damage to one E cell enhances the probability of damage in its neighbor. Such "bystander" effects have been observed in cultured cell systems and are thought to be propagated by reactive oxygen species and cytokines. An RNAi and mutant screen was initiated to identify genes required for the signaling. The screen includes genes for DNA repair systems, cell junctions, extracellular matrix components and signal transduction. So far, we have shown that mutations or knock-down in components of the non-homologous end joining DNA repair pathway (*cku-70*, Y47D3A.4; *cku-80*, R07E5.8; and *lig-4*, C07H6.1) and the MAP kinase *sek-1* (R03G5.2) result in strong (up to 7-fold) overall sensitization of the cells but do not influence their relative sensitivity patterns. An up-to-date summary will be presented. Precision irradiations of body segments with alpha particles confirms that middle and anterior E cells react to damage in posterior E cells but irradiation of the head from the posterior margin of the pharynx does elicit a response from the intestine. Work is in progress to conduct single cell irradiations with a particle microbeam at Texas A&M University to test individual cells and the topology of the putative bystander signaling.

375C

Hsp-16.2 overexpression produces a pathological phenotype in *C. elegans*. **S. Alavez**, G. J. Lithgow. Buck Institute, 8001 Redwood Blvd, Novato, CA, 94945.

Studies in model organisms, including the nematode C. elegans, clearly demonstrate that enhanced resistance to different forms of stress is a major factor for extended lifespan. In addition, as animals age, they often exhibit an attenuated stress response (1). Overexpression of stress response genes, such as those encoding heat-shock proteins, is sufficient to enhance stress resistance and slow aging in worms and fruit flies (2-4). For example, we previously demonstrated that the ubiquitous overexpression of the small heat-shock protein-16 (HSP-16) improves stress response and increases lifespan in C. elegans (3) in an insulin-signaling-dependent manner. Whether elevated HSP levels affect mammalian lifespan is the subject of ongoing studies; it is possible that HSP overexpression could also have some detrimental effects in more complex metazoans. For example, HSP gene expression is elevated in many cancers during malignant progression. At these high levels, HSP family members play an essential, facilitating role in cancer by permitting autonomous growth through the accumulation of overexpressed and mutated oncogene products and by inhibiting programmed cell death. We have made the striking observation that overexpressing the fusion protein HSP-16.2::GFP in C. elegans, in addition to increasing stress resistance and lifespan (~35% and 100%, respectively, as compared with control strain), causes somatic pathological protuberance to develop in the animal's head. The pathology presents a low incidence (~ 3%) and occurs between the pharynx and the nerve ring. The incidence of this pathology, as well as thermotolerance and lifespan increase, is reverted for GFP RNAi bacterial feeding. The pathology occurs between the pharynx and the nerve ring and contains DAPI-positive nuclear material consistent with a cellular expansion. Moreover, we find BrdU positive staining in theses pathologies and in the surrounding worm tissue consistent with DNA synthesis. We propose that the increased expression of the pro-longevity factor HSP-16.2 also results in the stochastic occurrence of tumor-like protuberances in the postmitotic tissue of the nematode C. elegans and that the study of this pathology could be relevant to study the relationship between disease and aging. 1.T. E. Johnson, G. J. Lithgow, S. Murakami, The journals of gerontology 51, B392 (Nov, 1996). 2.G. Morrow, M. Samson, S. Michaud, R. M. Tanguay, FASEB J 18, 598 (Mar, 2004). 3.G. A. Walker, G. J. Lithgow, Aging Cell 2, 131 (Apr, 2003). 4.H. D. Wang, P. Kazemi-Esfarjani, S. Benzer, Proceedings of the National Academy of Sciences of the United States of America 101, 12610 (Aug 24, 2004).

376A

Caloric restriction induces elevated expression of *sir-2.1* in *C. elegans*. **Sophie Bamps**, Fiona Savory, Julia Wirtz, Duncan Lake, Ian A. Hope. Institute of Integrative and Comparative Biology, University of Leeds, Leeds, LS2 9JT, UK.

Caloric restriction results in lifespan extension in yeast, worms, *Drosophila* and mammals. Yeast studies showed extension of lifespan in response to caloric restriction involves *sir-2* the founding member of a widely conserved gene family encoding the sirtuins, enzymes with histone deacetylase activity. Like in yeast, *Drosophila* and mammals, over-expression of the *C. elegans* sirtuin gene *sir-2.1* leads to extension of life span and deletion of the gene shortens life span (Tissenbaum & Guarente, 2001; Wang & Tissenbaum, 2006). Previously, a C-terminal translational *gfp* reporter fusion with *sir-2.1* under the control of a 300 nucleotide intergenic promoter fragment (in strains HT809, HT810) was reported to reveal expression of *sir-2.1* in most of the nerve cells in the head and the tail, and in the hypodermis (Wang & Tissenbaum, 2006). Recombineering allowed us to insert both a *mCherry* reporter gene just before the stop codon of *sir-2.1* and a *gfp* reporter just before the stop codon of R11A8.6, within a 29.3kb fosmid genomic DNA clone. R11A8.6 is the first gene and *sir-2.1* is the downstream gene in a two gene, 4.4kb operon. The *mCherry* expression pattern under normal growth conditions, with abundant food, indicated that *sir-2.1* is indeed expressed, as previously described, in many nerves in the head. However, GFP was also seen, along with mCherry, in the gut and in head muscles. This reveals that expression of *sir-2.1* is broader than previously described, fitting the expectation that the SIR2.1 function in influencing lifespan would be required in most cell types. Furthermore, the broader expression arises from transcription starting with the upstream gene of the *sir-2.1* operon. SIR-2.1::mCherry is nuclear, consistent with the proposed function in controlling gene expression, while R11A8.6::GFP is cytoplasmic, consistent with the enzymatic role of the glutathione-S-transferase encoded by R11A8.6. Finally, and importantly, the expression of *sir-2.1::mCherry* and *R11A8.6::gfp* rises dramatically upon starvation. No such increase is seen with *sir-2.1::gfp* under the control of the intergenic promoter fragment suggesting that elevated *sir-2.1* expression upon starvation depends on transcription across the operon from the upstream gene. Curiously, under starvation conditions, SIR-2.1::mCherry is not nuclear-localized, but is localized in puncta in the cytoplasm. Elevated transcription of *sir-2.1* also corresponds with elevation of expression of *sir-2.1* orthologues in other organisms upon starvation. We thank Heidi Tissenbaum for strains HT809, HT810. Tissenbaum & Guarente, Nature, 2001. 410, 227 Wang & Tissenbaum, Mech. Ageing Dev. 2006. 127, 48.

377B

The Early-Onset Torsion Dystonia Gene Product, torsinA, is a Mediator of ER Stress and Protein Homeostasis. **P. Chen**, A.J. Burdette, J.C. Porter, J.C. Ricketts, N. Roberts, S.A. Fox, L.A Berkowitz, K.A. Caldwell, G.A. Caldwell. Dept Biological Sci, Univ Alabama, Tuscaloosa, AL.

The capacity of cells to carry out their various functions is wholly dependent upon efficient protein synthesis, processing, trafficking, and degradation. In this context, dysfunction or deficit of proteins that monitor and ensure the proper maintenance of cellular homeostasis can have serious consequences in terms of disease onset, penetrance, or progression. A single codon deletion (GAG = ΔE) in the gene encoding human torsinA causes a non-degenerative neurological disorder termed early-onset torsion dystonia. TorsinA, an endoplasmic reticulum (ER) resident protein, belongs to the diverse AAA+ (ATPases Associated with a variety of cellular Activities) family. Here we use a well established ER stress model in the nematode C. elegans (hsp-4::GFP) to investigate the role of torsinA in maintaining protein homeostasis in the ER. Using GFP as an ER stress reporter, we found that transgenic nematodes expressing wild type (WT) human torsinA exhibited a striking reduction in ER stress caused by tunicamycin, while animals with either ΔE or a mixture of WT/ΔE torsinA failed to do so. Interestingly, ΔE and WT/ΔE torsinA worms exhibited higher ER stress than WT torsinA animals, even in the absence of tunicamycin treatment. In addition, mutations within torsinA ER signal sequence and N-terminal hydrophobic region abolished its capacity to maintain an ER stress threshold against cellular stressors, indicating the importance of its localization to the ER for this activity. Furthermore, although torsinA possesses very low ATPase activity, the ATPase domain appeared important for ER stress suppression, as two different mutations within the ATPase domain, K108A and E171Q, resulted in loss of stress suppression. A single nucleotide polymorphism (SNP), D216H, diminishes the penetrance of dystonia from 30-40% (WT/ΔE) to 3% (D216H/ΔE) in patients. In C. elegans, coexpression of ΔE torsinA with D216H torsinA significantly protected worms from ER stress caused by tunicamycin, thereby recapitulating the effect on penetrance observed with patients. In order to find genetic interacting factors of torsinA, a small scale of RNAi screen for altered ER stress levels in ΔE torsinA worms, was carried out. Positives from the screen included glutaredoxin, H3 histones, and 13 core components and regulators of Ras signaling. Taken together, our data indicate that torsinA serves to maintain protein homeostasis in the ER, shedding new light on the pathophysiology of torsion dystonia.

378C

Tissue-specific Proteostasis Networks in C. elegans. **Daniel Czyz**[1,4], Eric Guisbert[1,4], Klaus Richter[3,4], Patrick McMullen[2], Richard Morimoto[1]. 1) Department of Biochemistry, Molecular Biology and Cell Biology. Northwestern University, Evanston, IL; 2) Department of Chemical and Biological Engineering. Northwestern University, Evanston, IL; 3) Center for integrated protein science (CIPS) and Department Chemie. Technische Universität München, Garching, Germany; 4) these authors contributed equally.

The cellular ability to restore protein folding during proteotoxic stress requires activation of the heat shock response (HSR), which involves the activation of heat shock factor-1 (HSF-1). HSF-1 is a transcription factor that is repressed in the absence of stress by the Hsp70 and Hsp90 molecular chaperones. Although the HSR is well-understood on the cellular level, little is known about its regulation in the context of an intact, multicellular organism. Therefore, we performed a genome-wide RNAi screen for negative regulators of the HSR using a stress-inducible reporter, hsp70p::GFP. We identified 40 genes that repress hsp70p::GFP expression in an HSF-1-dependent manner. Knock-down of these genes also induces another HSF-1-dependent reporter, hsp-16.2p::GFP. Most of the identified genes fall into functional classes involved in protein synthesis, folding, transport, and degradation. Whether these genes directly or indirectly regulate HSF-1, they are influencing proteostasis, or protein homeostasis. HSF-1 is ubiquitous and most of the identified genes are broadly expressed; however, we found that the reporters are induced in a tissue-specific manner. Hence, we named the identified genes Tissue-Specific Proteostasis Regulators (TSPRs). We show that TSPRs are enriched in self-interactions and cluster into distinct networks that correlate with their tissue-specific reporter induction. We used these networks to successfully predict additional TSPRs. Together, these results suggest that specific networks regulate proteostasis in distinct tissues by repressing HSF-1 activity in the absence of stress.

379A

KGB-1, a JNK-like MAPK, negatively regulates FOS-1 transcription factor in stress response. **Ayuna Hattori**, Tomoaki Mizuno, Naoki Hisamoto, Kunihiro Matsumoto. Department of Molecular Biology, Graduate School of Science, Nagoya University, Nagoya, Japan.

The JNK MAP kinase (MAPK) pathway plays a pivotal role in the various stress responses in evolutionarily diverse species. In *C. elegans*, a JNK-like MAPK pathway composed of MLK-1 MAPKKK, MEK-1 MAPKK, and KGB-1 MAPK controls stress response. However, other components functioning together with this pathway have not been well characterized. Recently, we identified *cdk-8* gene, encoding CDK8 module subunits of Mediator complex, as a novel factor functioning together with the KGB-1 pathway. The *cdk-8* mutation caused a partial sensitivity to heavy metal stress and enhanced the stress sensitivity of *kgb-1* mutants. These results suggest that CDK-8 regulates the stress response in parallel to the KGB-1 pathway. The fact that JNK phosphorylates transcription factors raise the possibility that KGB-1 also regulates transcription factors. To identify the novel downstream factors regulated by KGB-1, we performed yeast two-hybrid screening using KGB-1 as a bait. As a result, we isolated FOS-1, a bZIP transcription factor homologous to human Fos, as a KGB-1 binding protein. Further biochemical analysis showed that FOS-1 is phosphorylated by KGB-1. In addition, the knock down of FOS-1 by RNAi suppressed the stress sensitivity of *kgb-1* mutants. These results suggest that KGB-1 negatively regulates FOS-1 through phosphorylation of FOS-1.

380B

The *C. elegans* ASK-p38 pathway regulates survival in anoxic conditions in a non-cell autonomous manner. **Teruyuki Hayakawa**, Kohsuke Takeda, Hidenori Ichijo. Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan.

Living organisms require oxygen to maintain their lives, however both excess and shortage of oxygen can be stressors to organisms depending on their biological context. Under anoxic conditions (i.e. completely deprived of oxygen), nematodes apparently decrease metabolism rate and arrest cell cycle. However, signaling pathways that regulate survival under anoxic conditions have not been elucidated. Apoptosis signal-regulating kinase (ASK) family proteins are MAP3Ks which activate the p38 and JNK pathways, and play pivotal roles in responses to stress such as oxidative stress in mammals. *C. elegans* possesses a single ASK family protein, named NSY-1, and its functions are well conserved with those of mammalian ASK1. Previous reports have shown that *nsy-1* loss-of-function mutation causes decrease in survival rates under oxidative stress and bacterial infection. Here we show that loss-of-function mutation of *nsy-1* increases survival rate under anoxic conditions. When animals were subjected to anoxic conditions (<0.2 % O2), *nsy-1* mutant animals showed higher survival rates than wild type ones. Mutant animals of the components of the p38 pathways, but not those of JNK pathways, showed higher survival rates. Consistent with these findings, we found that PMK-1, a p38 MAPK ortholog, was activated in response to anoxia and that the PMK-1 activation in response to anoxia was suppressed in *nsy-1* mutants. These results suggest that the NSY-1-PMK-1 pathway plays important roles in anoxic responses in C. elegans. Exogenous expression of NSY-1 in either of the hypodermal, intestinal, or neuronal tissue suppressed the increase in survival rate under anoxic conditions in *nsy-1* mutants, suggesting the non-cell autonomous roles of NSY-1.

381C

Effect of selective serotonin reuptake inhibitors (SSRIs) antidepressants on thermal stress resistance in *Caenorrhabditis elegans*. **R Keowkase**, JE Moreton, Y Luo. Department of Pharmaceutical Sciences, University of Maryland Baltimore School of Pharmacy, Baltimore, MD.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and is the most common form of dementia in elderly people. Currently, there are no drugs with disease-modifying properties. Moreover, increasing evidence suggests that AD is often accompanied by depression. Antidepressants such as selective serotonin reuptake inhibitors (SSRIs) are widely used in patients with AD. We found that both SSRIs fluoxetine and citalopram increased thermal stress resistance. To investigate the mechanism underlying the effect of fluoxetine and citalopram on thermal stress resistance, the mutant worms deficit in serotonergic (*ser-1*, *ser-4*) and insulin/insulin-like growth factor-1 (IGF-1) signaling pathway (*daf-16*) were used. The result showed that effects of fluoxetine and citalopram on thermotolerance depended on the insulin/IGF-1 signaling pathway and DAF-16 FOXO/forkhead transcription factor. Furthermore, the action of these drugs on thermal stress resistance is not associated with serotonergic signaling pathway as we first expected. Thus, in addition to their known therapeutic target serotonin, both fluoxetine and citalopram are able to modulate insulin/IGF-1 signaling pathway to provide thermal stress resistance. These findings give us a perspective that some antidepressants used in AD patients might have additional therapeutic benefits besides their efficacies to ameliorate depressive symptoms.

382A

Can environmental chemicals which affect mammalian reproduction have an impact on *C. elegans* physiology? **Cristina Lagido**[1], Debbie McLaggan[1], Andrew Frost[1], Maria R. Amezaga[1], Stewart M. Rhind[2], Paul A. Fowler[1], L. Anne Glover[1]. 1) Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, UK; 2) Macaulay Land Use Research Institute, Craigiebuckler, Aberdeen AB15 8QH, UK.

Environmental chemicals (ECs), including endocrine disrupting compounds and heavy metals can persist in the environment and have detrimental effects on reproductive, immune and neurological functions and induce cancers[1]. Assessment of risk posed by ECs is often based on their known individual toxicity and carcinogenicity rather than their endocrine disrupting effects and yet, effects are often associated with much lower concentrations than those resulting in toxic or carcinogenic effects. For example, prolonged exposure of pregnant sheep to a "real-world", complex mixture of ECs in pastures and soils fertilized with sewage sludge, had an adverse effect on fetal ovary development[2]. The application of sewage sludge to agricultural soils emphasises the need to better evaluate potential risks to public health.

We have developed a new tool to rapidly assess effects of environmental and physiological stress. We generated luminescent ATP sensor *C. elegans* strains through constitutive expression of the firefly luciferase gene and showed that their light output declines in response to knockdown of respiratory genes or treatment with xenobiotics[3]. We validated luminescence as a toxicological endpoint by showing that the decline in bioluminescence upon exposure to the model toxicant Cd was a sensitive indicator of toxicity, comparable to other conventional sublethal endpoints, such as reproduction and development[4].

Using our luminescent ATP sensor *C. elegans* strain, we have recently determined that a sublethal exposure to sewage sludge extract has a concentration-dependent detrimental effect on the worm's energy status. We also established that sewage sludge affected worm reproduction. We aim to investigate whether conserved pathways are involved in these responses in order to understand potential exposure effects.

[1]Rhind, S.M. (2008) Reprod Dom Anim 43 (Suppl. 2), 15-22. [2]Fowler, P.A. *et al.* (2008) *Mol Hum Reprod* 14 (5), 269-280. [3]Lagido *et al.* (2008) *BMC Physiol* 8(1) :7; Lagido *et al.* (2001) *FEBS Letters* 493, 36-39. [4]Lagido *et al.* (2009) *Toxicol Sci.*

383B

Investigating the role of PNC-1 in SIR-2.1 regulation and stress response. **Stephanie E. Lange**, Leah Y. Liu, Wendy Hanna-Rose. Dept of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA.

SIR-2.1 is an important lysine deacetylase that has been implicated in aging and stress response. The enzymatic activity of SIR-2.1 is NAD^+ dependent and is feedback inhibited by 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 may be reflected by the downstream modulation of *sod-3* expression. To test this, we are examining SIR-2 overexpression-mediated *sod-3*::GFP expression in combination with PNC-1 overexpression and loss-of-function. Our initial results suggest that the interaction is more complex than this simple model, and PNC-1 may regulate *sod-3* via SIR-2.1-dependent and -independent pathways. Consistent with a more complex role for PNC-1 in regulation of *sod-3*, we have preliminary evidence of oxidative stress regulation of PNC-1. Pnc1p is also upregulated in response to stress in yeast.[1] These data indicate a role for PNC-1 in stress response more broad than solely via regulation of SIR-2.1.

[1] Anderson et al. 2003. *Nature* 423(6936):181-5 [2] Berdichevsky et al. 2006. *Cell* 125(6):1165-77.

384C

Environmental and genetic mechanisms associated with prolonged anoxia survival in *Caenorhabditis elegans*. **Bobby LaRue**, Pamela Padilla. Department of Biological Sciences, University of North Texas, Denton, TX.

Oxygen deprivation is a key component of ischemic damage associated with cardiovascular disease and traumatic blood loss. These health issues will be better understood if the mechanisms that prevent oxygen-deprivation induced cell death are elucidated. Previously we showed that wild-type *C. elegans* survive one day of anoxia(<.001 kPa O2)but cannot survive three days of anoxia; in these experiments the animals were raised at 20C, maintained on OP50 bacteria and exposed to anoxia at 20C. Adult animals, grown at 20C, and then exposed to one day of anoxia and heat stress (28C) cannot survive indicating that the combination of thermal stress and anoxia decreases viability. Our recent findings demonstrate that preconditioning at 25C of wild-type animals will induce prolonged anoxia survival (three days). This thermal preconditioning effect is enhanced when animals are grown on RNAi control media,suggesting a synergetic affect between RNAi control media and thermal preconditioning. To determine if,in addition to thermal stress, the stress associated with dietary restriction will induce a preconditioning effect on animals exposed to anoxia,we used the dietary restriction mutant *eat-2(ad1116)*;we found that dietary restriction also induces anoxia survival. These results indicate that preconditioning environments can enhance anoxia survival in wild-type animals. Additionally, genetic mutations elicit a long-term anoxia survival phenotype. Several *daf-2* alleles as well as mutations in *glp-1 (glp-1(q158),glp-1(e2141)*) induce a long-term anoxia survival phenotype (20C). We are interested in identifying the molecular changes in *glp-1* and *daf-2* mutant animals that lead to a long-term anoxia survival phenotype. Both *glp-1* and *daf-2* mutations are known to have a longevity phenotype and the pathways involved with such are well understood. Therefore, we used RNAi to screen through several known genes required for the longevity phenotype in either *glp-1* or *daf-2* animals; these experiments will allow us to determine genetic pathways and genes required for anoxia survival and further elucidate components of signaling pathways required for longevity and/or stress resistance. We found that *aak-2*,the catalytic subunit of AMP activated protein kinase (AMPK) is required for the anoxia survival phenotype in *daf-2(e1370)*,25C preconditioned N2,and in a DAF-16 dependent fashion,*glp-1(e2141)*;the other AMPK catalytic subunit (*aak-1*) is not required,suggesting that *aak-1* and *aak-2* have distinct functions. Together,our findings indicate that stress and longevity pathways (Insulin-like, notch, TOR and AMPK signaling) overlap and may exhibit cross talk that is modulated depending upon the specific stress presented to the organism.

385A

Chromatin remodeling factors regulate HIF-1-independent hypoxia response. **Jihyun Lee**, Junho Lee. Research Center for Functional cellulomics, Institute of Molecular Biology and Genetics, Department of Biological Sciences, Seoul National University, Seoul, Korea.

Small heat shock proteins are induced by various stresses. The C. elegans genome contains 4 major HSP-16 proteins. Two of the *hsp-16* genes (*hsp-16.1* and *hsp-16.2*) in *C. elegans* responded to hypoxia, while the other two genes (*hsp-16.48* and *hsp-16.41*), which share the promoter regions with their counterparts, did not. The comparison of the promoter sequences of *hsp-16.1* revealed a new conserved regulatory element (block I) consisting of CAC(A/T)CT that was required for the hypoxia response, but not for other stress responses such as heat or ethanol. This hypoxia response of *hsp-16.1* does not require *hif-1* function, a hypoxia inducible factor, indicating that its induction may be mediated by a new mechanism.

HMG-1.2, a high mobility group box-containing protein, was identified as a block I binding protein. Downregulation of *hmg-1.2* by RNA*i* led to suppression of *hsp-16.1* induction under hypoxia. Among genes that are predicted to interact with HMG-1.2, proteins involved in chromatin structures were responsible for *hsp-16.1* induction at the hypoxic condition. It has been demonstrated that *tax-6*, an ortholog of Calcineurin A, was induced by hypoxia in a *hif-1*-independent manner. Others have shown that hypoxia induced intracellular Ca^{2+} and that HIF-1 is not involved in Ca^{2+}-dependent activation of hypoxia genes. Reduction function of *tax-6* decreased the hypoxia response of *hsp-16.1*.

Relatively little is known about regulatory pathways independent of HIF. It is possible that the calcium signaling and chromatin remodeling process are involved in *hif-1*-independent hypoxia response of *hsp-16.1*.

386B

Hyperosmotic stress leads to rapid perturbation of ATP levels: a possible role in osmosensing. **Debbie McLaggan**, Cristina Lagido, Anne Glover. Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, Scotland, UK.

We are interested in the early events in *Caenorhabditis elegans* following hyperosmotic stress. The molecular mechanisms by which animal cells detect volume changes and transduce those signals into effector responses still remain to be elucidated despite significant progress in the understanding of effector responses. We have established a reproducible, quantitative and scalable bioluminescent assay for the metabolic effects of the first few minutes at high osmolarity. We use our highly luminescent transgenic *C. elegans* strain PE255 *(feIs5) X* constructed through the constitutive and generalised expression of the bioluminescent enzyme firefly luciferase (1). We have previously demonstrated that light levels provide a sensitive measure of the ATP levels obtained by RNAi silencing of respiratory chain genes (1). In the context of the study of stress response, luminescence allows effective detection of transient changes in ATP pools in *C. elegans* that were previously not measureable. Following a hyperosmotic shock, we have observed a transient increase in luminescence of our biosenosr *C. elegans*, indicative of a transient increase in ATP. The increase in luminescence did not occur in the presence of the respiratory inhibitor sodium azide, indicating that *de novo* ATP synthesis is required for the observed increase. In addition there was no increased uptake of bioluminescence components upon osmotic upshock as determined by monitoring pharynx pumping. We have determined that the extent of light output is proportional to the extent of osmotic perturbation. This effect also appears to be independent of the initial osmolarity of the medium. Following water loss, the earliest subsequent event that has been determined is the dose dependent increase in WNK1 (with-no-K (lysine) Kinase-1) activation in mammalian cells (2). WNK kinases have important roles in regulating salt and water transport in the mammalian kidney. *C. elegans* has WNK-1 and other downstream homologues that are required for acute volume recovery in *C. elegans* (3). These have been postulated to function in a manner similar to mammalian WNK1 kinase signalling pathway (4). The ultimate aim of our studies is to determine whether the transient increase in intracellular ATP is involved in WNK mediated signaling of hyperosmotic water loss. (1) Lagido, C. *et. al.* (2008) BMC Physiology 8 (1):7. (2) Zagorska, A. *et. al.* (2007) J. Cell Biol. 1176:89-100. (3) Choe, K.P. and Strange, K. (2007) Am. J. Physiol. 293:C915-C927. (4) Choe, K.P. and Strange, K. (2007) FEBS J. 274:5782-5789.

387C

Hydrogen Sulfide Protects Against Hypoxia. **Dana L. Miller**, Mark B. Roth. Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA.

It has been recently demonstrated that hydrogen sulfide (H_2S) can protect animals against damage and death associated with decreased O_2 availability. For example, H_2S protects mammals against otherwise lethal hypoxia and improves outcome in mammalian models of severe blood loss, myocardial infarction, aortic occlusion and hepatic ischemia/reperfusion. The mechanisms by which H_2S exerts beneficial effects in mammals are unknown. We have developed a *C. elegans* model investigate the genetic factors that contribute to damage associated with ischemia in animals and to define the molecular mechanisms that mediate the beneficial effects of H_2S. Here we show that, as in mammals, H_2S protects against hypoxia in *C. elegans*. We have found that specific hypoxic conditions induce aggregation of polyglutamine-containing proteins. The range of O_2 concentrations that cause aggregation is expanded by mutations in the highly conserved hypoxia-inducible transcription factor, *hif-1*. These data suggest that perturbations in protein homeostasis occur when the cellular response to hypoxia is overwhelmed. Adaptation to H_2S protects against hypoxia-induced disturbances of protein homeostasis. Even transient exposure to H_2S early in development is sufficient to protect against hypoxia-induced aggregation of polyglutamine proteins. The H_2S-induced changes that protect against hypoxia are distinct from those that cause increased lifespan and thermotolerance. These data show that exposure to H_2S results in persistent physiological effects that can influence responses to changing environmental conditions.

388A

Condition-adapted stress and longevity gene regulation by C. elegans SKN-1/Nrf. Riva P. Oliveira[1,3], Kieran Dilks[2], **Jess Porter Abate[1]**, Jessica Landis[2], Jasmine Ashraf[2], Coleen T. Murphy[2], T. Keith Blackwell[1]. 1) Section on Developmental and Stem Cell Biology, Joslin Diabetes Center; Department of Pathology, Harvard Medical School; Harvard Stem Cell Institute, Boston MA; 2) Lewis-Sigler Institute for Integrative Genomics, Department of Molecular Biology, Princeton University, Princeton NJ; 3) Departamento de Ciências Biológicas, Universidade Federal de Ouro Preto, Brazil.

In C. elegans the transcription regulator SKN-1 is important for oxidative stress resistance and acts in multiple longevity pathways, including insulin/IGF-1-like signaling (IIS). SKN-1 is the ortholog of mammalian Nrf proteins, which induce Phase 2 detoxification genes in response to stress. Here we have used expression profiling to identify genes and processes that are regulated by SKN-1 under normal and stress-response conditions. Under normal conditions SKN-1 upregulates numerous genes involved in detoxification and related functions. Surprisingly, SKN-1 also downregulates a set of genes that reduce stress resistance and lifespan, including the IIS genes ins-7 and pdk-1. In each case, many of these genes appear to be direct SKN-1 targets. The metalloid sodium arsenite induces skn-1-dependent activation of certain detoxification gene groups, some of which were not SKN-1-upregulated under normal conditions. Another stressor, an organic peroxide, also triggers induction of a discrete Phase 2 gene set, but additionally stimulates a broad SKN-1-independent response. We conclude that under normal conditions SKN-1 has a wide range of functions in detoxification and other processes, including modulating mechanisms that reduce stress resistance and lifespan. In response to stress, SKN-1 and other regulators tailor transcription programs to meet the challenge at hand. Our findings reveal striking complexity in SKN-1 functions and in the regulation of systemic detoxification defenses.

389B

Dose-dependent roles for the *C. elegans* HIF-1 hypoxia-inducible factor in stress resistance and aging. **Jo Anne Powell-Coffman**, Yi Zhang, Dingxia Feng, Zhiyong Shao, Zhiwei Zhai. Department of Genetics, Development, & Cell Biology, Iowa State University, Ames, IA.

Oxygen homeostasis is essential to metazoan life, and animals have evolutionarily conserved strategies for adapting to changing levels of environmental oxygen levels during development, homeostasis and disease. The hypoxia-inducible factor (HIF) heterodimeric transcription factors are the central regulators of oxygen-sensitive gene expression in animals as diverse as humans and *C. elegans*. HIF stability and activity are regulated by environmental oxygen levels and by reactive oxygen species, and HIF complexes control the expression of batteries of genes that enable adaptation to hypoxic stress. We and others have shown that *C. elegans* HIF-1 and its regulators have important roles in adaptation to hypoxia, neuronal development, pathogen resistance, aerotaxis behavior, and egg laying [Trent et al., Genetics 1983; Darby et al. PNAS 1999; Jiang et al., PNAS 2001; Shen et al., JBC 2005; Pocock and Hobert, Nature Neuro 2008; Chang and Bargmann PNAS 2008]. We have begun to elucidate the roles of HIF-1 in the *C. elegans* stress response network, and we have discovered that HIF-1 overexpression results in dose-dependent extension of average lifespan. Interestingly, we find that *hif-1* deletion mutations also increase *C. elegans* longevity, but via different genetic pathways. We have investigated interactions between HIF-1 and other stress responsive transcription factors and have discovered feedback loops in which SKN-1 modulates HIF-1-dependent gene expression. Collectively, these findings provide a greater understanding of how HIF-1 is integrated into the *C. elegans* stress response network. These studies have been supported by grants from NIGMS (GM078424) and by the ISU Center for Integrated Animal Genomics.

390C

Determining the Role of the Small Heat Shock Protein HSP12.6 in C. elegans. **L. Ramsay[1,2]**, E.G. Stringham[1,2]. 1) Department of Biology, Trinity Western University, Langley, British Columbia, Canada, V2Y 1Y1; 2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada, V5A 1S6.

The small heat shock protein HSP12.6 is a target of DAF-16/FOXO and may contribute to influence lifespan (Murphy et al., 2003). In addition, HSP12.6 is thought to be acted upon by the JNK-1 signaling pathway. However, western analysis of C. elegans lysates was performed using the polyclonal anti-HSP12.6 antibody revealed that JNK-1 is not required for DAF-16 directed HSP12.6 expression. To monitor HSP12.6 expression in vivo, transgenic strains carrying a translation fusion of phsp12.6::HSP12.6::DSRED were constructed. Upon heat shock at 33º for 2 hours, the observed expression patterns of these translational fusion lines seem to be in muscle cells and axons in the head, tail, and vulva. In addition, there may be low levels of constitutive expression in non-heat shocked worms in the same areas of the body. A strain will be integrated and placed in the background of different mutants and/or reporters of genes in the DAF-16/Insulin-like signalling (DAF-16/IIS) pathway. This will help to elucidate the expression and function of HSP12.6 in response to stress in conjunction with previously proposed pathways. For example, as a downstream target of the DAF-16/IIS pathway, it is expected that heat stress induced HSP12.6 expression will be observed following the translocation of a DAF-16:GFP reporter into the nucleus. Also, different stages of worms will be viewed to determine if there are any changes in expression between separate stages of the life cycle. We also wish to determine the comparative role of HSF-1 versus DAF-16 in transcription of *hsp12.6*. Do they work together to transcribe *hsp12.6*, or does *hsp12.6* even need HSF-1 to function in response to heat stress? Therefore, expression of phsp12.6::HSP12.6::DSRED in an HSF-1 null mutant will be determined. In order to confirm previous results that JNK-1 is not required for DAF-16 directed HSP12.6 expression, *in vivo* expression pDAF-16::GFP, pJNK-1::GFP and/or phsp12.6::HSP12.6::DSRED will be observed in the background of various mutants. As HSF-1 is indicated to be involved in immunity of *C. elegans*, it is of interest to see if HSP12.6 has a role in response to the human pathogen *Pseudomonas aeruginosa*. In addition, it would be interesting to determine if there is a phenotype in affected worms in response to pathogens during different stages of their life cycle. In order to know if certain genes are working in the same pathway, the immune response in single and double mutants will be examined by conducting survival assays on *P. aeruginosa*, and by examining worms exposed to pathogens for expression of HSP12.6.

391A

Transgenerational Transmission of Low Dose Radiation Mediated Hormesis in C. elegans. **Renuka Sivapatham**[1], Maithili C Vantipalli[2], Arnold Kahn[2], Judith Campisi[2], Gordon J Litgow[2], Anders Olsen[1]. 1) Department of Molecular biology, University of Aarhus, Gustav Wieds vej 10, 8000 Aarhus C, Denmark; 2) Buck Institute for Age Research, 8001 Redwood Boulevard, Novato CA 94945.

Brief exposure to a mild stress causes induction of stress gene expression leading to enhanced stress responses, improved maintenance and repair and in some cases lifespan increase. This phenomenon is termed hormesis and has been observed in several species. For example, we previously demonstrated that short periods of mild heat stress in early life increase both mean and maximum lifespan of the soil nematode C. elegans. Similar hormetic responses have been described for many other stressors. Here we present data showing that treatment of the nematode with low-doses of ionizing radiation (IR) significantly increases resistance to sub-sequent heat stress. Importantly, we find that the progeny of nematodes treated with IR are resistant to heat stress compared to progeny of untreated controls. This novel transgenerational effect is intriguing since stress resistance in the progeny generation has not been described for IR or any other stressors. We find that this effect appears to be specific for IR and for example treatment with N-ethyl-N-nitrosourea (ENU) or mild heat stress does not have any transgenerational effect. Interestingly, this increase in stress resistance by IR is independent of insulin signaling, a well known pathway determining stress resistance and lifespan in C. elegans. Likewise, the tumor suppressor p53 (cep-1) is not required for low-dose IR induced stress resistance in the progeny generation. We are expanding our genetic analysis to include other well known DNA damage / checkpoint proteins as well as DNA repair genes. We have also undertaken microarray analysis of the progeny of worms exposed to IR to establish which gene expression changes are responsible for the increase in stress resistance. Since many long-lived worms are also resistant to stress, we are currently investigating whether low dose IR increases the lifespan of the nematode.

392B

Studies of the relationship between *pcs-1* and *hmt-1* revealed the role of coelomocytes in heavy metal detoxification. Marc S. Schwartz, Andy Chen, Joseph Benci, Devarshi Selote, **Olena K. Vatamaniuk**. Dept Crop & Soil Sci, Cornell Univ, Ithaca, NY.

The chronic exposure of humans to heavy metals, either occupational or from food and air, leads to their accumulation in tissues and causes various diseases, including neurodegenerative conditions, dysfunction of vital organs, and cancer. Protecting and detoxifying organisms in metal-polluted environments are contingent on our understanding of the effective cellular detoxification mechanisms. Our past studies have established that *pcs-1* and *hmt-1*, encoding phytochelatin (PC) synthase and a half molecule ATP binding cassette (ABC) transporter respectively, are acutely required for the detoxification of Cd^{2+}, but despite what was thought previously, do not act in concert in heavy metal detoxification (Vatamaniuk et al 2001, 2005). Here, we show that in addition to Cd^{2+}, *pcs-1* and *hmt-1* confer tolerance to As^{3+} and Cu^{2+}. Consistent with the notion that *pcs-1* and *hmt-1* operate in distinct metal detoxification pathways, *pcs-1;hmt-1* double mutants are more sensitive to these metals than *pcs-1* or *hmt-1* single mutants, and are expressed in distinct tissues: *hmt-1* is expressed in intestinal cells and head and tail neurons, whereas *pcs-1* is expressed in pharyngeal grinder, pharyngeal-intestinal valve, somatic and vulval muscles. Interestingly, *pcs-1* and *hmt-1* are co-expressed in coelomocytes. This finding raised the intriguing possibility that coelomocytes are involved in heavy metal detoxification. We tested this hypothesis, by analyzing the heavy metal sensitivity of the coelomocyte-deficient mutant strain, NP717 (from Dr. H. Fares laboratory, University of Arizona). In doing so we established that NP717 worms have increased sensitivity to $Cd^{2+}$and Cu^{2+}. After 4.5 days of culturing at 20^0C 100% of N2 worms reached adult stage regardless whether cultured on the medium with or without 50 μM $CdCl_2$ or 200 μM $CuCl_2$. NP717 worms have reached adults stage on the medium devoid of heavy metals as well. In contrast, when grown in the presence of Cd^{2+} or Cu^{2+}, 54% and 63% of NP717 respectively were delayed at early-developmental stages. These worms either died, not being able to reach an adult stage, or, died shortly after becoming adults and gave none or fewer progeny. These data demonstrate that coelomocytes, the functions of which in worms have not been well-understood, play an essential role in heavy metal detoxification.

393C

Lifespan extension in calcineurin-defective mutants requires autophagy genes in *C. elegans*. **Meenakshi Dwivedi**[1,2], Karunambigai Kalichamy[1], Magdalena Wiacek[1], Joohong Ahnn[1]. 1) Department of Life Science, Hanyang University, Seoul, South Korea; 2) Research Institute for Natural Sciences (RINS), Hanyang University, Seoul, South Korea.

Lifespan extension in any organism literally means a delay in the aging process. Many of the pathways found to be involved in lifespan regulation in different organisms are dependent on the autophagy pathway or autophagic genes. In *Caenorhabditis elegans*, the target of rapamycin (TOR) pathway, which is responsible for sensing nutrients (inhibited by dietary restriction) and the insulin/IGF-1 pathway, which responds to different environmental cues, are well known pathways involved in lifespan extension and are reportedly dependent on the autophagy. In addition, it has recently been reported that lifespan extension in mutants with lowered mitochondrial respiration and *cep-1* mutants (orthologue of *p53*) is also dependent on autophagic genes. The results of a study that we conducted also suggested that calcineurin regulates lifespan through the autophagy genes. Calcineurin (CaN) is a serine/threonine phosphatase, activated by Ca^{2+}/calmodulin (Ca^{2+}/CaM). CaN is known to regulate various cellular responses in different organisms. In this study, we report that calcineurin defective strains exhibit enhanced autophagy as assessed by the increased formation of GFP::LGG-1 associated autophagic structures in the seam cells. In addition to enhanced autophagy in calcineurin loss-of-function/null mutants, extended lifespan was also observed in *cnb-1(jh103)* calcineurin B null mutant and *tax-6(ok2065)* loss-of-function mutant of calcineurin A when compared to wild-type and *tax-6(jh107)* gain-of-function mutants. The RNAi knock-down of two essential autophagy genes, *bec-1* and *atg-7*, suppress both the number of autophagic puncta and the lifespan extension observed in the calcineurin-defective mutants. The suppression of lifespan extension to the levels similar to wild-type animals fed with control vector only was observed in both *cnb-1(jh103)* and *tax-6(ok2065)* mutants treated with *bec-1* RNAi. Thus, for the first time we suggest that pathway operating for lifespan extension in calcineurin mutants converge at the autophagic pathway. This activated autophagic pathway in calcineurin mutants may be due to the disruption in calcium signaling pathway, which remains to be elucidated.

394A

Exploring Aluminium Toxicity in *C. elegans*. **Kathryn E. Page**[1,2], Keith N. White[2], Catherine R. McCrohan[2], Gordon J. Lithgow[1]. 1) Buck Institute for Age Research, Novato, CA 94945; 2) Faculty of Life Sciences, University of Manchester, Manchester M13 9PT.

Aluminium (Al) is a highly abundant crustal metal with known toxic effects in multiple biological systems. *Caenorhabditis elegans* (*C. elegans*) is widely used for toxicity and aging studies due to its small size, large progeny numbers, short lifespan, and simple methods of genetic manipulation. *C. elegans* is a good model to study the toxic mechanism of Al in animals, and has been widely used for the study of environmental metal toxicology. Here we show Al effects fertility, and development progression, and that these phenotypes are transferable to the progeny of exposed worms. The exposures were carried out on agar plates with the Al mixed with concentrated OP50 which was then fed to the worms. Al negatively affects *C. elegans* developmental progression at 0.1 to 300 mM Al food concentration, and fertility at 1 mM Al food concentration. The developmental delay phenotype caused by Al exposure can be passed on to the next generation. It is possible that the mechanism of Al toxicity is similar to that of other toxic metals.

395B

The Effects of Manganese and Mn Homeostasis on Pathogen Infection in *C. elegans*. **Weixun Li**[1], Jeong Hoon Cho[2]. 1) Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, 133-791, Korea; 2) Science Education, College of Education, Chosun University, Korea.

Nramp1 (Natural resistance-associated macrophage protein-1) is a manganese transporter in macrophage functionally conserved in bacteria and eukaryotes. Manganese (Mn), which is required in small amounts functions as a cofactor for most antioxidants, and also known as a superoxide scavenger. It is also known that mouse defective in Nramp1 is susceptible to pathogen. Three Nramp homologues, *smf-1*, *smf-2*, and *smf-3*, have so far been identified in the nematode, *Caenorhabditis elegans*. GFP promoter assay revealed intestinal expressions of *smf* genes from early embryonic stages and these expressions were maintained through the adult stages. Furthermore, *smf* deletion mutants and *smf* RNAi have increased sensitivity to oxidative stress, EDTA and excess manganese. Interestingly, defective SMFs cause hypersensitivity to the pathogen, *Staphylococcus aureus* but not to the non-pathogenic *Bacillus subtilis*, indicating that manganese uptake is essential for the innate immune system. We are further investigating the roles of Manganese and other Mn homeostasis related genes, such as the Golgi calcium/manganese ATPase, *pmr-1*, and the vacuolar H+-ATPase, *vha-8* in the worms' defense system. We propose that *C. elegans* intestinal lumen provide a good and a simple model for macrophage phagosome especially in Mn-mediated innate immune systems.

396C

Expression of hepatitis B viral antigens induces growth retardation of C. elegans. **Yi-Yin Chen**[1], Wei-Ning Hung[1], Li-Wei Lee[2], Szecheng J. Lo[1,2]. 1) Graduate Institute of Biomedical Science, Chang Gung University, Taoyuan city, Taiwan; 2) Department of Life Sciences, Chang Gung University, Taoyuan city, Taiwan.

Hepatitis B virus (HBV) is a human hepatotropic virus. Its infection is associated with various liver diseases, including acute and chronic hepatitis and hepatocelluar carcinoma. Pathogenesis induced by HBV remains elusive. Here, we report HBV surface antigen (HBsAg) expression in various tissues of C. elegans caused different degree of retardation and reduction of brood-size. Transgenic worms expressing major HBsAg driven by the fibrillarin (fib-1) promoter had the most pronounced effect on brood-size and growth rate; those expressing major HBsAg driven by ges-1 or myo-2 had the moderated effect; those expressing HBsAg by neuron promoter (mec-7) had no significant effect. Since in transgenic mouse model and human cell line have demonstrated that the expression of HBsAg induced ER-stress, the effect of lower brood-size and growth retardation caused by HBsAg expression in pharyngeal and intestinal cells might be also resulted from ER-stress. To determine the temporal effect of HBsAg, a heat-shock induction system was employed. Transgenic worms expressing HBsAg at L4 stage had the effect while those HBsAg expressing in L1, L2 or L3 worms had less effect. Transgenic worms expressing another HBV core antigen (HBcAg), which is located at the nucleus, also caused brood-size reduction. How this antigen reduced brood-size remains to be investigated.

397A

Identification of modulators of RNA-dependent toxicity in myotonic dystrophy. **Susana M. Garcia**[1,2], Gary B. Ruvkun[1,2]. 1) Department of Molecular Biology, Massachusetts General Hosp, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Instability in nucleotide repeats leading to repeat expansions has been associated with an increasing number of degenerative disorders. Included in this group are nucleotide (CTG and CCTG) repeat expansions in non-coding regions, in which pathogenesis was shown to be mediated by RNA. The toxic effect caused by these mutant RNA transcripts highlights the capability of RNA to act as a dominant pathogenic species. Expansions of CTG or CCTG repeats in the non-coding regions of unrelated genes have been linked to myotonic dystrophies. Myotonic dystrophies (DMs) are autosomal, dominantly inherited multisystemic disorders characterized by progressive skeletal muscle loss, myotonia and cardiac conduction defects. DMs constitute the most common form of adult muscular dystrophy with a worldwide incidence of 1:8000. The presence of transcripts containing expanded CUG repeats leads to aberrant RNA-protein interactions. These pathogenic transcripts are predicted to accumulate and act in a transdominant fashion leading to misregulation of developmentaly-regulated alternative splicing factors. Whether additional RNA-binding proteins are affected is not known.

We are interested in the genetic factors and pathways that modulate this RNA-dependent pathogenic process and in particular the potential involvement of small-RNA pathways as regulators of expanded RNA toxicity. To identify the complement of factors that modulate CUG transcript toxicity and lead to cellular misregulation, we generated *C. elegans* strains expressing GFP containing different CTG-repeat lengths in the 3'UTR, of body wall muscle cells. Animals expressing GFP containing 3'UTR CUG repeats exhibit lower GFP expression which decreases progressively with aging. Furthermore, animals expressing GFP containing very long CUG repeats in their 3'UTR exhibit toxic phenotypes. We will take advantage of the age-dependent decrease in GFP expression in animals that express 3'UTR CUG repeats to screen, by RNAi, for the complement of genes that enhance or suppress this phenotype. The identification of these factors will allow a better understanding and characterization of the underlying RNA toxic mechanisms associated with repeat toxicity.

398B

A *C. elegans* Krüppel-like factor, KLF3 acts on fatty acid synthesis related signal transduction pathway to regulate their activity. Jun Zhang[1], Chuan Yang[3], Christopher Brey[2], Randy Gaugler[2], Chen-Han Huang[3], **Sarwar Hashmi[1]**. 1) Developmental Biology, New York Blood Center, New York, NY; 2) Biochemistry and Molecular Genetics, New York Blood Center, New York, NY; 3) Rutgers University, New Brunswick, NJ.

Type 2 diabetes (T2D) is a system disease involving changes in both conserved cores (pathway/network of glucose/lipid metabolism) and adaptive conduits for nutrient (food) intake, storage, and sensing. In full-blown T2D, insulin resistance and β-cell failure emerge owing to chronic pathogenic insults to metabolic networks and enduring perturbations of energy homeostasis. The family of Krüppel-like factors (KLFs), a conserved and important class of transcription factors, has been implicated to regulate lipogenesis and adipocyte differentiation in mammals. However, how fat storage is coordinated in response to positive and negative feedback signals is still poorly understood. To address mechanisms underlying fat storage we study a *Caenorhabditis elegans* Krüppel-like transcription factor, *Ce-klf-3* and demonstrate that KLF-3 is a key regulator of fat metabolism in *C. elegans*. The *Ce-klf-3* is highly expressed during worm's development and predominantly present in intestine, the site for fat digestion, absorption, storage, and utilization. We found a strong positive correlation between *klf-3* expression and a change in fat deposition in worm's intestine. Significantly, a *klf-3(ok1975)* loss-of-function mutation, characterized by 1.6 kb deletion spanning exon 2 to 3, increased fat accumulation in the intestine and caused severe defects in worm's reproduction. Although *klf-3* mutants seemed grossly similar to wild-type worms in appearance and in life span, they became completely sterile toward adulthood. Notably, mutant worms displayed extensive deposition of large amount of fat in the intestine, implicating a causal link of fat accumulation to reduce fertility. Our study also demonstrates that KLF-3 is critical for maintaining normal fatty acid composition and it does so by regulating genes involved in fatty acid desaturation pathway. Strikingly, *klf-3* mutant animals also impaired expression of genes devoted to fatty acid β-oxidation pathways. We present the first clear in vivo evidence supporting essential regulatory roles for KLF-3 in fat storage linking fat metabolism and reproduction in *C. elegans*. Such a casual relationship in the worm opens up a window to look into comparable disease states in human diabetes, i.e., obesity-conditioned insulin resistance and lipotoxicity-induced β-cell failure.

399C

A worm model of Tau pathology in Alzheimer's Disease using physiologic expression levels. **B.E. Jones**, S. Ackroyd, T. Sherman, K. Nehrke. Medicine, Nephrology Unit, University of Rochester School of Medicine, Rochester, NY.

Tau pathology is a well established characteristic of Alzheimer's disease. The role of Tau in initiation and progression of Alzheimer's disease however, remains an area of intensive scientific investigation. To date, animal models of Tau's role in the disease have used standard molecular biology techniques resulting in hyperphysiologic levels of Tau protein expression. Using a newly developed system for single copy genomic integration of a transgene in C. elegans1, we were able to create a model in which Tau is expressed at physiologic levels under the control of the mec-7 promotor in touch cells. The well defined morphology and behavioral output of these neurons make them ideal candidates for the investigation of a role for Tau prior to the appearance of major structural abnormalities such as neurofibillary tangles (NFTs). Our results indicate that physiologic levels of wild-type Tau expression do not cause functional deficits in the C. elegans gentle touch response. However, expression of a Tau mutant that is truncated at the C-terminus to mimic the caspase-cleaved form is sufficient to cause a progressive, age-related touch deficit despite the apparent lack of NTFs or concurrent cell death. This result suggests that soluble forms of mutant Tau play a significant role in the emergence of cellular dysfunction and that continued insult from soluble Tau over time exacerbates the problem. Interestingly, the touch deficit seems to be significantly worse in anatomical regions innervated by long axonal projections versus those innervated by short projections. This observation suggests that longer neuronal projections are more vulnerable to the aberrant effects of mutant Tau. The mechanism underlying this apparent susceptibility however, needs further elucidation. This genetic model will allow us to test how physiologic events such a protein processing influence Tau toxicity without the confounding detrimental effects of protein overexpression, and to assay the early contributions of Tau to neuronal dysfunction prior to cell death. Adding to the power of our system is the presence of a photoconvertible fluorescent protein tag (Dendra) that will allow us to explore differences in cellular turnover of our Tau constructs in various processing mutants (ie ubiquitin protease system vs. autophagy). Our ultimate aim is to define the mechanisms contributing to Tau turnover in neurons and to identify the functional consequences that occur as a result of aberrant Tau processing. 1. Frøkjær-Jensen C., Davis W., Hopkins C., Newman B., Thummel B., Olesen S.P., Grunnet M., Jorgensen E.M. (2008). Nature Genetics 40, 1375–1383.

400A

Loss of the *C. elegans* progranulin homolog, PGRN-1, results in decreased programmed cell death and an age-dependent response to cellular stress. **Aimee Wen Yi Kao[1]**, Ayumi Nakamura[1], Robin Eisenhut[1], Joshua Bagley[2], Robert Farese[3], Cynthia Kenyon[2]. 1) Dept. of Neurology, Univ of California San Francisco, San Francisco, CA; 2) Dept of Biochemistry and Biophysics, Univ of California San Francisco, San Francisco, CA; 3) Gladstone Institute of Cardiovascular Disease, San Francisco, CA.

Frontotemporal lobar degeneration (FTLD) is the second most common cause of dementia in those under the age of 65. Mutations in the human progranulin (PGRN) gene have recently been shown to be causal for both inherited and sporadic forms of FTLD (Baker *et al*., 2006, Hutton *et al*., 2006). Progranulin is a complex, multifunctional, secreted trophic factor that is expressed in a variety of tissues including neurons and astrocytes (Ahmed *et al*., 2007). Although PGRN is involved in development, wound healing, inflammation and tumor growth, its function in the nervous system is poorly understood (Eriksen and Mackenzie, 2008). In order to better understand the biological function of progranulin, we decided to study its homolog in *C. elegans*. Similar to the mammalian protein, the *C. elegans* progranulin homolog, PGRN-1, is expressed by the intestine and a subset of neurons. *pgrn-1* mutant worms appear grossly normal and live a normal lifespan compared to control worms. However, we have found that *pgrn-1* mutants have a decreased number of programmed cell deaths during development. In addition, young adult *pgrn-1* mutants display resistance to osmotic, thermal and ER stress compared to wild-type worms while older worms are sensitive to osmotic stress. The stress resistance phenotype can be rescued by expression of either worm or human progranulin. We are currently examining whether these constructs also rescue the cell death phenotype. Unlike mutations in the insulin-IGF-1 receptor gene, *daf-2*, *pgrn-1* mutations do not cause resistance to genotoxic or oxidative stress, suggesting a novel pathway for selective stress resistance. This dichotomous response to certain stressors—resistance while young and increased susceptibility with age—may shed light on why individuals with progranulin mutations develop FTLD symptoms late in adulthood and may explain the variable age of disease onset seen with identical mutations. Interestingly, our findings suggest that susceptibility to neurodegeneration may be determined during a developmental period when excess or unneeded neurons undergo programmed cell death.

401B

Using *C. elegans* to target kinases regulating muscle dystrophies and protein degradation. **Susann Lehmann**, Nate Szewczyk. School of Graduate Entry Medicine and Health, University of Nottingham, Derby City General Hospital, DE22 3DT, UK.

Dystrophic muscle structures and/or muscle protein degradation can severely affect health in human beings and also worms suffering from ageing, dietary restriction, starvation, exposure to environmental toxins, denervation, or endocrine disorders. Past studies have shown that kinases can regulate these pathologies in both man and worm. Kinases are active areas of research for drug development with antibodies and inhibitory drugs available for at least 20% of the human kinome. It has been reported that 80% of worm kinases are orthologous to human kinases. Thus, worm muscle pathology models could be a platform to identify kinases regulating human muscle pathology and existing drugs could be tested for therapeutic value. A strain containing a lacZ body wall muscle reporter established to read out on *unc-54* synthesis and cytosolic muscle protein degradation and two strains containing GFPs established to identify dystrophies of myofibres and mitochondria/nuclei were employed. These strains were first treated chronically, in replicate, using Ahringer's RNAi feeding vectors with positive results re-examined acutely. To date, 15 March 2009, 100 out of 450 kinases have been examined since 1 October 2008. To evaluate the RNAi method standard developmental phenotypes were scored and compared to published experiments utilizing the same RNAi vectors. 66% of genes showed phenotypes consistent with those previously published. However, in the majority of cases both our and past results were that the animals appeared wild-type. The inconsistent results fall in three categories, each accounting for roughly 12% of results: wild-type for us; wild-type previously; different developmental defects for us than previously. With respect to dystrophies and degradation we find as many as 17% of kinases appear to regulate myofibre morphology, 16% mitochondrial morphology, 1% nuclear morphology, and 20% muscle cytosolic protein degradation. To date, 78% of observations obtained in chronically treated animals are also observed in acutely treated adult animals. These results suggest that up to 120 of the worm 450 kinases may have an effect on muscle in the form of dystrophy or protein degradation. Although there are limitations, RNAi in *C. elegans* appears to be an efficient method to discover kinases which regulate muscle dystrophies and degradation. Further work will be required to elucidate the signalling mechanisms by which these kinases promote muscle dystrophy and/or protein degradation. Identification of signalling mechanisms should then enhance comparison of the regulation in worm and man and possibly further inform on the relative utility of specific inhibitory drugs. This work is funded by NIH NIAMS AR054342.

402C

Biochemical and Genetic Analysis of Parkinson's Disease-associated and Stress Response Proteins in C. elegans Models of Manganism. **Jennifer LeVora**[1,3], Raja Settivari[2], Richard Nass[2,3,4]. 1) Medical Neuroscience Program; 2) Department of Pharmacology and Toxicology; 3) Stark Neuroscience Research Institute; 4) Center for Environmental Health, Indiana University School of Medicine, Indianapolis, IN, 46202.

Parkinson's disease (PD) and manganism are characterized by motor deficits and damage to the substantia nigra, and dopamine or its metabolites are believed to contribute to both disorders. Proteins in which mutations have been linked with familial Parkinson's disease have also been proposed to contribute to the pathogenesis. In these studies we show that manganese (Mn^{2+}) causes dopamine (DA) neuronal death in *C. elegans*, and most of the known PD-associated genes, as well as specific glutathione transferases (GSTs) and heat shock proteins, are significantly upregulated following a 30 min exposure to Mn^{2+}. DA neuron vulnerability to Mn^{2+} is partially dependent on the divalent metal transporter-1 (DMT-1) orthologue SMF-1, since knockdown or deletion of the putative Mn^{2+} transporter partially protects against the neurodegeneration. Indirect immunofluorescence (IF) studies indicate that SMF-1 is expressed in DA neurons (as well as other cells), consistent with its role in contributing to Mn^{2+}-induced DA neurodegeneration. SMF-1 also significantly contributes to Mn^{2+}-induced reduction of mitochondrial membrane potential as determined by the fluorescent dye Tetramethylrhodamine ethyl ester (TMRE). IF studies also indicate that putative *C. elegans* GSTs play a significant role in inhibiting Mn^{2+}- or PD-associatiated neurotoxin-induced DA neurodegeneration, as knockdown or deletion of the GSTs increase DA neuron vulnerability in our PD models. Overall these studies suggest that *C. elegans* is a powerful model system to explore the molecular basis of DA neuron vulnerability in human manganism. *Support Contributed By: NIH R011ES014459, NIH R01ES010563, and DOD (RN).*

403A

The novel ciliary functions of Arf-like small GTPases. **Y. Li**, J. Hu. Nephrology and Hypertension, Mayo Clinic, Rochester, MN.

Cilia serve as motile or sensory devices on most eukaryotic cells surface. Defects in either cilia biogenesis or cilia function contribute to a wide spectrum of human diseases, also termed ciliopathies. ADP-ribosylaton factor (ARF)-like (ARL) proteins belong to the Ras superfamily of small GTP-binding proteins involved in diverse cellular functions. Comparative genomics study and molecular analysis indicate that, of dozens Arf/Arl proteins, three ARLs, ARL-3, ARL-6, and ARL-13B, are conserved ciliary proteins over the course of evolution from nematode to human. Remarkably, arl-13b and arl-6 (also called BBS-3) are each identified as one of the causal genes for Joubert Syndrome and BBS syndrome, respectively; while the molecular mechanisms underlying the functions of ARLs in cilia as well as the disease pathogenesis remain elusive. Using C. elegans as a model, we demonstrated that ARL-13B, ARL-6, and ARL-3 all localize to cilia per se and play critical roles in regulating cilia biogenesis and/or cilia sensory function in C. elegans. ARL-13B localizes primarily to ciliary middle segment and this localization is dependent on its C-terminus. We found PKD-2 ciliary mislocalization and mating behavior defects in arl-13b; arl-3 double knockout worms are worse than those in single knockouts. However, various ultrastructural cilia defects observed in arl-13b knockout worms can be partially rescued by arl-3 deletion. In addition, a constitutively active ARL-3 protein exacerbates the ultrastructural cilia defects in arl-13b animals. Taken together, our data suggest an intriguing working model that ARL-13B and ARL-3 act in concert in cilia sensory functions, but surprisingly, function antagonistically in cilia biogenesis process.

404B

Effects of Embryonic ethanol exposure in C. elegans. **Conny H C Lin**, Yun Lin, Catharine Rankin. Brain Research Ctr, Univ British Columbia, Vancouver, BC, Canada.

In North America, approximately 1%; of newborn children are affected with fetal alcohol spectrum disorder (FASD). FASD costs Canadians $5.3 billion annually, and the financial burden is even higher in the United States. The symptoms of FASD include a spectrum of physical, neurological, behavioral, and cognitive abnormalities; the range of symptoms is highly heterogeneous between affected individuals. We developed a novel model of FASD to explore parametric manipulations of dose and exposure duration utilizing the advantages of Caenorhabiditis elegans. We investigated the effects of 4 ethanol exposure durations at 3 distinct times during embryonic development with a range of ethanol concentrations at 2 different exposure frequencies on hatch rate, reproductive onset, body size, and lifespan. Further, we also explored some relevant neurological and behavioural effects of FASD from selected ethanol exposure patterns. For physical abnormalities, we found that 1) early development (gastrulation) was the most vulnerable stage, 2) multiple ethanol exposures produced a worse outcome than a single exposure, and 3) highly heterogeneous phenotypes following the same exposure still occurred in this homogeneous model system; which suggests that the phenotypic plasticity may largely be due to differential developmental compensation among exposed individuals. For neurological and behavioral abnormalities, the learning and memory for habituation to mechanical stimuli was intact in adult worms. Animals exposed to 60min of 20%; ethanol during gastrulation showed enhanced responses to a mechanical stimulus. Preliminary examinations of subsets of neurons suggested that there were displaced or possibly missing cell bodies for several neurons in the head region. This study showed that ethanol exposure during the embryonic development of C. elegans produced various physical and behavioural abnormalities comparable to other well-established models of FASD. Further investigations into the molecular and neurological causes of these abnormalities will provide new insights into the mechanistic causes of some FASD symptoms.

405C

Transgenic *C. elegans* expressing the fungal prion protein HET-s as a model for the study of amyloid infectivity and toxicity. **Berangere Pinan-Lucarre**[1], Yujie Qiao[1], Sven Saupe[2], Monica Driscoll[1]. 1) Rutgers U., Piscataway, NJ., USA; 2) CNRS UMR5095/Bordeaux 2 U., France.

Amyloids are protein fibers rich in beta sheet structure that are associated with numerous neurodegenerative diseases, including Alzheimer disease. Amyloids have two major biological properties. First, they can be infectious--meaning that they can spread the altered amyloid conformation to the same protein or even among proteins of distinct primary sequence (the latter phenomenon is known as cross-polymerization). Infectious amyloids are called prions. Second, amyloids can be toxic, whether they are infectious or not. Both properties have tremendous fundamental and biomedical impact.

[Het-s] is a prion of the fungus *Podospora anserina* involved in a defense cell suicide mechanism named heterokaryon incompatibility, which disables mixing of different strains by somatic fusion, an ubiquitous feature in filamentous fungi. The HET-s protein exists in a soluble state or in an aggregated, prion state named [Het-s]. The [Het-s] prion is not toxic by itself in *P. anserina* or in yeast. However cell death is rapidly triggered by the lethal interaction between 2 alleles of the *het-s* locus: *het-S* (het-"large S") and *het-s* (het-"small s") when the HET-s protein adopts the prion form. This prion has been extensively characterized and it is the only prion for which tridimensional structure is available to date. The critical point is that in the [Het-s] system, transgenic expression of *het-s* allows propagating a non-toxic prion while co-expression of *het-s* and *het-S* generates toxicity. We constructed strains expressing *het-s* or the prion domain only *het-s(218-289)* (the 218-289 fragment of the protein) as GFP fusions under the control of the ubiquitous promoter *dpy-30*. The fluorescence of *pdpy-30het-s::gfp* appears to be mainly diffuse in the cytoplasm while it shows strong aggregation in *pdpy-30het-s(218-289)::gfp* strains. We constructed strains expressing *het-s(218-289)::gfp* in the touch neurons using *mec-4* promoter and observed that the fusion protein is mostly aggregated into a large fiber spanning the cell body and the proximal part of the axon. These aggregates remain to be shown as infectious [Het-s] amyloids. In future experiments, we will aim to generate neuronal toxicity through the co-expression of *het-s(218-289)* and *het-S*.

This *C. elegans* model for prion studies, filling a gap between simple fungal systems and highly complex mammalian systems, might allow a better understanding of the molecular and cellular basis of amyloid infectivity and toxicity.

406A

Pharmacogenetic and biochemical analysis of Parkinson's disease-associated proteins in *C.elegans*: effects on mitochondrial function and dopamine neuron vulnerability. **Raja S Settivari**, Jennifer LeVora, Richard Nass. Dept. of Pharmacology and Toxicology, Center for Environmental Health, Stark Neuroscience Institute, Indiana University School of Medicine, Indianapolis, IN, USA.

Parkinson's disease (PD) is a progressive neurodegenerative disorder largely characterized by the loss of dopaminergic neurons. Although the etiology of the disease is unknown, it is believed to be multifactorial, with both genetic and environmental contributions that result in oxidative damage and mitochondrial dysfunction. We previously established novel *C. elegans* models for Parkinson's disease that now allow for the evaluation of PD-associated orthologues and other proteins in their role in dopamine (DA) neuron vulnerability in response to endogenous and exogenous insults, and provides opportunities to identify and characterize novel PD therapeutic targets and leads. Here we describe our expression and biochemical investigations of WT and mutant PD- and DA neuron-associated proteins, and their effects on mitochondria function and DA neurons under basal conditions and following exposure to neurotoxins. qPCR gene expression studies of PD- and DA neuron-associated proteins show significant changes in expression of most of the familial PD genes. Biochemical analysis shows that mutations within some of the PD-associated genes in *C. elegans* affect mitochondrial membrane potential (as measured by the fluorescent dye TMRE), respiration capacity (as measured by oxygen consumption), and cellular dopamine and glutathione concentrations. Mutations within several PD- and DA neuron-associated proteins increase the vulnerability of DA neurons to mitochondria-targeted neurotoxins, and anti-oxidant flavonoids that may increase mitochondrial function significantly protect against DA neurodegeneration. We have also generated antibodies to over 40 *C. elegans* proteins, including PD- and DA neuron-associated proteins, and our Western blot analysis and indirect immunofluorescence suggest differential protein expression in our PD models. Overall these studies suggest that mutations within DA neuron- and PD-associated genes result in increases in oxidative stress and mitochondria dysfunction, and further validates *C. elegans* as a powerful genetic model to explore PD. Support Contributed By: NIH R011ES014459, NIH R01ES010563, DOD and Collaborative Research Grant (Purdue/IUSM) (RN).

407B

Ataxin-3 protein context and cell-specific factors modulate polyQ-mediated neuronal aggregation in a *C. elegans* model of Machado-Joseph disease. **A. Teixeira-Castro**[1,2], R. Morimoto[2], P. Maciel[1]. 1) ICVS/Hlth Sci Sch, Univ Minho, Braga, Portugal; 2) DBMBCB, Northwestern University Evanston, IL, USA.

Understanding the basis for neuronal subtype-specific protein aggregation is of central importance for several human neurodegenerative diseases, including Machado-Joseph disease. In this study, we developed a novel Ataxin-3 (ATXN3) pathogenesis model in *Caenorhabditis elegans* and examined the aggregation profile of human ATXN3 by performing FRAP analysis, in live neuronal cells. We found that full-length ATXN3 aggregates only at high Q-length, not found in human patients, whereas C-terminal ATXN3 causes aggregation and neurotoxicity at a threshold length of ≈75 glutamines. Analysis of specific neurons in *C. elegans*, reveals that the ventral nerve cord motor neurons are highly affected. Interestingly, certain sensory neurons of the head contain aggregated foci only when the polyQ-stretch is expressed within ATXN3 protein flanking sequences. Moreover, co-expression of full-length human pathological ATXN3 (below aggregation threshold) with an aggregated species capable of initiating the nucleation events, aggravates the aggregation phenotype and new ATXN3-polyQ co-aggregates are formed also in the sensory neurons of these animals, which are not affected when the two species are expressed alone. These results provide direct evidence that protein context and cell-specific factors are major modifiers of polyQ pathogenesis.

408C

Mutations in heparan sulfate 6O-sulfotransferase (*HS6ST1*) cause Kallmann Syndrome and normosmic Idiopathic Hypogonadotrophic Hypogonadism. **J. Tornberg**[1], K. Keefe[3], L. Plummer[3], J. Hall[3], X. Hoang[3], R. Quinton[3], S. Seminara[3], V. Hughes[4], PS. Tsai[4], H. Habuchi[5], K. Kimata[5], N. Pitteloud[3], H. Bülow[1,2]. 1) Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY; 2) Dominick P. Purpura Dept Neuroscience, Albert Einstein Col Medicine, Bronx, NY; 3) Dept Medicine, Massachusetts General Hospital (MGH), Boston, MA; 4) Dept Integrative Physiology, University of Colorado Boulder, CO; 5) Institute for Molecular Science of Medicine, Aichi Medical University, Japan.

Heparan sulfates (HS) are abundant extracellular matrix polysaccharides modified by a specific set of enzymes that introduce complex patterns of epimerization and sulfations to sugar residues. Although HS serve as cofactors in many developmental signaling pathways, HS modification patterns have not been implicated with human development or disease. Idiopathic hypogonadotropic hypogonadism (IHH) caused by abnormal GnRH neuron development is a heterogeneous genetic disorder associated with anosmia (Kallmann syndrome [KS]) or normal olfaction (nIHH). We identified the *C. elegans* heparan sulfate 6O-sulfotransferase (*hst-6*) as a locus that suppressed a *kal-1/KAL1*/anosmin-1 gain-of-function phenotype. *KAL1*/anosmin-1 is the first gene implicated in KS in humans. Here, we report the identification of missense mutations in HS 6O-sulfotransferase type 1 (*HS6ST1*) in IHH families with variable degrees of olfactory dysfunction and GnRH deficiency. *In vitro* experiments demonstrate that the *HS6ST1* mutants display reduced enzymatic activity. Further, these mutants were unable to rescue the *hst-6* loss-of-function phenotype in relation to the *kal-1* gain-of-function phenotype in *C. elegans*. Finally, mice lacking the *Hs6st-1* gene exhibit GnRH neuron migration defects with incomplete penetrance. Our results implicate *HS6ST1* in the pathogenesis of KS and nIHH and demonstrate the importance of HS modification patterns for human neural development.

409A

Molecular and genetic analysis in a novel model of methylmercury neurotoxicity. **Natalia VanDuyn**[1], Raja Settivari[1], Suvi Asikainen[4], Martina Rudgalvyte[4], Garry Wong[4], Richard Nass[1,2,3]. 1) Dept of Pharmacology and Toxicology; 2) Center for Environmental Health; 3) Stark Neuroscience Institute, Indiana University School of Medicine, Indianapolis, IN; 4) Depts of Biosciences and Neurobiology, Kuopio University, Kuopio, Finland.

Methylmercury (MeHg) exposure from occupational, environmental and food sources is a significant threat to public health. The toxicant easily passes the blood brain barrier and can cause severe psychological and neurological problems. Although MeHg poisonings have been studied for decades, the molecular determinants involved in the cellular pathology are largely unknown. We have developed a novel model of methylmercury toxicity in the genetically tractable nematode *C. elegans*. We show that MeHg exposure confers animal death at low toxicant concentrations, and that the sensitivity is partially dependent on growth medium. Consistent with vertebrate studies, growth in the presence of selenium dramatically rescues the MeHg-induced death, and this effect is dependent on a live bacteria food source. Developmental defects are also observed as older larvae exposed to non-lethal doses of MeHg produce progeny that arrest as embryos *in utero*. Furthermore, exposure of younger larvae to similar concentrations of MeHg results in over a 2-fold delay in development. MeHg-induced reductions in pharyngeal pumping and egg-laying suggest toxicant-induced changes in neuronal signaling. Whole genome microarray and real-time PCR results show a strong induction of specific heat shock proteins and glutathione-S-transferases following exposure to MeHg. Gene expression studies also indicate a significant induction of genes involved in electron transport, cell growth and protein folding. MeHg also differentially affects the regulation of a miRNA targeting a serpentine receptor as well as other genes involved in growth and neurodevelopment, and further support possible toxicant-induced dysfunction in neurotransmitter signaling. Overall these studies suggest that *C. elegans* is a powerful model system to explore the molecular basis of MeHg toxicity. Support Contributed By: NIH R011ES014459 and Vanderbilt University Toxicology Program Grant (RN).

410B

Evolutionary perspectives in innate immunity in *C. elegans*. **C. ML. Boehnisch**[1], D. Wong[2], R. May[1], Th. Roeder[3], H. Schulenburg[3]. 1) University of Birmingham, Birmingham, United Kingdom; 2) Centre d'Immunologie de Marseille-Luminy, Université de la Méditerranée, France; 3) Zoological Institute, Christian-Albrechts-Universitaet , Kiel, Germany.

In its natural habitat, *C. elegans* continuously encounters a diversity of microorganisms, many of which are potential pathogens. These interactions shaped and are still shaping the architecture of defence mechanisms. In our study we address aspects of defence evolution, e.g. inducible immune response, and the importance of gene duplications and subsequent diversification of defence components such as the antimicrobial lysozymes.

Using the pathogenic bacterium *Bacillus thuringiensis* we characterized the transcriptional immune response. The induced response, which was studied in three different strains, appears to consist of two main parts: a universal anti-pathogen response and a *B. thuringiensis*-specific response. The induced response led to an up-regulation of known or putative immune effector genes including some lysozymes.

Lysozymes are very important antimicrobial peptides not only in C. elegans but in many other organisms as well. In contrast to most organisms, however, the genome of *C. elegans* contains at least 15 functional lysozyme genes. By combining phylogenetic data with expression patterns induced by different pathogens we demonstrate that this extraordinary diversity has resulted from gene duplication followed by functional differentiation.

In conclusion, genetic diversity in innate immunity,and complex host transcription profiles induced by parasites serve to ensure survival not only of *C. elegans* but of other host organisms as well.

411C

C. elegans invertebrate-type lysozyme genes: an investigation into their expression, inducibility and function. **Maria J. Gravato-Nobre**, Teresa Marsay, Suet L Wong, Dave Stroud, Jonathan Hodgkin. Dept Biochemistry, Univ Oxford, Oxford, United Kingdom.

C. elegans is a bacterial-feeder that spends its entire life cycle in decaying organic matter. In such an environment laden with infectious microorganisms, the worm depends on an effective digestive system capable of filtering, degrading and hydrolysing bacterial cells, and an inducible immune response capable of secreting potent antimicrobial effectors. Lysozymes have an important dual role acting both in digestion and defence. In addition to 10 classical lysozyme genes (*lys-1-lys-10*) the *C. elegans* genome encodes 5 invertebrate type lysozymes (*ilys-1-5*) whereas the related species *C. briggsae*, *C. remanei* contain only two and three *ilys* genes, respectively. Microarray analysis has previously shown that a cluster of three invertebrate lysozymes in *C. elegans*, *ilys-3*, *ilys-2* and to a lesser extent *ilys-1*, are upregulated in response to infection by *M. nematophilum*. To provide further insights into the role of *C. elegans* i-lysozymes *ilys-1* to *ilys-5*, we have undertaken different approaches that combine genome sequencing of many wild isolates, reporter constructs and RNAi assays. We find that all the *Cel ilys* genes are generally expressed in the worm's digestive tract. However, their exact expression pattern differ slightly, with notably distinct pattern of pharyngeal expression which suggests that these genes might have evolved separate functions. To investigate if the *Cel ilys* genes play a role in host-pathogen defence response, we developed inducibility assays using a number of different bacterial pathogens The differential up-regulation of the *Cel ilys* genes will be reported. In addition, we have analysed knockdowns and knockouts of *ilys* genes and will discuss natural variation in the *ilys-1* locus.

412A

Systemic and cell intrinsic roles of Gαq signaling in the regulation of innate immunity, oxidative stress response and longevity in *Caenorhabditis elegans*. **Trupti Kawli**[1], Man-Wah Tan[1,2]. 1) Department of Genetics, Stanford University, Stanford, CA; 2) Department of Microbiology and Immunology, Stanford University, Stanford, CA.

Regulation of longevity, immunity and stress resistance can profoundly affect organismal survival. We show that a signaling module formed by the G protein alpha subunit, Gαq and its downstream signal transducer phospholipase C, PLCβ can affect these processes in *C. elegans*. We find that Gαq and PLCβ mutants are sensitive to pathogen and oxidative stress, yet surprisingly are long lived. Immunomodulatory and lifespan extension effects of Gαq and PLCβ are mediated through controlled neuropeptide secretion and downstream regulation of insulin/IGF signaling pathway. In addition to its effects on insulin signaling, the Gαq and PLCβ signaling module functions cell autonomously to affect the activity of the p38 MAPK pathway, an important component of *C. elegans* immune and oxidative stress response. p38 MAPK activity in the intestine is regulated by diacylglycerol levels, a product of PLCβ's hydrolytic lipase activity. Genetic analysis shows that lifespan is largely determined by insulin signaling, whereas MAPK signaling is the primary regulator of oxidative stress in PLCβ mutants. The pathogen sensitivity phenotype of Gαq and PLCβ mutants is a summation of the beneficial effects of reduced neuroendocrine insulin signaling activation and detrimental effects of reduced intestinal MAPK activation. We propose a model whereby the Gαq and PLCβ signaling module differentially regulates pathogen sensitivity, oxidative stress and longevity through cell intrinsic and systemic effects on p38 MAPK and insulin/IGF signaling.

413B

A genome scale gene expression analyses reveals that *Burkholderia pseudomallei* suppresses *Caenorhabditis elegans* immunity via targeting a GATA transcription factor. **Song-Hua Lee**[1], Man-Wah Tan[2], Sheila Nathan[1,3]. 1) Faculty of Science & Technology, Universiti Kebangsaan Malaysia, Bangi, Selangor, Malaysia; 2) Department of Genetics and Department of Microbiology and Immunology, Stanford University School of Medicine, Stanford, U.S.A; 3) Malaysia Genome Institute, UKM-MTDC Smart Technology Centre, Bangi, Malaysia.

The gram-negative soil bacteria *Burkholderia pseudomallei* is the etiological agent of melioidosis in humans and animals, causing significant mortality and morbidity in South-east Asia and northern Australia, where it is endemic. The innate immune system is the first line of defense against this pathogen but the mechanisms of innate immunity to this pathogen remain to be fully elucidated. *B. pseudomallei* can infect the nematode *Caenorhabditis elegans* and upon ingestion complete mortality is observed by 48 hours. Immune responses of *C. elegans* to pathogens involved several conserved transcription factors, including the FOXO/DAF-16 and GATA/ELT-2 transcription factors. To investigate host immune responses to *B. pseudomallei* infections, we performed a genome-wide transcriptome analysis on age-matched adult worms infected for 2, 4, 8 and 12 hours. Our analyses revealed that approximately 6% of the *C. elegans* genes were transcriptionally regulated when compared to uninfected animals. Enriched among induced genes included those in the metabolism functional category, such as genes encoding cytochrome P450 and UDP-glucuronosyltransferase, as well as defence-related genes. In contrast, genes involved in the aging process, lipid metabolism and response to oxidative stress were robustly repressed upon *B. pseudomallei* infection. Interestingly, the proportion of genes that were suppressed increased with time of infection, suggesting that suppression of host defence genes may be an important virulence mechanism employed by *B. pseudomallei*. Another gram-negative human pathogen *Pseudomonas aeruginosa* is able to suppress *C. elegans* immunity by ejecting the transcription factor DAF-16 from the nucleus of intestinal cells (Evans et al. 2008). We showed that immune suppression by *B. pseudomallei* is distinct from that of *P. aeruginosa*. Instead, it involves the targeting of an intestinal GATA transcription factor ELT-2 and the down regulation of ELT-2 transcriptional targets. Evidence for this novel mechanism of host immune suppression will be reported.

414C

Investigation of four C-type lectin genes: *clec-17, 60, 70* and *86*. **Delia M. O'Rourke**, Jonathan Hodgkin. Biochemistry, University of Oxford, Oxford, United Kingdom.

Many genes encoding C-type lectins (*clecs*) are induced following infection of *C.elegans* with either Gram positive or Gram negative bacteria, as part of the worm's innate immune response. Different infections induce both shared and infection specific *clec* genes (H. Schulenburg 2008). The function of these genes is currently unclear: they may be involved in pathogen recognition and they may also have a role as antibacterial effectors. We are using a number of approaches to study four *clecs* induced in response to infection by *Microbacterium nematophilum*, *clec-17, clec-60, clec-70* and *clec-86* (O'Rourke et al 2006). RNAi knockdown or genetic knockouts of *clec-17, 60* and *86* affect *C.elegans* defense against *M.nematophilum*. We are constructing reporters to examine where and when the *clec* promoters are activated, making TAP TAG vectors and raising antibodies to examine protein location, post-translational modification and to identify interacting proteins. We are also attempting to express these *clecs* in heterologous systems with the aim of obtaining pure protein for structural analysis and for *in vitro* assays of antibacterial activity. We will present progress and data from these approaches. Schulenburg et al Immunology, 2008, 213(237-250) O'Rourke et al Genome Res, 2006, 16(1005-1016).

415A

Investigation of the mechanism of infection induced tail swelling. **Frederick A. Partridge**, Jonathan Hodgkin. Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

When *C. elegans* and certain other rhabditid nematodes are infected by various bacterial pathogens, notably the coryneform *Microbacterium nematophilum*, they respond by altering the development of the hindgut, producing a swollen tail. This is a protective innate immune response [1]. Our lab has previously shown that tail swelling involves activation of the ERK MAPK cascade in the rectal epithelial cells. The mechanism of tail swelling has been hard to address in genetic screens as mutants that abolish infectability are much more common than mutants affecting the response to infection. To avoid this problem we have stably expressed activated MAP kinase pathway genes in the hindgut, which phenocopies tail swelling in the absence of infection. This has allowed us to screen for modifiers of tail swelling without the confounding effects of susceptibility to infection. Results of RNAi and forward genetic screens will be presented.

[1] Nicholas and Hodgkin (2004) Curr Biol **14** 1256-61.

416B

A High Throughput Approach Towards *Vibrio cholerae* Pathogenesis in *Caenorhabditis elegans*. **Surasri N Sahu**, Hediye N Cinar. Division of Virulence assessment, Food and Drug Administration, Laurel, MD.

Vibrio cholerae (VC), the causative agent of cholera in humans, is responsible for devastating epidemics and pandemics across the world. The major virulence factor underlying the pathogenesis of cholera is cholera toxin (CT). However, CT negative VC non-O1 and non-O139 strains and CT deleted vaccine mutant strains are still capable of causing disease symptoms through mechanisms that are currently unclear. *Vibrio cholerae* cause lethality, growth retardation and escape behavior in *Caenorhabditis elegans* via cholera toxin (CT), and toxin co-regulated pili (TCP) independent process (1, 2). Absence of the CT and TCP response in *C. elegans* model may help to reveal the role of other toxins of VC that might otherwise be masked by these major virulence factors. CVD110, a *V.cholerae* vaccine strain, lacking several virulence factors such as zonula occludens toxin (*zot*), accessory toxin (*ace*), hemolysin (*hly A*) and cholera toxin A subunit gene (*ctx A*), showed attenuated killing in *C. elegans* (3,4). We are conducting microarray experiments to define host immune response genes expressed upon exposure to VC virulence factors. Differential expression profiling of *C. elegans* exposed to wild type VC versus CVD110 are being done using Affymetrix expression microarrays. Results of these experiments will be presented. (1) Vaitkevicius K. et al. PNAS, 103 (2006) 9280-9285 (2) Cinar HN. et al. 16 th International C. elegans Meeting, 2007 (3) Michalski J. et al. Infection and Immunity 61 (1993) 4462-4468 (4) Cinar HN. et al. Aging Stress and Pathogenesis Meeting, 2008.

417C

Immune and Behavioral Responses to Pathogens Share a Common Mechanism of Signaling in *C. elegans*. **Robert P. Shivers**[1,2], Tristan Kooistra*[1], Stephanie W. Chu[1,3], Daniel J. Pagano[1], Dennis H. Kim[1]. 1) Departent of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139; 2) Departent of Basic Sciences, The Commonwealth Medical College, Scranton, PA. 18411; 3) Graduate School of Biomedical Science, University of Massachusetts, Worcester, MA 01655.

A small number of evolutionarily conserved core signaling pathways of innate immunity, such as Toll-like receptor signaling pathways and mitogen-activated protein kinase (MAPK) cascades, are utilized in host organisms to respond microbial infection. Proteins containing the Toll-Interleukin-1 Receptor (TIR) domain are notably associated with innate immune signaling pathways and are present in the microbial response pathways of organisms from Dictyostelium to humans. In the nematode *Caenorhabditis elegans*, TIR-1, an ortholog of the mammalian TIR domain protein SARM, is required for immunity, acting upstream of a conserved NSY-1-SEK-1-PMK-1 MAPK pathway that is orthologous to the mammalian ASK1-MKK3-p38 MAPK pathway. *C. elegans* also responds to pathogenic bacteria with serotonin-dependent behavioral avoidance and aversive learning. We have determined that the *C. elegans* SARM-ASK1-MKK3 module has tissue-specific roles in both innate immune and behavioral responses to pathogens. We show that SARM-ASK1-MKK3 signaling regulates intestinal immunity cell autonomously in response to infection. In addition, we demonstrate that SARM-ASK1-MKK3 activity in the sensory nervous system is required for serotonin-dependent aversive behavior in response to pathogenic bacteria. These data demonstrate a commonality that these protective responses to pathogens share in signaling through the SARM-ASK1-MKK3 module and underscore the ancient origins of TIR domain protein-dependent signaling mechanisms in diverse physiological responses that are triggered by the interaction of microbes with the host.

418A

Over-activation of FOXO transcription factor, DAF-16, causes pathogen susceptibility in *Caenorhabditis elegans*. **Varsha Singh**, Alejandro Aballay. Dept Molec Genetics Microbiol, Duke Univ, Durham, NC.

DAF-16 is a FOXO transcription factor involved in regulation of metabolism and stress response in *C. elegans*. Previous studies have shown that overexpression of DAF-16 increases lifespan and resistance to pathogens . Here we report that hyper-activation of DAF-16 may lead to deleterious consequences during infection with bacterial pathogens. We also provide evidence that a stress-inducible Heat Shock Factor (HSF)-1 is utilized to control DAF-16 activitation by promoting its export from nucleus. Effect of HSF-1 appears to be mediated through Hsp70 which is also induced and translocated to nucleus during stress. Aquaporin upregulation is partly responsible for enhanced susceptibility to pathogens. Susceptibility due to DAF-16 activation could also be rescued by increasing osmolarilty of the exogenous medium indicating that osmotic balance is an important aspect of infection. In summary, while overexpression of DAF-16 is beneficial, its sustained activation leads to enhanced susceptibility to pathogens. *C. elegans* utilizes chaperones to promote DAF-16 nuclear export in a timely manner and prevent susceptibility.

419B

Induction of the *C. elegans* immune responses toward *Pseudomonas aeruginosa* precedes bacterial colonization. **Kwame Twumasi-Boateng**[1], Matthew Gill[2], Michael Shapira[1,3]. 1) Graduate Group in Microbiology, University of California, Berkeley; 2) The Buck Institute for Age Research; 3) Department of Integrative Biology, University of California, Berkeley.

Challenging *Caenorhabditis elegans* with bacterial and fungal pathogens results in an inducible, protective immune response. In organisms as diverse as vertebrates and fruit flies, various pattern recognition receptors recognize distinguishing features of pathogens and control the downstream responses to them. The specificity of the *C.elegans* immune response to different pathogens, also suggests upstream recognition; however, the mechanisms responsible for initiating these responses remain unknown. "Slow killing" of *C. elegans* by Pseudomonas aeruginosa is an infection-like process in which worms death is associated with bacterial proliferation in their gut. The worm protects itself by mounting a robust response to this infection. To associate subsets of this response with the degree of gut colonization, we exposed *C. elegans* to GFP-expressing *P. aeruginosa* and employed fluorescent microscopy and COPAS™ worm sorting to separate non-colonized worms from fully-colonized ones. Genome-wide analysis of gene expression in the two groups revealed that non-colonized worms displayed an immune response which is at least as robust as their colonized counterparts. A follow up analysis using quantitative RT-PCR to monitor expression of selected genes showed that responses can be elicited by UV-killed intact bacteria but cannot be induced by secreted bacterial factors. Our results demonstrate that colonization is not a requirement for the immune response, and further, suggest the ability of *C. elegans* to recognize pathogen associated molecular patterns.

420C

Vitamin metabolism in a knockout mutant of the folate tranporter *folt-1*. **Jason Rothman**[1], Wei-Siang Liau[1], Balasubramaniem Ashokkumar[2,3], Hamid Said[2,3], LaMunyon Craig[1]. 1) Biological Sciences, California State Polytechnic University, Pomona, CA; 2) Veterans Affairs Medical Center, Long Beach, CA; 3) Departments of Medicine and Physiology/Biophysics, University of California, Irvine, CA.

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 and to be involved in uptake of exogenous folate by worms. A knockout mutation of the gene, *folt-1(ok1460)* causes sterility due to defective oogenesis. Germ cell proliferation is drastically reduced, and knockout hermaphrodites contain few, if any, mature oocytes. On occasion, they also harbor several fertilized eggs containing defective embryos. Rarely, knockout hermaphrodites produce a small number of viable progeny with a brood size numbering less than 10. The knockout phenotype appears oogenesis specific because knockout worms produce functional sperm and knockout males are able to sire cross progeny. The lifespan of *folt-1* hermaphrodites is severely diminished, suggesting that FOLT-1 functions in the soma as well as in the germline. Here, we investigate the effects of vitamin supplementation and vitamin uptake in *folt-1* knockout worms. For thiamine, knockout worms upregulate thiamine uptake, and thiamine supplementation improves germline proliferation but does not correct the defects of the folt-1 knockout on embryonic development. We will describe similar investigations of several vitamins and argue that *C. elegans* vitamin metabolism is responsive to dietary availability.

421A

PES-9, the C. elegans homologue of human carnosinase is a putative model of human disease. **Katerina Sebkova**[1], Johana Nakielna[2], Zdenek Kostrouch[2], Marta Kostrouchova[1]. 1) Laboratory of Molecular Biology and Genetics, Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University, Prague 2, Czech Republic; 2) Laboratory of Molecular Pathology, Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University, Prague 2, Czech Republic.

Carnosine (beta-alanyl-histidine) is found in high quantities in brain, muscles and blood plasma but its physiological functions are largely unknown. Carnosine is synthesized enzymatically by carnosine synthase and its close analogue is a product of GABA metabolism. Carnosine has been shown to be able to scavenge reactive oxygen species and its intake may have a protective role in the development of Alzheimer disease. Carnosine has the potential to decrease protein glycation and is able to chelate divalent metal ions. On the other hand, elevated levels of plasma carnosine caused by a defficiency of serum carnosinase, one of the two dipeptidases proven to degrade carnosine, is connected to a variable picture of neurological disorders. The symptoms linked to high concentration of carnosine in human blood include: hypotonia, developmental delay, mental retardation, degeneration of axons, sensory neuropathy, demyelinization, gray matter anomalies, and myoclonic seizures. Here, we studied the expression and function of pes-9, the C. elegans homologue of vertebrate carnosinases CNDP2 and CNDP1. Inhibition of pes-9 by RNA interference causes embryonic lethality and L1 larval arrest with a broad spectrum of body morphology defects. The results indicate that the functional genomics of pes-9 may serve as an experimental model for elucidation of a rare metabolic disease. Acknowledgement: We thank Drs. A. Fire for vectors and host used in RNAi and M.W. Krause for support and advice. The work was supported by grants 304/08/0970 and 304/07/0529 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

422B

In vivo monitoring of gene transcription in the innate immune response. **Barbara Squiban**, Jonathan Ewbank. CIML, Marseille, France.

Infection of *C. elegans* with the fungus *Drechmeria coniospora* provokes the rapid induction of the antimicrobial peptide gene *nlp-29*. Transgenic worms with a p*nlp-29*::GFP reporter gene exhibit green fluorescence after infection. A needle prick also results in a very rapid increase in GFP expression. Initially, the fluorescence is restricted to the site of injury and then extends to the entire major epidermal cell syncytium, hyp7. This result is intriguing since all the >100 nuclei in hyp7 share the same plasma membrane and cytoplasm. The spread of fluorescence might reflect the diffusion of GFP following gene activation in the nucleus that is closest to the wound site, or an increase in the number of nuclei expressing the reporter gene. To characterize better this response, one needs to be able to assay specific gene transcription, at the single nucleus level. N. Broude's group has described a method that could be adapted to achieve this. It is based on GFP complementation regulated by the interaction of a split RNA-binding protein with its corresponding RNA aptamer. In it, eIF4A is split into two, and each fragment fused to split fragments of GFP. Coexpression of the two protein fusions (A-F1 and B-F2) in the presence of a transcript containing the specific RNA aptamer results in GFP fluorescence. Thus, the spatial and temporal changes in fluorescence seen within cells should reflect the dynamics of transcript production, localization and degradation. Unfortunately, when the A-F1 and B-F2 proteins were expressed together in worms, a strong fluorescence was observed, both in the cytoplasm and in the nucleus. The fluorescence appeared static in its distribution and was independent of the presence of the aptamer. One explanation is that, in *C. elegans*, the A-F1 and B-F2 molecules aggregate irreversibly. Attempts to circumvent the problem by diluting the injected transgenes were unsuccessful. We have therefore turned to a less sophisticated, indirect approach, based on the observation that a MAB-9::GFP fusion protein is localized to a very limited number of epidermal cell nuclei. Jon Audhya kindly provided us with a mCherry-histone reporter construct. We have placed this under the control of certain *nlp* promoters, and have generated transgenic lines that show no fluorescence in the absence of injury, but have red nuclei after wounding. We are therefore now in a position to follow the dynamics of gene expression at the single nucleus level within the epidermal syncytium. Thanks to J. Hodgkin and all members of his lab, who hosted JE during early stages of the project. Pujol et al (2008) Curr Biol 18:481 Valencia-Burton et al (2007) Nature Methods 4:421. Wollard & Hodgkin (2000) Genes & Dev 14:596.

423C

A mutation in the mitochondrial ATP synthase subunit 9/c of *Caenorhabditis elegans* results in oligomycin resistance, unusual mitochondrial morphogenesis and increased germ line cells apoptosis. N. Breuil[1], F. Farina[1], L. Riffault[1], M. Bolotin-Fukuhara[1], M. Lemullois[2], M. Pinto[1], **E. Culetto[1,3]**. 1) Université Paris-Sud, CNRS UMR 8621, 91405 Orsay Cedex, France; 2) Université Paris-Sud, IBAIC, CNRS UMR 8080, 91405 Orsay Cedex, France; 3) Université Paris-Sud, CNRS-CGM, 91198 Gif-sur-Yvette, France.

Mitochondria provide ATP synthesis and integrate signaling pathways that are key determinants of metazoan development. These roles are performed through a permanent cross-talk between mitochondrial and nuclear genomes. One way to approach the understanding of this crosstalk is to study the consequences of mutated components of the mitochondrial respiratory chain that are genetically encoded by both genomes. We have used the nematode *C. elegans* to manipulate this crosstalk and perturb it through the mitochondrial ATP synthase inhibitor oligomycin. *C. elegans* exposure to oligomycin, leads to hermaphrodite paralysis and lethality whereas wild type males and hermaphrodites with fertilization mutations (*fem-1*, *tra-1* and *glp-4*) are better able to cope with oligomycin toxicity. This result indicates that oligomycin resistance defines a novel sex-specific difference in *C. elegans*. Using forward genetic screen, we identified mutant strains that displayed resistance to the acute toxicity of oligomycin. One of the mutations responsible for this phenotype, *ff267*, is nuclear dominant and was mapped to the left arm of the chromosome III. Fine SNP mapping and sequencing of this mutant allele demonstrated that the mutation corresponds to a single substitution of an evolutionary conserved residue in the ATP9/c subunit of the mitochondrial ATP synthase complex. Using electron and fluorescence microscopy, we observed that the mitochondria morphogenesis is altered in the *atp-9* mutant leading to elongated mitochondria reticulum with abnormal ultrastructural defects. This result suggests that ATP-9 could participate in the mitochondria reticulum morphology. Moreover, the *atp-9(ff267)* mutant results in cold sensitive defects of somatic gonad formation and germ line development leading to fertility alteration which could be enhanced by modifying cholesterol concentration. The mutant has additional defects such as life span modification, oxidant stress increase and abnormal germ line cells apoptosis which is reminiscent of putative link between mitochondria activity and cell cycle checkpoint. The *atp-9(ff267)* mutant recapitulates pleiotropic phenotypes observed for different *C. elegans* mitochondrial mutants and could be a useful system to modelize mitochondrial dysfunction.

424A

Structure/function analysis of TDP-43 neurotoxicity in *C. elegans*. **Peter E.A. Ash[1]**, Leonard Petrucelli[1], Harald Hutter[2], Tassa Saldi[3], Virginia Fonte[3], Christine M. Roberts[3], Christopher D. Link[3]. 1) Dept. of Neuroscience, Mayo Clinic Florida, Jacksonville, FL; 2) Dept. of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada; 3) Inst. of Behavioral Genetics, Univ. of Colorado at Boulder, Boulder, CO.

TDP-43 is a conserved RNA binding protein with known roles in mRNA splicing and stability. Cytoplasmic deposition of TDP-43 has been linked to multiple neurodegenerative diseases, including ALS and frontotemporal lobar dementia (FTLD). We have engineered pan-neuronal expression of human TDP-43 protein in C. elegans, with the goal of generating a convenient in vivo model of TDP-43 neurotoxicity. Full-length (wild type) human TDP-43 expressed in C. elegans is nuclear as is observed in human cells. Transgenic worms with neuronal human TDP-43 expression exhibit an uncoordinated phenotype and have abnormal motorneuron synapses. By using this uncoordinated phenotype as a read-out of TDP-43 neurotoxicty, we have investigated the contribution of specific TDP-43 domains as well as TDP-43 sub-cellular localization to toxicity. Deletion of either RNA recognition domain (RRM1 or RRM2) completely blocks neurotoxicity, as does deletion of the C-terminal region. These deleted TDP-43 variants still accumulate in the nucleus, although their subnuclear distribution is altered. In contrast, N-terminal deletions result in the formation of toxic cytoplasmic aggregates. Mutation of the TDP-43 nuclear localization signal (NLS) results in cytoplasmic deposition of full-length TDP-43, which is not toxic. Mutations that alter two TDP-43 caspase cleavage sites (D89/219E), however, do not reverse TDP-43 toxicity. Our results demonstrate that TDP-43 neurotoxicty can result from either nuclear activity of the full-length protein or accumulation of cytoplasmic aggregates composed of C-terminal fragments. These results suggest that there may be (at least) two different mechanisms of TDP-43 neurotoxicity.

425B

Age-dependent modification of expanded CAG toxicity in simple C. elegans models. **Arnaud Tauffenberger**[1,2,3], Samar Bel-Hadj[2], Alex Parker[1,2,3]. 1) Pathologie et biologie cellulaire, Université de Montreal, Montreal, Quebec, Canada; 2) CRCHUM, Hopital Notre-Dame, Montreal, Quebec, Canada; 3) Centre of Excellence in Neuromics, Montreal, Quebec, Canada.

Arnaud Tauffenberger1,2,3, Samar Bel-Hadj1, Alex Parker1,2, 3 1CRCHUM, 2Centre of Excellence in Neuromics, 3Department de pathologie et biologie cellulaire, Université de Montréal, Montréal, Québec, Canada The expansion of CAG trinucleotides coding for glutamine is the cause of at least nine late-onset neurodegenerative diseases. The expanded CAG (expCAG) diseases may have some pathogenic mechanisms in common as the disease threshold for all of these disorders is around 40 CAG repeats and they display misfolded intracellular protein aggregation phenotypes. We have turned to the model organism C. elegans to explore two areas of interest: 1. Age-dependent toxicity: Although the expCAG diseases show anticipation, the size of CAG repeat only partially correlates with the variation in age-of-onset. Evidence suggests that even for these single trait neurodegenerative diseases, disease onset and progression may have additional environmental and genetic components. Using C. elegans transgenic worms expressing expCAG in neurons we are exploring the links between aging and late-onset neurodegeneration. We hypothesize that genes regulating longevity, stress response, their associated signaling pathways and downstream effectors may encode new therapeutic targets for age-dependent proteotoxicity in humans. 2. Mechanisms of expCAG toxicity: Long homopolymeric CAG nucleotide stretches are unstable and may be prone to translation errors. Mistranslation of expCAG to alternate reading frames may produce hybrid proteins with additional toxicity. We have created transgenic strains to investigate this phenomenon. A summary of our data and recent findings will be presented.

426C

HAT's off: a novel and powerful class of nematicides for soil-transmitted nematode infections. **Yan Hu**[1], Alan Kelleher[1], Sophia Georghiou[1], Edward G. Platzer[2], Cheng-Yuan Kao[1], Chang-Shi Chen[1], Raffi V. Aroian[1]. 1) Division of Biological Science, Univiversity of California, San Diego, La Jolla, CA, 92039-0322, U.S.A; 2) Dept. of Nematology University of California Riverside, CA 92521-0415, U.S.A.

Soil-transmitted nematodes, hookworm, *Ascaris*, and *Trichuris* (HAT), are amongst the most prevalent human parasites, infecting more than 1 billion of the poorest peoples around the world. We have limited number drugs for treating these parasites, and, for practical reasons, only one (albendazole) is commonly used in mass drug administration (MDA). All our current drugs have limitations as used in MDAs, including moderate efficacy against hookworms, low efficacy against *Trichuris*, and threat of drug resistance. Therefore there is need for new human anthelmintics (anti-worm drugs) that are safe, effective and broad spectrum in scope. Our laboratory is working to develop Crystal (Cry) proteins produced by soil bacteria *Bacillus thuringiensis* (Bt) as a new class of anthelmintics. Bt Cry proteins are commonly used as insecticides in agriculture, are safe to humans, and have a mechanism of action (form pores in invertebrate intestine) different from nematicides currently use. Several Cry proteins have been identified that target nematodes, including *C. elegans* and plant-parasites. We are now using the mouse intestinal parasitic nematode, *Heligmosomoides polygyrus*, as a model for human disease. Here we will present our latest data showing the efficacy of Cry proteins against this rodent parasitic nematode, as well as our system to improve the anthelmintic activity of Cry proteins by looking for point mutant Cry variants that are hyperactive against *C. elegans*.

427A

Caenorhabditis elegans as a model animal to study the homologues genes function in Trichinella. **Yurong Yang**, Wei Jian, Weiwen Qin. Biol Dept, Rm #216, Xiamen Univ, Xiamen, Fujian, FUJIAN, China.

Trichinella is an important animal parasitic nematode in the world. As a pathogen, every year more than millions people were under the health threat. The larvae intrude the skeletal muscle tissue of host and formed encysted muscle larvae, and the adult worms parasite in the same host intestine, so the larvae and adult worm parasite in different tissue of the same animal host, and did not need to change the host. A lot of protective antigens have been found, but most of these antigens genes function and the mechanism have not been clearly elucidated. The Free living nematode, Caenorhabditis elegans has been shown a good model to study the basic biological processes and genes function in vivo system. C.elegans as one of the important model animals has been applied to the research on animal parasitic nematodes, as having a short life cycle, small adult size, easy maintenance in a large numbers and tractability. With genome information, resources available and research techniques like RNAi and in situ hybridization, the nematode shows a powerful tool to the study of the molecular biology of the animal parasitic nematodes. In this study, we use the EST sequence from trichinella to blast in the worm base and found several homologues genes in C.elegans which known are involved in the early embryo development. We cloned these homologues genes from trichinella and study these genes function in C.elegans by using RNAi. The results shown knock down these homologues genes in C.elegan can affect the embryo development in C.elegans, also affect the lifespan of wild type worm. The expression of these homologus genes in Trichinella were study further. In this paper, a full-length of cDNA encoding a putative Rho-family small GTPase gene cdc-42 was isolated from Trichinella spiralis. The uninterrupted open reading frame (ORF) of TsCDC-42 encodes a predicted protein of 147 amino acids and containing a highly conserved domain of CDC-42. The highest identity of TsCDC-42 with CDC-42 from Drosophila is 67%, and with the CDC-42 homologue of C.elegans is 62%, and the similarity is up to 71%. Phylogenetic analyses revealed TsCDC-42 is closely related to the molecule inferred from the cdc-42 gene of C.elegans. The transcript of TsCDC-42 was analyzed during different stage of the worm.The present study demonstrated that C.elegans is a good parasitic nematodes substituted model to study the function and mechanism of antigen genes in Trichinella. The application of C.elegans in parasitic nematodes can assist to identify the homologues genes function in parasites. Furthermore, it can be of great help to screen the candidate genes in trichinella to the aspects of the parasitic nematodes control.

428B

Identification of nematotoxic fungal lectins and their respective target glycans in *C. elegans* . **Alex Butschi**[1], Markus Künzler[2], Markus Aebi[2], Micheal O. Hengartner[1]. 1) Institute of Molecular Biology, University of Zurich, Zurich, Switzerland; 2) Institute of Microbiology, ETH Zurich, Zurich Switzerland.

Lectins are carbohydrate-binding proteins that do display no enzymatic activity towards the recognized sugars. Higher fungi express a variety of lectins with different substrate specificities; their function is usually unknown. In bacteria, plants, and recently in animals, lectins have been shown to play a role in the defense against pathogens and parasites. Here we show that several lectins of the homobasidiomycete *Coprinopsis cinerea* and other higher fungi display toxicity towards *Caenorhabditis elegans* and thus may be part of a lectin-mediated defense system of higher fungi against predators and parasites. In a forward genetic screen using transposon mutagenesis, several mutant *C. elegans* strains exhibiting resistance against specific lectins were isolated. All identified transposon insertions were located in genes involved in the biosynthesis of specific glycans. These glycans represent the *in vivo* ligands for the respective fungal lectins and their binding is a prerequisite for the observed toxicity. As an example, a mutation in the *fut-8* gene encoding the fucosyltransferase responsible for the α-1,6-core fucosylation of *C. elegans* N-glycans conferred resistance towards the *C. cinerea* galectin CGL2, but not towards RedA, another lectin from the same mushroom, that has been shown to bind α-1,3-core fucosylated N-glycans. Conversely, a mutation in *fut-1*, the gene coding for the *C. elegans* α-1,3-core fucosyltransferase conferred resistance towards RedA, but not towards CGL2. These results demonstrate that this type of genetic screen in *C. elegans* is an excellent way to identifiy the target glycan structures of nematotoxic fungal lectins. Although the mechanism of toxicity is currently unclear, we hypothesize that clustering of multivalent glycoconjugates by oligomeric lectins is a key event triggering cell and organismic death. The identification of specific target glycans of these lectins might thus be of interest with regard to pest control.

429C

We remember: using *C. elegans* to combat forgotten nematode diseases. **Yan Hu**[1], Shu-Hua Xiao[2], Raffi. V. Aroian[1]. 1) Division of Biological Science, Univercity of California, San Diego, La Jolla, CA, 92093-0322, U.S.A; 2) National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, Shanghai, 200025, People's Republic of China.

Intestinal parasitic nematodes or helminths are the most prevalent neglected tropical disease agents in the world today, infecting more than 1 billion of the poorest people around the world. These namatodes infection place a tremendous burden on infected populations, in particular children and pregnant women. Tribendimidine, a derivative of amidantel, is the only new human anti-nematode drug (anthelmintic) to be developed and used clinically in the last 30 years. However, little is known about its mechanism of action and by what means, if at all, the parasites can deliver resistance to the drug. *C. elegans* is particularly well-suited for such mechanism & resistance studies and in fact has been used to determine the mechanism of action of virtually all anthelmintics used clinically. Here we will describe a forward genetic screen for *C. elegans* mutants resistant to tribendimidine. In this screen, we obtained ten tribendimidine-resistant alleles among the progeny of 5100 F1 mutagenized genomes. These alleles fall into four complementation groups. Genetic mapping, drug studies, complementation testing, and sequencing of DNA indicate these tribenidmidine resistant alleles actually mutate genes previous identified in screen for resistance to a known anthelmnitic family. Furthermore, we demonstrate that *C. elegans* mutants isolated based on their resistance to this known anthelmintic family are also resistant to tribendimidine. Our results, which have important implications for clinical use, demonstrate tribendimidine does not define a new class of anthelmintic but is a member of one anthelmintic class current in usage Our lab also has been pioneered studying Crystal proteins made by *Bacillus thuringiensis* to use as next-generation anthelmintics. In the process of studying tribendimdine, we decided to use to *C. elegans* to further explore different anthelmintic classes. Here we will describe these experiments. We have tested various anthelmintics against *C. elegans* mutants resistant to different classes of anthelmintics (nAChR agonists, benzimidazoles, Cry proteins) to see how resistance to one class correlates with susceptibility to another class. Furthermore, we have exposed *C. elegans* to different drug combinations and calculated the synergistic effects of these combinations. Our data demonstrate a surprising and encouraging finding: one specific combination of anthelmitics recreates characteristics associated with some of the best combination therapies used to treat diseases like HIV.

430A

Knock-down of mucin-like genes using RNAi changes lectin staining in adult hermaphrodites of *Caenorhabditis elegans*. **Keith G Davies**[1,2], Maria Gravato-Nobre[1], Jonathan Hodgkin[1]. 1) Biochemistry, University of Oxford, Oxford, OX1 3QU, UK; 2) Plant Pathology and Microbiology, Rothamted Research, Harpenden, AL5 2JQ, UK.

In animal parasitic nematodes a family of glycosylated proteins called mucins is a component of the cuticle's surface coat and these proteins are thought to be important in the evasion of the immune response. Mucins are a family of proteins of high molecular weight with greater than 50 percent of their mass being made up of glycans. They are rich in the amino acids proline, serine and threonine and can either be secreted or membrane bound. Eleven mucin-like genes (*H43E16.1, F59A6.3, cpg-1, let-653, F35E12.7, cwp-4, K06A9.1, H02F09.3, C12D12.1, C26G2.2* and *F16F9.2*) were knocked down by RNAi and screened for changes to glycans present in the mouth, pharynx, grinder, surface coat, vulva and rectal regions using four different lectins (wheat germ agglutinin, WGA; Concanavalin A, Con A; *Tetragonolobus purpurea* agglutinin, TPA; Peanut agglutinin, PNA). Results showed different patterns of lectin binding to each of the structures scored and the binding was variable between replicated individuals. Generally, lectin binding was low across wild type worms with the exception of TPA which appeared to have a higher level of recognition to the cuticle and rectum. The lectins WGA, Con A and TPA bound to the vulva of wild type worms more successfully than PNA. Knocking down mucin-like genes with RNAi affected lectin binding patterns and the effects differed between the different structures scored. For example, knocking down *H43E16.1, F59A6.3, C26G2.2* and *F16F9.2* increased the binding of PNA to the grinder but whereas *H43E16.1, F59A6.3* also showed increasing binding of PNA to the cuticle, *C26G2.2* and *F16F9.2* were no different from wild type worms. Conversely, knock-downs of *F35E12.7, cwp-4, K06A9.1, H02F09.3*, which increased the binding of PNA to the cuticle showed binding similar to wild type worms in the grinder, whereas, *C26G2.2* and *F16F9.2*, which had increased binding to the grinder, were no different from wild type worms. The cuticle forms an important barrier between a nematode and its environment and reducing mucin-like proteins appears to affect the surface coat of *Caenorhabditis elegans* in complex ways.

431B

Development of a *C. elegans* model for a hereditary laye-onset cerebellar ataxia (LOCA). **Julie Demers-Lamarche**[1,2], Samar Bel Hadj[1], Isabelle Thiffault[1,2], Bernard Brais[1,2,3], Alex J. Parker[1,3,4]. 1) Centre de recherche du CHUM; 2) Département de biologie moléculaire, Universite de Montreal; 3) Centre of Excellence in Neuromics; 4) Departement de pathologie et biologie cellulaire, Universite de Montreal, Montreal, Quebec, Canada.

Recessive ataxias are a heterogeneous group of neurodegenerative diseases. Late-onset ataxias have largely been considered as either milder forms of dominant ataxias or sporadic diseases seemingly not caused by genetic factors. Genotyping of our present cohort, 58 affected cases belonging to 34 families, allowed us to link a form of late-onset cerebellar ataxia (LOCA) with a region on the chromosome 2. Sequencing of genes in the candidate region uncovered mutations in a gene coding for ubiquitin-like protein not previously known to be mutated in a disease. We investigated the effect of the inhibition of the C. elegans homologues on the phenotype. We showed that RNAi silencing in both wild-type N2 and neurosensitive rrf-3 strains reduced lifespan. The RNAi experience on N2 strain did not activate a number of in vivo stress reporters including sod-3::GFP, hsp-4::GFP, hsp-6::GFP and hsp-60::GFP. The identification of recessive mutations in a ubiquitin-like gene in LOCA cases suggested that abnormal protein folding also plays a role in this new form of late-onset neurodegenerative disorder so we reasoned that the RNAi gene homologue should affect proteotoxicity. We tested this hypothesis with transgenic worms expressing a polyglutamine-tract of 40 residues fused to YFP. The RNAi accelerated the mean onset of paralysis of these animals compare to control and the appearance of Q40::YFP aggregation puncta. We are now generating a transgenic C. elegans strain for this late-onset ataxia.

432C

Identifying host factors required for Sindbis virus replication using *C. elegans.*. **Johannes H Decker**, Ellen Bradley, Charles Rice, Margaret MacDonald, Shai Shaham. The Rockefeller University, New York, NY.

Alphaviruses cause equine and human illness characterized by fever, rash, arthritis, encephalitis and even death. Recent outbreaks of an illness resembling dengue fever, caused by the alphavirus Chikungunya, underscore the potential of these viruses to inflict human suffering. Sindbis virus is a well studied *Alphavirus*. While much is known about viral factors important for Sindbis replication, little is understood about roles played by host factors. To uncover host factors important for Sindbis replication, we generated a transgene containing a heat-inducible promoter (HIP) fused to DNA encoding a modified Sindbis virus, in which all structural proteins were replaced by GFP. GFP expression from this transgene only occurs following viral replication. The HIP::Sindbis(GFP) transgene was introduced and integrated, together with a HIP::mCherry reporter, into wild-type animals. Animals subjected to a 45 min heat shock at 34C express mCherry within 50 min, whereas GFP is seen only after 6 hours, consistent with viral replication. Furthermore, a ribonuclease protection assay of RNA from heat-shocked animals suggests generation of a replication-dependent subgenomic viral fragment containing GFP. Although evident, expression of GFP from the HIP::Sindbis transgene is variable, and of low intensity. We reasoned that since the virus passes through a dsRNA replication intermediate, that low GFP expression may be due to degradation by RNAi. Indeed, animals lacking *rde-4*, a gene essential for RNAi, express GFP more consistently and at higher levels. GFP was expressed robustly only in muscle cells of the pharynx, vulva, uterus, and body wall. Remarkably, Sindbis replication in vertebrates also exhibits muscle tropism, suggesting that *C. elegans* may be a suitable model for studying host components promoting replication. To uncover such host factors, we performed EMS mutagenesis on *rde-4*; HIP-Sindbis(GFP) animals, and screened for reduced GFP expression. We identified 33 mutants, 12 of which retained a functional heat-shock response, and were viable and fertile. We are cloning the genes affected in these mutants. In parallel, we are also crossing our *rde-4;* HIP-Sindbis(GFP) line to muscle-defective mutants to examine whether genes required for muscle function are required for viral replication. Similar approaches to study replication of other viruses in *C. elegans* have been previously described, however, it is not yet clear whether host factors described in those studies are required for viral replication in native hosts. Our discovery that Sindbis exhibits similar tropism for replication in *C. elegans* and vertebrates suggests, perhaps, that similar host factors are indeed involved.

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All three *C. elegans* MAP kinase pathways are required for resistance to intestinal infection by *Saccharomyces cerevisiae*. Meijiang Yun, Shinya Iguchi, Charu Jain, Reeta Prusty Rao, **Samuel Politz**. Dept Biol/Biotech, Worcester Polytechnic Inst, Worcester, MA.

Few antifungal agents are available to combat human pathogenic fungal infections. We are using *C. elegans* as a host for the budding yeast *S. cerevisiae* in order to study simultaneously the genetics of pathogen resistance and virulence in these model organisms. We have observed the interaction of RFP-labeled *S. cerevisiae* with *C. elegans*. Wild-type worms grown from the L4 stage on RFP-labeled yeast accumulated RFP-labeled yeast cells in the intestinal lumen, which became progressively more distended and full of yeast cells over a period of 1-3 days. We had noted previously that a Deformed anal region (Dar) phenotype was induced by yeast in some individuals. However, in contrast to the interaction of *C. elegans* with *M. nematophilum*, RFP yeast fluorescence did not accumulate at the anus or in the rectum, even in individuals that displayed Dar. In survival assays, wild-type *C. elegans* grown from hatching in the presence of *S. cerevisiae* showed a slight, but significant lifespan reduction compared to controls grown on *E. coli* OP50 alone ($p = 0.006$, TD50 reduced from 11.99 to 10.16 days). In order to test genetic requirements for this apparent partial resistance, we tested mutants altered in genes of the *C. elegans* MAP kinase pathways, which are known to be required for resistance to several pathogenic bacteria which cause intestinal infection and killing. Of the mutants tested, only *jnk-1(gk7)* (JNK pathway MAPK) and *nsy-1(ag3)* (p38 pathway MAPKKK) mutants did not show reduced survival in the presence of yeast. In contrast to the *jnk-1* mutant results, mutation of *kgb-1*, which encodes another *C. elegans* JNK-like MAPK, did result in significantly reduced lifespan when grown on yeast. p38 pathway mutants altered in *sek-1* (MAPKK) and *pmk-1* (MAPK) were susceptible to yeast infection as well. The ERK pathway mutant *mek-2(n1989)* (MAPKK) also showed reduced survival in the presence of yeast. Our results suggest that all three MAP kinase pathways are required for full resistance to yeast. We intend to further investigate the requirements for resistance to *S. cerevisiae* by testing mutants altered in additional MAP kinase pathway components as well as candidate genes from other pathways implicated in immunity.

434B

The role of MIG-15 in polarization and maintenance of polarization of the Q cells. **Jamie Chapman**, Erik Lundquist. Department of Molecular Biosciences, University of Kansas, Lawrence, KS.

MIG-15 is a Nck-interacting kinase that has been found to act with the Racs and integrin in axon pathfinding (Poinat *et al*., 2002; Shakir *et al*., 2006). Hedgecock and Guo first described Q cell descendant migration defects in *mig-15* mutants. The bilateral Q neuroblasts, born between the V4 and V5 lateral seam cells, undergo initial anterior and posterior polarizations and migrations before dividing to produce neurons that will continue in their long-range anterior and posterior migrations. We found that mutations in *mig-15* reduced the size of the initial Q cell protrusions, but did not affect the direction of the initial polarizations. After this initial polarization at a time when in wild type the Q cells have migrated, the Q cells often fail to migrate and fail to maintain their initial proper polarizations, sending ectopic protrusions in both anterior and posterior directions. In order to examine the role of MIG-15 in the polarization and maintenance of polarization of the Q cells, we began looking at candidate genes that might be working with MIG-15 in these processes. Baum and Garriga (1997) showed that INA-1/α-integrin mutants have defects in Q cell descendant migration. Additionally, the C-terminus of MIG-15 has been shown to physically interact with the cytoplasmic domain of PAT-3/β-integrin (Poinat *et al*., 2002). Therefore, we began to examine the role of INA-1 in the Q cell polarization and maintenance of polarization. Analysis of the Q cells in *ina-1* null mutants showed that *mig-15* and *ina-1* mutants phenotypically resembled each other during the initial polarization stage, with both mutants containing Q cell protrusions that were reduced in size, but formed in the correct direction. Looking later at the migration stage when *mig-15* mutants fail to migrate and begin to send out ectopic protrusions, *ina-1* mutants also failed to migrate, but did not send out ectopic protrusions and remained polarized in the correct directions. Further analysis of the Q cell descendants displayed other similarities between *mig-15* and *ina-1* mutants, in which AQR and PQR both reversed directions of migration and failed to fully migrate along their normal migratory pathways. A double mutant of *mig-15* with *ina-1* resembled *ina-1* mutants alone for AQR/PQR migration, and overexpression of INA::GFP increased the AQR/PQR migratory defects in a hypomorphic *mig-15* background. Taken together, these results suggest that MIG-15 could be acting with integrin to regulate the polarization and migration of the Q cells and their descendants. In contrast, the maintenance of polarization appears to be independent of *ina-1*, suggesting that MIG-15 might be working with other molecules to maintain the polarity of the Q cells.

435C

CDK-5 regulates polarized trafficking of dense core vesicles. **Patricia R. Goodwin**[1,2], Peter Juo[1]. 1) Dept Physiology, Tufts Univ, Boston, MA; 2) Program Neuroscience, Tufts Univ, Boston, MA.

Neuropeptides are neuromodulators that regulate classical neurotransmitter activity in mammals and invertebrates. Neuropeptides are released from dense core vesicles (DCVs), which can undergo polarized trafficking to the axon and release at synaptic sites. In *C. elegans*, neuropeptide release facilitates cholinergic synaptic transmission at the neuromuscular junction (1). DCVs can be visualized *in vivo* using fluorescently-tagged neuropeptides (such as INS-22::Venus)(2,3). In polarized DA motorneurons, DCVs are primarily localized to the axon in the dorsal nerve cord by the kinesin motor UNC-104/Kif1A (1-4). In this study, we show that cyclin dependent kinase-5 (CDK-5) functions in cholinergic motorneurons to regulate the polarized trafficking of DCVs to axons. In mammals, CDK-5 and its cyclin-like activator, p35, regulate multiple cellular functions including axon outgrowth and synaptic transmission, and dysregulation of CDK-5 contributes to several neurodegenerative disorders. Here we show that in *cdk-5(gm336)* or *p35(tm648)* loss-of-function mutants, the abundance of DCVs in the axons of DA motorneurons decreases with a coincident increase in the abundance of DCVs in the dendrite. This increase in dendritic DCVs can be rescued by expressing wild type *cdk-5* cDNA in cholinergic neurons. The effect of CDK-5 on polarized DCV trafficking is relatively specific since the presynaptic localization of synaptic vesicle marker SNB-1::GFP and active zone marker SYD-2::GFP is not altered in cdk-5 mutants. To investigate why DCVs accumulate in the dendrites of *cdk-5* null mutants, we performed time-lapse imaging of mobile INS-22::Venus puncta in motorneuron dendrites. DCVs are highly mobile and move in a saltatory fashion in both anterograde and retrograde directions (4). Our data indicate that in the DA motorneuron dendrite of *cdk-5* mutants, there is an increase in the total number of DCVs moving in the anterograde direction and an increase in total number of stationary DCVs, compared to wild type controls. There is no change in the number of DCVs moving in the retrograde direction. The ectopic DCVs observed in the dendrites of *cdk-5* mutants do not appear to be transported there by their normal axonal motor, UNC-104/Kif1A, because deletion of *cdk-5* in an *unc-104* null background still results in an increase the number of DCVs in the dendrite. We hypothesize that CDK-5 regulates polarized DCV trafficking by inhibiting the inappropriate loading of DCVs onto an unidentified dendrite-directed motor. References: (1)Jacob & Kaplan. J Neurosci 2003 (2)Sieberth et al. Nature 2005 (3)Sieberth et al. Nat Neurosci 2006 (4)Zahn et al. Traffic 2004 (5)Dhavan and Tsai. Nat Reviews 2001.

436A

Frizzled receptor antagonism in neuronal polarity

. **Mark Gurling**, Chun-Liang Pan, Gian Garriga. Dept. of Molecular and Cell Biology and Hellen Wills Neuroscience Institute, UC-Berkeley, Berkeley, CA. 94720.

Wnt glycoproteins signal through Frizzled receptors to regulate many aspects of neural development such as neuronal migration and polarity[1,2,3,4]. The two Frizzled receptors LIN-17 and MIG-1 appear to play antagonistic roles in certain developmental contexts[3,5]. In worms mutant for the Frizzled receptor LIN-17, the polarity of the PLM mechanosensory neuron is reversed. Mutations in *lin-44*, which encodes one of the Wnt proteins, caused similar PLM phenotypes. Synapses made by the anterior process in wild-type animals are now made by the posterior process of mutant PLMs[1,2]. A similar phenotype is seen when another Frizzled, MIG-1, is over expressed in the PLM suggesting an antagonistic relationship between *lin-17* and *mig-1*. Consistent with this hypothesis, loss of MIG-1 suppresses the polarity reversal of *lin-17* and *lin-44* mutants. We propose two explanations for how excess MIG-1 may cause a reversal of PLM polarity. In model 1, increased MIG-1 signaling antagonizes LIN-17 signaling. In model 2, excess MIG-1 competes with LIN-17 for the Wnt ligand LIN-44, thereby reducing signaling through LIN-17, and possibly another Wnt receptor. Model 1 predicts that loss of the MIG-1 Wnt ligand should suppress the PLM polarity defect caused by excess MIG-1. Loss of *cwn-1*, *cwn-2* or *mom-2* does not ameliorate the polarity phenotype. This lack of a genetic interaction suggests that MIG-1 might bind LIN-44 to prevent it from interacting with LIN-17, inconsistent with a MIG-1 signaling role proposed in model 1. We are attempting to distinguish between these two models by determining which domains of MIG-1 are necessary and sufficient to generate the PLM polarity defect.

1. Prasad BC, Clark SG. (2006) Development 133: 1757-1766
2. Hilliard MA, Bargmann CI. (2006) Developmental Cell 10: 379-390
3. Pan C-L, et al. (2006) Developmental Cell 10: 367-377
4. Maloof JN, Whangbo J, et al. (1999) Development 126: 37-49
5. Forrester, W.C., Kim, C., Garriga, G. (2004) Genetics 168: 1951-1962.

437B

Wnt and Frizzled molecules regulate dendrite formation in *C. elegans*. . **Leonie Kirszenblat**, Divya Pattabiraman, Brent Neumann, Massimo A. Hilliard. Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

Neurons exhibit distinct morphological domains, axons and dendrites, which are essential for functional wiring of the nervous system. While many molecules involved in axon development have been discovered, there is little known about the molecules and mechanisms required for dendrite formation. To understand how dendrites develop in *C. elegans* we have chosen to study the oxygen sensory neuron, PQR. The PQR neuron has its cell body positioned in the left lumbar ganglion on the posterior-lateral body. From the cell body two processes extend. A single dendrite extends posteriorly towards the tail ending with a cilium in the pseudocoelom. On the opposite side, an axon extends anteriorly joining the ventral nerve cord and terminating in the midbody region. PQR is born post-embryonically during the L1 stage, allowing easy visualization of dendrite and axon development using the *gcy-36::GFP* transgene.

We have used a candidate-mutant approach to identify genes involved in dendrite formation. The secreted Wnt ligand, LIN-44, regulates neuronal polarity in PLM mechanosenosory neurons in the *C. elegans* tail. We found that LIN-44 specifically regulates dendrite formation in PQR. In *lin-44* mutants, the PQR dendrite was often shortened or absent. This defect appeared in the initial stages of development (at the L1 stage) and persisted until adulthood, suggesting that the phenotype results from a failure in dendrite development rather than the presence of ectopic pruning. Interestingly, the PQR axon was not affected in these animals, indicating a dendrite-specific effect of LIN-44 on this cell. LIN-44 is expressed by hypodermal cells in the tip of the tail, a position that is posterior to the PQR cell body. The PQR dendrite develops towards the region where LIN-44 is expressed, and thus this ligand may act as an attractant cue for the growing dendrite.

LIN-17/Frizzled acts as a receptor for LIN-44 in regulating neuronal polarity and other biological processes. We found that *lin-17* mutant animals presented defects in PQR dendrite development similar to those observed in *lin-44* mutants: PQR dendrite was shortened or absent, while the axon was unaffected. Thus, as for *lin-44*, the *lin-17* gene has a dendrite specific effect.

Our results suggest that Wnt molecules and Frizzled receptors are key molecules required for PQR dendrite formation.

438C

Molecular Mechanisms that Control Dendritic Branch Formation in *C.elegans*. **Cristina Aguirre-Chen**[1], Hannes E. Buelow[1,2], Zaven Kaprielian[1]. 1) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

The establishment of proper dendritic arborization patterns is a key phase of neuronal development that is required for the proper functioning of the nervous system. However, the molecular mechanisms that control dendritic branch formation are poorly understood across species. The *C.elegans* PVD neurons are a pair of putative mechanosensory neurons that respond to harsh mechanical stimuli. In both midlarval- and adult-staged animals, the PVD neurons exhibit an elaborate branching pattern. Aside from the finding that *mec-3*, a LIM homeodomain-containing gene, is required for the elaboration of PVD-associated arbors, the mechanisms that regulate branch formation in these neurons remain unclear. To identify novel regulators of PVD branching, both a small-scale, candidate-based (140 genes) and a large-scale RNAi screen of the approximately 3000 genes on Chromosome IV were carried out in a PVD reporter background. In total, twelve genes were retrieved that either promote or suppress the formation of PVD branches. The focus of our current studies is the *bicd-1* gene, whose RNAi phenotype indicates a role for it in suppressing PVD branch formation and whose homologs have been shown to play a crucial role in the localization of mRNAs and organelles via its interactions with dynein. Preliminary characterization of the *bicd-1* deletion allele, *ok2731*, confirms that this mutant phenocopies the enhanced branching phenotype exhibited by *bicd-1* RNAi-treated animals. Additionally, a *bicd-1* transcriptional reporter line that we recently generated displays robust and rather specific labeling of both the PVD and FLP neurons, the two most highly branched neurons in the *C. elegans* nervous system, as well as the H-shaped excretory cell. Currently, we are carrying out cell type-specific rescue experiments to determine the site of action of *bicd-1* and double mutant analyses to identify genes that genetically interact with *bicd-1*.

439A

zyg-8 Function in *C. elegans* Motor Neuron Development. **Renee A. Baran**, Garland Tang, Farah Shirazi. Dept Biol, Occidental Col, Los Angeles, CA.

Mutations in alpha-tubulin, doublecortin, and LIS1 block neuron migration and cause lissencephaly in humans. Doublecortin/DCX and doublecortin-like proteins bind microtubules and function to promote microtubule assembly and stability while LIS1 mediates interactions with the dynein motor complex. Doublecortin proteins have also been shown to localize to neurite endings and influence axon outgrowth and dendrite arborization (Friocourt et al., 2003; Deuel et al., 2006; Cohen et al., 2008). *zyg-8*, the sole *C. elegans* member of the doublecortin family, encodes a doublecortin-like protein required for positioning the mitotic spindle during embryonic divisions (Gonzcy et al., 2001). We utilized conditional *zyg-8* mutants to study the role of *zyg-8* in later stages of neuronal development. *zyg-8(b235ts)* mutants were shifted to the nonpermissive temperature as late embryos or early L1 larvae prior to synapse formation, and the GABAergic motor neurons of mutant adults were analysed for expression of the synaptic vesicle marker SNB-1::GFP, active zone marker UNC-10::RFP, and an axon-restricted microtubule plus-end binding protein. *zyg-8* mutants exhibited defects in synapse morphology similar to gain-of-function *tba-1* mutants (R. Baran & Y. Jin). SNB-1::GFP puncta were irregular in size and spacing and GFP was mislocalized in the commissures. Synapses were sometimes missing from the most distal regions of axons along the dorsal nerve cord, a phenotype also observed in *tba-1(ju89)* mutants. These results suggest that *zyg-8* may play a significant role in larval and adult motor neurons in maintaining axon and synapse integrity.

440B

Wnt-directed asymmetry of the bHLH factor LIN-32 regulates cell fate specification in the ray sublineage. **Renee M. Miller**[1], Douglas S. Portman[1,2]. 1) Ctr. Neural Dev. & Disease, Univ Rochester, Rochester, N.Y; 2) Dept. Biomedical Genetics, Univ Rochester, Rochester, N.Y.

The post-embryonic ray sublineage of the *C. elegans* male presents an appealing and tractable system in which to uncover the mechanisms by which a single precursor cell gives rise to multiple unique differentiated daughters. During the third larval stage, the Rn precursor cell undergoes three rounds of division to yield a hypodermal cell (Rn.p), an RnA neuron (Rn.aaa), an RnB neuron (Rn.apa), an Rnst glial-like cell (Rn.app), and a cell death (Rn.aap). Ultimately, the RnA, RnB, and Rnst cells comprise the nine bilateral pairs of ray sensilla used by the male for the detection of and copulation with hermaphrodites. The basic helix-loop-helix transcription factor LIN-32, the *C. elegans* homolog of atonal, is required for the generation of male rays. We have previously demonstrated that LIN-32 can function at multiple points during the ray sublineage to independently specify RnA, RnB, and Rnst fates, in addition to its early pro-neural role in the Rn.a ray neuroblast. The expression of LIN-32 in the ray sublineage is asymmetric, with higher expression initiated and maintained in the anterior daughter following cell division. This asymmetry is controlled by Wnt signaling because in *lin-17 (frizzled)* mutant males, the anterior and posterior daughter cells express equivalent levels of LIN-32::GFP. To test the hypothesis that asymmetric LIN-32 expression actively patterns ray neuron fates, we devised two different ways to disrupt asymmetry by expressing LIN-32::GFP at equivalent levels in all branches of the ray sublineage. First, we examined the fate of RnA and RnB neurons, and the RnSt, in adult *lin-17* mutant males using the integrated array *fsIs18 (trp-4::mCh (RnA) + pkd-2::CFP (RnB)*). Using two different weak alleles of *lin-17*, we observe a significant shift toward the anterior fate, suggesting that LIN-32 acts in the anterior branch of the ray sublineage to correctly specify neuronal fate. In a complementary approach, we used the heat shock promoter to turn on LIN-32::GFP in all cells during the ray sublineage. We similarly find that animals heat shocked during this critical period sometimes lack Rnst cells and have altered expression of ray neuron markers, frequently demonstrating a higher number of RnA-like neurons. Together, our results support the idea that a Wnt signal received by LIN-17(fz) represses the expression of the bHLH factor LIN-32 in the posterior daughter during the execution of the ray sublineage. Thus, a single bHLH factor can act both early on to specify a neural precursor cell and later to actively pattern the fates of its progeny.

441C

A screen for regulators of sexually dimorphic motor neuron development. **Zachary Palchick**, Avantika Jalan, Daniel London, Rachel Stephenson, Sonya Krishnan, Jennifer Ross Wolff. Department of Biology, Carleton College, Northfield, MN.

Sexually dimorphic neurogenesis in *C. elegans* depends on the coordinated activities of Hox transcription factors and the sex-determination cascade culminating in the transcription factor TRA-1. The hermaphrodite VC motor neurons, which regulate egg laying, and the male CA and CP motor neurons, which innervate mating muscles, arise from analogous precursor cells (Pn.aap), yet adopt unique targets, neurotransmitter expression, and morphology. A striking sexual dimorphism in this lineage is the male-specific cell division of P3.aap-P8.aap, each of which generates a CA neuron that expresses the peptidergic marker *ida-1::gfp* and a CP neuron that expresses the serotonergic marker *tph-1::gfp*. In males, one of two Hox proteins, LIN-39 or MAB-5, must be present to promote this division, and LIN-39 is additionally required to specify serotonergic fate in CP.[1] In hermaphrodites, P3.aap-P8.aap do not divide, but require LIN-39 to survive and differentiate into VCs.[2] While it is clear that motor neuron specification is sexually dimorphic, it is not known how LIN-39 and MAB-5 interact with TRA-1 to generate CAs and CPs in males, but VCs in hermaphrodites. We hypothesize that the asymmetric CA/CP neurogenic division is promoted in males by LIN-39 and MAB-5. In hermaphrodites, TRA-1 opposes this Hox-mediated division to promote VC motor neuron fate. To test our hypothesis, we seek genes whose loss of function disrupts this division. We predict that these genes will include cofactors of LIN-39 and MAB-5 that promote asymmetric neuronal division and targets of TRA-1 that make this division sex-specific. In a pilot RNAi screen, we have found that the Hox gene *nob-1* is required with *mab-5* and *lin-39* for male-specific neurogenesis in the Pn.aap lineage. *nob-1(RNAi)* males fail to express *tph-1::gfp* in CPs, but do express *ida-1::gfp*, suggesting that CAs are present. Previous work has shown that *nob-1* is required for the asymmetric division of the hypodermal cell T.[3] Our preliminary results suggest that *nob-1* may similarly promote the asymmetric division that differentiates CP from CA. We are exploring interactions among *nob-1*, *lin-39*, and *mab-5*, as well as the roles of other *nob-1*-interacting genes, such as *psa-3*/Meis and Wnt pathway components, in promoting asymmetric neuronal division in males. We continue to seek additional regulators of the CA/CP generating division in a genetic screen. Studying these regulators will elucidate how Hox-mediated patterning intersects with sexual regulation to specify neurons that control sexually dimorphic behavior. [1]Salser, SJ, et al., Genes & Development 7:1714-1724; [2]Clark, SG, et al. Cell 74: 43-55; [3]Arata, Y., et al. Developmental Cell 11: 105-115.

442A

hlh-19 a *C. elegans* achaete-scute like gene is expressed in the M4 pharyngeal motor neuron. **Aixa Alfonso**[1], Oscar Ramirez[1], Ryan Doonan[2]. 1) Dept Biological Sci, Univ Illinois @ Chicago, Chicago, IL; 2) Dept Biology, Univ College London, London, England.

The achaete-scute family of proneural genes has been extensively characterized in *Drosophila*, and valuable information on the role of these genes in promoting neurogenesis has been obtained. The role of these transcription factors in *C. elegans* neurogenesis remains relatively uncharacterized. As part of our effort to understand the role of *hlh-3* in the differentiation of the hermaphrodite-specific motor neurons (HSNs) (Doonan et al., 2008), we set out to determine the expression pattern of three other achaete-scute-like genes *hlh-6*, *hlh-12*, and *hlh-19*. To determine the identity of the cells that express these proteins, we generated promoter fusion reporter genes. Our first goal was to characterize if they were expressed in the nervous system and whether expression overlapped that of *hlh-3*. We, as well as others, have shown that two of these genes are not expressed in the nervous system: *hlh-6* expression is limited to the pharyngeal gland cells (Raharjo and Gaudet, 2007) and *hlh-12* is expressed in the gonadal leader cells (Tamai and Nishiwaki, 2007).

Here we report that *hlh-19* is expressed in the nervous system. Multiple neurons express the reporter, notably the pharyngeal neuron M4 and what appears to be a subset of sensory neurons in the head of both males and hermaphrodites. Expression is first detected at the comma stage, and continues throughout postembryonic development and adulthood. Interestingly, the reporter is also expressed in the stomatointestinal and body wall muscles of males but not hermaphrodites. We are currently determining the identity of the neurons in the head. Our future goal is to study the role of *hlh-19* in the specification and differentiation of the *hlh-19* positive neurons. We are currently investigating the consequences of missing gene function using a 218 bp deletion allele of *hlh-19* obtained from the National Bioresource Project, Japan. *hlh-19 (tm3105)*, if translated is missing most of the putative DNA binding domain and dimerization region of the protein. Preliminary analysis of *hlh-19 (tm3105)* revealed no obvious phenotype associated with M4 dysfunction however, the mutant is homozygous viable.

References: (1) Doonan, Hatzold, Raut, Conradt, and Alfonso 2008. Mechanisms of Development 125:883-893 (2) Raharjo and Gaudet 2007. Developmental Biology 302:295-308 (3) Tamai and Nishiwaki 2007. Developmental Biology 308:562-571.

443B

Genetic analysis of dopamine neuron type specification in the postdeirid. **Sriharsh M Gowtham**, Maria Doitsidou, Oliver Hobert. Dept. of Biochemistry and Biophysics, Columbia University, New York, NY.

The dopaminergic neurons PDE left and PDE right are a pair of sensory neurons in the posterior part of the worm. They provide a convenient model for elucidating the genes required for the differentiation of dopaminergic neurons. We investigate the role of known genes that act in PDE lineage specification as well as novel factors, retrieved from a forward genetic screen, that show an abnormal PDE phenotype (1). The genes of interest include *unc-86*, *ast-1*, *vab-15*, *dopy-1*, *dopy-4* and *dopy-6*. We are trying to address the time and site of action of these genes by assessing the fate of various cells of the PDE lineage in the corresponding mutants. In addition we are conducting epistasis experiments to uncover possible interactions between the genes of interest and we are characterizing their expression patterns in wild type as well as in mutant animals.

(1): Doitsidou et al. Nature Methods, October 2008.

444C

Specification of chemosensory neuron fate by the *C. elegans* NKX/HMX homolog MLS-2. **Kyuhyung Kim**, Rinho Kim, Piali Sengupta. Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA.

The *C. elegans* chemosensory nervous system provides an opportunity to investigate development of sensory neuron types at a single cell resolution. The bilateral AWC neurons are members of the amphid sensory organs in the head, and respond to odorants (Bargmann and Horvitz, 1991; Chalasani et al., 2007), as well as temperature (Kuhura et al., 2008; Biron, Wasserman et al., 2008). We previously showed that the CEH-36 OTX/OTD homeodomain transcription factor plays a major role in specification of AWC neuron identity, and is expressed only in the AWC and ASE chemosensory neuron types in adult animals (Lanjuin et al., 2003). To identify additional molecules required for development of the AWC neurons, we screened for mutants in which expression of *ceh-36*p*::gfp* was altered. In *oy88* mutant animals expression of *ceh-36*p*::gfp* was affected in the AWC, but not the ASE neurons. Genetic mapping, genomic rescue, and sequencing of mutant animals showed that *oy88* is an allele of the *mls-2* NKX class of homeodomain transcription factors. MLS-2 has previously been shown to regulate proliferation, cell division, fate specification and differentiation in the M linage (Jiang et al., 2005). In addition, MLS-2 is required for the development of the CEPsh glia (Yoshimura et al., 2008).

Further analyses showed that the expression of multiple terminal differentiation markers was abolished in 50% of AWC neurons in *mls-2* mutant animals. *mls-2* appears to act upstream of *ceh-36* in the genetic pathway regulating AWC neuronal identity. Moreover, 70% of *mls-2* mutant animals showed ectopic expression of ASH-specific markers. Loss of AWC-specific expression and ectopic expression of ASH-specific markers are not correlated, suggesting that MLS-2 acts independently in these two lineages. We did not detect any obvious defects in the expression of other amphid neuron-specific markers. Currently we are performing spatial and temporal rescue experiments, mosaic analyses, and lineage analysis to further characterize the roles of MLS-2 in chemosensory neuron development. Furthermore, using promoter analyses and bioinformatics searches, we are attempting to determine whether MLS-2 directly regulates *ceh-36* expression, and identify additional targets of MLS-2 regulation in chemosensory neurons.

445A

MYST family member *lsy-12* is required for correct specification of the ASE chemosensory neurons. **M. Maggie O'Meara**[1], Oliver Hobert[1,2]. 1) Department of Genetics and Development, Columbia University, New York, NY; 2) Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

A large-scale genetic screen for mutants that effect the bilateral specification of the ASE chemosensory neurons revealed six mutants falling into the same complementation group that are required for correct specification of ASEL fate. Acting upstream of a bistable feedback loop required for initiation of ASEL/R fate, *lsy-12* also appears to act as an ASEL inducer, having the ability to drive ASEL cell fate. Mapping, cloning, rescue, and RT-PCR experiments confirm that *lsy-12* is a single gene product coded from R07B5.8 and R07B5.9 locus. The combine gene is a member of the MYST histone acetyltransferase family. This implicates a role in chromatin modification, which may be necessary early in development as a mark of the lineage distinction necessary for the bilaterally asymmetric cell fates of the ASEL and ASER neurons.

446B

Re-imposing left-right neuronal symmetry: *hlh-14* and its requirement in the ABalppp/ABpraaa neuronal lineages. **Richard J. Poole**[1,2], Enkelejda Bashllari[1,2], Oliver Hobert[1]. 1) Department of Biochemistry & Molecular Biophysics, Columbia University, New York; 2) Equal contribution.

The bilaterally symmetric gustatory neuron pair ASEL/ASER displays directional asymmetric expression of several cell fate markers and senses and discriminates distinct inputs. The adult laterality of the ASE neurons is specified embryonically at the 4-cell stage and is dependent on the asymmetric lineage origins of ASEL and ASER (Poole and Hobert 2006). Despite their asymmetric origins, the ASE neurons are to a large extent molecularly and morphologically symmetric. Moreover, from the ABalppp/ABpraaa stage onwards the asymmetric lineages that give rise to ASE become bilaterally symmetric across the left-right axis and go on to produce identical pairs of neuronal and sheath cells on the left and right sides of the worm. How is bilateral symmetry re-imposed on these lineages and more specifically in the case of ASE what leads to the expression of the zinc finger transcription factor *che-1*, the terminal selector of ASE cell fate, in two cells from asymmetric lineages?

To address these issues we have performed a genome-wide RNAi screen for regulators of ASE cell fate (see abstract by Bashllari, Poole and Hobert). From this RNAi screen we have isolated *hlh-14/achaete-scute*. We find in *hlh-14* mutants that ASE cell fate is completely lost, including the expression of *che-1*. Intriguingly *hlh-14* expression is first observed at the ABalppp/ABpraaa stage, the stage at which the bilateral symmetry is re-imposed on these lineages. This expression persists to the comma stage in posterior lineage branches but is progressively lost from more anterior branches. Examination of other terminal cell fates within this lineage indicates that while the posteriorly situated ASE neurons are completely dependent on *hlh-14* anteriorly situated cells are not. Surprisingly, cells situated within the center of the lineage are partially dependent on *hlh-14*. This suggests redundancy with a second gene in the center and anterior branches of the lineage. Cell lineage analysis indicates that when cells lose their fate in *hlh-14* mutants they are transformed to hypodermal cells. Taken together our data suggest *hlh-14* is required to establish neuronal cell fate in the posterior branches of the ABalppp/ABpraaa lineage as part of a mechanism that re-imposes bilateral symmetry on these lineages. We are currently investigating what regulates neuronal fate in the anterior branches of the lineage, how *hlh-14* expression is regulated and whether *hlh-14* plays additional roles in determining the terminal cell fate of the neurons in the posterior of the lineage.

447C

The C.elegans Tailless/TLX transcription factor nhr-67 controls neuronal identity and left/right asymmetric fate diversification. **Sumeet Sarin**, Celia Antonio, Baris Tursun, Oliver Hobert. Howard Hughes Medical Institute, Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY.

Mechanisms of cell fate determination require complex signaling and gene regulatory processes, which together endow the nervous system with an impressive amount of diversity. In *C. elegans*, neurons are classified into subtypes based on variable morphology, projection patterns, and the combinatorial expression of transcription factors, neurotransmitters, receptors and ion channels. The molecular logic leading to such subtype specification is poorly understood and the ASE gustatory neurons provide a useful model to understand such processes. While the ASE neurons share a number of features, they are functionally and genetically asymmetric across the L/R axis. The progression from an initially symmetric to a bistable, asymmetric system is an intriguing feature of this specification event. We show here that the *C. elegans* Tailless/TLX-type orphan nuclear receptor, *nhr-67*, acts in a reiterative manner to determine the identity and subsequent left/right asymmetric subtype specification of the ASE neurons. *nhr-67* controls a number of ASE sensory features without affecting general neuronal characteristics and positively regulates the ASE-selector gene and Zn-finger transcription factor, *che-1*. Subsequent to its induction of overall ASE fate, *nhr-67* promotes right/left diversification by transcriptionally activating the Nkx6.1-type transcription factor, *cog-1*. In ASE right (R), this induction leads to repression of the ASE left (L) regulatory program. In ASEL, the cog-1 transcript is downregulated by the microRNA, *lsy-6*, leading to left fate. Interestingly, *nhr-67* holds a broader role in nervous system development as it is required for the specification of a number of neurons, illustrating the diverse use of this type of transcription factor.

448A

Determining the genetic profile of two types of *C. elegans* mechanosensory neurons: the touch receptor neurons and the FLP neurons. **Irini Topalidou**, Alexander Bounoutas, Martin Chalfie. Dept Biol Sci, Columbia Univ, New York, NY.

Cells acquire many of their characteristics during differentiation by expressing specific subsets of genes. The identification of this select gene pool is an important means of understanding cell fate determination. We used *gfp* cell sorting and cDNA microarrays to identify genes that are upregulated in the touch receptor neurons (TRNs) and FLP neurons, both of which require MEC-3 for their differentiation. We identified channel and receptor subunits, transcription factors, and calcium sensors among the 198 genes expressed at high levels in the TRNs. Using existing mutants and/or feeding RNAi, we tested the role in gentle touch sensation of most of the genes (163/189) not previously identified as needed for touch sensitivity. Eight of the genes were needed for optimal touch sensitivity; genes encoding the transcription factor ALR-1, the PON-1 homolog K11E4.3, and the GABA receptor LGC-37 were among them. We demonstrated that ALR-1 is needed in the TRNs throughout development to regulate the expression of touch neuron-specific genes. Further analysis on the role of K11E4.3 in touch sensation will be given elsewhere in this meeting (Y. Chen and M. Chalfie). Interestingly, most of the *mec* genes (i.e: *mec-18*) that were previously identified as TRN-specific were among the 203 genes upregulated in the FLP neurons. QPCR analysis for *mec-18* mRNA in animals with genetically-ablated TRNs showed that *mec-18* mRNA is indeed expressed in these animals, mainly at the early larvae stages, although no immuno-detectable MEC-18 is produced. Finally, a short-lived *gfp* driven by the *mec-18* promoter ($P_{mec\text{-}18}$*praja::gfp*) produces fluorescence in young FLP neurons. We conclude that both post-transcriptional and transcriptional regulation play important roles in differentiating the touch receptor neurons from the FLP neurons.

449B

The *aristaless/Arx* homolog *alr-1* regulates the fate of the touch receptor neurons by acting as a transcriptional activator. **Irini Topalidou**, Martin Chalfie. Dept Biol Sci, Columbia Univ, New York, NY.

The Drosophila homeobox gene *aristaless (al)* participates in the development of wings, legs and aristae. In humans, mutations of the *al* homologue (*Arx*) cause multiple forms of X-chromosome linked mental disorders. The *C. elegans aristaless/Arx* orthologue (*alr-1*) regulates the development of chemosensory and GABAergic neurons and is needed for the integrity of the amphids. Using cell sorting and cDNA microarrays, we identified *alr-1* among the genes upregulated in embryonic touch receptor neurons (TRNs). GFP promoter and translation fusions for *alr-1* are expressed in five of the six TRNs (ALM, PLM and AVM, but not PVM) throughout development. This expression depends on MEC-3. ALR-1 is functionally important in the TRNs, since *alr-1* mutants are variably insensitive to gentle touch. Expression of *alr-1(+)* in the TRNs rescues this touch insensitivity. Using temperature-sensitive *mec-8*-dependent splicing, we expressed *alr-1* in a temperature-dependent manner and demonstrated that *alr-1* is needed for touch sensitivity throughout development. Expression of genes needed for TRN function, including *mec-3*, are down- regulated in *alr-1* mutants in all touch neurons except PVM. We used Quantitative PCR (QPCR) to show that mRNA levels of the affected genes were ~50% of wild-type levels, a result that may explain the touch-insensitive phenotype of the *alr-1* mutants. In contrast, the TRN-expression of the pan-neuronal genes *unc-119* and *sng-1* was unaffected in *alr-1* mutants, arguing against ALR-1 acting as a general enhancer of transcription in the TRNs. These observations lead to a model in which ALR-1 positively enhances MEC-3 and, perhaps, UNC-86 activity and/or expression to regulate the fate of the TRNs. MEC-3 is needed for its own continued expression; ALR-1 may contribute to this maintained expression. Consistent with this hypothesis, ALR-1 acts as a transcriptional activator in an *in vivo* yeast expression system, by directly inducing expression from the *mec-3* promoter and from the TRN-specific *mec-18* promoter. Mutations identified in the ARX-homeobox of patients with mental retardation abolish ALR-1-dependent gene expression.

450C

Identifying genes regulating postembryonic development of DD neurons by microarray. **Yingchuan Qi**, Yishi Jin. HHMI, Div. of Biological Sci., UC San Diego, La Jolla, CA.

Six “dorsal D”, or DD, GABAergic ventral cord motor neurons are born in the late stage of the embryonic development. Upon hatching the axonal patterns of DDs are identical to those in older larvae and adults, and the presynaptic terminals are formed along the ventral process of the DDs. While maintaining their axonal pattern, DDs undergo synaptic remodeling by switching their presynaptic terminals from innervating the ventral muscle to innervating the dorsal muscles towards the end of the L1 stage. The timing of this DD remodeling is under the control of the heterochronic gene *lin-14*[1]. In loss-of-function mutants of *lin-14*, the synaptic remodeling of DDs takes place precociously at early L1.

To understand the mechanisms underlying DD synaptic remodeling, we carried out a microarray analysis on isolated embryonic DDs from wild type and *lin-14(lf)*. About 3,900 genes showed differential expression between wild type and *lin-14(lf)* mutants. The genes defining GABA properties were not altered in *lin-14(lf)*, indicating that *lin-14* does not control DDs’ GABAergic fate specification. The number of genes up-regulated in *lin-14(lf)* was about the same as the number of genes down-regulated in *lin-14(lf)*. In particular, we found that a set of acetylcholine receptor genes were up-regulated in *lin-14(lf)* mutant. We have focused further analysis on two genes, *acr-12* and *acr-14*, both of which are expressed in L1 DDs[2]. Loss of function mutations of *acr-12* or *acr-14* cause no obvious DD developmental defects. However, when we expressed the hyperactive form of each acetylcholine receptor gene in DDs, we observed impaired neurite outgrowth of DDs. We found similar neurite outgrowth defects in *lin-14* null animals. We are testing a hypothesis that the activity of DDs is maintained at a low level to allow initial development of DDs during embryogenesis and early L1 stage and that altering the activity level impairs the DD postembryonic development.

1.Hallam, S. J. & Jin, Y. Nature 395, 78-82 (1998).
2.Cinar, H., Keles, S. & Jin, Y. Curr Biol 15, 340-6 (2005).

451A

A Structure-Function Analysis of the LAR-Receptor Tyrosine Phosphatase in Synaptic Development. **Johnie J. Gallagher**, Lindsey Roe, Brian D. Ackley. Molecular Biosciences, The University of Kansas, Lawrence, KS.

The Leukocyte-common antigen related (LAR) receptor regulates different facets of neural development, including cell migration, axon guidance and synapse formation. We have demonstrated that the *C. elegans* LAR homologue, *ptp-3* functions at neuromuscular junctions to maintain the proper morphology of synaptic domains, including the vesicle clustering region and the active zone. The *ptp-3* locus encodes for at least two distinct proteins, LAR-A and LAR-B, which differ in the extracellular domain, and our analyses indicate that PTP-3A is the only isoform active at synapses. To understand how PTP-3A functions at synapses we have undertaken a structure-function analysis. We have deleted predicted protein motifs from the PTP-3A specific part of the extracellular domain and are testing their ability to rescue the synaptic morphology defects present in *ptp-3* null animals. We have targeted the 3 N-terminal immunoglobulin (Ig) repeats, as well as two of the fibronectin type III (FNIII) repeats. The Ig repeats of another type IIa RPTP, PTPσ, were shown to bind specifically to heparan sulfated proteoglycans, where as the 5th FNIII repeat of vertebrate LAR was found to be able to immunoprecipitate the laminin-nidogen complex *in vitro*. We will present data looking at the localization of the deletion constructs, their effect on the size and shape of active zones and vesicle clustering domains and on behavioral measures of synaptic transmission.

A second question we are asking is the function of the intracellular phosphatase domains. A PTP-3A transgene lacking the intracellular domain (ICD) is incapable of rescuing the synaptic defects. To know if this is a structural role for PTP-3A at the synapse or whether the catalytic phosphatase activity was necessary, we have generated mutations in the phosphatase domains. We have found that in an in vitro phosphatase assay that mutations in the first phosphatase domain (C1) result in approximately a 10-fold loss of activity relative to the intact C1 domain. The second phosphatase domain in LAR-like RPTPs has been shown to be catalytically inactive. We have mutated this domain to have the necessary residues to be active. This protein shows approximately 60-fold more activity in the phosphatase assay. We will generate lines of animals with either the hypoactive or hyperactive version of PTP-3A in the *ptp-3* null background and evaluate the effect on synaptic morphology and function.

452B

A genetic approach towards structure function analyses of Heparan Sulfate. **Alexandra A. Mirina**, Raja Bhattacharya, Robert A. Townley, Matthew Koh, Hannes E. Buelow. Genetics, Albert Einstein Col Med, Bronx, NY.

Heparan sulfates (HS) are polysaccharides of the cell surface and extracellular matrix that are attached to proteins to form HS proteoglycans (HSPG). The relatively simple heparan polymers undergo complex modifications involving de-acetylation, epimerization and sulfations to generate structural motifs that mediate the diverse functions of HSPGs. There is both biochemical and genetic evidence that these HS motifs have instructive functions in modulating ligand/receptor interactions during cell-cell communication. However, structure function analyses of HS domains in vivo has to date been limited due to the absence of an experimental system to characterize HS sugars with sufficient resolution in a genetically tractable system. We have developed an approach to directly correlate HS structure with function in vivo. To this end we have engineered transgenic animals that express an affinity tagged HS proteoglycan under a cell specific promoter. We have affinity purified the HS proteoglycan and, subsequently analyzed the sugars from this defined proteoglycan and tissue. In a complementary approach, we have begun to phenotypically characterize the strains misexpressing tagged HS proteoglycans. We observe that hypodermal expression of a tagged HSPG (syndecan-1) in strains lacking 2-O (hst-2) or 6-O (hst-6) sulfation leads to a severe body morphology defect not unlike the dumpy (dpy) phenotype. Using point mutant alleles of the transgene, we show that the synthetic dpy phenotype is dependent on the putative sugar attachment sites in the HS proteoglycan indicating that the synthetic phenotype is dependent on HS sugars. To gain further insight into the genetic pathways that govern the HS sugar dependent phenotype we have conducted a genetic screen for loci that produce a synthetic dpy phenotype in the syndecan-1 misexpressing strain. To date we have screened 14,000 haploid genomes and isolated 116 mutants. We expect the synthetic dpy mutants to define loci that control the synthesis of specific HS motifs or their effectors. This integrated biochemical and genetic approach will allow us to determine the relationship between HS structure and function in vivo.

453C

The Role of Intracellular Trafficking in Modulating Ciliary Structure and Function in *C. elegans*. **D. B. Doroquez**[1], A. Olivier-Mason[1], A. Sarkeshik[2], J. R. Yates III[2], P. Sengupta[1]. 1) Dept of Biology, Brandeis University, Waltham, MA; 2) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

Primary cilia are organelles that serve as environmental sensors, and are present on nearly all cell types in vertebrates. Each cilium consists of a central microtubular axoneme surrounded by a membrane. The structure and biogenesis of these organelles are highly conserved from algae to humans, allowing for parallel studies of cilia in many model systems. Defects in cilia biology are implicated in multiple diseases, including polycystic kidney disease, Bardet-Biedl syndrome and sensory pathologies. *C. elegans* is an ideal system to study cilia with its experimental tractability and the ability to analyze its 60 ciliated sensory neurons. These cilia are essential for sensory functions, such as chemosensation and olfaction. Individual olfactory neurons exhibit highly specialized cilia structures that are essential for their unique sensory functions. Cell-specific mechanisms of intraflagellar transport (IFT) and sensory signaling contribute to ciliary structural and morphological diversity. In particular, sensory signaling is required to modulate the specialized architecture of AWB olfactory neuron cilia and this modulation is dependent on vesicular trafficking. Little is known about the regulation of vesicular trafficking in cilia formation and maintenance. Defects in trafficking are likely to affect cilia structure and function due to altered transport and localization of ciliary signaling molecules resulting in defective cellular homeostasis. The overall goal of this project is to study the role of intracellular trafficking in the generation and maintenance of cilia morphology in *C. elegans*. In order to identify components involved in *C. elegans* cilia biology, we have taken a proteomics-based approach. We have identified proteins associated with different IFT complex proteins and motors via mass spectrometry. Many predicted IFT-associated components were identified in this analysis, suggesting that this approach may allow us to identify new cilia-related components [Olivier-Mason *et al.* abstract]. We have identified vesicular trafficking proteins associated with IFT complex proteins. We are characterizing the roles of identified vesicular trafficking proteins in the regulation of cell-specific cilia biogenesis, the localization of ciliary transmembrane proteins, and the maintenance of sensory signaling. These experiments will elucidate the role of vesicular transport in the regulation of cilia structure, and provide new information about how these sensory organelles are built and maintained. These studies may provide insights into the basic biology underlying ciliopathies.

454A

Regulation of neuronal development by ARX/ALR-1 in mammals and *C. elegans*. **Martin Wojtyniak**, Masami Shima, Susan Birren, Piali Sengupta. Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA.

Human neuronal development is a complex process where much remains to be uncovered. The mouse has served as an excellent system for modeling human disorders, however the complexity of a mammalian brain does not lend itself to facile genetic studies. In contrast, *Caenorhabditis elegans* offers a powerful system to model the genetic basis of neuronal development due to its small and well-defined nervous system. The conservation of genetic pathways, despite divergent phenotypic outputs, allows us to utilize *C. elegans* to explore more complex disorders evident in higher mammals. The *C. elegans Aristaless* homolog, *alr-1*, shares a high degree of homology with the mammalian *Arx* gene. Mutations in the *Arx* homeobox gene have been linked to a spectrum of neuronal disorders in humans: X-linked lissencephaly with ambiguous genitalia (XLAG), mental retardation, and epilepsy. Moreover, studies of the developing mouse brain suggest that ARX plays a role in the development and migration of GABAergic interneurons. *alr-1* mutant animals exhibit defects in the specification of GABAergic motoneurons and in the development of a subset of chemosensory neurons. In light of this phenotypic conservation, we aim to investigate the genetic pathways associated with *alr-1*, and reiterate those findings in a mouse model to better understand the roles of *Arx* and *alr-1* in neuronal development. Given that murine ARX can rescue several neural defects in *alr-1* deficient animals and the range of *Arx*-mediated disorders is tightly coupled to specific mutations, we can address the etiology of several neural disorders by exploring their genetic pathways using *C. elegans* as a model system.

455B

A Toolkit and Robust Pipeline for the Generation of Fosmid-Based Reporter Genes. **Baris Tursun**, Luisa Cochella, Inés Carrera. Dept Biochemistry, Columbia Univ, New York, NY.

An ultimate fluorescent reporter to study spatiotemporal gene expression patterns in transgenic animals should contain all *cis*-regulatory information to recapitulate every aspect of endogenous gene expression. Genomic clones such as BACs and fosmids cover large genomic regions thereby capturing all cis-regulatory information of a target gene within BAC or fosmid. Homologous recombination based recombineering of fosmids to insert tags such as gfp at the target gene locus represents the most straightforward method to generate the best possible reporter for studying gene expression. We have generated an extensive vector toolkit for recombineering to be use with fosmids for generating reporter constructs in *C. elegans*, whose genome is almost entirely covered by an available fosmid library. Our toolkit allows for insertion of fluorescent proteins (GFP, YFP, CFP, VENUS, mCherry) and affinity tags (FLAG, HA, BLRP) at specific target sites within fosmid clones in a virtually seamless manner. It also provides novel cassettes to introduce a SL2-spliced intercistronic region between the gene of interest and the fluorescent protein. This cassette creates a reporter controlled by all 5' and 3' *cis*-acting regulatory elements of the examined gene by avoiding the direct translational fusion between the two. With this configuration, the onset of expression and tissue specificity of secreted, sub-cellular compartmentalized or short-lived gene products can be easily detected. Moreover, we developed a simple and highly efficient protocol to easily establish a pipeline for the generation of reporter fusions for *C. elegans* expression studies. The simplicity, speed and robustness of this recombineering procedure have resulted in the routine use of this strategy for expression studies in our lab.

456C

Imaging C. elegans by Optical Projection Tomography. **M. Rieckher**[1], H. Meyer[2], U. Birk[2], J. Ripoll[2], N. Tavernarakis[1]. 1) Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece; 2) Institute of Electronic Structure and Laser, Heraklion Crete, Greece.

Small sample spatial in vivo imaging techniques such as confocal microscopy, micro MRT (μMRT), Selective Plane Illumination Microscopy (SPIM), or contrast enhanced techniques, such as DICM (Differential Interference Contrast Microscopy) are common tools for imaging fluorescent expression in the nematode Caenorhabditis elegans. However, these methods have limited capacity for high resolution, rapid, whole body 3D microscopic imaging and/or imaging of multiple contrast agents. The recently developed approach of Optical Projection Tomography (OPT) enables 3D visualization of whole specimens up to several millimetres in sizes as it has already been shown in zebra fish, chick and mouse embryos. This is achieved by applying a filtered back projection algorithm on images taken from equidistant angles of a rotating specimen with magnification dependent resolution, down to 1-5 μm. We applied a modified OPT setup for 3D imaging of C. elegans expressing GFP in specific neurons. We demonstrate that this novel technique allows rapid acquisition of whole-animal fluorescent expression patterns in the nematode with high accuracy. OPT visualization can be easily adapted to image multiple tissues and cell types, with a variety of chromophores, that allow multi-colour projections, in the nematode.

457A

Genetic control of axon guidance and branching: what have we learned from *png-1*? **Nasrin Babadi**[1], Claudia Arauz[2], Maria Gallegos[3], Antonio Colavita[1]. 1) Neuroscience Dept., University of Ottawa, Ottawa, ON, Canada; 2) CMM Dept., University of Ottawa, Ottawa, ON, Canada; 3) Dept. of Biology California State University, East Bay Hayward, CA.

Understanding the cellular and molecular mechanisms governing axon guidance and branching is a central issue in developmental neurobiology. PNGases are highly conserved genes that are involved in the proteasomal degradation of misfolded glycoproteins during ER-associated degradation. We have previously reported the role of *C. elegans* PNGase, *png-1* in regulation of axon branching at the vulva. *png-1* mutants displayed excessive ectopic branching defects in the VC4 and VC5 motorneurons at the vulva. Additionally, *png-1* mutants displayed overextension and branching defect in the DVB and AVL motorneurons. To further understand the role of *png-1* in the regulation of axon guidance and branching, we conducted several genetic screens looking for enhancers/suppressors of *png-1* associated branching defects. We identified *sax-2* and *sax-1*mutants that enhanced *png-1* branching in VC4 and VC5. Similarly, these mutants enhanced *png*-1 overextension and branching defects at the DVB and AVL. These findings suggest that *png-1* and *sax-1/sax-2* act in parallel genetic pathways to regulate axon guidance and branching. Furthermore, we analyzed homologues of genes involved in the ER-associated degradation in C.elegans for defects similar to *png-1*. We identified *rad-23* to display overextension and branching defects in the DVB and AVL motorneurons only. Currently we are completing the characterization of these genes and we believe our finding will shed new light on mechanisms involved in axon guidance and branching.

458B

THE PAPS TRANSPORTER PST-1/LET-462 IS REQUIRED FOR HEPARAN SULFATION AND IS ESSENTIAL FOR VIABILITY AND NEURAL DEVELOPMENT. **Raja Bhattacharya**[1], Robert Townley[1], Katherine Berry[2], Hannes Buelow[1]. 1) Genetics, Albert Einstein Col Medicine, Bronx, NY; 2) Dept. of Biochemistry and Molecular Biophysics, Columbia University Medical Centre,New York, NY.

Kallmann Syndrome (KS) is a genetically heterogeneous disease combining anosmia and hypogonadism. The X-linked form of KS is caused by lesions in the KAL1 gene, which codes for a secreted molecule with similarities to neural cell adhesion molecules. In a modifier screen of an axon branching phenotype induced by kal-1, the C. elegans homologue, we obtained four strong suppressor mutants, three of which were previously mapped to genes coding for enzymes involved in heparan sulfate modifications (Bülow and Hobert, 2004). We describe here the characterization of the fourth strong suppressor mutation, the recessive allele ot20. Using a cosegregating temperature sensitive lethality we mapped ot20 to a 100kb interval on the proximal left arm of chromosome V. RNA interference and transgenic rescue experiments suggest that a gene named PAPS transporter (pst-1) is responsible for both the suppression of branching and the lethality. Furthermore, ot20 failed to complement all four available alleles of let-462, which mapped to a similar region and we have found mutations in both ot20 and alleles of let-462 within the pst-1 coding region. Sulfations of sugars, such as heparan sulfates (HS) or tyrosines require the universal sulfate donor PAPS (phosphoadenosyl-phosphosulfate) to be transported from the cytosol into the Golgi. Metazoan genomes encode two putative PAPS transporters (PAPST1 and PAPST2), which have been shown in vitro to preferentially transport PAPS across membranes. We have identified the C. elegans orthologs of PAPST1 and PAPST2 and named them pst-1 and pst-2, respectively. We show that pst-1 is essential for viability in C. elegans and can act non-autonomously to mediate its essential functions. pst-1 is contributed maternally and is specifically required during late embryonic and early larval stages. pst-1 null mutants arrest at the 3-fold stage of elongation probably due to aberrant muscle-hypodermis interactions. Additionally, pst-1 is required for specific aspects of nervous system development rather than formation of the major neuronal ganglia or fascicles. The neuronal defects correlate with reduced complexity of HS modification patterns as measured by direct biochemical analysis. Combining the biochemical results with epistatic analysis of HS modifying enzymes mutants we provide the first in vivo evidence that pst-1 is primarily involved in heparan sulfation. Our results suggest that pst-1 functions in metazoans to establish the complex HS modification patterns that are required for viability and the development of neuronal connectivity with high fidelity.

459C

Do orthologs of the yeast RAM pathway mediate Wnt signaling in neuronal polarity? **Shih-Chieh Chien**, Julie Oppermann, Mark Gurling, Gian Garriga. Dept of Mol & Cell Biol, Univ California, Berkeley, Berkeley, CA.

Wnts regulate cell migration and polarity along the *C. elegans* A/P axis. For example, Wnts control the polarity of the mechanosensory neuron ALM[1,2]. Although single *cwn-1*, *cwn-2* or *egl-20* Wnt mutants display normal ALM polarity, the polarity of the ALMs is often reversed in *cwn-1*; *cwn-2* or *cwn-1*; *egl-20* double mutants. We find that ALM polarity also requires the Frizzled receptor MOM-5 and the Dishevelleds DSH-1 and MIG-5. While Frizzled and Dishevelled proteins mediate the effects of Wnts in many developmental contexts, how these molecules signal to control neuronal polarity is unclear. We find that the MIG-15 kinase and potential components of a MIG-15 signaling pathway might be novel Wnt effectors in neuronal polarity.

MIG-15, the *C. elegans* ortholog of Nck-interacting kinase (NIK) in mice and Misshapen in *Drosophila*, was shown to function in cell migrations that also require Wnt function[3]. We found that *mig-15* mutants exhibit a low frequency of ALM polarity defects that was enhanced by a mutation in *cwn-1*, suggesting that MIG-15 could mediate the effects of Wnts in ALM polarity. In *S. cerevisiae*, Kic1p, a distant relative of MIG-15, acts in the RAM signaling pathway to regulate polarized cell growth[4]. We asked whether *C. elegans* orthologs of RAM signaling molecules also regulate ALM polarity. Through RNAi and mutant analysis in a *cwn-1* sensitized background, we identified *mop-25.2* and *sax-2* as regulators of ALM polarity. The gene *mop-25.2* is the ortholog of yeast Hym1p, which physically interacts with Kic1p, and SAX-2 is the ortholog of the RAM scaffold protein Tao3p. SAX-2 acts in the ALM to establish its polarity. We were surprised to find that *cwn-1*; *sax-1* mutants did not have an ALM polarity phenotype. SAX-1 is the ortholog of the NDR kinase Cbk1p and a target of the Kic1p kinase. We are currently testing whether the other *C. elegans* NDR kinase WTS-1 provides an overlapping function in ALM polarity in the absence of SAX-1. We are now testing whether the RAM pathway homologs act downstream of the Wnts in ALM polarity.

[1]Hilliard MA, Bargmann CI. (2006) *Dev. Cell* 10: 379-390.
[2]Prasad BC, Clark SG. (2006) *Development* 133: 1757-1766.
[3]Chapman JO, Li, H & Lundquist EA. (2008) *Dev. Biol*. 324: 245-257.
[4]Nelson B *et al*. (2003) *Mol. Biol. Cell* 14: 3782-3803.

460A

RACK-1 Controls Axon Pathfinding. **Rafael Sênos Demarco**, Erik A Lundquist. Molecular Biosciences, University of Kansas, Lawrence, KS.

During development, neurons extend axonal processes to appropriate targets in the nervous system. In the growth cone, dynamic actin cytoskeleton reassembly creates protrusions (filopodia and lamellipodia) that guide axonal growth.

Our lab has previously characterized UNC-115 as an actin-binding protein required for axon pathfinding. UNC-115 is downstream of the Rac GTPases RAC-2 and CED-10, two of the three C. elegans Rac homologs that act redundantly in axon pathfinding. When performing a yeast-two-hybrid assay with a conserved region with unknown function of UNC-115, seven cDNAs encoding the Receptor for Activated C Kinase (RACK-1) were isolated. RACK-1 is a 7-WD repeat molecule that interacts with protein kinase C and Src signaling, and is involved in cell shape in other systems.

Initial genetic analysis suggests that RACK-1 might act in the UNC-115 pathway in axon pathfinding. Few PDE axon pathfinding defects were found in *rack-1(tm2262)* alone or in double mutant combinations with *unc-115(ky275)* or *ced-10(n1993)*. However, double mutants of *rack-1* and *mig-2(mu28)*, the other Rac GTPase, displayed PDE pathfinding defects. In addition, the constitutively active Rac GTPases CED-10 and RAC-2, which produce ectopic lamellipodial and filopodial projections, were partially suppressed by *rack-1(tm2262)*, while constitutively active MIG-2 was not. Also, a myristylated version of UNC-115, which is thought to be overactive, synergized with *rack-1(tm2262)*. These results suggest RACK-1 might be inhibiting UNC-115's activity downstream of CED-10 (and possibly RAC-2).

Further studies will better dissect the interactions of RACK-1 with UNC-115, as well as with the other putative interacting molecules Src and PKC.

461B

Ras-interacting protein 1 homologue RIN-1 is a novel effector protein of CED-10/Rac that regulates neuronal cell and axon growth cone migration. **M. Doi**[1], Y. Kubota[2,3], H. Minematsu[4], K. Nishiwaki[2,3], M. Miyamoto[4,5]. 1) Neuroscience Research Institute, AIST; 2) RIKEN Center for Developmental Biology; 3) Dept. of Bioscience, Kwansei-Gakuin Univ; 4) Dept. of Biology, Graduate School of Science; 5) Center for Supports to Research and Education Activities, Kobe Univ., Japan.

Neuronal cell migration and axon guidance both require proper regulation of the actin cytoskeleton as the cell responds to several extracellular guidance cues. A member of the small GTPase family Rho/Rac is one of the intrinsic factors in actin remodeling. Similar to other small GTPase families, the GTP-bound form of Rac interacts with effector proteins to be delivered to its appropriate membrane domain and function properly. In *C. elegans*, the CED-10/Rac functions in the migration of several types of cell and in axon pathfinding in cooperation with the MIG-2 Rho-like GTPase. CED-10 also regulates the phagocytosis of apoptotic cells. However, the precise molecular mechanisms by which CED-10 interacts with its effector proteins and regulates specific cellular responses to guidance cues are not well understood. In this study, we identified RIN-1 as a novel CED-10/Rac effector that is involved in cell migration and axon guidance. Using a constitutive active-form of CED-10 as bait, we performed a yeast two-hybrid screening and found that the *C. elegans* homologue of Rin1 is physically bound to CED-10. Rin1 was originally identified as a Ras-interacting protein in mammalian cells, but its function in *C. elegans* has not been reported. In our in vitro assays, the *C. elegans* RIN-1 protein specifically bound to the GTP-form of CED-10, but not to the CED-10 GDP-form. Furthermore, RIN-1 did not physically interact with other members of the Rho/Rac family, suggesting that RIN-1 may function as a specific effector protein for CED-10. To confirm this, we examined whether *rin-1* mutants have similar phenotypic defects with *ced-10* or *mig-2* mutants. The *rin-1* single mutants did not show obvious defects, however, the *rin-1; mig-2* double mutants had significantly severe defects in axon pathfinding of the AVM neuron, neuronal cell migration, and dorsal morphology of the body compared to the defects observed in the *mig-2* single mutants. This suggests that RIN-1 and MIG-2 act redundantly in actin remodeling during these migration events, which is similar to CED-10 and MIG-2 redundancy. However, we also found that rin-1 is not involved in several CED-10 signaling pathways, such as the migration of the distal tip cells and the phagocytosis of apoptotic cells. Together with genetic analyses using guidance mutants, our results strongly indicate that RIN-1 acts as an effector of CED-10 in specific cells or in actin remodeling in response to a restricted guidance molecule.

462C

A candidate gene approach to identify additional genes that function in the sax-1/sax-2 pathway to regulate mechanosensory neurite termination. **Maria E. Gallegos**, Padma Karamchedu, Priya Chandramouli, Pranti Das. Dept Biol, Cal State Univ, East Bay, Hayward, CA.

In *C. elegans*, *sax-1* and *sax-2* regulate neurite termination and the maintenance of neuron morphology. Specifically, in *sax-1* and *sax-2* loss-of-function mutants, the neurite of the posterior mechanosensory neuron (PLM) extends beyond its normal site of termination anterior to the ALM cell body. In addition, ectopic neurites extend from the cell soma of most if not all neurons, a mutant phenotype that worsens as the animal ages. *sax-1* and *sax-2* are likely orthologs of budding yeast RAM pathway members, *CBK1* and *TAO3*, respectively. Additional members of this pathway include *KIC1*, *SOG2/HYM1*, and *MOB2*.

We are taking a reverse genetic approach to determine the role that putative RAM pathway members and candidate downstream effectors play in PLM neurite termination and maintenance of neuron morphology in *C. elegans*. Specifically, we have used single and combinatorial RNAi to knockdown gene activity of putative *MOB2* and *KIC1* orthologs. Genes examined in this RNAi study include *gck-1*, *gck-4*, *cst-1/cst-2*, *T12B3.4* and *F38H4.10*. We are also using deletion allels to test the role that candidate downstream effectors, *rab-8* and *rab-10*, play in PLM neurite termination. *rab-8* and *rab-10* are most closely related to yeast *SEC4*, a RAB GTPase involved in polarized secretion. Interestingly, yeast Cbk1p binds Sec2p, the guanyl-nucleotide exchange factor for Sec4p. Results will be presented.

463A

The role of ephrin reverse signaling in guidance of the amphid commissure. **Emily N. Grossman**, Andrew D. Chisholm. Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093.

Eph receptor tyrosine kinases and their ephrin ligands function in cell contact mediated signaling in many developmental and physiological processes. Because ephrin ligands are cell surface proteins, signaling can operate in forward, reverse, or bidirectional modes. Some ephrins lack intracellular domains and are tethered to the cell membrane by glycosylphosphatidylinositol (GPI) anchors. It is not well understood how GPI-linked ephrins mediate reverse signals. *C. elegans* provides an excellent model to dissect the mechanism of reverse signaling via GPI-linked ephrins. C. elegans encodes a single Eph receptor (VAB-1) and four GPI-linked Ephrin (EFN) ligands. Eph signaling has many functions in C. elegans, including regulation of embryonic neuroblast movements (George et al, 1998) and ventral guidance of the amphid commissure. Genetic analysis implies that both the neuroblast movements and amphid commissure guidance involve reverse signaling. To further understand the mechanism of reverse ephrin signaling in the context of a simple axon choice point we have begun to dissect its role in ventral guidance of the amphid commissure (AC). Axons of the AC extend ventrally before turning anteriorly into the nerve ring. Using a panel of markers for amphid neurons we confirmed that vab-1 null mutants display 50-60% penetrant defective ventral AC targeting, whereas vab-1 kinase-dead mutants display 10-20% defects. These results suggest that bidirectional signaling is involved, with reverse signaling playing the major role. efn-1 mutants display ~30% guidance defects, whereas efn-2 and efn-3 have more minor and possibly redundant roles. Interestingly, some vab-1 mutants have differential effects on different axons, suggesting different cells may have different requirements for ephrin signaling. Although the general expression patterns of VAB-1 and the ephrins have been described, we do not know which cells they function in to target the amphid axons. We are using cell type specific rescue to test whether VAB-1 or its ligands are required in the amphid neurons, in putative guidepost cells, or in both. Recent work in vertebrate axon guidance has implicated neurotrophin receptors as signaling partners for GPI-linked ephrins. Although the C. elegans genome does not appear to encode orthologs of the low-affinity p75 neurotrophin receptor, it does contain a distant relative of the high-affinity Trk receptor tyrosine kinases. We are testing whether this Trk-related kinase might operate in the ephrin reverse signaling pathway.

464B

A screen for genes paralleling the function of HSPGs during *C. elegans* nervous system development. **Stephan Gysi**[1,2], Ronald Egli[1], Lucia Reh[1], Christa Rhiner[3], Michael Hengartner[1,2]. 1) Institute of Molecular Biology, University of Zurich, Zurich Switzerland; 2) Neuroscience Center Zurich, Zurich, Switzerland; 3) Centro Nacional de Investigaciones Oncologicas, Madrid, Spain.

During nervous system development axons navigate through the extracellular matrix (ECM) to their target. Axons obtain the information where to grow from guidance cues located in the ECM or on cells neighbouring the growth path of the axon. A number of these guidance cues have been characterized in the past. However, there is increasing evidence that there are other molecules around that fine-tune the response of the axon to the various cues it senses. Heparan sulfate proteoglycans (HSPGs) are proteins that carry long sugar side chains. HSPGs have been shown to interact with a wide variety of morphogenes in vertebrates and invertebrates. We have previously described the role played by the HSPG Syndecan (SDN-1) during nervous system development in *C. elegans*. It is, however, still unknown through what mechanism SDN-1 exerts its function. Furthermore there is genetic evidence that there is at least one other HSPG core protein acting in parallel to SDN-1. With the aim to find genetic interactors of SDN-1, we performed forward genetic screens. We have screened about 20'000 genomes and isolated 10 candidate mutations. FLP and SNP mapping revealed that they map to 8 different intervals. Candidate *op481* is mapping to the middle of chromosome three to an interval not containing any previously described axon guidance gene. Sequencing revealed that *op481* carries a premature stop codon in the gene *zfp-1*. We will show a first characterization of the function of *zfp-1* in axon guidance. Additionally epistasis analysis of the different candidates will show whether we identified multiple parallel signalling pathways.

465C

The Genetics of Axon Regeneration. **Marc Hammarlund**. Program in Cellular Neuroscience, Neurodegeneration and Repair and Department of Genetics, Yale University, New Haven, CT.

Axon regeneration in response to nerve injury has the potential to restore function, but the genetic requirements for this process are poorly understood. We screened for genes required for regeneration and discovered a key regulator of axon regeneration in *C. elegans* (1). The dual-leucine zipper MAPKKK DLK-1 functions via the MAPKK MKK-4 and the p38 MAP kinase PMK-3 to regulate regeneration in response to injury. In the absence of this pathway, regeneration does not occur. Moreover, increasing the activity of this pathway increases regeneration above normal levels. Our data suggest that after axon injury, activation of this MAP kinase cascade is required to switch the axon to a plastic state capable of growth. Other genes found in our screen may identify additional mediators of regeneration.

(1) Hammarlund et al. 2009, *Science*.

466A

C. elegans axon branch formation is regulated by multiple proteoglycans. **Martin L. Hudson**[1], Vivian Yee[2], Andrew D. Chisholm[3], Brian D. Ackley[1]. 1) Molecular Biosciences, University of Kansas, Lawrence, KS 66045; 2) MCD Biology, University of California, Santa Cruz, CA 95064; 3) Division of Biological Sciences, University of California, San Diego, La Jolla, CA 92093.

KAL-1/anosmin has roles in cell migration and axon branching, but the molecular mechanisms behind its function in these processes are poorly understood. Over-expression of KAL-1/anosmin in *C. elegans* AIY interneurons leads to a highly penetrant axon branching phenotype. This phenotype is strongly suppressed by heparan sulfate (HS) biosynthesis mutants. There are two possible mechanisms for HS biosynthesis mutations suppressing KAL-1 induced axon branching. Either HS modification of KAL-1 is required for induction, or an HS-decorated receptor regulates the observed axon branching phenotype. KAL-1 does not appear to be a heparan sulfate proteoglycan (HSPG), suggesting the axon branching may be due to KAL-1 interacting with an HSPG.

To address these possibilities we examined mutations in syndecan and glypicans, which are HSPGs known to physically interact with KAL-1. Using genetic epistasis experiments, we have shown that *sdn-1*/syndecan mutants do not suppress KAL-1 induced axon branch formation in AIY interneurons, but can suppress ectopic branching when over-expressed cell-autonomously. *gpn-1*/glypican mutants actually increase KAL-1 induced axon branch formation, but again, can cell-autonomously suppress when expressed specifically in AIY interneurons. Only *lon-2*/glypican mutants suppress axon branch formation directly. *sdn-1, gpn-1, lon-2* triple mutants recapitulate HS biosynthesis mutants, suggesting no other HSPGs are involved in this process. Based on these data, we propose a model where GPN-1/glypican and syndecan negatively regulate KAL-1 function cell autonomously, where as LON-2/glypican functions downstream as a positive effector of KAL-1 activity. Although *lon-2* mutants can suppress branching, we do not know whether this is a cell autonomous or non-cell autonomous effect. In addition, there is no direct physical mechanism for KAL-1 to interact with the cytoskeleton and drive axon branch formation. Of the known KAL-1 interaction partners, only SDN-1 has a cytoplasmic region yet syndecan mutants do not suppress branching. This suggests that a KAL-1 co-receptor is required to transduce the axon branching signal. We are performing biochemical screens to identify other KAL-1 binding proteins potentially involved in this pathway and that may function as a KAL-1 co-receptor. Experiments are also ongoing to establish whether LON-2/glypican functions cell autonomously.

467B

The contactin homolog *rig-6* is involved in axon guidance and branching in *C. elegans*. **M. Katidou[1,2]**, N. Tavernarakis[1], D. Karagogeos[1,2]. 1) IMBB, FORTH, Heraklion, Crete, Greece; 2) University of Crete, Medical School Heraklion, Greece.

The Immunoglobulin Superfamily (IgSF) is a conserved family of proteins, playing a leading role in many developmental processes, including nervous system patterning. The contactin subgroup of the IgSF consists of glycosylphosphatidylinositol (GPI) anchored glycoproteins, known to be essential for axon growth, guidance and fasciculation, neuronal migration and myelination in vertebrates. Aiming to further understand the role of contactins, we are characterizing RIG-6 (C33F10.5), the only member of the contactin subfamily in *C. elegans*. We have determined *rig-6* spatiotemporal expression pattern; it is expressed in head neurons, ventral cord motorneurons and commissures, HSN and CAN neurons, in muscle cells, spermatheca and hypodermis. *rig-6* expression begins in embryonic stages, and it is maintained throughout adulthood. To further investigate the role of RIG-6, we have used *rig-6* RNAi knockdown to study effects on neuronal development and axonal migration. Our data show that downregulation of *rig-6* expression leads to the formation of ectopic branches in ALM axons. Moreover, we have observed abnormal crossing of axons in the ventral nerve cord (VNC) To over-express *rig-6*, we have generated a plasmid that encompasses the promoter, the complete coding sequence and the UTRs of the gene. Transgenic animals show cross defects in the VNC, revealing that the level of expression of *rig-6* is critical for normal axon guidance in the VNC. In addition, commissures are misguided and tend to form branches. Several behavioral abnormalities have been observed in the gain of function mutants of *rig-6*, namely in locomotion, defecation and fertility. Mutations in UNC-53, a cytoskeleton binding protein involved in anteroposterior cell migration and axon guidance, cause the formation of ectopic branches in ALM axons, abnormal commissure branches as well as cross defects in the VNC. To test whether *unc-53* and *rig-6* function in the same pathway, we have used *rig-6* RNAi in *unc-53(n152)* mutant animals. *rig-6* downregulation enhances the formation of commissure branches in *unc-53(n152)* mutants. Moreover, *rig-6* overexpression in *unc-53(n152)* animals leads to increased frequency of commissure branches and cross defects compared to *unc-53(n152)* or *rig-6* gain of function mutants alone. Our data suggest that *rig-6* affects axon guidance and branching in *C. elegans*. UNC-53 could act downstream of RIG-6, converting an extra-cellular signal to an intracellular response.

468C

Analysis of novel motor axon guidance mutants in C. elegans. Z. Naqvi[1], S.S. Sybingco[2], M. Bueno de Mesquita[1], R. Rezania[1], G. Kholkina[3], V. Sertetchnaia[3], L. Ngumbullu[3], A. Guigova[3], S.H. Park[4], **M. T. Killeen[1,2,3,4]**. 1) Program in Molecular Science, Ryerson University, 350 Victoria St., Toronto, ON. M5B 2K3, Canada; 2) Graduate Program in Biology, York Unviversity, 4700 Keele St., Toronto, ON M3J 1P3, Canada; 3) Dept Chemistry & Biol, Ryerson University, 350 Victoria St., Toronto, ON M5B 2K3, Canada; 4) Dept of Occupational and Public Health, Ryerson University, 350 Victoria St., Toronto, ON M5B 2K3, Canada.

A genetic enhancer screen has been conducted for motor axon guidance defects in an *unc-5(e53)* mutant of *C. elegans*. The screen is an F2 screen following EMS treatment of *unc-5(e53)* animals transgenic for *unc-129::gfp*. The mutants have a defect where some of the motor neurons fail to exit their cell bodies in the ventral cord. Five mutants have been found in the screen to date. Three of the mutants were mapped to three different locations on the genome by snip-SNP analysis. One mutant has increased motor axon defects in the DA and DB classes of motor neurons in both *unc-5(e53)*, and *unc-6(ev400)* backgrounds, suggesting that the gene functions in a pathway parallel to the *unc-6*/netrin pathway for motor axon guidance. The mutant strain has no apparent defects in the guidance of the motor axons but has a low penetrance of mechanosensory neuron defects in an otherwise wild-type background. The mutant strain has a low brood size and has an additional phenotype of distortion in the dissected gonad. Microinjection experiments have been performed and a rescue fosmid identified. We are currently sequencing the genes contained on the fosmid to find a mutation. In addition, we are cloning the genes contained on the fosmid to effect rescue of the mutant phenotypes. *unc-5(e53)* animals are also being treated with dsRNA to phenocopy the mutant defects.

469A

unc-3 is necessary for axon pioneering and guidance in C. elegans. **Grace S Kim[1]**, Meng Xu[1], John G. White[2], David H. Hall[1]. 1) Neuroscience, Albert Einstein Col Medicine, Bronx, NY; 2) Anatomy, University of Wisconsin, Madison, WI.

Genetic and phenotypic studies of unc-3 mutants have shown that unc-3 is required for proper organization of ventral cord processes (1,2). The defasciculation and wiring defects seen in unc-3 mutants have been attributed to disruption of pathfinding in the pioneer neurons during embryonic development. We have used Elegance, a 3D reconstruction software program developed at AECOM (3), to analyze the deformed morphology of the unc-3 (e151) ventral cord to unprecedented detail at the EM level. Both the e151/e151 and e151/Df animals display severe axon guidance errors that affect some pioneers (AVKs) and some motor neurons more strongly than the command interneurons. These results support the essential role of the unc-3 gene in axon pioneering and guidance. We present the pattern of severe axon guidance errors in terms of the mispositioning of specific axons, their deviance from their normal neighborhoods, and their synaptic wiring defects. This study, along with molecular genetic studies identifying the unc-3 gene product as a CeO/E transcription factor, should guide future studies of specific axon guidance cues at the molecular level and provide further insight into the role of the mammalian O/E family members. This study used annotated unc-3 TEM datasets received from John White (MRC/LMB and U. Wisconsin) that are now part of the MRC archive in the Hall lab. This work was supported by NIH RR12596 (to DHH) and an AECOM summer research fellowship (to GSK). 1.Wightman, B., Baran R. and Garriga, G. (1997) Development 124: 2571-2580. 2.Prasad, B.C. et al. (1998) Development 125: 1561-1568. 3.Emmons, S.W. et al. (2009) at this meeting.

470B

A screen to identify genes required for axon regeneration. **Paola Nix**, Linda Hauth, Michael Bastiani. University of Utah, Salt Lake City, UT.

An understanding of when and how neurons regenerate is an important key to understanding several neurological disorders. How do neurons recognize breaks and how do they regulate and execute regrowth? To identify genes required for neuronal regeneration we exploited the fact that neurons in *unc-70* mutants continuously break and regenerate. Thus, most commisures in *unc-70* adults are the product of regeneration. We performed an RNAi-based screen for genes that block D neuron regeneration in *unc-70*. We expected that RNAi of genes required for regeneration would reduce the number of commisures typically observed in *unc-70* mutants.

To date, we have identified over 60 candidates that affect the ability of neurons to regenerate. These candidates represent many different functional groups including signaling proteins, transcription factors, cytoskeletal, cell surface or secreted proteins, molecules involved in cellular metabolism and other uncharacterized proteins. Several candidate genes are required for axon outgrowth and pathfinding during development. Understanding how these genes affect regeneration will hopefully provide new therapeutic targets for restoring neuronal function following injury or disease.

As a result of our screen we have shown that DLK-1/MAP kinase signaling is essential for axon regeneration. Loss of DLK-1 and the downstream components, MKK-4 and PMK-3, completely eliminate regeneration, while DLK-1 overexpression improves regeneration. During the course of our analysis of DLK-1 we have begun to characterize specific cellular events that take place during regeneration. We have identified a critical period around the time of axotomy in which DLK-1 must be expressed to support regeneration. We have begun to analyze age-related differences and show that overexpressing DLK-1 in old adults makes them "younger" in terms of their ability to regenerate. In addition, by overexpressing DLK-1 we can induce regeneration in cells that are normally unable to regenerate. Finally, we have identified a developmental role for *dlk-1*: to mitigate neuronal damage in response to stress encountered in the wild. We show that *dlk-1* null mutants subjected to heat shock during development carry a large number of axon stumps that fail to regenerate, whereas most wild-type animals recover from heat shock without serious defects. These results provide unique insights into the process of regeneration itself.

471C

A combination of morphological landmarks and diffusible cues regulate dendritic arborization in PVD sensory neurons. **Cody J. Smith**[1], Joseph D. Watson[2], Clay W. Spencer[1], Millet Treinin[3], Byeong Cha[1], David M. Miller, III[1]. 1) Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Program of Neuroscience, Vanderbilt University, Nashville, TN; 3) Department of Physiology, Hadassah Medical School–Hebrew University, Jerusalem, Israel.

Sensory neurons that detect noxious stimuli (nociceptors) display highly branched dendritic arbors adjacent to the skin. The variable complexity of these structures has stymied efforts to identify molecular determinants of dendritic morphogenesis. To simplify this problem, we are using live-cell imaging and genetic methods in C. elegans to study a single type of nociceptive neuron with a stereotypical dendritic architecture. The PVD neuron (L+R) adopts a series of orthogonal branching decisions that envelops the animal in a net-like array of sensory processes directly beneath the hypodermis. Time-lapse imaging with fluorescent markers has revealed that PVD dendrites adopt a 90-degree turn to fasciculate with sub-lateral nerve cords. In contrast, PVD dendrites withdraw upon intracellular contact with an apparent self-avoidance mechanism that insures maximum coverage of the sensory field. These responses suggest that PVD dendritic outgrowth and branching are guided by extracellular signals. We generated a microarray profile of PVD to identify transcripts with potential roles in dendritic morphogenesis. Genetic ablation of the PVD-enriched axon guidance receptors UNC-5 (e152) and UNC-40/DCC (e271) disables the self-avoidance mechanism but does not perturb PVD fasciculation with sub-lateral nerve cords. A similar phenotype was obtained for the axon guidance cue UNC-6/netrin and its downstream effector UNC-34/ena (e315). On the basis of these results, we propose a novel mechanism in which extracellular UNC-6 interacts with UNC-5 and UNC-40 to mediate dendritic self-avoidance. Ongoing experiments are designed to establish the location of UNC-5 and UNC-40 receptors in growing PVD processes and to define the spatial origin of the UNC-6 cue.

472A

The non-classical cadherin *fmi-1* mediates pioneer-follower axon guidance in the ventral nerve cord. **Andreas Steimel**, Harald Hutter. Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada.

The ventral cord axon tracks are established through sequential outgrowth of pioneer and follower axons. The PVP axon pioneers the left axon track closely followed by the PVQ axon. In an EMS screen for animals with defects in ventral cord axon guidance we isolated the *fmi-1* allele *rh308* (1). *fmi-1(rh308)* animals display strong PVP and PVQ axon guidance defects. Interestingly the pioneer-follower relationship between PVP and PVQ axons is disrupted in *fmi-1(rh308)* animals. PVQ axons cross the ventral midline independently of the PVP axons or even leave the ventral cord. They stop prematurely in virtually all *fmi-1(rh308)* animals. Further *fmi-1* alleles were isolated in genetic screens for defects in synapse formation and HSN axon guidance in the Jin and Garriga Lab respectively. In 68% of *fmi-1(rh308)* animals HSN axons fail to join the ventral cord axon tracks and circle around the vulva. Moreover HSN axons stop before reaching the nerve ring in nearly all *fmi-1(rh308)* animals. Interneuron axons that extend along pioneers in the right axon track are affected in 31% of *fmi-1(rh308)* animals.

Fmi-1 is the homologue of Drosophila Flamingo and vertebrate CELSR1, 2 and 3, which are known to function in neuronal development (2). FMI-1 is characterized by a unique domain composition with eight cadherin repeats, laminin G and EGF modules and a G-protein coupled receptor domain.

A 2.6 kb *fmi-1* promoter GFP construct is mainly expressed in neurons including PVP, PVQ and HSN. Expression starts during gastrulation before axons grow out, persists throughout all larval stages and decreases noticeably in adults. To determine the subcellular localization of FMI-1 we fused the *fmi-1* transcript to GFP. FMI-1::GFP is predominantly localized to axons and rescues PVQ defects in *fmi-1(rh308)* animals.

Mosaic analysis revealed that *fmi-1* acts cell-autonomously in PVP and PVQ axons. Interestingly *fmi-1* is necessary in both PVP and PVQ axons for correct PVQ axon guidance. An *fmi-1* construct containing only the cadherin repeats in the extra-cellular domain can nearly completely rescue PVP-independent PVQ axon outgrowth. These data suggest a model, where FMI-1 acts as homophilic adhesion molecule between pioneer and follower neurons to ensure that follower axons extend along the pioneer axons.

1. Hutter et al., Dev Biol 284 (2005) 260-272; 2. Takeichi, Nat Rev Neurosci. 2007 Jan;8(1):11-20.

473B

The Role of 3-O Sulfation of Heparan Sulfate in Neuronal Development in *C. elegans.* . **Eillen Tecle**, Hannes Buelow. Department of Genetics, Albert Einstein College of Medicine, Bronx, NY.

Our lab is interested in how Heparan Sulfate (HS) modifications regulate neuronal connectivity and patterning in *C. elegans*. HS is a highly modified un-branched glycosaminoglycan exhibiting substantial molecular diversity due to multiple modifications such as sulfations, epimerization and acetylation. HS modifications have been documented to have specific and instructive roles (Bülow and Hobert, 2004; Bülow et al., 2008) in neuronal development leading to the hypothesis of a HS code that regulates nervous system patterning. However, the role of the 3-O sulfation modification of HS, introduced by heparan sulfotransferase 3 enzymes (HST-3s), has not been established. Vertebrate genomes code for at least seven members of the HST-3 gene family that are grouped into two distinct classes. A subset of the vertebrate HST-3s display temporarily and spatially restricted expression patterns in the developing and post-natal brain, however, very little is known about the *in vivo* function of these enzymes in neuronal development due to functional redundancy. We have identified one gene coding for a predicted HST-3 of each class in the *C. elegans* genome: *hst-3.1* and *hst-3.2*. Analysis of neuronal patterning in null mutants of *hst-3.1* and *hst-3.2* indicates that both genes are required for synaptic branch formation in a subset of *C. elegans* neurons. In addition, *hst-3.2* may play a role in axon termination. These phenotypes are reminiscent of mutations in genes regulating synaptic maturation and/or function. Therefore, on going experiments are focused on determining if loss of *hst-3.2* and/or *hst-3.1* results in synaptic disorganization and if either gene acts in known synaptic pathways. We have recently elucidated that several other null mutants of HS modification enzymes and HS core proteins share the phenotypes that we identified in null mutants of *hst-3.1* and *hst-3.2*. We are investigating the interaction of HS modifications and core proteins, via double and triple mutant analysis, in various cellular contexts. Furthermore, we are determining the cellular focus of action of the HS 3O-sulfotransferases. This analysis will provide invaluable insight into possible mechanisms by which the HS code regulates neuronal development and patterning.

474C

Characterization of *ot21*: enhancer of the Kallmann Syndrome gene *kal-1* induced axonal branching phenotype in AIY interneurons. **J. Tornberg**[1], J. Maydan[3], D. Moerman[3], H. Bülow[1,2]. 1) Dept Molecular Genetics, Albert Einstein Col Medicine, Bronx, NY; 2) Dominick P. Purpura Dept Neuroscience, Albert Einstein Col Medicine, Bronx, NY; 3) Dept Zoology, University of British Columbia, Vancouver, Canada.

Kallmann Syndrome (KS) is a genetically heterogenous disease with the most defining features of hypogonatrophic hypogonadism and anosmia, which are believed to be the result of neural targeting and migration defects. The X-linked form of this disease accounts for approximately 10% of KS cases and is caused by mutations in the anosmin-1/KAL1 gene. We have previously shown that over-expression of the *Caenorhabditis elegans* homolog of anosmin-1/KAL1 in AIY interneurons causes a highly penetrant, dosage-dependent, and cell autonomous axonal branching phenotype. In a modifier screen we have isolated the *ot21* enhancer mutation, which leads to significantly longer axonal branches (Bülow *et al.*, 2002 PNAS). This enhancement is specific for the *kal-1*-dependent branching phenotype in AIY because *ot21* fails to enhance the ectopic neurites elicited by mutations such as *ttx-3* and *sax-2* which cause similar phenotypes in AIY interneurons. Phenotypic analysis of *ot21* revealed axonal defects in DVB and HSN motor neurons as well as specific behavioral defects. Cloning and molecular characterization of the *ot21* mutation revealed a missense mutation in a gene important for axonal and synaptic development consistent with a potential role of *kal-1* in neural targeting.

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A conserved SWIM domain protein regulates axon guidance in *C. elegans*. **Z. Wang**[1,2], Y. Hou[3], Z. Wu[1,2], A.D. Chisholm[1], Y. Jin[1,2]. 1) Division of Biological Sciences, UC San Diego, La Jolla, CA 92093, USA; 2) Howard Hughes Medical Institute; 3) Department of MCD Biology, UC Santa Cruz, CA95064.

We are interested in the molecular pathways that guide axon outgrowth during development and in adults. We will report our analysis of the role of PQN-55 (prion-like-Q/N-rich-domain-bearing protein 55), a member of a conserved novel protein family (see the abstract by Hou et al, 2007 International Worm Meeting), in axon guidance and regeneration in *C. elegans*. This protein family contain an N-terminal SWIM domain, followed by four domains conserved among the family members. The SWIM domain is defined by its characteristic Zinc-binding CxCxnCxH sequence, and has been linked to transcriptional regulation and several signal transduction pathways. P*pqn*-55-GFP reporters are expressed widely in neurons, epithelial cells, and muscles from early embryogenesis onward. Analysis of the localization of functional GFP-tagged PQN-55 in neurons suggests PQN-55 may be associated with endosomes. *pqn-55* loss-of-function mutants are overall healthy but show mild abnormalities in egg-laying behavior and locomotion. Axon guidance is generally normal in these mutants. However, we observed that some AVM touch neurons display impaired ventral axon guidance. The ventral projection of AVM is known to be under the control of the repellent SLT-1/SAX-3 pathway and the attractive UNC-6/UNC-40 pathway. Interrupting either pathway causes ventral guidance defects of AVM axons. We are currently determining whether PQN-55 functions in one of these known guidance pathways. We will also report our studies on the role of PQN-55 in adult axon regeneration.

476B

Identifying the role of wdr-23 in regulating the abundance of synaptic proteins. **Trevor Charles Griffen**, Derek Sieburth. Zilkha Neurogenetic Institute, Keck School of Medicine, USC, Los Angeles, CA.

wdr-23 was identified in an RNAi screen for genes that cause resistance to the paralytic effects of the acetylcholine esterase inhibitor aldicarb[1]. WDR-23 is a conserved protein composed of seven WD-40 repeats predicted to form a β-propeller. *wdr-23* is expressed broadly, including in cholinergic motor neurons. WDR-23A::GFP expressed in neurons is seen in both the nucleus and cytosol. We have characterized a wdr-23 deletion allele, tm1817, provided by the Mitani lab. wdr-23 mutants are scrawny, slow growing, and resistant to aldicarb, but not resistant to the paralytic effects of the muscle agonist levamisole. We have fully rescued the movement, growth and aldicarb defects by re-expressing WDR-23A::GFP cDNA under its endogenous promoter.

To determine the function of *wdr-23*, we have examined the changes in synapse number and structure in *tm1817* mutants. We did not observe changes in synapse number, as measured by the of the number of UNC-10::GFP or SNB-1::GFP containing cholinergic synapses, suggesting that synapse formation is normal in these mutants. To examine synaptic function, we expressed SNB-1::GFP under the *unc-129* promoter and observed an increase in both peak synaptic fluorescence and axonal fluorescence. Recent evidence suggests that WDR-23 interacts with the DDB-1/CUL-4 ubiquitin ligase complex to regulate the abundance of target proteins (such as SKN-1)[2]. We have determined that there is an increase in endogenously expressed SNB-1 in *wdr-23* mutants. These results suggest that WDR-23 is either regulating the stability of SNB-1 or the expression of both *snb-1* and *unc-129*. We are testing the possibility that WDR-23 acts together with DDB-1/CUL-4 to regulate the expression or degradation of *snb-1, unc-129* and other synaptic proteins. We will determine if this regulation occurs via regulation of SKN-1 by the WDR-23/DDB-1/CUL-4 ubiquitin ligase complex or another mechanism.

[1]Sieburth D et al. Nature (2005) [2]Choe K, Przybysz A & Strange K. Mol Cell Biol (2009).

477C

Expression and localization of the synaptic adhesion protein neuroligin. **Jerrod Hunter**[1,2], Greg Mullen[1], John McManus[1], Jessica Heatherly[1,3], Angie Duke[1], Jim Rand[1,2,3]. 1) Genetic Models of Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104; 2) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; 3) Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Neuroligins were first identified as mammalian postsynaptic cell adhesion molecules that bound specifically to presynaptic membrane proteins called neurexins. There are four neuroligin genes in mammals, and mutations in the human genes encoding neuroligin 3 and neuroligin 4 are associated with autism spectrum disorders (Jamain et al., 2003; Laumonnier et al., 2004; Yan et al., 2005). *C. elegans* has a single neuroligin gene, *nlg-1* (C40C9.5). Using a transgenic transcriptional reporter, we found that *nlg-1* is expressed in a subset of neurons in *C. elegans* adults, including ~20 cells in the ventral nerve cord, and ~20 cells in the head. We identified the *nlg-1*-expressing cells in the ventral nerve cord as the cholinergic VA and DA motor neurons. The *nlg-1*-expressing cells in the head do not fall neatly into a single neuron class or neurotransmitter type, and include the AWA and AWC odorsensory neurons, the AIY, RIA, and URB interneurons, and the RMH and URA motor neurons. We also observe *nlg-1* expression in the midbody PVD mechanosensory and HSN motor neurons, and faint expression in body wall muscles. To examine subcellular localization of the NLG-1 protein, we generated a transgenic NLG-1::YFP fusion protein under the control of the *nlg-1* promoter. We believe that this fusion protein is functional because it rescues all *nlg-1* mutant behaviors (see abstract by Heatherly et al.). We observed NLG-1::YFP fluorescence in neuronal processes, localized chiefly to synaptic regions, especially the nerve ring and the nerve cords. Confocal microscopy revealed that NLG-1::YFP is present at synapses, and is offset from synaptic vesicle and active zone proteins. The C-terminus of the NLG-1 protein contains a Type II PDZ-binding motif which is necessary for proper subcellular localization. We are currently trying to identify the gene products required for proper localization of NLG-1. (Supported by a grant from Autism Speaks).

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Investigating the Phosphatydylinositol signaling underlying subcellular localization of synapses. **T. Kimata**[1], A. Kuhara[1], Y. Tanizawa[2], I. Mori[1]. 1) Div Biol Sci, Nagoya Univ, Nagoya, Japan; 2) Present add: MRC, Cambridge, UK.

Although it is well known that synapses are formed at specific subcellular regions, the molecular mechanism underlying subcellular localization of synapses has not been well characterized. RIA type of interneurons in *C. elegans*, which are essential for the thermotaxis (1), have a vast number of synapses in a single neurite where all presynapses are localized to its proximal region and most postsynapses are localized to its distal region. This characteristic pattern of synapses makes RIA as a good model for the analysis of subcellular localization of synapses. We have reported that *ttx-7* mutant lacking Inositol Monophosphatase (IMPase) shows abnormal localization of synaptic proteins in RIA, which leads to thermotaxis defect(2).However how IMPase regulates the subcellular localization of synapses has not yet been revealed.

Through the screen for the suppressor mutations of *ttx-7*, we identified that the loss of function mutation in *egl-8* gene encoding phospholipase C β(PLCβ) strongly suppresses the synaptic defect of *ttx-7* mutant. Expressing *egl-8* cDNA using RIA specific promoter rescued this suppression but the expression in AIY interneurons did not. EGL-8/PLCβ cleaves PtdIns(4, 5)P_2 into IP_3 and DAG, indicating the importance of the signaling utilizing PtdIns(4, 5)P_2 or IP_3, DAG in the subcellular localization of synapses. The IP_3 or DAG related mutants such as *age-1*, *ipp-5*, *dgk-1*, *dgk-2*, did not show the synaptic defects in RIA(2). We however found that a mutation in *unc-26* gene encoding a C. elegans ortholog of human synaptojanin 1 known to remove the phosphate from PtdIns(4, 5)P_2 partially suppresses the synaptic defect of *ttx-7* mutant. Taken together, we hypothesized that PtdIns(4, 5)P_2 is a key regulator for the patterning of synapses in RIA interneurons. We are now attempting to visualize PtdIns(4, 5)P_2 in RIA interneurons. Through the study, we hope to provide a new insight on the molecular mechanism regulating the subcellular localization of synapses.

1)Mori and Ohshima, Nature, 1995
2)Tanizawa et al., Gens Dev, 2006.

479B

Screen for regulators of RAB-5 endosomal compartments in synapse formation. **Sharon B. Sann**[1], Matthew M. Crane[2], Alicia Arney[1,3], Hang Lu[2], Yishi Jin[1,3]. 1) Division of Biological Sciences, UC San Diego, La Jolla, CA 92093; 2) Interdisciplinary Program in Bioengineering, Georgia Institute of Technology, Atlanta GA 30332; 3) Howard Hughes Medical Institute.

Synaptic vesicles are required for release of neurotransmitters and subsequent communication between neurons. The synaptic vesicle membrane proteins that facilitate fusion with the plasma membrane are transported from the cell body in synaptic vesicle precursors. These precursors must undergo a maturation process before they become functional synaptic vesicles, and this process likely involves fusion with endosome-like compartments within the synaptic terminal. The Rab-5 GTPase is associated with canonical early endosomes and is known to be present in presynaptic terminals. Previous research has shown that biasing the cycling state of Rab-5 towards a primarily GTP-bound form appears to lead to increased association of Rab-5 with endocytic membrane within the synaptic terminal, resulting in a concomitant decrease in the numbers of synaptic vesicles.[1]

To further understand the role of Rab-5 in neurons, we have conducted forward genetic screens for mutations that disrupt YFP::RAB-5 expression in the GABAergic motor neurons. We performed the screens in two ways. One was in an *unc-104* mutant background, and the other used an automated screen[2] in a wild-type background. We isolated about 20 mutants. Preliminary characterizations suggest that they define several non-complementation groups. We have mapped and cloned one mutant and found that it affects the *C. elegans* Rabx-5, a guanine exchange factor (GEF) of RAB-5. *Rabx-5* mutants exhibit moderate aldicarb and levamisole resistance. Quantitative imaging analysis of YFP::RAB-5 reveals specific alterations in synapses and cell bodies. In contrast, *rabx-5* mutations have no effects on expression of constitutively active Rab-5-GTP (YFP::Rab-5(Q78L)). These results support that RABX-5 is a functional GEF for Rab-5 in neurons. We have observed similar changes in synaptic expression of Rab-5 in another rab effector, rabaptin *rabn-5*.

The identification of *rabx-5* validates our screen as a powerful way for revealing molecules involved in membrane trafficking during neuronal development. We are currently examining the role of these proteins on synaptic vesicles and golgi dynamics. We are also exploring the other mutations revealed in this screen.

1 Brown H, Van Epps H, Goncharov A, Grant B, Jin Y (2009). Dev Neurobiol 69(2-3):174-90.
2 Chung K, Crane MM, Lu H (2008). Nat Meth 5(7):637-643.

480C

unc-4 antagonizes a Wnt signaling pathway upstream of *ceh-12*/HB9 to specify synaptic choice in the *C. elegans* motor circuit. **Rachel L Skelton**[1], Judsen Schneider[1], Stephen Von Stetina[2], Kathie Watkins[1], David M Miller III[1]. 1) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN; 2) Huntsman Cancer Institute, University of Utah, Salt Lake City, UT.

Neural function depends on the creation of synapses between specific neurons. In *C. elegans*, this important developmental decision is regulated by the UNC-4 homeodomain protein. UNC-4 functions in VA motor neurons to block the adoption of inputs normally reserved for VB sister cells. This wiring defect in *unc-4* mutants disables backward locomotion. Thus, we have proposed that UNC-4 preserves normal VA inputs and backward movement by inhibiting VB gene expression. This model was substantiated by our previous finding that ectopic expression of the VB-specific gene, *ceh-12* (HB9 homeodomain protein), results in the miswiring of VA motor neurons in the posterior ventral nerve cord. Now we have used cell-specific microarray profiling and independent mutant screens to establish that ectopic expression of CEH-12/HB9 in posterior VA motor neurons depends on a response to a local source of EGL-20/Wnt. Our results show that *unc-4* antagonizes a Wnt signaling pathway involving *egl-20*/Wnt, *mom-5*/Frz, and *mig-1*/Frz that is required for *ceh-12* expression in VA motor neurons. We propose that UNC-4 represses *mom-5*/Frz expression, thereby rendering VA motor neurons unresponsive to EGL-20/Wnt. RNAi and genetic experiments have revealed additional Wnt signaling components that interact with the *unc-4* pathway, including the Frizzled receptor, *lin-17*, *lin-44*/Wnt, *dsh-1*/Disheveled, *pry-1*/Axin, and *pop-1*/TCF-LEF. Further investigation will determine if these canonical Wnt pathway components function upstream of *ceh-12* or in a parallel pathway. This work has revealed a sensitive mechanism for exploiting diffusible Wnt ligands for precise patterning of connectivity in the *C. elegans* ventral nerve cord. The existence of comparable Wnt gradients in the vertebrate spinal cord could reflect similar but as yet unexplored roles for Wnt signaling in vertebrate motor circuit assembly.

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Regulators of synaptic remodeling in *C. elegans* are revealed by analysis of UNC-55 transcriptional targets. **Sarah C. Anthony**[1], Joseph D. Watson[2], Bill Walthall[3], David M. Miller III[1]. 1) Vanderbilt University Medical Center, Nashville, TN; 2) University of North Carolina School of Medicine, Chapel Hill, NC; 3) Georgia State University, Atlanta, GA.

Neurons form functional circuits by adopting polarized morphologies with separate axonal and dendritic compartments. These domains may be reorganized in response to injury or developmental cues. Though this remodeling feature is evolutionarily conserved, the mechanism is poorly understood. One example of this phenomenon occurs in the GABAergic motor circuit of *C. elegans*. Dorsal D (DD) motor neurons initially form inhibitory neuromuscular junctions (NMJs) with ventral muscle. At the end of the first larval stage, DDs reverse polarity to synapse with dorsal muscle. This DD polarity switch is coincident with the birth of VD motor neurons, which make NMJs with ventral body muscle. In *unc-55* mutants, VD motor neurons are remodeled to mimic the dorsal polarity of DD motor neurons (White et. al., 1978). UNC-55 is a COUP family nuclear hormone receptor normally expressed in VD motor neurons (Zhou and Walthall, 1998). Thus, UNC-55 appears to function as a transcriptional switch to turn off synaptic remodeling genes in VD motor neurons. We propose that UNC-55-regulated targets are likely to fulfill crucial roles in synaptic remodeling. To identify these genes, we employed the mRNA tagging method to compare gene expression profiles of wild-type and *unc-55* mutant L2 GABAergic motor neurons. 188 transcripts enriched >2-fold in the *unc-55* profile represent candidate synaptic remodeling genes. RNAi of these UNC-55 regulated transcripts revealed >40 genes that partially suppress the Unc-55 remodeling defect ($p<0.01$), as visualized by rescue of GABAerigc NMJs on the ventral side with the synaptic marker *juIs1(punc-25::SNB-1::GFP)*. Most striking among these candidates is the homeobox transcription factor IRX-1/Iroquois, which suppresses both the loss of ventral NMJs as well as the Unc-55 backward movement defect. Other suppressors of the Unc-55 synaptic remodeling phenotype include specific ion channels, cytoskeletal components, and cell-cell signaling molecules. The wide range of potential functions encoded by these UNC-55 regulated genes is indicative of a complex synaptic remodeling pathway. Ongoing experiments are designed to confirm that these genes lie in the UNC-55 pathway and to investigate their cellular role in the mechanism of synaptic remodeling.

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Genome-wide RNAi analysis of neuronal cell fate and left/right asymmetry in *C. elegans*. **Enkelejda Bashllari**[1,2], Richard Poole[1,2], Oliver Hobert[1]. 1) Department of Biochemistry & Molecular Biophysics, Columbia University Medical Center, New York, NY; 2) Equal contribution.

The developmental programs that lead to the specification of individual neuron types in a nervous system remain incompletely understood. Forward and reverse genetic screens have served as a valuable tool to identify genes involved in neuronal fate specification. We describe here the results of a genome-wide RNAi screen to uncover factors involved in the specification of a single neuronal sub-type in the nematode *C. elegans*. In wild-type animals, the two morphologically bilaterally symmetric gustatory neurons ASE left (ASEL) and ASE right (ASER) undergo a left/right asymmetric diversification in cell fate, manifested by the differential expression of a class of putative chemoreceptors and neuropeptides. Using an asymmetrically expressed ASEL-specific GFP reporter we have screened ~14,000 RNAi targeted genes for novel factors that play a role in the development, specification and maintenance of ASE neuronal identity.

Thus far, we have identified 254 genes whose knockdown by RNAi produces a loss of the ASEL fate or a gain of an ectopic ASEL; only 5 of these were previously known to affect ASE development. Further analysis of the uncovered genes places them in several distinctive phenotypic categories; genes that affect early blastomere identity (*par-3*, *mex-5*, etc) whose division defects produce lineage transformations expected to create ectopic ASEs, overall neuronal cell fate specification factors such as the transcription factor *achaete-scute/hlh-14* (see abstract by Poole, Bashllari and Hobert) and classical asymmetry genes such as *F27D4.2* which specify neuronal sub-type by left/right asymmetry within a neuron class. Their molecular identity places these genes in several categories with the ones of most interest to us being proteins involved in signaling, RNA-binding, transcription and chromatin regulation. The subsequent detailed characterization of the identified genes in the aforementioned categories is expected to provide more insight into the complex genetic architecture of neuronal cell fate specification and sub-type left/right asymmetric decision.

483C

The role of endogenous RNAi components and DAF-16 in neuronal development of C. elegans. **Lisa Kennedy**, Alla Grishok. Columbia University Medical Center, New York, NY.

We are investigating the roles of RNAi-induced transcriptional gene silencing (RNAi-TGS) in the development of Caenorhabditis elegans. We have found that a RNAi-promoting chromatin factor Zinc Finger Protein 1 (ZFP-1), a dsRNA-binding protein of the Dicer complex, RDE-4 and DAF-16, a conserved FOXO family transcription factor, are involved in the control of migration, axonal outgrowth and cell fate of the hermaphrodite-specific neurons (HSNs). The hermaphrodite-specific neurons (HSNs) are a pair of bilaterally symmetric serotonergic motor neurons that innervate the vulval muscles and stimulate hermaphrodites to lay eggs. The HSNs are generated 400 minutes after fertilization in the tail of the embryo when each of two HSN/PHB precursors divides to give rise to an HSN and a PHB phasmid chemosensory neuron. Ten minutes after being born, the HSNs migrate to the center of the embryo where they flank the gonad primordium. In order to visualize the hermaphrodite-specific neurons (HSNs) in various mutant backgrounds, a tph-1::gfp reporter, a transcriptional fusion that drives the expression of GFP in serotonergic neurons, was used to detect HSNs in young adult worms. The HSNs in the rde-4 (ne301) mutants fail to migrate fully out of the tail 50% of the time, whereas in the zfp-1 (ok554) and daf-16 (mu86) mutants the frequency of partial migration out of the tail is 13% and 18%, respectively. A daf-16; zfp-1 double mutant exhibits the most severe HSN migration defects since the majority of those HSNs that have not migrated out of the tail remain the most posterior as compared to the single mutants of zfp-1, daf-16 and rde-4. In addition to HSN migration defects, the daf-16; zfp-1 mutant strain exhibits the most penetrant defects in HSN axonal outgrowth and HSN lineage abnormalities (extra HSNs) as compared to the single mutants zfp-1, rde-4, and daf-16, which only occasionally exhibit these additional phenotypes. We predict that the chromatin factor ZFP-1 and the dsRNA binding protein RDE-4 negatively regulate neuronal genes required for proper migration during development through RNAi-TGS. The gene daf-16 has been identified as a positive regulator of zfp-1 and therefore may work through ZFP-1 during neuronal development and/or in parallel to negatively regulate common targets.

484A

Uncovering the heparanome in *C. elegans* and the factors that create it. **Matthew Attreed**[1], Toin van Kuppevelt[2], Hannes Bülow[1]. 1) Genetics, AECOM, Bronx, NY; 2) Department of Biochemistry, Nijmegen Center for Molecular Life Sciences, University Medical Center Nijmegen, Nijmegen, The Netherlands.

The heparanome is composed of the entirety of heparan sulfate (HS) in *C. elegans*. HS are unbranched, highly modified polysaccharide chains which are attached to core proteins to form proteoglycans. We have previously shown that HS modifications display specific and instructive functions during neural development, possibly due to the interaction of ligands and receptors in the extracellular space. The HS glycosaminoglycans (GAGs) are modified by enzymes, which can add sulfates, remove acetyl groups, and epimerize the conformation of sugars in the molecule. These HS modifications are introduced non-randomly and non-uniformly to form functional domains of HS modification patterns. While we know the enzymes that introduce individual modifications, nothing is known about what regulates the enzymes to create defined HS domains. Our work sets out to first determine the temporal and spatial expression of defined HS patterns, and second, to devise a genetic approach to identify the genes that are required to establish the HS domain patterns. To this end, we have conducted immunohistochemical (IHC) studies of the heparanome using single chain variable fragment (scFv) antibodies that recognize specific HS modification patterns. We find that antibodies that recognize different HS patterns stain distinct anatomical structures, sometimes with extraordinary cell specificity, suggesting that worms harbor a complex 'sugar landscape' in the extracellular space. We are currently in the process of defining (i) the individual modifications that are required for staining with a given antibody, and (ii) the temporal and spatial expression pattern of HS domains. To facilitate a forward genetic approach to identify genes involved in the creation of defined HS modification patterns, we have developed a novel approach that allows, for the first time, in vivo labeling of defined sugar modification patterns. This will greatly speed up the screening process and should allow the use of high throughput screening approaches to identify the genes, which create functional domains on HS.

485B

A second paraoxonase-like gene is expressed in the touch receptor neurons. **Yushu Chen**, Martin Chalfie. Department of Biological Science, Columbia Univ, New York, NY.

Paraoxonases (PONs) are a family of enzymes with a wide range of activities, including drug metabolism, deoxidization of lipids, and detoxification of organophosphates. The mec-6 gene in Caenorhabditis elegans encodes a paraoxonase-like protein that is part of the MEC-4 channel complex that transduces gentle touch in the touch receptor neurons (TRNs) and is required for neurodegeneration caused by gain-of-function mutations in DEG/ENaC genes (e.g., mec-4 and deg-1). C. elegans has four other PONs-like genes: k11e4.3, e01a2.7, k05f6.11 and e01a2.10 whose products remain uncharacterized. We found that K11E4.3::YFP translational fusion is expressed in the TRNs and a few other neurons. In the processes of the TRNs, K11E4.3::YFP is distributed as puncta that co-localize with MEC-6 puncta. K11E4.3 is required for touch sensitivity in sensitized backgrounds, specifically in mec-6ts animals at the permissive temperature or mec-6 hypomorphic alleles that do not, on their own, produce touch insensitivity. These data suggest that the touch transduction complex may have more than one of these proteins. We are currently examining the expressing of all the C. elegans PON-like genes. Two, k05f6.11 and e01a2.10, are expressed in the hypodermis.

486C

Genetic and functional analysis of left/right asymmetric neuron size. **Andrew D. Goldsmith**[1], Sumeet Sarin[1], Oliver Hobert[2]. 1) Department of Genetics & Development, Columbia University, New York, NY; 2) Department of Biochemistry & Molecular Biophysics, Columbia University, New York, NY.

Nervous system are thought to be bilaterally symmetric on a structural level, but strongly lateralized (left/right asymmetric) on a functional level. However, in vertebrates, a morphological correlate for laterality has been observed. In several seemingly bilateral brain regions, left/right asymmetric brain function has been correlated with differences in soma size in groups of cells in the left vs. right hemisphere. The genetic basis for this asymmetry is unknown. The functionally asymmetric *C. elegans* gustatory neurons, ASE left (ASEL) and ASE right (ASER) allow us to address this question. We have recently discovered that ASEL and ASER differ substantially in size: ASER is consistently larger than ASEL. This asymmetry has been observed in both the cell soma and dendritic diameter. This difference in size is genetically controlled by a pathway of gene regulatory factors that we have previously shown to control the functional laterality of the two ASE neurons. The effector genes of this regulatory pathway, i.e. the genes that control difference in neuronal cell sizes, are, however, unknown.

We have undertaken a candidate gene approach to identify these effector genes. We are in the process of visualizing the ASE left/right asymmetric size difference with an ASE-expressed *gfp* reporter transgene in a large panel of genetic mutant backgrounds. This panel includes genes in pathways that have been previously reported to control cell size in other systems, including Myc, Insulin-signaling, TGFb-signaling, various oncogene/tumor-suppressor gene orthologs and other genes. Results of this mutant analysis will be reported. With these mutants at hand we plan to undertake a functional analysis of neuronal size differences.

487A

A new role for UNC-40/DCC and UNC-6/Netrin in synaptic partner choice. Joori Park[1,2], Akshi Goyal[2], Philip Knezevich[2], Shante O'Hanlon[2], Mekala Rahman[2], Kang Shen[1], **Miri VanHoven[2]**. 1) Howard Hughs Medical Institute, Stanford University, Stanford, CA; 2) San Jose State University, San Jose, CA.

The human central nervous system is composed of approximately 100 billion neurons interconnected into precise circuits by 100 trillion synaptic connections. These circuits are required for nervous system functions including perception, thought and behavior. Much is known about the early steps in circuit formation in which neurites extend to target regions containing the correct synaptic partners. Much less is known about how individual neurons choose the correct synaptic partner when they reach a target region with many neurons. This is an important area of study, as altered synaptogenesis is thought to play a role in neurological disorders such as schizophrenia and autism. To understand how correct synaptic partner choice is mediated, we have developed a genetically encoded fluorescent trans-synaptic marker to visually label synaptic contacts between individual neurons of interest in complex environments called NLG-1 GRASP, for Neuroligin-1-mediated GFP Reconstitution Across Synaptic Partners. We have also labeled pre- and postsynaptic neurites with the red mCherry fluorophore. Together, these markers enable us to instantly assess correct synaptic partner choice by visualizing neurite contact and synaptogenesis between pre- and postsynaptic neurons of interest in live animals, making it feasible to use genetic methods to discover genes mediating this fundamental process. In addition, we have developed these markers in *C. elegans*, the only model organism for which there is a complete synaptic map, making it ideal for the study of synaptic partner choice. Using this marker, we have found that two proteins previously studied for their role in cell migration and axon guidance in other systems, UNC-40/DCC (Deleted in Colorectal Cancer) and UNC-6/Netrin, have a novel role in mediating synaptic partner choice between sensory neurons and interneurons in the *C. elegans* ventral nerve cord, and our current focus is on further characterizing this role.

488B

Automated Screening for Mutants Defective in ASE Asymmetric Neuronal Fate Specification. **Feifan Zhang**, Oliver Hobert. Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, New York, NY 10032.

We use an automatic screening method to isolate mutants in which ASE neurons fail in asymmetric cell fate specification. The ASE neurons are a pair of chemosensory neurons that are morphologically symmetric yet functionally asymmetric. The left cell (ASEL) is the primary sodium sensor, while the right cell (ASER) is primarily responsible for potassium and chloride detection. Disruption of this asymmetry results in chemosensory discrimination defects. Previous screens have identified a "bistable feedback loop" that controls the ASEL/R laterality. In order to look for other factors that might be embedded into this complex yet incomplete network, we use the COPAS Biosort system (Union Biometrica, "worm sorter") to automate our screen, which allows us to screen through a large amount of genomes within a much smaller amount of time compared to labor-intensive manual screens. The worm sorter is very powerful in picking up mutants in which the GFP expression varies from that of the wild type population by as little as one single neuron. Using cell-specific GFP reporters, we are able to isolate mutants that have either lost GFP expression in ASEL ("ASEL off") or gained ectopic expression of GFP in ASER ("2 ASEL"). After 8 rounds of four-to-five-hour screen we have identified 21 "ASEL off" mutants from 48,000 genomes and after 15 rounds of similar screen we have isolated 40 "2 ASEL" mutants from 90,000 genomes. Our complementation tests, mapping and characterization of those mutants are currently in progress.

489C

The two-immunoglobulin domain protein ZIG-3 mediates maintenance of neuronal architecture . **Claire Bénard**, Nartono Tjoe, Thomas Boulin, Oliver Hobert. Dept Biochem, Columbia Univ, New York, NY.

Proper neuronal connectivity throughout the life of an animal is essential for nervous system function. Dedicated mechanisms maintain the architecture of the worm's nervous system after its structure is established embryonically. This task is not trivial considering the mechanical stress inflicted upon the nervous system by the motions of the animal, the dramatic increase in body size, the remodeling of parts of its anatomy, and the addition of new neurons. Research in *C. elegans* has implicated four molecules in this process of neuronal maintenance: the two-Ig domain protein ZIG-4, the FGF receptor EGL-15(5A), the L1-like SAX-7 protein, and the giant DIG-1 protein that contains multiple cell-cell interaction and adhesion domains. These proteins provide crucial information, through an as yet unknown mechanism, for neuronal structures to maintain the precise position that they acquired during embryonic development.

Eight genes encode two-Ig domain containing proteins in *C. elegans*. Given the role of *zig-4* in neuronal maintenance, we have undertaken the systematic analysis of each of the other seven *zig* genes and have discovered that additional members of the *zig* gene family mediate maintenance of neuronal architecture. In particular, we have uncovered that similar to *zig-4*, the gene *zig-3* is required for axon maintenance. In *zig-3* mutants, ventral nerve cord axons become displaced during the early L1 stage after they had acquired their proper positioning during embryogenesis. The axons that are affected in *zig-3* mutants overlap with those affected in *zig-4* mutants. Both *zig-3* and *zig-4* encode similar proteins that are secreted and non-autonomously maintain axon position. *zig-3* and *zig-4* are located very close on the genome and are highly similar by sequence, which might suggest recent gene duplication. However, our molecular genetic analysis reveals that *zig-3* and *zig-4* are not redundant, as each single mutant displays similar defects that are not further enhanced in a double mutant combination. Morevoer, overexpression of one gene does not compensate for the loss of the other. These findings highlight the exquisite molecular specificity that these immunoglobulin proteins confer to the mechanism of axon maintenance in the ventral nerve cord. Also, these results suggest that the unique combinations of particular ZIG proteins and other maintenance molecules in specific cellular contexts may be at the core of the mechanism that brings about robust maintenance.

490A

Mapping and cloning of *ju496* an enhancer of *nid-1* synaptic morphology defects. **Karanda Jean Hildebrand**, Brian D. Ackley. Molecular Biosciences, University of Kansas, Lawrence, KS.

Nidogen is a conserved basement membrane protein that is involved in multiple developmental processes. *nid-1* mutant animals exhibit a diffusion of synaptic vesicles and active zone components, indicating it is required for proper synaptic morphology. Previously we have demonstrated that nidogen functions at synapses via the LAR receptor, *ptp-3*, and the intracellular scaffold molecule, *syd-2*. To identify other molecules that might function in this pathway we performed a genetic modifier screen in *nid-1* deficient animals, using the *rpm-1*(*ju44*) allele as a sensitizer. We isolated multiple enhancers and a suppressor of *nid-1*. One of the alleles, *ju496*, demonstrated a robust enhancement of the *nid-1* phenotype. We found that *ju496* mutant animals exhibit synaptic organization defects, axonal guidance defects, and muscle attachments defects, and have strongly uncoordinated movement. Based on genetic mapping, *ju496* is on LG X. Bulk segregate analysis and deficiency tests put *ju496* between -2.1 and 0.6. Based on our current snip-SNP mapping results, *ju496* is located to the between *dpy-7* and *dpy-6*. Currently we are finely mapping the region where this gene resides through the use of SNP mapping. Once the genetic mapping of *ju496* is complete, we will begin the genetic characterization of this gene.

491B

STN-2/γ-syntrophin mediates SAX-7/L1CAM function in maintaining neuronal positioning by linking SAX-7 to DYS-1/dystrophin. **shan zhou**, karla opperman, lihsia chen. Dept. of Genetics, Cell Biology & Development and the Developmental Biology Center, university of minnesota, minneapolis, MN.

Cell adhesion receptors are essential in multiple processes that range from cell migration to maintaining tissue integrity. As transmembrane proteins, cell adhesion molecules (CAMs) convert extracellular signals into cellular responses via cross-talk with cytoskeletal and intracellular signaling networks. The mechanisms by which CAMs relay these signals are still not clear, particularly with those belonging to the immunoglobulin (Ig) superfamily. L1CAMs are single-pass transmembrane IgCAMs that are conserved in metazoans. Mammals have four L1CAMs (L1, NrCAM, CHL1, and Neurofascin) that are required for nervous system functions. Polymorphisms in NrCAM and CHL1 are associated with autism and schizophrenia while mutations in L1 cause the neurological CRASH disorder, the symptoms of which include mental retardation and spastic paraplegia. The roles of L1CAMs underlying these diseases are not clear. *C. elegans* has two L1CAMs: SAX-7 and LAD-2, which is a non-canonical L1CAM with a divergent cytoplasmic tail. We previously showed SAX-7 and LAD-2 have non-overlapping functions with LAD-2 mediating axon guidance and SAX-7 maintaining the positions of motor neurons along the ventral nerve cord. We determined that for proper function, SAX-7 requires intracellular interactions that include STN-2/γ-syntrophin. Syntrophins are known to associate with dystrophin, an actin-binding molecule that is best characterized for its role in maintaining muscle integrity. Mutations in dystrophin result in Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy. In addition to progressive muscle weakness, many DMD patients also exhibit cognitive deficits, including mental retardation, thus revealing as-yet-unidentified neuronal roles for dystrophin. We hypothesize that STN-2/γ-syntrophin mediates SAX-7 function in maintaining neuron positions by linking SAX-7 to dystrophin; linkage to dystrophin likely provides SAX-7 anchorage to the actin cytoskeleton, thus leading to increased SAX-7-mediated cell adhesion. Consistent with the hypothesis, our analysis revealed that dys-1 genetically interacts with sax-7 and plays a role in maintaining neuronal positioning. Furthermore, our protein recruitment assays in HEK293 cells strongly support a molecular interaction between SAX-7 and DYS-1 that requires the presence of STN-2 as a linking molecule. Lastly, we show that STN-2 is required in neurons to mediate SAX-7 function in maintaining neuronal positioning. Taken together, these results point to a novel role for dystrophin in maintaining neuronal positions via its molecular interaction with L1CAMs.

492C

G-protein signaling modulates the activity state of the ASH sensory neurons. **Rachel T. Wragg**, Gareth P. Harris, Vera M. Hapiak, Holly J. Mills, Amanda Korchnak, Sarah B. Miller, Richard W. Komuniecki. Dept Biological Sci, Univ Toledo, Toledo, OH.

Monoamines modulate key behaviors in both vertebrates and invertebrates, but our understanding of the mechanisms underlying aminergic modulation is still in its infancy. In *C. elegans*, food/5-HT stimulate aversive responses to dilute octanol through three distinct 5-HT receptors that appear to operate at different levels within the ASH-mediated locomotory circuit. In contrast, octopamine inhibits 5-HT dependent increases in responses to dilute octanol. The expression of SER-5 and F14D12.6 in the ASHs is essential for this serotonergic stimulation and octopaminergic inhibition, respectively (Harris *et al*., 2009, J. Neuroscience 29, 1446-1456; Wragg *et al*., 2007, J. Neuroscience 27:227-244). Based on the expression of full-length translational fusions, both receptors appear to be expressed in axons of the ASH and presumably function to modulate neurotransmitter release. In the present study, we have used combination mutants and the ASH RNAi knockdown and overexpression of key G-protein signaling molecules to better understand the pathways downstream of these receptors in modulating NT release. Animals with increased Gαs or Gαq signaling (*gsa-1gf*, *acy-1gf*, *pde-4lf* and *egl-30gf*) exhibited elevated basal responses to dilute octanol. As predicted from these results, the ASH RNAi knockdown of *gsa-1*, *acy-1* or *kin-2*, using the *Psra-6* promoter, abolished 5-HT dependent increases in aversive responses to dilute octanol. In contrast, the ASH knockdown of Gαo (*goa-1*) had no effect on basal or 5-HT stimulated responses to dilute octanol, but completely abolished OA inhibition through a pathway requiring F14D12.6. Together, these results suggest G-protein signaling differentially modulates the responsiveness of the ASHs to dilute octanol. These studies are continuing to examine the role of SER-5 and F14D12.6 signaling in the ASHs.

493A

C. elegans WSP-1 is a putative synaptic transmission regulator at the neuromuscular junction. **Yuqian Zhang**, Terry kubiseski. Biology, York University, Toronto, ON, Canada.

The interest of our lab is to investigate the role of RhoGTPase family in neuronal development. RhoGTPases are involved in modulating the actin cytoskeleton and promoting polarity in a number of different cells. The three mostly studied RhoGTPases are Rho, Rac and Cdc-42. Neuronal Wiskott-Aldrich syndrome protein (N-WASP) is a downstream effector of CDC-42 in central nervous system and a key regulator of actin reorganization. It has been reported that in human intractable epilepsy patients, both N-WASP and CDC-42 are significantly upregulated, which indicates either a cause or consequence (1). We have determined that CDC-42 appears to regulate synaptic transmission based on the uncoordinated phenotype of cdc-42 mutant and the fact that cdc-42 homozygous worms showed hypersensitivity to acetylcholine esterase inhibitor : aldicarb and furthermore, an “epileptic like” behavior on plates containing PTZ, a GABA receptor antagonist (2). C. elegans wsp-1 has two splicing transcripts, a major transcript which is the homologue of mammalian N-Wasp, and a minor transcript containing a region unique to C. elegans wsp-1 (3). To study the role of wsp-1 in neuronal development, we obtained a strain named NG324, which has a portion of the major transcript deleted. We found that this wsp-1 mutant is also hypersensitive to aldicarb and were able to rescue this phenotype with somatically expressed transgene wsp-1. We propose that WSP-1 is also a synaptic regulator at the neuromuscular junction. But wsp-1mutant did not show obvious phenotype on PTZ plates, which suggests that the interaction of WSP-1 to CDC-42 may not be essential for CDC-42 function in GABAnergic synaptic transmission. To better understand the role of wsp-1, we are generating double mutants with known roles in synaptic transmission, one we have made is unc13(e51); wsp-1(gm324). We found that deletion of the major transcript of wsp-1 in unc13 mutant can switch its aldicarb sensitivity from resistant to sensitive, which indicates that WSP-1 may negatively regulate the neurotransmitter release. We are currently performing transgene rescue experiment to understand the exact function site of wsp-1 in synaptic transmission. References 1.Xiao F, Wang XF, Li JM et al. (2008) Overexpression of N-WASP in the brain of human epilepsy Brains Research 1233:168-175 2.Locke C, Berry K et al. (2008) Paradigms for pharmacological characterization of C. elegans synaptic transmission mutants J Vis Exp 18: 837 3.Sawa M, Suetsugu S et al. (2003) Essential roles of the C. elegans Arp2/4 complex in cell migration during ventral enclosure Journal of Cell Science 116:1505-1518.

494B

Sac1p- and Fig4p-like lipid phosphatases act in the nervous system of *Caenorhabditis elegans*. **Wiebke A Sassen[1,2]**, Eugenia Butkevich[1], Dieter Klopfenstein[1]. 1) DFG Research Center Molecular Physiology of the Brain, Goettingen, Germany; 2) Goettingen Graduate School for Neurosciences and Molecular Biosciences.

The SAC domain is a lipid polyphosphatase domain conserved in most eukaryotes. It dephosphorylates the headgroup of Phosphatidylinositolphosphates (PIP), a class of phospholipids which is a key regulator of membrane trafficking.

The SAC domain was first described in the Yeast lipid phosphatase Sac1p which is a transmembrane protein at the ER and Golgi regulating the amount of PI(4)P. A second SAC domain-containing phosphatase named Fig4p is recruited by adapter proteins to the Yeast vacuole membrane where it converts $PI(3,5)P_2$ to PI(3)P. Both enzymes are essential for membrane trafficking and secretion.

In mammalia, the SAC domain is found in the Sac1p homologs SAC1 and SAC2 and in a Fig4p homolog. SAC1 complements the deletion of *sac1* in Yeast. All three proteins are found in neurons, one of which, FIG4 has been implicated in Charcot-Marie-Tooth neuropathies.

Like mammals, *C. elegans* owns two Sac1p homologs F30A10.6 and W09C5.7 and a single Fig4p homolog C34B7.2. F30A10.6 is related to mammalian SAC1 whereas W09C5.7 seems to be the SAC2 ortholog of *C. elegans*. Thus, the nematode is a perfect model system to study the function of these three lipid phosphatases in higher eukaryotes.

C34B7.2 is known to be mainly expressed in the nervous system localizing to synaptic vesicles. RNAi-knockdown causes a decrease in acetylcholine release at neuromuscular junctions. We verified this phenotype for C34B7.2 and in addition also reveal it for F30A10.6. Accordingly, both phosphatases play a role in synaptic vesicle trafficking. In contrast, the knockdown of W09C5.7 did not influence the release of neurotransmitters at neuromuscular junctions. The expression patterns of W09C5.7 and F30A10.6 are unknown.

To study functional conservation of the *C. elegans* phosphatases we expressed HA-tagged proteins in a Yeast *sac1* deletion strain. We show that F30A10.6 can rescue several phenotype characteristics of the Yeast *sac1* knockout, while W09C5.7 and C34B7.2 failed to do so. Consequently, we suggest to change the Accession number F30A10.6 to the gene name *sac-1*.

495C

Beta subunits CCB-1 and CCB-2 modulate voltage-dependent calcium currents in *C. elegans* muscle cells. **Maelle Jospin**. Université de Lyon 1, UMR CNRS 5123, Villeurbanne, France.

As in other organisms, voltage-gated calcium channels mediate the calcium influx necessary for activation of contractile proteins in *C. elegans* muscle cells. Muscle voltage-gated calcium channels are typically composed of an alpha1 pore-forming subunit and three accessory subunits, named beta, alpha2/delta and gamma. We previously showed that voltage-dependent calcium currents are mainly supported by the alpha1 subunit EGL-19 in *C. elegans* muscle cells (Jospin et al., 2002, J Cell Biol 159:337-348). In the present study, we investigate the role of the two putative beta subunits, *ccb-1* and *ccb-2*, on the properties of this current. For this purpose, we recorded voltage-dependent calcium currents in *C. elegans* muscle cells, using *in situ* patch clamp techniques, from wild type, *ccb-2(ok862)* and *ccb-1(RNAi)* worms. Preliminary results show that voltage-dependent calcium currents are smaller in *ccb-1(RNAi)* mutants compared to that of the wild type. In contrast, *ccb-2(ok862)* muscle cells exhibit currents of bigger amplitude compared to that of the wild type. We conclude from these results that both beta subunits modulate voltage-dependent calcium currents in muscle cells and that they act in an antagonist manner.

496A

The Molecular Basis of Ethanol Response in *C. elegans*. **Jill Bettinger**, Jennifer Gardner, Mia Bolling, Emily Smail, Andrew Davies. Dept Pharmacology/Toxicology, Virginia Commonwealth, Richmond, VA.

We study acute responses to ethanol intoxication, with the goal of identifying the mechanisms by which neurons are affected by and respond to ethanol. Initial (naïve) sensitivity to ethanol is widely variable in humans, and has been correlated with lifetime propensity to abuse alcohol. Initial sensitivity is a complex phenotype that results from at least two different components; the instantaneous responsiveness of neurons to ethanol, and the development of acute functional tolerance, which is an immediate (within minutes) adaptation to the effects of the drug. Worms are a good model for the effects of ethanol on neurons; they respond to similar doses as those that intoxicate humans, and they display both components of initial sensitivity. Our laboratory has taken a two-pronged approach to identifying the molecules that render neurons responsive to ethanol. First, we have performed forward genetic screens for animals with altered development of acute functional tolerance. We have identified at least 10 complementation groups of genes that disrupt acute tolerance. One of the mutations was in the putative transcription factor *ctbp-1*, which is expressed in a small number of neurons, suggesting that we can identify an anatomical locus for the development of acute tolerance. Second, we are screening mutations in candidate neuronal genes for effects on initial sensitivity to ethanol. We are performing an exhaustive characterization of all genes known to be involved in synaptic transmission, a suspected target of ethanol's action.

497B

Screening for targets of ethanol that mediate effects on locomotion in *C. elegans*. I. Martin, R.I. Friedberg, K.S. Meyers, C.R. Burnette, J.C. Bettinger, **A.G. Davies**. Pharmacology & Toxicology and Psychiatry, Virginia Commonwealth University, Richmond, VA.

The acute sensitivity of a human individual to ethanol's intoxicating effects correlates negatively with predisposition towards alcoholism. We seek to understand mechanisms of ethanol intoxication in *C. elegans* to identify candidate genes and pathways that may vary in human individuals with differing acute responses to alcohol. Using forward genetics, we previously have identified the BK potassium channel encoding gene, *slo-1*, as a significant mediator of the acute effects of ethanol on locomotion and egg-laying behaviors. Given that mutant animals carrying complete loss-of-function mutations in the *slo-1* gene show ethanol-induced decreases in the speed of locomotion, we surmise that there must be additional mediators of ethanol's effects in this organism.

By screening for new mutations that enhance the ethanol resistance of a *slo-1* null mutant we expect to identify genes that act additively to, and independently of, *slo-1* to mediate the effects of ethanol on locomotion. So far we have screened ~6000 randomly mutagenized haploid genomes and identified 15 mutant strains that appear to have reduced sensitivity to the effects of ethanol on locomotion compared with the *slo-1* null mutant parent animals. The increase in ethanol resistance generated in the *slo-1* mutant background by most of the new mutations varies between 10-30% depending on the strain. In addition, several of the isolated strains appear to increase the basal (untreated) speed of the *slo-1* mutant animals, which is decreased relative to wild-type animals. These changes in basal speed appear to occur without altering the degree of ethanol resistance provided by the *slo-1* mutation. These particular mutations are unlikely to affect mechanisms of ethanol intoxication but may prove useful in understanding the role of SLO-1 in regulating locomotion, as they appear to compensate to some extent for loss of SLO-1 function. Mapping of several of the mutations that appear to alter ethanol sensitivity is underway with the aim of identifying genes using a positional cloning strategy. Funded by ABMRF and NIAAA.

498C

UNC-73 RhoGEF-2 Isoforms regulate locomotion in *C. elegans*. **Shuang Hu**, Robert Steven. University of Toledo, Toledo, OH.

The sinusoidal movement of *C. elegans* is generated by coordinated contraction and relaxation of the body wall muscles. This activity is controlled by excitatory, inhibitory and modulatory signals from the nervous system. We are interested in the role that the Rho GTPase pathway plays in this signaling. The *unc-73* gene encodes eight isoforms that contain up to two RhoGEF domains. In the nervous system the UNC-73 RhoGEF-1 domain specifically activates Rac GTPases in the process of axon guidance, while the RhoGEF-2 domain specifically activates Rho to influence neurotransmission (Steven et al, 1998, 2005). *unc-73* RhoGEF-2 domain mutants have a lethargic and egg-laying phenotype similar to $G\alpha_q$ pathway mutants and UNC-73E, a RhoGEF-2 isoform, is a $G\alpha_q$ (EGL-30) effector acting in parallel with PLC_β (Williams et al, 2007). However, we have determined that activation of the $G\alpha_s$ pathway suppresses the slow locomotion phenotype of the *unc-73* RhoGEF-2 domain mutants while the activated $G\alpha_s$ pathway does not suppress the $G\alpha_q$ lethargic phenotype (Reynolds et al, 2005). Here we attempt to better define the role of the UNC-73 RhoGEF-2 isoforms in neurotransmission and their relationship with the $G\alpha_q$ and $G\alpha_s$ pathways. UNC-73E expression in the cholinergic motor neurons fails to rescue the locomotion defects of RhoGEF-2 domain mutants. However, knocking out UNC-73 RhoGEF-2 isoforms in the cholinergic motor neurons by cell specific RNAi causes a decrease in locomotion rate and an unexpected coiler phenotype. To examine the functions of other genes in the cholinergic motor neurons, we expressed *egl-30*, *unc-31*, and *rho-1* RNAi constructs driven by the same *unc-17* cholinergic motor neuron promoter. *punc-17::unc-31(RNAi)* animals have a slow and coiled appearance similar to *punc-17::unc-73(RNAi)* animals. Our results suggest that UNC-73 plays an important role in the regulation of locomotion in the cholinergic motor neurons, and this regulation may involve the release of neuromodulators through UNC-31. *unc-73* RhoGEF-2 mutants do not show significant drug resistance in an aldicarb resistance assay, indicating that UNC-73 is unlikely to have a general role in neurotransmitter release. However, additional preliminary data from a coelomocyte uptake assay indicate neuropeptide release decreases in an *unc-73* RhoGEF-2 domain mutant, suggesting UNC-73 is involved in neuropeptide release. We are currently making double mutants to more closely examine the relationship between *unc-73* and the $G\alpha_q$ and $G\alpha_s$ pathways. These studies will help determine the role of UNC-73 and Rho GTPase signaling in neurotransmission and the regulation of locomotion.

499A

Forward Genetic Analysis of the Synaptic $G\alpha_s$ Pathway. Barret C. Phillips, Stacey L. Edwards, **Kenneth G. Miller**. Genetic Models of Disease Program, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Neurons communicate with each other and with muscle cells at synapses. A long term goal of this lab is to bridge the gap between synaptic function and the control of behavior by determining how signal transduction pathways regulate the activity of synapses. Our studies focus on the neuromuscular synapses that control locomotion in *C. elegans*. Genetic studies have shown that the integrated activities of at least 4 major, conserved $G\alpha$ pathways control synaptic activity to produce the *C. elegans* locomotion behavior. Within this network, the neuronal $G\alpha_s$ pathway is an especially critical, but poorly understood, link between synaptic function and behavior- driving both primordial synaptic functions, such as *C. elegans* locomotion, and higher synaptic functions such as sleep, learning, and memory. However, the key proteins through which the $G\alpha_s$ pathway acts to mediate these functions remain poorly understood or simply unidentified. To address this, we have undertaken forward genetic screens to isolate mutants that suppress the strong phenotypes of *kin-2(ce179)* mutants, containing hyperactivated Protein Kinase A. These mutants exhibit hyperactive locomotion, slow growth, and aldicarb hypersensitivity resulting from overactivation of the $G\alpha_s$ pathway in both muscle cells and neurons. In Screen A, we are using the COPAS Biosort to plate 3 adult *kin-2(ce179)* (F1 progeny of EMS-mutagenized animals) per well on 24-well plates and screening for adult F2 mutants with improved growth and/ or sluggish locomotion. In Screen B, we are plating 200 *kin-2(ce179)* L1s (F2 progeny of mutagenized animals) per well on 24-well plates containing 0.1 mM Aldicarb and screening for the presence of F3 and F4 progeny after 7d at room temperature. We have nearly completed the screening cycles and have begun crossing the suppressor mutations away from the parent strain, mapping, and complementation analysis. Of special interest are several classes of locomotion-impaired mutants isolated in both screens that do not correspond to previously identified *unc* genes. Despite the large number of genes known to confer aldicarb resistance, a group of 11 mutants from screen B included only 1 allele of the synaptic vesicle priming protein UNC-13 (which was only borderline resistant to 0.1 mM Aldicarb as a double with *kin-2(ce179)*), 3 alleles of *egl-10* (RGS for $G\alpha_o$), and 7 mutants that do not correspond to known aldicarb resistance loci. The semi-clonal design of screen A allowed us to isolate 10 sterile paralyzed *kin-2(ce179)* suppressors, 2 of which are null mutants of the $G\alpha$ GEF RIC-8 and at least one of which is not *ric-8*.

500B

Acetylcholine-Gated Chloride Channels (ACCs) are widely expressed in the nervous system suggesting a central role for fast inhibitory cholinergic neurotransmission in C.elegans. **Claudia M Wever**, Patrick Janukavicius, Igor Putrenko, Joseph A. Dent. Deptartment of Biology, McGill University, Montreal, Quebec, Canada.

The cloning and characterization of the acetylcholine-gated chloride channels (ACCs) in C. elegans was the first indication that nematodes employ acetylcholine as a fast (ionotropic) inhibitory neurotransmitter. We previously characterized two subunits, ACC-1 and ACC-2, which form homomeric acetylcholine-sensitive chloride channels and a third subunit, ACC-3 that forms heteromeric channels with ACC-1 (Putrenko et al., 2005). However, there are eight subunits in the clade to which ACC-1, -2 and -3 belong. To better understand the role of acetylcholine in C. elegans behaviour, we have been characterizing the expression patterns of the eight ACC-like subunits and characterizing their pharmacology in Xenopus oocytes. To perform electrophysiological analysis of the ACC-like subunits, we have injected subunit RNA into Xenopus oocytes and exposed these injected oocytes to different concentrations of acetylcholine, recording the electrical changes that are elicited. We have shown that K10D6.1, a previously uncharacterized subunit in the ACC clade, forms a functional homomeric channel that responds to acetylcholine, with an EC_{50} of 20.43 μM ACh(± 6.13). We have also found that F47A4.1, another uncharacterized subunit, appears to interact with ACC3 to form a heteromeric acetylcholine-gated channel with an EC_{50} of 250 μM ACh (± 47). The expression patterns of the ACC-like subunits were determined by microinjection of promoter-GFP fusion constructs. All of the subunits are expressed in roughly 20 neurons in C.elegans. Among the many neurons in which ACC-1 is expressed is MC, a neuron critical for pharyngeal pumping. As MC is a cholinergic motor neuron, ACC-1 expression may implicate the ACC subunits in autocrine feedback inhibition. ACC-3 is expressed in many neurons as well, including AWB, a chemosensory neuron. F47A4.1, is expressed in PVD, a mechanoreceptor neuron and AFD, a thermosensory neuron. It is also expressed in HSN, which is critical for egg laying. Expression in HSN is of particular interest because of the known roles of acetylcholine in regulation of HSN by the VC neurons. Various subunits also express in ventral cord motor neurons. The wide variety of neurons in which the different ACC subunits are expressed highlights the vast potential of acetylcholine as an inhibitory neurotransmitter in C.elegans. We have obtained deletion strains for ACC-1, ACC-2, ACC-3 and ACC-4. All these strains are viable with no obvious phenotypes. We are investigating subtle behavioural phenotypes associated with deletions in these subunits. This work is supported by NSERC and Chemtura Co.

501C

The cell adhesion molecule RIG-3 regulates neuropeptide secretion. **Kavita Babu**, Zhitao Hu, Joshua Kaplan. Dept Molecular Biol, Massachusetts General Hosp, Boston, MA.

Cell Adhesion molecules (CAMs) play important roles at the synapse. They are required for maintaining the integrity of the synaptic junction and promoting the stability of the synapse by linking the pre- and post-synaptic membranes. Target recognition, signal transduction, differentiation of pre- and post-synaptic specialisation's and regulation of synaptic structure and function are important functions of CAMs (Curr Opinion in Cell Biol. 2003, 15:621-632). To identify CAMs that regulate the body wall neuromuscular junction (NMJ) we have done a screen for changes in aldicarb sensitivity following RNAi of 186 putative cell adhesion molecules (Trends in Genet 1999, 15:M33-37, J Cell Sci 2004, 117:1167-70). Aldicarb is an acetylcholine esterase inhibitor that causes hypercontraction of muscles in wild type animals. Mutants with defects in synaptic transmission have altered rates of paralysis to aldicarb. One of the positives from the screen was RIG-3 (neuRonal IGCAM-3), which is a GPI anchored Ig domain molecule. Inactivation of rig-3(by RNAi or knockout allele) caused hypersensitivity to aldicarb. A rig-3 promoter construct was expressed in ventral cord motor neurons, as well as many other neurons. Expression of rig-3 transgenes in cholinergic neurons or in body muscles rescued the aldicarb defect, whereas expression in GABA neurons failed to rescue this defect. Thus, RIG-3 function in cholinergic neurons or in body muscles regulates aldicarb responsiveness. To determine the cause for the aldicarb phenotype, we analyzed several synaptic markers and recorded post-synaptic currents in rig-3 mutants. We found no significant changes in the rates of endogenous cholinergic or GABAergic transmission (EPSC and IPSC, respectively) in rig-3 mutants. Similarly, the distribution of presynaptic markers (SYD-2::GFP and GFP::SNB-1) were unaltered in both cholinergic and GABAergic motor axons. Taken together, these results suggest that synaptic vesicle (SV) fusion occurs normally in these mutants. By contrast, several results suggest that rig-3 mutants have decreased neuropeptide secretion. When two fluorescently-tagged neuropeptide (INS-22::YFP and NLP-21::YFP) markers were expressed in the cholinergic DA motor neurons, we observed increased dorsal cord puncta fluorescence and decreased coelomocyte fluorescence in rig-3 mutants. These results suggest that the aldicarb hypersensitivity observed in rig-3 mutants is a secondary consequence of decreased neuropeptide secretion.

502A

eat-6 is involved in modulation of excitatory neurotransmission by serotonin. **Elena G. Govorunova**, Mustapha Moussaif, Andey Kullyev, Thomas V. McDonald, Ji Y. Sze. Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

Mutations in a human homolog of the *C. elegans eat-6* gene encoding a Na^+, K^+ ATPase alpha subunit (Davis et al., 1995) has been associated with familial hemiplegic migraine. Migraine is the most common neurological disorder linked to low serotonergic disposition. We sought to study pathophysiological mechanisms of migraine and their relation to serotonin signaling using *C. elegans* as a genetic model. In *C. elegans*, exogenous serotonin inhibits ACh neurotransmission at the body-wall neuromuscular junctions (Nurrish et al., 1999). Worms carrying the *ad467* mutation in the *eat-6* gene show hypersensitivity to the ACh esterase inhibitor aldicarb (Doi and Iwasaki, 2008), and are strongly resistant to exogenous serotonin in the aldicarb assay. RNA*i* of *eat-6* renders worms hypersensitive to aldicarb, similar to that observed in the *eat-6(ad467)* mutant. Electropharyngeograms recorded from these worms reveal changes in the electrical activity of the pharynx, partially overlapping with those found in the *eat-6* mutant. Interestingly, overexpression of *eat-6* in the cholinergic neurons in the WT background confers hypersensitivity to aldicarb, suggesting that both an increase and a decrease in EAT-6 activity result in excessive ACh neurotransmission. Overexpression of *eat-6* in the body-wall muscles causes hypersensitivity to the AChR agonist levamisole, indicating that *eat-6* acts both pre- and postsynaptically in modulation of ACh neurotransmission at the body-wall neuromuscular junctions. The *tph-1* mutant that lacks serotonin shows modest hypersensitivity to aldicarb. Analyses of the *tph-1* mutants expressing the WT *tph-1* gene in specific serotonergic neurons suggest that serotonin released from the ADF neurons stimulates ACh neurotransmission, whereas serotonin released from the NSM and RIH/AIM neurons inhibits it. We hypothesize that the *eat-6* mutant is deficient in the inhibitory serotonin signaling by the NSM and RIH/AIM neurons. The role of *eat-6* in modulation of excitatory neurotransmission by serotonin may explain the link between serotonin signaling and migraine in human patients.

503B

Dopamine and octopamine regulate cholinergic neurotransmission. **Satoshi Suo**, Joseph Culotti, Hubert Van Tol. SLRI, Samuel Lunenfeld Research Institute, Toronto, ON, Canada.

Animals assess food availability in their environment by sensory perception and respond to the absence of food by changing hormone and neurotransmitter signals. In Caenorhabditis elegans, octopamine is released from the RIC neurons in the absence of food and activates the cyclic AMP response element binding protein (CREB) homologue CRH-1 in the cholinergic SIA neurons through the octopamine receptor SER-3. In contrast, dopamine signaling is activated only in the presence of food. We showed previously that dopamine suppresses octopamine signaling through two Gi/o-coupled D2-like dopamine receptors DOP-2 and DOP-3. The D2-like receptors work in both the octopaminergic neurons and the octopamine-responding SIA neurons, suggesting that dopamine suppresses octopamine release as well as octopamine-mediated downstream signaling. Therefore, the decrease of dopamine signaling in the absence of food activates octopamine signaling.

In this study, we examined the effects of dopamine and octopamine on acetylcholine signaling. We first found that the dopamine-deficient cat-2 mutant is mildly resistant to aldicarb, a cholinesterase inhibitor. dop-2;dop-3 double mutants also exhibited aldicarb resistance. These results suggest that impaired dopamine signaling results in reduced acetylcholine signaling. Aldicarb resistance of cat-2 mutants was suppressed by tbh-1, which is required for octopamine synthesis, indicating that activation of octopamine signaling in the absence of dopamine causes the aldicarb resistance. Octopamine likely works through SER-3 and CRH-1 since ser-3 and crh-1 similarly suppressed aldicarb resistance of cat-2 mutants. Collectively, the results suggest that octopamine signaling negatively regulates cholinergic neurotransmission whereas dopamine signaling positively regulates it by suppression of octopamine signaling.

504C

The role of EGL-8 at the *C.elegans* neuromuscular synapse. **Rachael E Ward**, Stephen J Nurrish. Cell Biology Unit, MRC Laboratory for Molecular Cell Biology, UCL, London, United Kingdom.

The membrane bound second messenger diacylglycerol (DAG) plays a key role in the regulation of neurotransmitter release in both mammals and the *C. elegans*. When activated by EGL-30 (Gαq), EGL-8 (Phospholipase C-β; PLC-β) hydrolyses phosphatidylinositol (4,5) biphosphate (PIP_2) to produce DAG and inositol 1,4,5-triphosphate (IP_3). It has previously been shown that EGL-8 is required in the motorneurons of the *C. elegans* to stimulate the production of presynaptic DAG and facilitate acetylcholine (ACh) release at the neuromuscular junction (NMJ), demonstrating that the DAG that modulates ACh release is produced by EGL-8. In motoneurones, DAG is spatially restricted to neurotransmitter release sites. What controls this specific localisation is, at present, unclear. One possible mechanism is that EGL-8 plays a role in DAG localisation. EGL-8/PLC-β contains a putative PDZ domain on its C-terminal, suggesting it may mediate the clustering of receptors and signalling molecules in the motoneuron. Indeed, the *Drosophila* PLC-β; NORPA, is known to form part of a signalling complex in the phototransduction signalling pathway that includes the scaffolding/PDZ-containing protein; INAD and the 7 transmembrane receptor; rhodopsin. In the present study, we are using histological and genetic techniques to investigate the putative role of EGL-8 in DAG localisation at the *C. elegans* motoneuron. We are taking advantage of a Mos1 site within EGL-8 to generate transgenic animals using Mos1-mediated mutagenesis. In addition, we will use ultrastructural and immunohistochemical techniques to demonstrate the localisation of components of the cholinergic signalling pathway within the motoneuron and their effects on synapse formation.

505A

Regulation of locomotion by PKC phosphorylation of UNC-18. Mark R. Edwards, Alan Morgan, Robert D. Burgoyne, **Jeff W. Barclay**. Sch Biomedical Sci, Univ Liverpool, Liverpool, United Kingdom.

Munc18-1 is an essential protein in exocytosis, primarily characterised via an interaction with the SNARE protein syntaxin. Phosphorylation of Munc18-1 by Protein Kinase C (PKC) reduces its binding affinity for syntaxin. Expression of a PKC phosphomimetic mutant Munc18-1 in mammalian cells reduces quantal size and accelerates the kinetics of individual fusion events. In C. elegans UNC-18 can be phosphorylated by PKC in vitro; however, the precise site(s) of phosphorylation are unknown, as are the in vivo effects of phosphorylation on C. elegans behaviour. We confirm that UNC-18 is indeed phosphorylated by PKC and demonstrate Ser322 as an in vitro phosphorylation site. We show that a putatively phosphomimetic mutant, S322E has reduced binding affinity for C. elegans syntaxin, UNC-64. To explore the phenotypic effects of PKC phosphorylation of UNC-18, the unc-18 null mutant e81 was transgenically rescued with mutants that were nonphosphorylatable (S322A) or were putative phosphomimetic (S322E). Both mutant worms were indistinguishable from transgenic rescues with wildtype UNC-18 when comparing thrashing in solution and sensitivity to aldicarb. Interestingly, blocking the PKC phosphorylation site (S322A) eliminated food-induced slowing of locomotion. UNC-18 S322A worms moved as slow in the absence of food as in the presence of food. This decrease in locomotion rate of S322A worms was correlated with a concomitant increase in the number of spontaneous reversals performed in the absence of food. We conclude that worm locomotion rate can be regulated via a signalling pathway involving PKC phosphorylation of the synaptic protein UNC-18 on Ser322.

506B

Characterization of Calcium Binding Kinase Interacting Protein F30A10.1. **R.C. Caylor**, B.D. Ackley. Department of Molecular Biosciences, The University of Kansas, Lawrence, KS 66045.

Nidogen is an evolutionarily conserved basement membrane protein that functions with the LAR receptor and intracellular scaffold molecule α-liprin to regulate synapse morphology. *nid-1 (cg119)* mutants exhibit diffuse synaptic vesicles rather than the punctuate structures observed in wild type. We identified *F30A10.1* as a novel nidogen suppressor in an RNAi screen. *F30A10.1* encodes a calcium binding kinase interacting protein. It is an ortholog of the vertebrate calcium and integrin binding protein (CIB1). A deletion allele, *tm1353*, was able to suppress the nidogen synaptic phenotype thus corroborating the RNAi results. An F30A10.1 full length GFP fusion demonstrated fluorescence beginning early in embryogenesis starting after gastrulation and throughout the animal's lifetime. GFP is primarily observed in the nervous system and musculature including the body wall muscles, pharyngeal, and vulval muscles.

Previously we have found that the calcium channel subunits *unc-2* and *unc-36* also suppressed the synaptic patterning defects in nidogen mutants. Alone *unc-2* and *unc-36* mutants have ~1.5 fold increase in the size of the vesicular puncta. The *tm1353* allele phenocopies *unc-2* and *unc-36*, and *unc-2 (e55); tm1353* and *unc-36 (e251);tm1353* worms do not show any significant increase in synapse size suggesting these molecules are working in a linear pathway. However, it was not apparent how *unc-2*, *unc-36* and *F30A10.1* function to suppress *nid-1*.

To identify calcium-dependent binding partners of F30A10.1, we immobilized F30A01.1 protein to sepharose beads. N2 lysate was run over the column in the presence of calcium. Bound protein was eluted with EDTA. Bands on an SDS gel were visualized by Coomassie and identified by mass spectrometry. One of the hits was the scaffolding protein RACK-1. *rack-1(tm2262)* mutants display diffuse synaptic puncta similar to nidogen mutants. *tm2262; tm1353* double mutants have synaptic areas that are smaller than both *tm2262* and *tm1353* mutants alone. These results suggest that RACK-1 and F30A10.1 might antagonize each other in synaptic development and/or maintenance. A simple model to explain our results suggests that *unc-2*/*unc-36* could be an activator of F30A10.1 and that RACK-1 inactivates Ca-bound F30A10.1. Our data also suggest that *rack-1* may be in a linear pathway with *nid-1*, and we are currently examining that possibility. Together these results begin to describe how two pathways that function antagonistically may sculpt the accumulation of synaptic vesicles in the proper size and region of axons.

507C

Sphingosine kinase regulation of the synaptic vesicle cycle. **Jason P. Chan**, Derek S. Sieburth. Zilkha Neurogenetic Institute, University of So. California, Los Angeles, CA.

Sphingosine kinase (SK1) catalyzes the phosphorylation of sphingosine to sphingosine-1-phosphate, a bioactive lipid implicated in exocytosis, calcium homeostasis and membrane dynamics. However, the functions of SK1 at synapses remains unclear. Recent findings from an RNAi screen for regulators of synaptic transmission in the C. elegans suggested that SPHK-1, the ortholog for mammalian SK1, acts in the synaptic vesicle cycle. Thus, to further examine the synaptic role of SPHK-1, we obtained mutants in sphingosine kinase from the KO consortium (ok1097). This allele contains deletions of exons I–V and part of exon VI, resulting in a protein lacking the catalytic domain of SPHK-1. We found that sphk-1 mutants are uncoordinated and show resistance to inhibitors of cholinesterase (RIC), suggesting a defect in ACh secretion at the neuromuscular junction (NMJ). Pan-neuronal re-expression of SPHK-1, but not a kinase dead SPHK-1, is sufficient to rescue the RIC phenotype. These findings suggest a presynaptic role of SPHK-1 and S1P in neurotransmission. Consistent with this, we show that SPKH-1 is expressed motor neurons and a functional GFP-tagged SPHK-1 fusion protein (SPHK-1::GFP) adopts a punctate pattern of fluorescence in motor neuron axons. The SPHK-1::GFP punctal fluorescence partially colocalizes with the synaptic vesicle proteins RAB-3 and SNB-1 and several periactive zone proteins. Thus, SPHK-1 may be a novel synaptic player that regulates neurotransmitter secretion. To determine the signaling pathways SPHK-1 acts in, we generated double mutants for sphk-1 and one of several signaling molecules (e.g. egl-30, goa-1, etc.) and examined their behavior on an aldicarb-induced paralysis assay. We find that SPHK-1 may function downstream of the Gαq/DAG signaling pathway. By examining the distribution of SPHK-1::GFP in several synaptic transmission mutants, we find that Gαq signaling regulates synaptic abundance of SPHK-1::GFP. To further address the functions of SPHK-1, we have begun to examine the how loss of SPHK-1 alters the distribution of a panel of fluorescently tagged synaptic markers. We find that that sphk-1 mutants exhibit an accumulation of synaptic vesicles and dense core vesicles at synapses (using tagged SNB-1, RAB-3, and INS-22) similar to that seen in exocytosis mutants. Finally, we describe ongoing electrophysiological experiments to test whether SPHK-1 regulates ACh secretion at the NMJ and other cellular analyses to better understand the mechanisms of SPHK-1 action. We propose that SPHK-1 is a previously uncharacterized protein player that regulates the synaptic vesicle cycle at the NMJ.

508A

Understanding the role of PKC-1 signaling pathway in regulating Dense Core Vesicle secretion. **Krishnakali Dasgupta**, Derek Sieburth. Zilkha Neurogenetic Institute , USC, Los Angeles , CA.

Synaptic vesicles (SV) and dense core vesicles (DCV) both exhibit calcium dependent neurotransmitter release. However, differences in their biogenesis, release properties, and recycling suggest that each type of organelle may use some unique proteins to carry out their functions. PKC-1, a protein kinase C ortholog in worms, is one such candidate for a unique DCV regulating molecule, since it has been shown to regulate neuropeptide secretion, while possibly not affecting SV release.(1) PKC-1 is most similar to the human eta and epsilon isoforms which are activated by the second messenger DAG via their C1 domains. In order to identify additional components that act in the PKC-1 pathway to regulate DCV secretion, we have used a constitutively active PKC-1 mutant which lacks the autoinhibitory domain and is predicted to be active in the absence of DAG. Expression of this transgene specifically in motor neurons causes hypersensitivity to an acetylcholinesterase inhibitor, aldicarb (Hic) and loopy locomotion. We screened for suppressors of either the loopy or Hic phenotype of animals expressing the constitutive PCK-1, and identified ~30 suppressors that restore movement or aldicarb response. We are in the process of mapping them and characterizing their effects on neuropeptide secretion. Presently, we have identified one aldciarb resistant candidate allele, vj3, which shows decreased uptake of NLP-21::YFP by coelomocytes and increased accumulation of DCVs in axons. However, vj3 mutants do not cause an accumulation of the SV marker at synapses, suggesting that vj3 mutants specifically reduce DCV secretion. vj3 maps to a small interval on LG I that does not contain any known neurotransmitter secretion genes. We are currently in the process of fine mapping, and tesing candidate genes for aldicarb resistant phenotypes by RNAi. 1.PKC-1 regulates secretion of neuropeptides; Derek Sieburth1, Jon M Madison & Joshua M Kaplan; Nature Neuroscience 10, 49–57 (2007).

509B

The Effect of Hypoxia on Neuronal Necrosis and Glutamate Receptor Trafficking. **Piya Ghose**[1,2], Eun Chan Park*[1], Christopher Rongo[1]. 1) The Waksman Institute, Department of Genetics, Rutgers University; 2) Graduate Program in Neuroscience, Rutgers University.

Neurons in the brain are sensitive to oxygen levels, undergoing necrosis within minutes after hypoxia (e.g., oxygen deprivation during ischemic stroke). This neuronal toxicity is mediated in part by the excitatory neurotransmitter glutamate, which is released at high levels during hypoxia, and the ionotropic glutamate receptors (GluRs) that are trafficked to postsynaptic membranes. However, the mechanistic relationship between hypoxia, GluR function and subcellular trafficking, and excitotoxic necrosis has not been fully elucidated. Here we examine the effects of hypoxia and mutations in hypoxia response factors on necrosis and GluR trafficking in C. elegans. In C. elegans, an excitotoxic-like necrosis can be modeled in vivo by expressing a constitutively active G alpha S subunit in neurons. Activated G alpha S modulates the activity of yet unknown ion channels, resulting in the necrosis of a subset of neurons that express it (1). The resulting intermediate necrosis phenotype affords us the ability to screen for factors that either enhance or suppress neuronal necrosis, including hypoxia. The C. elegans GluR GLR-1 is expressed in these same neurons, where it functions to mediate locomotion reversal behavior (2,3) and is trafficked to synaptic connections (4). We find that hypoxic treatment enhances the necrosis caused by activated G alpha S expression, and alters the trafficking of GLR-1 in these same neurons. We anticipate that our studies will further elucidate the mechanisms of neuronal damage after hypoxia. By identifying the molecular mechanisms that regulate GluR signaling and necrosis in response to hypoxia, we hope to provide useful therapeutic targets for the prevention of nervous system damage after hypoxia exposure.(*co-first author)

References

1. Berger, A.J. et al., G alphas-induced neurodegeneration in Caenorhabditis elegans. J Neurosci. 18,2871-80 (1995). 2. Hart, A.C. et al., Synaptic code for sensory modalities revealed by C. elegans GLR-1 glutamate receptor. Nature. 378,82-5 (1995). 3. Maricq, A.V. et al., Mechanosensory signalling in C. elegans mediated by the GLR-1 glutamate receptor. Nature. 378,78-81 (1995). 4. Rongo, C. et al., LIN-10 is a shared component of the polarized protein localization pathways in neurons and epithelia. Cell 94,51-759, 1998.

510C

Identifying genes required for the miR-1 and MEF-2 induced retrograde synaptic signal. **Sabrina Hom**[1,2], Seungwon Choi[1,2], Zhitao Hu[1,2], Katherine Thompson-Peer[1,2], Joshua Kaplan[1,2]. 1) Dept Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept of Genetics, Harvard Medical School, Boston.

A fundamental question pertaining to the regulation of synaptic activity concerns how pre- and post-synaptic sites coordinate function. While retrograde signaling has been well-documented in the regulation of synaptic activity, little is known about the molecular mechanisms underlying this phenomenon. We have recently demonstrated that the muscle-specific microRNA miR-1 regulates aspects of both pre- and post-synaptic activity at the NMJ, a process that is thought to involve the generation of a retrograde signal resulting in diminished excitatory presynaptic release (Simon et al., 2008). This signal is dependant on the transcription factor MEF-2, indicating that transcriptional regulation is a key component in the retrograde pathway. We have carried out a microarray analysis of mir-1 mutants that reveals a list of about 100 candidate genes that are up- or down-regulated in the mir-1 background. To distinguish those genes potentially involved in the activation of the MEF-2 dependant retrograde signal, we are currently carrying out a small-scale RNAi screen to identify suppressors of the mir-1 induced signal. In identifying and characterizing components of the retrograde signaling pathway, we hope to clarify the roles of miR-1 and MEF-2 in regulating synaptic activity.

511A

The *flamingo* protocadherin regulates cell migration, axon guidance and synapse formation in both cell-autonomous and non-autonomous fashion. **Elvis Huarcaya Najarro**[1], Alexandr Goncharov[2], Yishi Jin[2,3], Brian Ackley[1]. 1) Molecular Biosciences, KU, Lawrence, KS; 2) Division of Biological Sciences, University of California, San Diego, San Diego, CA 92093; 3) Howard Hughes Medical Institute.

The flamingo/starry night protocadherin has been demonstrated to function in the planar cell polarity pathway and separately in axon target recognition. We find that mutants for the *C. elegans* homolog of flaming, *fmi-1/cdh-6*, demonstrate multiple defects in the patterning of the nervous system. Mutations in *fmi-1* cause abnormal distribution of synaptic vesicle clusters in the GABAergic neurons, misrouting of the DD and VD neurons and positioning defects in the DD cells. Additionally, electron microscopy analysis showed that mutations in *fmi-1* cause a failure in synaptic target recognition. In order to determine where *fmi-1* functions, we carried out expression analysis of *fmi-1* by using the putative *fmi-1* promoter to drive expression of GFP and co-injecting the type D-type motor neuron marker P*unc-25*::mCherry or cholinergic neuron marker P*acr-2*::mCherry. These studies suggest that *fmi-1* is expressed in the DD GABAergic, and most, if not all of the cholinergic motorneurons, as well as other cells. However, we find no evidence that the postembryonically derived VD neurons express *fmi-1*. Since the axon pathfinding defects in D-type motor neurons are primarily observed in VD neurons, this observation raises the possibility that *fmi-1* functions in a non-cell autonomous manner. Further, knockdown of *fmi-1* using dsRNA expressed specifically in the cholinergic neurons caused synaptic defects in the VD, but not DD neurons. This indicates that *fmi-1* likely mediates signaling between the cholinergic and GABAergic neurons to establish the requisite patterning of these neurons. For at least the VDs, *fmi-1* appears to act non-autonomously. Results from the Hutter lab and Garriga lab indicate *fmi-1* acts cell autonomously in patterning of interneurons and the migration of the HSN respectively. We'd like to thank M. Zhen for the isolation of two *fmi-1* alleles and N. Alvarez for some preliminary characterization of the axon guidance defects.

512B

Optogenetics tools to dissect small neuronal networks and neuropeptide signalling systems. **Steven J Husson**[1,2], Jana Liewald[1], Christian Schultheis[1], Martin Brauner[1], Karen Erbguth[1], Thorsten Schedletzky[1], Liliane Schoofs[2], Alexander Gottschalk[1]. 1) Institute of Biochemistry, Johann Wolfgang Goethe-University Frankfurt, Germany; 2) Functional Genomics and Proteomics, K.U.Leuven, Belgium.

Light-gated ion channels or pumps such as the blue light-activated depolarizing Channelrhodopsin (ChR2) and the yellow light-driven hyperpolarizing Halorhodopsin (HR) allow optical activation or inhibition in muscles and neurons of live and behaving *C. elegans* (Zhang et al., 2007). Furthermore, inward currents evoked by either ChR2 or HR, as well as muscle currents in response to activating ChR2 in motor neurons, can be directly measured by electrophysiology, while photo-evoked body contraction or elongation of the animal could be monitored at the behavioural level (Nagel et al., 2005; Liewald et al., 2008). These state-of-the-art technologies pave the way for further functional dissection of individual neuronal networks in a detail that is not possible in higher organisms. Doing so, we are investigating some defined neuropeptidergic signalling pathways. Neuropeptide release can be triggered by photo-activating the respective neurons in an acute fashion while effects on behaviour can be observed at the same time. This way, we can correlate neuropeptide action with acute behavioural changes or effects, about which very limited knowledge is currently available in any system. While higher organisms display millions of contributing neurons, only a handful of neurons take part in individual neuronal networks in *C. elegans*. This opens the possibility to study the contribution of each neuron to the function of a small network, for example involved in nociception. The huge advantage of our optogenetics tools is that we can specifically stimulate the sensory input neurons, while other potentially contributing neurons are kept silent. The involvement of different ion channels, receptors or neurotransmitters can be assessed by using different genetic backgrounds, while the physiological properties of each individual neuron will be monitored by electrophysiology. **Liewald JF**, Brauner M, Stephens GJ, Bouhours M, Schultheis C, Zhen M, and Gottschalk A (2008) Optogenetic analysis of synaptic function. Nat Methods, 5, 895-902. **Nagel G**, Brauner M, Liewald JF, Adeishvili N, Bamberg E, and Gottschalk A (2005) Light activation of channelrhodopsin-2 in excitable cells of Caenorhabditis elegans triggers rapid behavioral responses. Curr Biol, 15, 2279-2284. **Zhang F**, Wang LP, Brauner M, Liewald JF, Kay K, Watzke N, Wood PG, Bamberg E, Nagel G, Gottschalk A, and Deisseroth K (2007) Multimodal fast optical interrogation of neural circuitry. Nature, 446, 633-639.

513C

Pharmacogenetic Analysis Reveals a Post-Developmental Role for Rac GTPases in *C. elegans* Dynein-Mediated GABAergic Vesicle Transport. **Bwarenaba B. Kautu**[1], Cody J. Locke[2], Kalen P. Berry[1], S Kyle Lee[1], Kim A. Caldwell[1], Guy A. Caldwell[1]. 1) Biological Sci, Univ Alabama, Tuscaloosa, AL; 2) Neuroscience Program, University of California San Francisco, San Francisco, CA.

The nerve cell cytoskeleton regulates many aspects of neuronal activity. Genetic perturbations of cytoskeletal dynamics can lead to a variety of cognitive and affective disorders. For instance, mutations in microtubule-associated proteins such as LIS1 and dynein, as well as actin-associated proteins such as Rac GTPases and integrins have been thought to underlie epileptic seizures in patients with cortical malformations. Because many developmental genes are pleiotropic in nature, it is plausible that post-developmental roles for these cytoskeletal regulators could be masked by developmental anomalies. Here we report that *C. elegans* with function-altering mutations in canonical Rac GTPase signaling pathway members demonstrated a robust behavioral response to a GABA receptor antagonist, pentylenetetrazole (PTZ). This phenotype is also exhibited by GABA and *lis-1* mutants. Furthermore, these mutants also exhibited hypersensitivity to an acetylcholinesterase inhibitor, aldicarb. Together, exposures to these complementary neural stimulants reveal deficiencies in inhibitory neurotransmission.

RNAi against hypomorphs revealed synergistic genetic interactions between the dynein motor complex and some, but not all, members of Rac signaling pathways. These genetic interactions are consistent with putative Rac-dependent interactions between actin and microtubule networks. In addition, we also found that Rac and integrin mutants (as well as RNAi against some members of Rac signaling pathway) displayed synaptic vesicle trafficking defects in GABAergic D-type motorneurons. Identical phenotypes have been observed previously in *lis-1* and dynein mutants, suggesting that Rac signaling pathway may converge with LIS-1 and dynein to modulate inhibitory GABAergic transmission and, therefore, neuronal synchrony, post-developmentally at *C. elegans* neuromuscular junctions (NMJs). In all, our findings strongly suggest a post-developmental role for an evolutionarily conserved cytoskeletal Rac signaling network, which cooperates with the dynein complex to mediate GABAergic vesicle transport.

514A

Characterization of *C. elegans* Snapin mutants. **Susan M Klosterman**, Ashley A Martin, Hetal Parekh, Anna O Burdina, Szi-Chieh Yu, Janet E Richmond. University of Illinois at Chicago, Chicago, IL.

Synaptic vesicle priming is dependent on the formation of SNARE complexes formed between synaptobrevin, SNAP-25 and syntaxin. The subsequent calcium-dependent release of these fusion-competent vesicles requires the recruitment of the calcium-sensor, synaptotagmin, a protein which is also required for efficient endocytosis. The interaction between the SNARE complex and synaptotagmin is thought to be enhanced by Snapin, a protein first identified as a SNAP-25 interacting molecule and subsequently shown to localize with synaptotagmin to dense core vesicles. Consistent with the proposed function of Snapin, chromaffin cells from Snapin knockout mice exhibit a decrease in the size of the readily-releasable dense core vesicle pool, commensurate with reduce Snapin/synaptotagmin-associated SNARE complex interactions. Whether Snapin plays a similar role in synaptic vesicle release has yet to be fully explored. To address this question, we have begun to characterize a *C. elegans* Snapin (SNPN-1) deletion mutant, obtained from the *C. elegans* knockout consortium and out-crossed in the lab. Preliminary aldicarb and electrophysiological data show a modest reduction in cholinergic transmission in this *snpn-1* mutant, where as synaptotagmin (SNT-1) mutants exhibit a more severe defect in both assays. We are currently exploring whether expression of SNPN-1 under the promoter for the vesicular ACh transporter (*Punc-17*) rescues the *snpn-1* mutant cholinergic transmission defect. As a first step in assessing whether SNPN-1 is involved in SNT-1 recruitment to SNARE complexes following synaptic vesicle priming, we have generated *snt-1;snpn-1* double mutants. Preliminary data suggest that the phenotype of the double mutant is no more severe than either single mutant suggesting these two proteins may act in the same pathway. We are currently conducting electrophysiological experiments to explore the calcium-dependence of the evoked phenotypes of these single and double mutants. In addition we are analyzing mutants prepared by high-pressure freeze fixation for ultra-structural EM analysis. Together these experiments should further define the role of interactions between SNPN-1 and SNT-1 and elucidate Snapin function with regard to synaptic transmission.

515B

Mechanism of Antagonistic Dopamine Receptor Signaling in *C. elegans*. **Kathryn N Maher**, Daniel Chase. Biochemistry and Molecular Biology, University of Massachusetts, Amherst, MA.

Defects in signaling by the neurotransmitter dopamine underlie a variety of neurological disorders including schizophrenia, drug addiction and Parkinson's disease. Despite the clinical importance of understanding how dopamine modulates neural activity, the molecular and cellular basis for its actions remains largely unclear. It is known that dopamine can act both synergistically and antagonistically through two classes of receptors (D1- and D2-like) and that these receptors can be coexpressed in neurons. However, it is not clear where the coexpressed receptors are localized within a single neuron or how their localization contributes to the signaling mechanism by which they modulate neural activity. In *C. elegans* D1- and D2-like dopamine receptors (DOP-1 and DOP-3, respectively) are coexpressed and antagonize each other in the cholinergic motor neurons. Our lab has identified several molecular components that act downstream of these receptors, which are conserved and expressed in the mammalian brain. I have taken advantage of the simple nervous system of *C. elegans* to investigate the intra-neural mechanism of antagonistic dopamine signaling. Specifically I am: 1) determining the subcellular localization of DOP-1 and DOP-3 receptors in the cholinergic motor neurons using functional fluorescent receptor protein transgenes in live animals and 2) examining the contribution of dopamine signaling through DOP-1 and DOP-3 receptors on cholinergic neural activity by measuring spontaneous and induced post synaptic currents in the muscles they innervate. Fluorescently labeled receptors expressed in cholinergic motor neurons have revealed that DOP-1 receptors localize to synaptic terminals whereas DOP-3 receptors do not. This indicates that the two receptors are likely modulating activity of this neuron through two different signaling pathways and suggests a model in which dopamine acts extrasynaptically through DOP-1 to induce neuron excitability at presynaptic sites while dopamine binds DOP-3 at non-synaptic sites to reduce excitability of the neuron. We will test this model of antagonistic dopamine signaling by using electrophysiological recordings of the receptor mutants.

516C

Molecular Analysis of Glutamate Transporters and Excitotoxic Neurodegeneration in *C. elegans*. **Itzhak Mano**[1], Robert Kalb[2], Monica Driscoll[3]. 1) Physiology & Pharmacology, Sophie Davis Biomedical School, City College, The City University of New York, New York, NY; 2) Department of Neurology, Children's Hospital of Philadelphia, University of Pennsylvania, Philadelphia, PA; 3) Molecular Biology & Biochemistry, Rutgers University, Piscataway, NJ.

Glutamate (Glu) is the main excitatory neurotransmitter in humans and a key transmitter in nematodes. Glu synaptic concentrations are carefully controlled by a special uptake system, the Glu Transporters (GluTs). Insufficient activity of GluTs (seen in stroke and a range of neurodegenerative diseases) causes Glu accumulation, exaggerated stimulation of the post-synaptic cell, and excitotoxic neurodegeneration. We study the regulation of normal and abnormal Glu neurotransmission at three levels:

1) We analyze the regulation of normal synaptic activity by GluTs: We find that nematodes use an unusual strategy to eliminate Glu that has been released into synapses. This strategy relays heavily on remote uptake of Glu, an intriguing approach to controlling synaptic function.

2) We study the molecular events that lead from the pathological accumulation of Glu in the synapse to the demise of the affected neurons. We combined Δ*glt-3*, a GluT-null mutation, with activated-Gαs* expression to trigger excitotoxicity. We find that neurodegeneration is mediated by Ca^{2+}-permeable Glu receptors of the AMPA subtype and by a calcineurin-regulated adenylyl cyclase. This novel functional interaction can be recapitulated using mammalian proteins expressed in *Xenopus* oocyes, suggesting a new view on potential neurodegenerative mechanisms in excitotoxicity.

3) We study the ability of stress-resistance signaling pathways to protect from excitotoxic neurodegeneration. We find that a central signaling pathway that confers stress resistance also reduces the susceptibility of neurons to excitotoxicity.

Together these observations highlight the ability of *C. elegans* studies to provide new insights into decisive events in the processes that mediate neuronal physiology and pathology, and to provide new inroads to understand the mechanism of critical human diseases.

517A

Membrane Trafficking in Cholinergic Neurons Lacking UNC-16 (JIP3). Stacey L. Edwards, Barret C. Phillips, Nicole K. Charlie, **Kenneth G. Miller**. Genetic Models of Disease Program, Oklahoma Medical Research Foundation, Oklahoma City, OK.

In a genetic screen for suppressors of *goa-1* ($G\alpha_o$) null mutants we recovered 3 alleles of *unc-16*, which encodes the *C. elegans* JIP3 ortholog. A recent pioneering study showed that impairing UNC-16 function increases the early endosome content of axons (Brown et al., 2009). Null mutations in UNC-108 (Rab2) also suppress *goa-1* null mutants and have early endosome–related membrane trafficking defects (Chun et al., 2008; Mangahas et al., 2008; Edwards et al. WM2009 abstract). To determine if *unc-16* and *unc-108* mutants have similar neuronal membrane trafficking defects, we examined the trafficking of the tagged pro-neuropeptide NLP-21-Venus in *unc-16* mutant neurons. In *unc-108* null mutants, early endosomes remove the Venus tag from immature dense core vesicles after pro-neuropeptide processing resulting in reduced levels of the Venus tag in neuronal somas (59% of wild type) and axons (33% of wild type). Although *unc-16* mutants also had reduced NLP-21-Venus levels in cell somas (29% of wild type) and axons (59% of wild type), the dissimilar ratios of cell soma-to-axonal Venus tag compared to *unc-108* mutants suggested different membrane trafficking defects. Further underscoring the differences between *unc-108* and *unc-16* mutants, we found that *unc-16* mutants have lysosomes in their axons, which we never observed in wild type or *unc-108* mutant axons. Lysosomes are the end-stage organelle of a pathway extending from early and recycling endosomes through late endosomes to lysosomes and also involving Golgi-derived vesicles. To determine the extent to which components of the endo-lysosomal system accumulate in *unc-16* mutant axons, we imaged the following markers expressed from integrated arrays in cholinergic neuronal cell somas and axons: Golgi (AMAN-2-Venus), early endosomes (YFP-RAB-5 and RFP-SYN-13), recycling endosomes (CFP-RAB-11), late endosomes (CFP-RAB-7), and lysosomes (CTNS-1-RFP). We found that the entire endo-lysosomal pathway accumulates at high levels in *unc-16* mutant cholinergic axons (5–7 fold higher than wild type for Golgi and early endosome markers, 1.8–fold higher for recycling endosomes, 4-fold higher for late endosomes, and infinitely higher for lysosomes, which are not observed in wild type). In *unc-16* mutant cell somas, only the RAB-5 early endosome marker was strongly depleted (decreased to 23% of wild type), while the cell soma levels of the other markers ranged from 60%–86% of wild type. We plan to use time-lapse imaging to determine if lysosomes aberrantly enter *unc-16* mutant axons or if they form within axons.

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Impaired Dense Core Vesicle Maturation in Mutants Lacking UNC-108 (Rab2). Stacey L. Edwards[1], Nicole K. Charlie[1], Janet E. Richmond[2], Jan Hegermann[3], Stefan Eimer[3], **Kenneth G. Miller**[1]. 1) Genetic Models of Disease Program, Oklahoma Medical Research Foundation, Oklahoma City, OK; 2) Dept. of Biological Sciences, University of Illinois at Chicago, Chicago, IL; 3) Center for Molecular Physiology of the Brain, European Neuroscience Institute, Goettingen, Germany.

Despite a key role for dense core vesicles (DCVs) in neuronal function, there are major gaps in our understanding of DCV biogenesis. We performed a genetic screen for *C. elegans* mutants with behavioral defects consistent with impaired DCV function and recovered five mutations in UNC-108 (Rab2). Electrophysiological analysis showed that acetylcholine release from small synaptic vesicles is normal in *unc-108* mutants. Genetic analysis showed that *unc-108* mutations impair a DCV function unrelated to neuropeptide release that, together with neuropeptide release, fully accounts for the role of DCVs in locomotion. EM analysis of DCVs in *unc-108* mutants, coupled with quantitative imaging of DCV cargo proteins, revealed that Rab2 acts in cell somas during DCV maturation to prevent the loss of soluble and transmembrane DCV cargo. In Rab2 null mutants, two-thirds of these cargo move to early endosomes via a PI(3)P–dependent trafficking pathway, whereas aggregated neuropeptides are unaffected. Other studies have shown a strong increase in tubulovesicular structures that correspond to early and recycling endosomes in *unc-108* mutant neuronal cell somas (Chun et al., 2008) and a long delay in the rate at which newly engulfed cell corpses move through an early endosome intermediate in *unc-108* mutants (Mangahas et al., 2008) We propose that, in the absence of Rab2, an expanded and/ or longer-lived early endosomal network interacts with maturing dense core vesicles and removes most of their soluble and transmembrane proteins before they exit the cell soma. These results, along with a complementary study (Sumacovic et al., submitted), reveal how neurons use the most highly conserved animal Rab protein to solve a challenging trafficking problem. Chun, D.K., J.M. McEwen, M. Burbea, and J.M. Kaplan. 2008. UNC-108/Rab2 Regulates Post-endocytic Trafficking in *C. elegans*. Mol Biol Cell. 19:2682-95. Mangahas, P.M., X. Yu, K.G. Miller, and Z. Zhou. 2008. The small GTPase Rab2 functions in the removal of apoptotic cells in *Caenorhabditis elegans*. J Cell Biol. 180:357-73. Sumakovic, M., Hegermann, J., Husson, S. J., Luo, L., Schwarze, K., Olendrowitz, C., Schoofs, L., Richmond, J. and S. Eimer. Submitted. UNC-108/RAB-2 and its effector RIC-19 are involved in dense core vesicle maturation in *C. elegans*.

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Transfer at a thermosensory synapse in *C. elegans*. **Anusha Narayan**, Gilles Laurent, Paul Sternberg. Division of Biology, Caltech, Pasadena, CA.

C.elegans is an attractive system for neural circuit analysis. To analyze the functional dynamics of circuits that control behavior, it is necessary to understand the underlying synaptic transformations. Thermotaxis is an established behavior in *C. elegans*[1-2]. We attempt to characterize the transfer function at a prominent synapse within the thermotactic circuit: between AFD, the primary thermosensory neuron[3], and AIY, its principal post-synaptic partner.

We drive expression of Channelrhodopsin-2(chR2), a light-activated cation channel [4], solely in AFD, using a cell-specific promoter, and use whole-cell patch-clamp recording techniques to measure the light-evoked synaptic response at AIY.

We are able to reliably activate the presynaptic cell AFD, evoking depolarizing potentials of up to 40 mV and inward currents of up to 15 pA. The postsynaptic response at AIY is small: less than 5 mV, with inward currents of less than 1 pA, and is graded and tonic, lasting the duration of the stimulus. The response reverses around 0 mV and seems to be frequency independent, showing no obvious short-term facilitation or depression.

Our results further validate the use of chR2 to stimulate neural activity, and indicate that this synapse has low gain, and transmits information from AFD to AIY with short latencies and high fidelity. It will be interesting to see how AIY integrates this information with other incoming streams, and to examine processing downstream in the thermotactic circuit.

1.Mori, I., H. Sasakura, and A. Kuhara, *Worm thermotaxis: a model system for analyzing thermosensation and neural plasticity.* Curr Opin Neurobiol, 2007. 17(6): p. 712-9.
2.Ryu, W.S. and A.D. Samuel, *Thermotaxis in Caenorhabditis elegans analyzed by measuring responses to defined Thermal stimuli.* J Neurosci, 2002. 22(13): p. 5727-33.
3.Clark, D.A., et al., *The AFD sensory neurons encode multiple functions underlying thermotactic behavior in Caenorhabditis elegans.* J Neurosci, 2006. 26(28): p. 7444-51.
4.Boyden, E.S., et al., *Millisecond-timescale, genetically targeted optical control of neural activity.* Nat Neurosci, 2005. 8(9): p. 1263-8.

520A

An RNAi screen for transcription factors involved in synaptic function. **Edward Pym**[1,2], Monica Feliu-Mojer[1,2], Amy Vashlishan[1,2], Joshua Kaplan[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

The differential regulation of gene expression, by transcription factors, is essential for both the development and function of the nervous system. During the development of the nervous system, transcription factors play many key roles in a diverse range of decisions, from early cell fate to axon pathfinding and synaptic connectivity. Once the nervous system is 'up and running' transcription factors take on many functional roles, from responses to environmental stresses such as ischemia, to LTP and synaptic homeostasis. It is now emerging that there are growing classes of neurological disorders characterized by aberrant transcriptional control of proteins in the nervous system, e.g. transcription channelopathies (1). To identify transcription factors that have roles in nervous system we plan to use C.elegans to undertake an RNAi screen, of all annotated transcription factors in the genome, for an altered sensitivity to aldicarb, an acetylcholine esterase inhibitor that causes hypercontraction of muscles in wild type animals. This approached has been successfully used before to isolate many new genes that regulate synaptic function both in traditional EMS mutagenesis screens (2) and in RNAi screens (3). The RNAi screen will be done in both F0 and F1 animals using the nre-1,lin15b strain that allows for enhanced RNAi. We have already performed a small pilot screen, that successfully identified two transcription factors previously un-annotated as having an altered aldicarb sensitivity; mua-1 and cnd-1. (1) Waxman (2001) Nat Rev Neurosci, vol. 2 (9) pp. 652-9. (2) Nguyen, M et al. (1995). Genetics, vol. 140 (2) pp. 527-35. (3) Sieburth, D et al. (2005) Nature, vol. 436 (7050) pp. 510-7.

521B

A visual screen for abnormal localization of cholinergic receptors tagged using homologous recombination. **Magali Richard**[1], Valérie Robert[1], Aurélien Duboin[2], Vincent Studer[2], Jean-Louis Bessereau[1]. 1) Biologie Cellulaire de la Synapse, Ecole Normale Supérieure, Paris, France; 2) Neurobiologie et Diversité Cellulaire, ESPCI, Paris, France.

Ionotropic acetylcholine receptors (AChRs) are pentameric ligand-gated ion channels that have been conserved throughout evolution. At the *C. elegans* neuromuscular junctions (NMJ) two types of AChRs mediate excitatory neurotransmission: N-AChRs, likely homomeric channels composed of the ACR-16 subunits, which are sensitive to nicotine, and L-AChRs, heteropentameric receptors activated by the drug levamisole. Several components involved in L-AChR formation and function have been identifed in the past using forward screens for mutants with decreased sensitivity to levamisole. However, resistance to levamisole is quite an indirect index of the numerous molecular mechanisms controling AChR composition, assembly, trafficking and localization. To study the biology of L-AChRs, we propose a novel approach based on the *in vivo* vizualization of tagged AChRs. Using the MosTIC technique recently developped in our laboratory, we have introduced, by homologous recombination, different fluorescent tags into the endogenous locus *unc-29*, which encodes one L-AChR subunit. We then used two different approaches to inactivate gene function (i) a chemical mutagenesis using EMS (ethylmethane sulfonate), (ii) RNA interference (RNAi) targeting 1300 genes selected for putative role in synaptic function. First, we will present mutants isolated in the EMS screen for AChRs clusters disappearance at the nerve ring. Second, we will describe a RNAi screen, for abnormal localization of AChRs at the NMJ, and the characterization of some putative candidates in which AChRs clusters seem to be altered. Third, we will demonstrate how microfluidic devices can improve the efficiency of visual screens.

522C

THE ROLE OF RAB SMALL GTPASES IN GLUTAMATE RECEPTOR TRAFFICKING. **Donglei Zhang**, Doreen Glodowski, Nora Isack, Christopher Rongo. The Waksman Institute, Department of Genetics, Rutgers University, Piscataway, NJ 08854.

Regulated endocytosis and trafficking of AMPA-type glutamate receptors (AMPARs) is critical for synaptic plasticity. However, the specific combinations of clathrin-dependent and -independent mechanisms that mediate AMPAR trafficking *in vivo* have not been fully characterized. To better understand AMPAR trafficking, we have been examining the trafficking of the AMPAR GLR-1 in *C. elegans* (1). GLR-1 is localized on synaptic membranes, where it regulates reversals of locomotion in a simple behavioral circuit (2). We previously identified two genes that regulate GLR-1 membrane recycling: RAB-10 and LIN-10 (2, 3). Animals lacking RAB-10, a small GTPase required for endocytic recycling of intestinal cargo, or LIN-10, a PDZ-domain containing protein, share the same phenotype: GLR-1 accumulates in large, internalized accretions and animals display a decreased frequency of reversals (3, 4). Interestingly, reducing clathrin-dependent endocytosis specifically suppresses the *lin-10* mutant phenotype, whereas reducing clathrin-independent endocytosis specifically suppresses the *rab-10* mutant phenotype. Thus, we hypothesize that LIN-10 and RAB-10 recycle AMPARs from intracellular endosomal compartments to synapses along distinct pathways. Moreover, we suspect that another Rab protein, analogous to RAB-10, functions in the LIN-10 pathway.

We have taken two approaches to identify additional cellular factors involved in LIN-10-mediated trafficking of GLR-1. First, we have performed a yeast 2-hybrid screen using LIN-10 as bait. We are characterizing the functions of the proteins identified in the screen to determine if they regulate AMPAR trafficking. Second, we are testing all of the known Rab genes in the genome for a role in GLR-1 trafficking, either by analyzing previously identified mutations or by generating mutant transgenes that express GDP- or GTP-locked versions of the Rabs. Our long-term goal is to further define the regulatory pathways involved in the movement and localization of AMPARs at synapses *in vivo*.

1. Hart, A.C. *et al*., *Nature* 378, 82-85 (1995) and Maricq, A.V. *et al*., *Nature* 378, 78-81 (1995).
2. Rongo, C. *et al*., *Cell* 94, 751-759 (1998).
3. Glodowski, D. *et al*., *Mol Biol Cell* 18, 4387-96 (2007).
4. Chen *et al*., *Mol Biol Cell* 17, 1286-97 (2006).

523A

Homeostatic regulation of GABA neuromuscular synapses. **Alyson L. Sujkowski**, Kathleen M. Davis, Brianne L. Sturt, Stavros Moraitis, Nizar Mohammed, Bruce A. Bamber. Department of Biological Sciences, The University of Toledo, Toledo, OH 43606.

Modulation of GABA synapse function affects the excitation-inhibition balance in neural circuits, and plays a significant role in human brain function. The GABA neuromuscular synapse in *C. elegans* is a simple, genetically accessible model to study this modulation. The GABA receptor in muscle is encoded by UNC-49, a ligand-gated chloride channel homologous to the mammalian $GABA_A$ receptor. Postsynaptically, GABA neuromuscular junctions exhibit two forms of homeostatic regulation. First, exposure to the GABA agonist muscimol causes flaccid paralysis. Worms eventually adapt to muscimol, adopting the shrinker phenotype typical of GABA-defective mutants. Adaptation is due to a 6-10 fold reduction of GABA receptor abundance, and a proportional reduction in muscle GABA sensitivity, caused by increased GABA receptor trafficking to the lysosome. Levamisole responses are unaffected by muscimol exposure, suggesting that this plasticity is specific for GABA receptors. Second, GABA receptor levels are reduced in *twk-18(cn110)* mutants. TWK-18 is a twin-pore potassium channel expressed in body wall muscles that exhibits steep temperature dependence of activity. *cn110* is a gain-of-function allele with proportionally higher K^+ currents across the full temperature range. *twk-18(cn110)* mutants move normally at 20°C, but show flaccid paralysis at 30°C, presumably because the elevated K^+ currents interfere with muscle contraction. GABA receptor levels are reduced 2-3 fold compared to wild type in *twk-18(cn110)* mutants raised at 20°C and 25°C, but not at 15°C, possibly as compensation for elevated muscle K^+ conductance at the higher temperatures. These two forms of plasticity suggest that receptor activation, chloride influx, and membrane excitation state can influence postsynaptic GABA receptor expression to establish and maintain appropriate cellular excitability. We are using pharmacological and genetic approaches to independently manipulate these parameters to better understand how GABA receptor abundance is controlled. We are also using microarrays and RNA interference screening to identify the relevant signaling pathways. Analysis of muscimol-treated worms also suggests that presynaptic GABA release may be modulated as well. Upon removal of muscimol, locomotion recovers several hours before muscle GABA responsiveness recovers, consistent with suppression and relatively fast recovery of presynaptic GABA release. We are presently characterizing the structure and function of presynaptic GABA neurons in muscimol-treated and *twk-18(cn110)* worms, to test the extent to which presynaptic and postsynaptic aspects of GABA synapse function are coordinately regulated.

524B

Investigation of the signaling pathways of VAV-1, a Rho family guanine nucleotide exchange factor, in *C. elegans*. **Amanda Fry**, Patrick Spooner, Kenneth Norman. Center for Cell Biology and Cancer Research, Albany Medical College, Albany, NY.

C. elegans VAV-1 is a guanine nucleotide exchange factor (GEF) for Rho/Rac GTPases and is homologous to the mammalian Vav proto-oncogenes. VAV-1 is widely expressed and has been shown to regulate rhythmic activities such as ovulation and defecation in *C. elegans*. VAV-1 regulates these rhythmic activities by modulating intracellular calcium signaling in part through inositol 1,4,5-triphospate receptors, but other signaling mechanisms must be at work downstream of VAV-1. Transgenic animals expressing constitutively active VAV-1 have uncoordinated rhythmic activities and locomotion. Consistent with VAV-1 modulating calcium, calcium oscillations are also disorganized in these transgenic animals. By screening a mutagenized strain of *C. elegans* that expresses constitutively active VAV-1 for suppression of their uncoordinated locomotion phenotype, we aim to identify other components of VAV-1 signaling pathways. Several putative suppressors of constitutively active VAV-1 have been identified (also see P. Spooner's poster). In order to determine the physical locations of the unidentified suppressor mutations in the *C. elegans* genome, single nucleotide polymorphism (SNP) mapping is currently being used. The location of one such suppressor mutation has been narrowed to genomic interval containing about 60 genes, and we are in the process of testing candidates for their ability to suppress constitutively active VAV-1. Additionally, recent evidence suggests that mammalian Vav2 and Vav3 are important for sympathetic nervous system function and hence control of normal heart function. Interestingly, we have found that *vav*-1 mutants display abnormal pharyngeal contraction and are hypersensitive to the acetylcholine esterase inhibitor, aldicarb. Worm strains that have impaired synaptic transmission are resistant to aldicarb whereas mutants with hyperactive synaptic transmission are hypersensitive to aldicarb. Therefore, these data suggest that VAV-1 likely functions to negatively regulate synaptic transmission. Our future studies will focus on identifying the mechanism underlying the synaptic transmission defect and cloning the suppressor mutations of constitutive VAV-1 activation.

525C

Transient food deprivation prolongs the mating potency of aged C.elegans males. **Xiaoyan Guo[2]**, L Reen Garcia[1,2]. 1) Howard Hughes Medical Institute; 2) Texas A&M Universtiy, College Station, TX.

Caloric restriction can extend lifespan in different organisms from yeast and C.elegans to mammals. We are interested in determining how caloric restriction can have a long lasting effect on sensory motor behaviors. In our study, we use C.elegans mating behavior as a model to test the long term effects of short term caloric restriction on sensory motor behavior. We put a Day 1, Day 3, Day 5, Day 7 adult him-5 male with one day 1 adult pha-1 hermaphrodite on a 0.5 mm mating lawn at 20°C. Pha-1 hermaphrodites are used because they can move just like wide type but can not lay viable progeny at 20°C, which means the presence of any progeny on the mating lawn indicates that mating has occurred. Two days later we score the plates for the presence of at least one progeny. In parallel, we allow L4 males to develop into adult in the absence of food for 20 hours, and then we put them back onto the plates with food. We do the same series crosses with these males as the fed ones. Our results show that on day1 both fed and 20 hours starved males have high efficiency to sire progeny: 93.3% and 86.9% respectively can fertilize a hermaphrodite. However, on day 3, 72.8% of males that were starved for 20 hours can mate and sire progeny, but only 48.3% of fed males can fertilize the hermaphrodite. For the following days, there is slight but not statistically significant difference between fed and 20 hours starved males. We also conducted a lifespan assay for the 20 hours starved and fed males. We found that there is no difference in their lifespan. We hypothesize that starved males can mate well on day 3 is not because they are physiologically younger than fed counterparts, but because starvation promotes the function of molecules that regulate mating. To find genes that are involved in this food-deprivation induced effect on day 3 mating, we assayed mutants that are involved in nutrient sensing, such as NAD-dependent histone deacytylase Sir-2.1 and FOXO-ortholog Daf-16. We found that the difference on day 3 mating efficiency induced by food deprivation is lost in both of these mutants, indicating that they are involved in this process. We are currently measuring what mating step degrades from day 1 to day 3. This will determine what neural and muscle circuits are sensitive to aging and will help us to identify how temporary food deprivation can have protective effects on behavioral circuits.

526A

Neuronal and Intestinal Protein Kinase D Isoforms Mediate Na^+ (Salt Taste)-induced Learning. **Y. Fu[1]**, M. Ren[1], H. Feng[1], Z. F. Altun[2], C. S. Rubin[1]. 1) Dept. Mol. Pharmacol., Albert Einstein Col. Med., Bronx, NY; 2) Dept. Neuroscience, Albert Einstein Col. Med., Bronx, NY.

To ensure survival and optimize reproduction, *C. elegans* must accurately sense attractive environmental stimuli while avoiding toxins, predators and starvation. Consequently, *C. elegans*' behavior towards attractive chemosensory signals (e.g., Na^+ ions) can be modified by associated cues (e.g., starvation) and previous experience. Behavioral plasticity, based on associative learning, is a conserved neurophysiological process, but knowledge of underlying signaling mechanisms is limited. Using a learning paradigm, we discovered that two protein kinase D (PKD) isoforms, DKF-2A and DKF-2B, are essential for Na^+-induced plasticity. Two promoters initiate transcription of the *dkf-2* gene. Spliced mRNAs encode 2 PKD isoforms: DKF-2A is expressed in intestinal cells; DKF-2B accumulates in neurons comprising chemosensory circuitry. DKF-2A/2B and other PKDs constitute a special class of protein kinase C (PKC) effectors that generate novel branches in diacylglycerol (DAG)-controlled signaling networks. Activated PKDs translocate from plasma membrane to intracellular locations and phosphorylate effectors that are not PKC substrates. In contrast to WT *C. elegans*, DKF-2 deficient animals are incapable of switching attraction to 25 mM Na^+ to aversion after preincubation with 100 mM sodium acetate (minus food). Reconstitution of *dkf-2(pr3)* null animals with a *dkf-2A*::DKF-2A-GFP or *dkf-2B*::DKF-2B-GFP transgene did not restore Na^+-induced learning. However, transgenic animals expressing DKF-2A-GFP and DKF-2B-GFP in intestine and neurons, respectively, were indistinguishable from WT *C. elegans* in behavioral plasticity. Thus, a Na^+-induced behavioral change is triggered by a binary detector system embedded in nervous tissue and the gut. This implies that PKD-regulated behavioral output reflects integration of signaling information acquired by both neurons and intestinal cells. The molecular basis for experience-dependent learning was clarified. EGL-8 (PLCβ homolog) produces DAG that controls PKC-mediated activation of DKF-2 isoforms.TPA-1, a DAG activated PKCδ homolog, exclusively controls *in vivo* activation (and functions) of DKF-2B in neurons and DKF-2A in intestine. Functions of the EGL-8-DAG-TPA-1-DKF-2 signaling module are not limited to plasticity and physiological outputs dependent on interactions between different cell types. The intestinal EGL-8-DAG-TPA-1-DKF-2A pathway independently promotes induction of ~85 mRNAs that mediate innate immunity in animals lacking DKF-2B. Neuronal DKF-2B mediates chemotaxis to volatile odorants in DKF-2A depleted *C. elegans*.

527B

Phosphodiesterases in the C. elegans AWC Neuron. **Scott Hamilton**, Noelle L'Etoile. Center for Neuroscience, University of California, Davis, Davis, CA.

Similar to calcium signaling, cyclic nucleotides have been shown to transduce complex intracellular signals that are temporally and spatially discrete. Phosphodiesterases (PDEs) are important regulators of intracellular levels of these second messenger cyclic nucleotides (cGMP and cAMP) that act by hydrolyzing the cyclic nucleotide to its corresponding monophosphate. We propose a central role for one or more PDEs in the AWC for chemosensation and adaptation signal transduction pathways. This is based on evidence that key cyclic nucleotide regulated proteins are required for these processes. For example EGL-4, a cGMP-dependent kinase, has been shown to be necessary and sufficient for adaptation (Lee et al., submitted 2009). DAF-11 and ODR-1, receptor-like guanylate cyclases, produce cGMP in the AWC and TAX-2/TAX-4 form cGMP-dependent channels that provide for calcium signaling. All of these factors are required for odor sensation by the AWC neurons. Thus, a role for cyclic nucleotide regulation by PDEs in AWC neurons is postulated. We are currently examining six of the seven known PDE and PDE-like genes (pdl-1, pde-1, pde-2, pde-4, pde-5 and pde-6) by conducting chemotaxis and olfactory adaptation behavioral screens on single and double mutants. We have isolated two mutants with interesting phenotypes: pde-2 (tm3098) is adaptation defective and pde-4 (ce268) is chemotaxis defective. Additionally, we will present morphological analysis for developmental defects in AWC neurites. Further behavioral, genetic, physiological and molecular analyses will clarify the central role of cyclic nucleotides in the biochemical processes of olfaction and adaptation in the AWC. A better understanding of PDEs and their effect on cyclic nucleotide levels will help shed light on how key neuronal and cardiac processes are regulated temporally and spatially.

528C

The Characterization of two Oriental Beetle Pheromone Insensitive Mutants *obi-1* and *obi-3* in *Pristionchus pacificus*. Jonathan Yaghoobian[1], Jessica Cinkornpumin[1], **Ray Hong[1,2]**. 1) Department of Biology, California State University, Northridge, CA; 2) Department for Evolutionary Biology, Max Planck Institute for Developmental Biology, Tuebingen, Germany.

Although biologists have been aware of nematode associations with insects for some time, the details of these interactions have received little attention. A systematic effort to identify the natural ecology of *Pristionchus* nematodes revealed species-specific host preferences for several beetle species, including the oriental beetle found in Japan and northeastern U.S. In *Pristionchus pacificus*, the first and only molecular component identified so far that is involved in odor signaling is the cGMP dependent protein kinase, *Ppa*-EGL-4. To obtain more upstream factors involved in insect pheromone attraction, we performed a non-saturating X-ray mutagenesis on *P. pacificus* for mutants that do not show attraction toward their host oriental beetle pheromone (ZTDO) using the conventional plate chemotaxis assay.

Of the seven *obi* candidates isolated, we selected two lines for further analyses due to their strong obi chemotaxis phenotype, viability, as well as linked morphological phenotypes after >3x outcrossing to wildtype. The two promising candidates were named *obi-1* and *obi-3* for Oriental Beetle pheromone Insensitive mutants. The chemosensory phenotype of *obi-1* and *obi-3* is specific for ZTDO and does not affect the chemoattraction toward other known *P. pacificus* attractants such as the plant volatile β-caryophyllene and the moth pheromone ETDA. Neither *obi-1* nor *obi-3* show dauer formation defect (daf-c) or temperature sensitivity. However, both *obi-1* and *obi-3* animals show higher frequency and amplitude turns than wildtype on OP50. Adult *obi-1* hermaphrodites are ~20%; longer than wildtype, with a body length-to-width ratio of 17 compared to 13 in the wildtype. An increase in egg retention (egl) also accompany this long phenotype (lon). Because the chemotaxis assay with cGMP treatment is extremely labor intensive, we used a linked long body morphological phenotype in *obi-1* to conduct subsequent mapping of F2 progeny from crosses between *obi-1* and the mapping strain Washington. Using 200 F_2 lines and molecular markers (Single Stranded Conformational Polymorphisms and Simple Sequence Length Polymorphisms), we were able to confine *obi-1* to a 153 kb region represented by 10 subcontigs of Supercontig 58. No known *C. elegans* long mutants map near the syntenic chromosome region in *P. pacificus*. *Obi-1*'s odor specific phenotype and genetic location suggest that it is not an allele of *egl-4* (Chr IV) and thus likely to be a parallel or upstream component of odor signaling in *P. pacificus*.

529A

Role of the phopholipase Cε homolog PLC-1 in the regulation of salt chemotaxis learning in *C. elegans*. **Ryo Iwata[1]**, Hirofumi Kunitomo[2], Yuichi Iino[1,2]. 1) Dept. of Biophys. and Biochem., Univ. of Tokyo, Tokyo, Japan; 2) Mol. Genet. Res. Lab., Univ. of Tokyo, Tokyo, Japan.

C. elegans shows plasticity of chemotaxis towards NaCl: when worms are starved in the presence of NaCl, their chemotaxis towards it decreases dramatically. We call this behavioral plasticity "salt chemotaxis learning".

Previous works have shown the involvement of the Go-Gq signaling pathway in this process. Hyperactivation of Gqα-DAG signaling causes learning defects. As Go-Gq signaling regulates synaptic transmission at the neuromuscular junction, it is hypothesized that the learning involves changes in neurotransmitter release from the ASER salt-sensing neuron. However, it is largely unknown how past experience modifies the Go-Gq signaling and neurotransmission.

Through a genetic screen for learning-defective mutants, we now identified a missense mutation *pe1237* in F31B12.2, a previously uncharacterized gene predicted in Wormbase. We generated a deletion mutant *pe1238*, which harbors a 1.9kb deletion in the F31B12.2 gene. Intriguingly, the *pe1238* mutant worms showed a behavior opposite to *pe1237* and avoided the NaCl source without preconditioning. Thus, this genetic locus defines a critical regulator of salt chemotaxis learning, which determines attraction to or avoidance of NaCl.

Despite the gene prediction of F31B12.2 in Wormbase, we identified a large transcript of 10kb by an RT-PCR analysis of mRNAs. This transcript spans C05A9.3, F31B12.2, *frm-9* and *plc-1*, and encodes an in-frame fusion of the four predicted gene products. The C-terminal half of the protein encoded by the transcript is PLC-1, the homolog of the mammalian phopholipase Cε (PLCε). PLCε is an isozyme of the phospholipase C (PLC) family which contains two Ras-associating domains and a CDC25 homology domain. Small GTPases of the Ras superfamily have been suggested to activate PLCε.

We are currently testing whether the large transcript functions in the regulation of salt chemotaxis learning by rescue experiments. Future work will focus on the activation mechanisms of the PLC-1 variant and how it regulates learning in combination with the Go-Gq signaling pathway.

530B

Cellular and molecular mechanism underlying *C. elegans* chemotaxis toward mild alkaline pH. **Takashi Murayama**, Mayuki FUJIWARA, Ichiro MARUYAMA. Molecular Neuroscience Unit, OIST, Okinawa, Japan.

Wild-type *C. elegans* is attracted to mild alkaline pH in environment. To investigate cellular and molecular modes underlying this chemosensory behavior, we have used agar plate assays with a linear pH gradient. Along pH gradients from pH 6.8 to pH8.5, wild-type worms were attracted toward higher pH regions, whereas *che-1* mutants defective in chemosensory ASE neurons were not. Laser ablation of ASEL and ASER indicated that ASEL is essential for the chemotaxis. Consistently, ASEL- or ASER-specific rescue of *dyf-3*, a mutant defective in sensory cilium structures, also indicated that ASEL is responsible for the chemotactic attraction. Furthermore, it was found that pH shifts from low to high, but not from high to low, activated ASEL when the neuronal activity was monitored by using a voltage-sensitive fluorescent protein. ASEL-specific rescue of *tax-4* improved the defect of the mutant in the chemotaxis toward mild alkaline pH. This result suggests that the TAX-2/TAX-4 cyclic-nucleotide gated ion channel is involved in the chemotaxis. When we searched candidate genes that regulate the TAX-2/TAX-4 ion channels by RNAi, knock-down of *gcy-14*, which encodes a cell-surface receptor-type guanylyl cyclase, significantly impaired the chemotaxis toward mild alkaline pH. The *gcy-14* gene is preferentially expressed in ASEL, and GCY-14 fused with GFP was localized to the tip of ASEL sensory endings, suggesting that GCY-14 acts as a sensor molecule in ASEL for mild alkaline pH.

531C

Contribution of cGMP-gated channels to olfactory plasticity. **Damien M. O'Halloran**, Xiao-Dong Zhang, Svetlana Altshuler, Julia Kaye, Tsung-Yu Chen, Noelle L'Etoile. Cntr Neurosci, Univ California, Davis, Davis, CA.

Adaptation is a fundamental property of sensory systems that enables animals to adjust to ongoing changes in the environment by decreasing their sensitivity to persistent stimuli. In *C. elegans* AWC neurons are responsible for sensation of a range of attractive volatile odors (Bargmann et al., 1993). Prolonged odor exposure leads to reversible decreases in the animal's attraction to that odor (Colbert and Bargmann, 1995; L'Etoile et al., 2002; Kaye et al., 2009). The odor specificity of this adaptation is determined by the feeding status of the animal. If a well-fed worm is exposed to benzaldehyde for 1hr it will adapt to both benzaldehyde and isoamyl alcohol (both sensed by AWC), this process is termed cross adaptation. In contrast, a starved worm will adapt to benzaldehyde and its response to isoamyl alcohol will remain intact (Colbert and Bargmann, 1997). The TAX-4/TAX-2 cyclic nucleotide-gated channel (cNG) is required for AWC olfactory responses (Coburn and Bargmann, 1996; Komatsu et al., 1999). TAX-4 is an alpha subunit that can form homomeric channels while TAX-2 is a beta subunit that requires TAX-4 to form a functional channel (Coburn and Bargmann, 1996; Komatsu et al., 1999). *C. elegans* encodes two additional predicted alpha subunits, CNG-1 and CNG-3 (Cho et al., 2004;2004b). In olfactory neurons and photoreceptors cNG channels are comprised of three alpha subunits and one beta subunit (Zheng and Zagotta, 2004). Here we report that CNG-1 is required in AWC to induce cross adaptation between benzaldehyde and isoamyl alcohol. The ability of food to induce this cross adaptation is dependent on the homodimeric kinesin motor, OSM-3. OSM-3 contributes to the formation of cilia distal segments (Evans et al., 2006). Distal segments are not observed in the AWC neurons and so the requirement for OSM-3 in our paradigm suggests a role for other sensory neurons apart from AWC. We also demonstrate that CNG-3 is required in AWC for adaptation to 30mins exposures to odor. Interestingly, the double mutants *cng-1;cng-3* are wildtype for both the food induced cross adaptation response and short term adaptation. We examine this further by modeling our mutants by expressing the cNG channel subunit cDNAs in HEK cells. We found that CNG-1 channel subunits can modulate the channel dynamics of TAX-2/TAX-4 channels. This CNG-1/TAX-2/TAX-4 composition may mimic the *cng-3* mutant, which is short-term adaptation defective. CNG-1 lengthens the time constant for the channel closing in response to removal of cGMP. The TAX-2/TAX-4/CNG-3 channel has very similar properties to that of the TAX-2/TAX-4 channel. We are currently investigating native channel conformations in the AWC neurons in *C. elegans* using FRET.

532A

Functional analysis of CASY-1, an ortholog of Calsyntenins/Alcadeins, which is essential for multiple forms of learning. **Hayao Ohno**, Daisuke D. Ikeda, Hirofumi Kunitomo, Yuichi Iino. Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo, Japan.

Caenorhabditis elegans shows various types of behavioral plasticity elicited by sensory stimuli. In salt chemotaxis learning, a learning paradigm previously reported by us, worms learn to avoid NaCl when the tastant is presented under starvation conditions. *casy-1* was identified by a genetic screen for mutants that fail to show salt chemotaxis learning. CASY-1 also has essential roles in other types of learning and information processing such as olfactory adaptation, temperature learning and sensory integration. Calsyntenins/Alcadeins, the mammalian homologs of CASY-1, are highly conserved cadherin-like type I transmembrane proteins expressed in the central nervous system. Biochemical studies have shown that Calsyntenins/Alcadeins form a tripartite complex with APP (amyloid precursor protein) and X11/X11L, and are proteolytically processed in a fashion similar to APP. Whereas a genetic association of human calsyntenin-2 and memory performance has recently been reported, molecular functions of the proteins remain largely unknown. Western blotting suggested that CASY-1 is cleaved near the transmembrane domain like its mammalian orthologs, and functional domain analysis of CASY-1 revealed that the N-terminal ectodomain is essential for salt chemotaxis learning, whereas the membrane-anchored C-terminal stump is dispensable. Of the extracellular domain, the cadherin domains are dispensable, but the LG/LNS domain alone is not sufficient. Cell-specific rescue experiments demonstrated that expression of CASY-1 in the ASER salt-sensing neuron is sufficient for salt chemotaxis learning (but not for olfactory adaptation), and expression in the AWC odorant-sensing neurons is sufficient for olfactory adaptation. These results suggest that the LG/LNS domain of CASY-1 modulates chemotaxis behavior by localizing at/near the surface of the sensory neurons that sense the chemical stimuli relevant to the behavior. To identify an interacting partner(s) of CASY-1, we performed yeast two-hybrid screens using the functionally essential LG/LNS domain of CASY-1 as a bait. So far we have identified 13 genes whose products potentially interact with the domain.

533B

C. elegans mutants defective in high-alkaline pH avoidance. **Shigeki Sanehisa**, Mayuki Fujiwara, Takashi Murayama, Ichiro Maruyama. Okinawa Institute of Science and Technology (OIST), Uruma, Okinawa 904-2234, Japan.

Cellular and molecular bases underlying nociception of high alkaline pH still remains to be explored. *C. elegans* senses higher alkaline pH ranges than ˜pH 10.5 as a noxious stimulus. On an agar plate with a pH gradient, wild-type worms avoided higher pH regions, and moved toward lower pH ranges. To understand the molecular and cellular basis of the high-alkaline pH avoidance, we have screened mutants defective in high-alkaline pH avoidance from animals treated with ethylmethanesulfonate (EMS), using the plate assay. Among five mutants isolated that could not avoid the high-alkaline pH, three of them showed normal chemotaxis behaviors toward water-soluble attractants such as NaCl and mild-alkaline pH, as well as normal osmotic avoidance behaviors. We will discuss about molecules and neurons responsible for the nociception of high-alkaline pH.

534C

Decreased TRPV Channel Function Restores Bitter Taste Response to *C. elegans grk-2* mutant animals. **Meredith J. Scheider[1]**, Elizabeth Hong[1], Angela Chaparro-Garcia[1], Marina Ezcurra[2], William R. Schafer[2], Denise M. Ferkey[1]. 1) Dept of Biological Sciences, University at Buffalo (SUNY), Buffalo, NY; 2) Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, UK.

Since *C. elegans* use chemical cues to navigate their environment, signaling through chemosensory G protein-coupled receptors (GPCRs) must be tightly regulated. One mode of regulation is via G protein-coupled receptor kinases (GRKs), which phosphorylate activated GPCRs to terminate signaling. Despite the previously described role of GRKs in GPCR signal downregulation, *C. elegans* lacking GRK-2 function are not hypersensitive to odorants. Instead loss of *grk-2* broadly disrupts chemosensation and animals are unresponsive to a number of chemical stimuli detected by ASH, AWA and AWC (Fukuto and Ferkey et al., 2004). Loss of GRK-2 function appears to disrupt signaling downstream of chemosensory GPCRs and upstream of calcium flux as *grk-2* mutant animals lack a stimulus-evoked calcium flux in response to ASH detected stimuli (Fukuto and Ferkey et al., 2004).

To identify components responsible for transducing or regulating GPCR signaling we performed a genetic screen for suppressors of the *grk-2* chemosensory defects. We screened 25,000 F2 animals and identified 8 mutations that restored *grk-2* response to the bitter tastant quinine (ASH). Genetic mapping of 3 mutants identified the TRPV-related channels OSM-9 and OCR-2. Loss of TRPV channel function in *grk-2* mutants restored response to bitter compounds generally, yet failed to restore response to other soluble tastants also detected by ASH. We are using cameleon to determine whether decreased TRPV channel activity restores stimulus-evoked calcium signaling in the ASH neurons, which would correlate with the restored bitter taste response. Furthermore, although ASH and AWA share a common signal transduction cascade, loss of neither TRPV channel restored AWA-mediated chemotaxis to *grk-2* mutant animals.

While the mechanism by which decreased TRPV channel function restores *grk-2* chemosensory behavior appears to be unique to the ASH-mediated avoidance of bitter tastants, our data supports the model that there are “cellular sensors” of signaling levels that recruit compensatory inhibitory proteins to dampen signaling and protect cells from overstimulation in the absence of GRK-2. Decreasing primary signal transduction, via decreased TRPV channel activity, may prevent activation of inhibitory pathways and allow chemosensory signaling and behavioral responses.

535A

Functional Analysis of Asymmetrically-Expressed GCY Proteins in *C. elegans*. **Heidi K. Smith[1]**, Christopher O. Ortiz[2], Oliver Hobert[3]. 1) Dept of Biological Sciences; 2) Ctr for Neurobio. and Behav; 3) HHMI and Dept. of Biochem and Molecular Biophysics, Columbia University Medical Center, New York, NY.

Chemosensation allows animals to evaluate their environment, detect food, other animals, and dangerous toxins while responding with appropriate behaviors essential to the animal’s survival. A robust chemosensory system can be generated even from a seemingly simple nervous system, such as that of *C. elegans*, which can detect and respond to a vast number of chemical cues. One important, but poorly understood, strategy used by *C. elegans* is to “lateralize” the function of some of its sensory neurons, such as the ASE neurons, thus increasing the discriminatory power of a system comprised of relatively few elements. We have found that the ASE neurons respond to several salt cues and these responses are asymmetric in terms of whether the left or the right ASE neuron responds to a specific salt cue (Ortiz *et al.*, submitted). Previous work in our lab has furthermore identified a number of guanylyl cyclase genes as having a role in the chemotaxis asymmetry of ASE (Ortiz *et al.*, submitted). Mutant analysis has revealed that individual *gcy* genes are specifically required for sensing particular ions. Such specificity could be conferred through either the protein’s receptor family ligand-binding region (RFLBR) in its extracellular domain or by the protein’s guanylyl cyclase (GC) domain in its intracellular region. We seek to identify the molecular mechanisms by which these asymmetrically expressed receptor type guanylyl cyclases confer the specificity that underlies this lateralization. In order to test these predictions, intra- or extra-cellular domains of individual GCY proteins were swapped, chimeric proteins were introduced into mutant background animals in a cell-specific manner, and then rescue of chemotaxis defects were tested. The individual GCY protein domains that confer the cellular specificity of ASE neurons, which enables them to mediate responses only to particular cues are identified by evaluating assay output. Results from such experiments allow for the characterization of the domain(s) essential for specificity. By identifying these molecular mechanisms, key predictions of the role that these proteins play in ASE neurons, putatively functioning either as chemoreceptors or, alternatively, as signal transducers, can begin to be tested. We will pursue further strategies for elucidating these molecular mechanisms as part of an overall effort in exploring the relationship between individual genes, their patterns of expression in specific cellular contexts, and the chemosensory behaviors exhibited by C. elegans in response to salt cues found in its environment.

536B

Behavioral decision-making during acidic pH avoidance in *C. elegans*. **T. Wakabayashi**, T. Togashi, R. Shingai. Iwate Univ, Morioka, Japan.

The behavior of *C. elegans* consists of four simple locomotory events, forward, backward, turn by deep bending and rest (Ref.1). Worms combine these four locomotions appropriately to achieve their complex behaviors.

C. elegans can avoid acidic pH less than 4.0 (Ref.2). Behavioral analysis of single worms, by using automatic worm tracking system, revealed that worms showed two kinds of avoidance responses during the acid avoidance behavior. 1) Stereotypic stop-back-turn-forward sequence was elicited when worms encountered into acidic region of pH gradient formed on agar plate (back avoidance), and 2) worms avoid acidic region by biased body bending superimposed on continuous forward movement (turn avoidance). Interestingly, choices between these two avoidance responses are not random. When worms encountered into the border of acidic region in a large angle (at around 90?), they chose back avoidance, and when they encountered in a small angle (less than 20?), they chose turn avoidance. Moreover, turn avoidance occurred at more neutral region than back avoidance. These results suggest that worms can recognize the difference between these two sensory contexts and make a decision how to avoid acidic region.

Four chemosensory neurons (ASE, ADF, ASH and ASK) were known to be required for the acid avoidance behavior in *C. elegans* (Ref. 2). *eat-4* gene encodes a vesicular glutamate transporter expressed in neurons including ASH and ASK, and is required for the function of these neurons (Ref. 3). *eat-4(ky5)* mutant showed reduced back avoidance and the defect is partially rescued by expressing wild-type eat-4 in ASK neurons. In contrast, *egl-3(n150)* and *egl-3(n729)* showed slightly increased back avoidance. EGL-3 is a proprotein convertase thought to downregulate the glutamate release in ASH (Ref. 4). Taken together, diminished glutamatergic transmission reduces back avoidance and enhanced glutamatergic signaling accelerates back avoidance, suggesting the role of glutamatergic pathway in the behavioral decision during acid avoidance.

(Ref. 1) Croll (1975) J. Zool. 176: 159-176.
(Ref. 2) Sambongi et al. (2000) Neuroreport 11: 2229-2232.
(Ref. 3) Lee at al. (1999) J. Neurosci. 19: 159-167.
(Ref. 4) Kass et al. (2001) J. Neurosci. 21: 9265-9272.

537C

A combination of salt and food conditions in the habitat affects preference for salt in *C. elegans*. **Hirofumi Kunitomo**, Yuichi Iino. Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, Tokyo, Japan.

Caenorhabditis elegans senses a wide variety of chemicals associated with food, danger, or other animals. Sodium chloride is generally considered as an attractive cue for the animal. We have previously reported, however, that the worms avoid NaCl when animals are pretreated with NaCl under starvation. This behavioral plasticity, salt chemotaxis learning, is regulated by the insulin/PI3-K signaling pathway that functions in ASER, the right member of the bilateral gustatory neurons, ASE (Tomioka et al. 2006). Here we show that cultivation of worms under trace amount of NaCl in the presence of food for several hours or longer also induces avoidance of NaCl. This type of plasticity of salt chemotaxis, tentatively called low salt conditioning, is stimulus-specific and reversible. Salt, but not osmolarity, in the growth media is required for maintaining preference of salt. To reveal the mechanisms of low salt conditioning, we assessed the behavior of the mutants that show defects in salt chemotaxis learning or in the development of specific neurons. Of the insulin signaling pathway components, *age-1* and *akt-1* were required for low salt conditioning, whereas *ins-1* and *pdk-1* were not. *casy-1*, an ortholog of calsyntenin essential for salt chemotaxis learning, was not involved in low salt conditioning. Interestingly, the mutants of *odr-7*, which is required for proper differentiation of the AWA olfactory neurons, showed severe defects in low salt conditioning but not in salt chemotaxis learning. These results indicate that salt chemotaxis is modulated by a combination of salt and food conditions in the habitat and the two plasticity paradigms, both resulting in avoidance of salt, are regulated at least in part by distinct genetic and neural mechanisms.

538A

Neuronal regulation of ascaroside response during mate response behavior in the nematode *Caenorhabditis elegans*. **Jagan Srinivasan**[1], Fatma Kaplan[2], Chirag Pungaliya[3], Arthur Edison[4], Frank Schroeder[3], Paul Sternberg[1]. 1) Biology Division, Caltech, Pasadena, CA 91125; 2) USDA-ARS, 1700/1600 S.W. 23rd Drive, Gainesville, FL 32608; 3) Boyce Thompson Institute, Cornell University, Ithaca, NY 14850; 4) McKnight Brain Institute, and National High Magnetic Field Laboratory, University of Florida, PO Box 100245, Gainesville, FL 32610-0245.

Small-molecule signaling plays an important role in the biology of *Caenorhabditis elegans*. We have previously shown that ascarosides, glycosides of the dideoxysugar ascarylose regulate both development and behavior in *C. elegans* [1]. The mating signal consists of a synergistic blend of three dauer-inducing ascarosides, ascr#2, ascr#3, and ascr#4. The ascarosides ascr#2 and ascr#3 carry different though overlapping information, as ascr#3 is more potent as a male attractant than ascr#2, whereas ascr#2 is slightly more potent than ascr#3 in promoting dauer formation. Using differential analysis of NMR spectra (DANS), we have now identified additional ascarosides in the *C. elegans* metabolome [2]. Biological testing of synthetic samples of these compounds revealed additional evidence for synergy and provided insights into structure-activity relationships. Two types of neurons, the ASK neurons and the male-specific CEM neurons, are required for male attraction by ascr#3. We are currently testing neuronal and genetic requirements for the response to the new ascarosides discovered using DANS.

References

1. Srinivasan J, et al. (2008) A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature 454:1115-1118.

2. Pungaliya et al. (2009) A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. PNAS in press.

539B

Tracking circadian activity in PDF and clock mutants. **Ellen Meelkop**, Liesbet Temmerman, Tom Janssen, Liliane Schoofs. K.U.Leuven, leuven, Belgium.

A wide variety of organisms use an internal biological clock to adapt their lifestyle to the periodically changing environment. A circadian clock has a period of 24 hours and controls a day- and night rhythm in behaviour and biochemistry. Since the first publication of a circadian clock in the nematode Caenorhabditis elegans in the year 2002, none of the “classical” circadian clock homologues, such as the period and timeless genes, could be linked to this worm’s clock. Until now, they are only proven to be involved in the developmental rhythm rather than the circadian rhythm of C. elegans. With our discovery of three pigment dispersing factor (PDF) peptides and three receptor homologues in C.elegans, the question raised as to whether or not these peptides and receptors are involved in the C. elegans clock, since PDF is considered to be the key output signal between the internal circadian clock and rhythmic behaviour in the fruit fly Drosophila melanogaster. We implemented the Goodman parallel worm tracker to measure the average movement speed of populations of different strains over several days. In contrast with previously described experiments, we succeeded in showing circadian rhythmicity in the activity of wild type nematodes under standard culture conditions (NGM plates with Escherichia coli OP50 as food source). In addition, rapid overgrowth of plates due to offspring was avoided without the use of FUDR or RNAi. As previously described, the activity of wild type nematodes is higher during the day compared to the night and this rhythm persists under constant dark conditions. Preliminary observations of pdf-1 mutants showed no clear aberrations except for an overall lower speed and higher percentage of non-moving individuals compared to wild type nematodes. These results do not resemble the behaviour of pdf mutant fruit flies, which lose their morning activity peak, show an advanced evening activity peak and which lose their rhythmicity after three days in constant dark conditions. This does not necessarily rule out the possible involvement of PDF in the C. elegans clock, as pdf-2 and pdfr-1 mutants remain to be tested. Currently, we are analysing circadian rhythms in pdf-1 and “classical” clock mutants.

540C

Pleiotropic roles of a calcium binding protein, calumenin in *C. elegans*. **Hyun-Ok Song**[1], Gunasekaran Singaravelu[2], Hyun Sung[3], Meenakshi Dwivedi[3], Soonjae Kwon Kwon[1], Do Han Kim[1], Joohong Ahnn[3]. 1) Department of Life Science, GIST, Gwangju 500-712, Republic of Korea; 2) Waksman Institute, Rutgers University, Piscataway, NJ 08854, USA; 3) Laboratory of Developmental Genetics, Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, Republic of Korea.

Calumenin is a Ca^{2+} binding protein located in the lumen of endoplasmic reticulum. Even though it has been implicated in various diseases, the *in vivo* functions of calumenin have not yet been reported. Here, we report the characterization of *C. elegans* calumenin loss of function mutant, *calu-1(tm1783)*. Biochemical analysis revealed that CALU-1 binds with Ca^{2+} and the *calu-1(tm1783)* mutant exhibited pleiotropic defects such as decreased brood size, reduced egg laying rate, sluggish locomotion, and small body size suggesting the multiple roles of calumenin in *C. elegans*. Consistent to its ability to bind Ca^{2+}, *calu-1(tm1783)* mutant shows reduced pumping rate and prolonged defecation cycle, which are known to be mediated by Ca^{2+} signaling in *C. elegans*. The knock-down of *calu-1* phenocopies *calu-1(tm1783)* mutant, suggesting that specific loss of CALU-1 is responsible for the observed defects. Interestingly, *calu-1(tm1783)* mutant has severe defects in cuticle showing aberrant annuli and deformed alae indicating the possible role(s) of calumenin in cuticle formation and/or maintenance. To our knowledge, this is the first report of genetic analysis of calumenin illustrating its *in vivo* functions.

541A

Calcineurin homologous protein is required for a proton-activated muscle contraction that occurs during defecation. Ashley Taylor[1], Kiri Ulmschneider[1], Bryne Ulmschneider[1], **Jamie Wagner**[1], Keith Nehrke[2], Maureen A. Peters[1]. 1) Biology Dept., Oberlin College, Oberlin, OH; 2) Medicine Dept., University of Rochester School of Medicine and Dentistry, Rochester, NY.

The rhythmic defecation behavior in *C. elegans* occurs with a period of ≈50 seconds and is controlled by oscillatory calcium signaling in the intestine. The first step of the digestive motor program, the posterior body wall muscle contraction, occurs without neuronal input. Instead, protons released from the intestine act as fast transmitters to elicit this contraction[1,2]. Protons are transported from the intestine into the pseudocoloemic space via the sodium-proton exchanger, PBO-4 (also called NHX-7). A drop in pseudocoloemic pH is thought to activate proton-gated ion channels (PBO-5 and 6) located on the posterior body wall muscles, resulting in their contraction. PBO-4 activity is precisely timed, but the mechanism underlying its activation is unclear. The *pbo-4* gene product, like its vertebrate counterparts, contains a large intracellular C-terminal tail with many binding sites for calcium-responsive proteins such as calmodulin, calmodulin kinase II, and calcineurin homologous protein. Any or all of these regulatory molecules may link calcium signaling to PBO-4 activity.

The mutation of a calcineurin homologous protein (*chp-1*), a co-factor for sodium-proton exchangers[3], results in a posterior body contraction mutant, *pbo-1*. *pbo-1(sa7)* mutants exhibit severe constipation, drastic reduction in posterior body contraction strength, low brood size and slow growth. SNP mapping and RNAi phenocopy both implicate Y71H2AL.1 as the gene mutated in *pbo-1(sa7)III*. Y71H2AL.1 encodes *chp-1*, a calcium responsive protein containing two EF-hand calcium binding motifs. *pbo-1(sa7)* is a non-conservative point mutation of a calcium-coordination residue in the first EF-hand. The recently isolated allele, *pbo-1(tm3716)*, eliminates the second EF-hand (provided by the National Bioresource Council). A *pbo-1* transcriptional reporter exhibits intestinal expression, positioning *pbo-1/chp-1* to respond to the intestinal calcium wave. *pbo-1/chp-1* is an excellent candidate for the molecule that coordinates the cyclic intestinal calcium wave and the initiation of posterior body contraction. Ref.: 1. Beg et al., (2008) Cell 132:149-60. 2. Pfeiffer et al., (2008) Current Bio. 18:297-302. 3. Malo and Fleigel, (2006) Can. J. Physio. Pharm. 84:1081-95.

542B

Modeling behavioral strategies with stochastic calculus. **Leon Avery**. Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX.

Behavioral and developmental choices made by any animal represent a strategy for surviving and reproducing in its environment. The essence of strategy is making decisions on the basis of incomplete information, decisions whose costs and benefits can't be accurately determined at the time they must be made. Worms make many such choices: the decision to leave low-quality food in search of higher quality, the decision to lay eggs or allow them to hatch internally, and the decision to become a dauer or remain a dauer are examples. Not coincidentally, most of these decisions involve food availability, perhaps the most important environmental variable to a worm. I am trying to quantitatively model such decisions, using mathematical tools developed for financial markets.

The L2/L2d decision is particularly interesting, because it appears to be unnecessary. An L2d can become either an L3 or a dauer, while an L2 can only become an L3. Since the L2d can do everything the L2 can do and more, why does the L2 exist? A likely answer is suggested by the work of Golden and Riddle. They showed that L1 → L2d → L3 pathway takes a few hours more than L1 → L2 → L3. Under ideal conditions a worm population doubles in 10-11 hours. (This number is calculated from published life-history traits, and is in approximate accord with lab experience.) A delay of 7 hours, therefore, reduces fitness by a factor of $2^{7/10.5} = 0.62$. Thus a worm that becomes an L2d pays a price of about 40% of its fitness for the option of eventually becoming a dauer. A worm should become an L2d if it can confidently predict that conditions will be so bad in the future as to cause a decrease of fitness of this magnitude. Most of what we know about dauer formation concerns how the worm evaluates environmental conditions. However, the L2d should also be preferred in highly uncertain environments, since it postpones the dauer decision into the future, when more accurate information will be available. This effect can be modeled using the tools of stochastic calculus, used to price options in financial markets. They predict that the L2/L2d decision should be strongly influenced not only by how good the environment is, but also by how volatile it is.

543C

Analysis of noise robustness in neural circuit of *C. elegans*. **Yuishi Iwasaki**. Department of Intelligent System Engineering, Ibaraki University, Hitachi, Ibaraki 316-8511, Japan.

Is a worm's behavior, which seems to be random walk, deterministic or not? Noise or fluctuation sometimes plays an important role to organize decision or choice behavior. Neurons in *C. elegans* are believed to be non-spiking and communicate by graded synaptic transmission. In this meaning, the nervous system of *C. elegans* is not a "digital" control system but an "analogue" control system which seems to be sensitive to noise. In general, noises (fluctuations) are classified into two types. One is external noises for individuals such as environmental noises. For examples, concentration fluctuation in chemicals for chemotaxis and thermal fluctuation for thermotaxis. The other is internal noises in living organisms. For examples, fluctuation in a neuron's membrane potential and noise in synaptic transmission.

To analyze the noise robustness in neural circuit of *C. elegans*, simulation is carried out using a stochastic differential equation, so-called Langevin equation. As a neural circuit to simulate the dynamics, I focus on that of chemotaxis in this work. The number of chemical synapses and gap junctions is determined from the two databases of the neural connectivity (Oshio et al., 2003; Chen et al., 2006). I analyze the response of the neural circuit against the noises. If the additive noises are supposed to be uncorrelated each other, the law of large numbers naively says that the influence of the noises decreases as the number of connected neurons (elements) increases. In *C. elegans*, however, the number of connected neurons for a given neuron is not so large since the total number of neurons in the whole nervous system is 302. Therefore the influence of the noises does not sufficiently vanish. This work was supported by MEXT No. 20115004.

544A

Characterization of seizure modifiers in worms lacking CaMKII function. **Allyson V. McCormick**[1], James H. Thomas[2], Brian Kraemer[3]. 1) Seattle Institute for Biomedical and Clinical Research, Seattle Veterans Affairs Medical Center, Seattle, WA 98108; 2) Department of Genome Sciences, University of Washington, Seattle WA 98195; 3) Seattle Veterans Affairs Medical Center, Geriatrics Research and Education and Clinical Center, Seattle, WA 98108.

Several *C. elegans* mutants have seizures with acute exposure to neurostimulants[1,2] (e.g. pentylenetetrazole). Loss-of-function alleles of *unc-43*, the worm homologue of CaMKII, have robust full-body convulsions easily induced by elevated temperature and drug exposure. To further describe worm seizures genetically, we have conducted pilot EMS screens for suppressors and enhancers of *unc-43* seizures.

Thus far, we have isolated fourteen suppressors and enhancers. Since *unc-43(lf)* animals have a range of phenotypes caused by a lack of function in specific tissues, modifiers have been classified by changes in seizures and a variety of other behaviors (e.g. spontaneous reversal rate). Characterization of genetic lesions is currently underway.

Though identification can be time consuming, a key advantage of forward genetic screens is to isolate single point mutations that create more subtle variations in gene function. This is exciting since so little is known about modifiers in human epilepsies. Such modifiers may contribute to drug resistance, seizure frequency and developmental timing.

[1]Williams, S.N. et al. (2004). *Hum Mol Genet* (13) 2043-59.
[2]Locke, C.J. et al. (2006). *Brains Res* (1120) 23-34.

545B

A species specific chemosensory cue can modulate body size in *C. elegans*. **Evan L. Ardiel**, Catharine H. Rankin. Dept Psychology, Univ British Columbia, Vancouver, BC, Canada.

Previous research on *C. elegans* has shown that sensory perception mutants are smaller than wild-type worms (Fujiwara et al., 2002). This suggests that sensory input from the environment regulates adult body size. But what are the relevant external cues? Work in our lab suggests that interactions with conspecifics are one source. We have found that worms reared in isolation are dwarfed compared to worms reared in colonies. Furthermore, adding another worm to the plate of an isolated worm rescues colony body size. *C. briggsae* are also dwarfed when reared in isolation, but the relevant conspecific cue appears to be species specific, as the presence of *C. briggsae* cannot rescue colony body size in an isolated *C. elegans*. Using sensory perception mutants with cell structure (*che-2, che-3, osm-3, daf-6*), signal transduction (*odr-1, odr-3, tax-4, osm-9*), and cell specification (*ceh-36, lim-4, odr-7*) defects and cell-type specific rescues, we have identified a subset of chemosensory neurons with a role in the modulation of body size in response to conspecifics. Because isolated worms exposed to the stable water-soluble or volatile chemical cues of a colony are still smaller than colony worms, we propose that a species specific contact pheromone triggers growth to a larger body size. Fujiwara, M., Segupta, P., & McIntire, S.L. (2002). Regulation of body size and behavioral state of *C. elegans* by sensory perception and the egl-4 cGMP-dependent protein kinase. Neuron, 366 (6), 1091-1102.

546C

Genome-wide analysis of thermotactic behavior controlled by CREB and its downstream genes in *Caenorhabditis elegans*. **Yukuo Nishida**, Takuma Sugi, Ikue Mori. Group of Molecular Neurobiology, Nagoya University, Japan.

Animals memorize environmental information for optimal behavior. In order to understand the molecular basis of memory formation, we took advantage of *C. elegans* thermotactic behavior, which was designated with the simple neural circuit, including processes of thermosensation and memory formation (Mori *et al.*, 2007). In the latest study, we revealed that heat-shock transcription factor acts as a body thermal sensor, thereby controlling thermotactic behavior (Sugi *et al.*, unpublished). This work shed light on the requirement of transcriptional event for thermotactic behavior. We then focused on the transcriptional factor *crh-1*, the ortholog of cAMP responsible element binding protein (CREB), because CREB is a representative example as a memory-regulated transcriptional factor. Yet, little is known about the molecular identities of CREB downstream genes, although the expectant role of CREB is to control the activation of the genes that are critical for memory formation.

We first examined the importance of *crh-1* in thermotaxis of *C. elegans*. When wild-type animals and *crh-1* mutants were cultivated at 17°C, both animals migrated to lower temperature regions on the thermal gradient. In the case of cultivation at 23°C, although wild-type animals migrated to higher temperature regions, the mutants dispersed on the thermal gradient. We further conducted the temperature-shifted thermotaxis assay. At 3 hours after shifting the cultivation temperature from 17°C to 23°C, although wild-type animals changed to migrate to the new temperature 23°C, the mutants changed to disperse on the thermal gradient. These results suggest that *crh-1* mutants exhibit defect in acquisition of higher temperature memory, further implying that transcriptional activity of CRH-1 is essential for memorizing a higher temperature.

To isolate the genes generating the temperature memory, we proceed to conduct the genome-wide microarray analysis in combination with the temperature-shifted thermotaxis assay. Then we plan to compare the gene expression changes between wild-type animals and *crh-1* mutants during the higher temperature acquisition process. In addition, cell-specific rescue experiments are underway to identify cells in which transcriptional activity of CRH-1 is required for thermotaxis. We will then examine the relationship between the known molecular pathway within neurons identified by the cell-specific rescue experiments of *crh-1* mutants and the pathway regulated by the isolated CRH-1 downstream genes. We conceive that further analyses of *crh-1* and its downstream genes provide comprehensive insights into the molecular basis for temperature memory of thermotactic behavior.

547A

MOLECULAR MECHANISM OF SALT TASTE. **OLUWATOROTI O UMUERRI**, Renate Hukema, Martijn Dekkers, Suzanne Rademakers, Gert Jansen. CELL BIOLOGY, UNIVERSITY OF ERASMUS MEDICAL CENTER, 3015 GE, Rotterdam, Netherlands.

NaCl is essential for homeostasis and physiological functions in many organisms. However, the molecular mechanism of NaCl detection is not well known. In mammals, the epithelial Na+ channel (ENaC) and the transient receptor potential ion channel of the vanilloid type 1 (TRPV1) have been shown to be involved in NaCl detection. Previous studies in C. elegans identified five genes involved in NaCl chemoattraction. These are tax-2 and tax-4 (cyclic nucleotide gated (CNG) channel subunits), tax-6 and cnb-1 (calcineurin A and B subunits) and ncs-1 (neuronal calcium sensor). Analysis of these mutants in our assay, in which we exposed the animals to a very steep NaCl gradient, showed reduced chemotaxis to NaCl. However we found that these mutants still showed significant attraction at higher NaCl concentrations. By analyzing the behaviour of double mutants, we found that chemotaxis to NaCl involves two genetic pathways. The first pathway involves two mitogen activated protein (MAP) kinases, nsy-1 and sek-1, and three genes that have been previously characterized, tax-2, tax-4 and tax-6. The second pathway involves tax-2, another CNG channel subunit, cng-3, the Gα protein odr-3, the TRPV channel subunit osm-9 and the guanylate cyclase gcy-35. We used cell specific rescue of the mutant genes, laser ablation of specific neurons and neuronal calcium imaging to find out where in the neuronal circuit of C. elegans these genes function. Thus far, the involvement of the main salt sensing neurons, ASE, has been confirmed. In addition, we found that the ADF neurons also play a role. We are currently performing a synthetic genetic screen to identify additional genes that play a role in NaCl chemotaxis. We are using odr-3 mutants to find mutants that affect the nsy-1/sek-1/tax-2/tax-4/tax-6 NaCl chemotaxis pathway.

548B

Coordinated Regulation of Foraging and Metabolism by RFamide Neuropeptide Signaling. **Merav Cohen**[1,4], Vincenzina Reale[2], Birgitta Olofsson[3], Andrew Knights[2], Peter Evans[2], Mario de Bono[1]. 1) MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK; 2) Inositide Laboratory, The Babraham Institute, Cambridge CB2 4AT, UK; 3) Department of Zoology, University of Cambridge, Downing Street, Cambridge CB2 3EJ, UK; 4) Department of Genetics, The Hebrew University, Jerusalem 91904, Israel.

Animals modify food-seeking behavior and metabolism according to perceived food availability. Here we show that, in the roundworm *C. elegans*, release of neuropeptides from interneurons that are directly postsynaptic to olfactory, gustatory, and thermosensory neurons coordinately regulates behavior and metabolism. Animals lacking these neuropeptides, encoded by the *flp-18* gene, are defective in chemosensation and foraging, accumulate excess fat, and exhibit reduced oxygen consumption. Two G protein-coupled receptors of the NPY/RFamide family, NPR-4 and NPR-5, are activated by FLP-18 peptides in vitro and exhibit mutant phenotypes that recapitulate those of *flp-18* mutants. Our data suggest that sensory input can coordinately regulate behavior and metabolism via NPY/RFamide-like receptors. They suggest that peptidergic feedback from interneurons regulates sensory neuron activity, and that at least some of this communication occurs extrasynaptically. Extrasynaptic neuropeptide signaling may greatly increase the computational capacity of neural circuits.

549C

Identification of Appetite Signals in *C.elegans*. **Justine Melo**, Gary Ruvkun. Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA.

C. elegans are discriminating feeders that will leave a food source in search of better alternatives if the current food source fails to support rapid growth and reproduction. The internal energetic and physiologic cues that signal an insufficient diet and induce lawn-leaving (ie. foraging) behavior in *C. elegans* are unknown. Reasoning that artificial inactivation of genes involved in basic physiology could be used to simulate dietary deprivation, a pilot screen of ~30 essential genes was conducted to determine whether RNAi could be used to induce foraging behavior. Inactivation of genes governing gut development and homeostasis, mitochondrial respiration and protein synthesis were found to induce robust foraging phenotypes. Additionally, animals inactivated for these genes exhibited the behavioral features of starved animals, namely rapid locomotion and low turning frequency. RNAi-treated animals responded normally to chemotactic cues, indicating proper nervous system function.

Having validated that RNAi-induced gene inactivations could be used to stimulate foraging behavior, I carried out a large-scale screen for internal signals of appetite in *C.elegans*. Because genes involved in basic physiology and metabolism seemed most likely to function in nutrient signaling, ~6,000 genes with annotated metabolic or essential functions were tested for their ability to stimulate foraging behavior upon inactivation by RNAi. Of these, ~250 genes exhibited a robust foraging response upon inactivation. Major classes of genes identified include those essential for basic energetic processes such as mitochondrial function and lipid, sugar and steroid metabolism. The protein synthetic machinery was another prominent target of the screen.

In order to identify the tissues in which foraging signals originate, a panel of strains has been constructed that restricts feeding-based RNAi to specific tissues, namely the intestine, nervous system, hypodermis, muscle or gonad. These strains are being used to retest the collection of ~250 foraging "hits" to identify those tissues involved in relaying appetite signals to the nervous system. In summary, the aim of these studies is to identify the biochemical and physiologic pathways that control metabolic sensation in *C.elegans*, and to generate an atlas of the tissues that relay such signals to the nervous system for the purpose of generating adaptive feeding behaviors.

550A

A Microfluidic Platform for High-throughput Calcium Imaging Assays. **Trushal Chokshi**[1], Nikos Chronis[2,3]. 1) Department of Electrical Engineering and Computer Science, University of Michigan, 1301, Beal Avenue, Ann Arbor, MI-48109; 2) Department of Mechanical Engineering, University of Michigan, 2350 Hayward Street, Ann Arbor, MI-48109; 3) Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI-48109.

We developed a microfluidic platform for performing high-throughput Ca+2 imaging of chemosensory neurons in C. elegans. The platform automatically loads and unloads single nematodes at the stimulation site and delivers a chemical odor to the nematode's nose. The platform is interfaced with an image analysis software that assists in collecting high-resolution Ca+2 imaging data without any manual intervention. The automated platform is an essential tool for obtaining repeatable, accurate imaging data from large populations of nematodes and therefore identifying significant statistical trends. We used the microfluidic platform to explore the impact of aging on the chemosensory properties of the ASH neuron. The ASH calcium transients in young nematodes were observed to be statistically different from older nematodes. We envision the use of the proposed microfluidic platform for high-throughput screening of age-resistant mutants or drugs that have anti-aging properties.

551B

High-throughput mostly-automated quantification of subtle behaviors. Nicholas Swierczek[1], Andrew Giles[2], Catharine Rankin[2], **Rex A. Kerr**[1]. 1) Janelia Farm Research Campus, Asburn, VA, U.S.A; 2) Department of Psychology, University of British Columbia, Vancouver, BC, Canada.

We have designed and built a tool, the Multi-Worm Tracker (MWT), that enables analysis of subtle behavioral defects at speeds suitable for forward genetic screening.

The Multi-Worm Tracker images a plate of worms with a 4 megapixel camera running at 10 Hz, delivers stimuli at user-specified intervals, and uses real-time image analysis to extract and save key parameters for each worm. We typically track dozens of worms on a single 5 cm plate; this allows rapid screening of baseline behaviors such as speed and turning frequency; of stimulus-driven behaviors such as reversals induced by tap to the plate; and of adaptations to repeated stimuli. Due to the low magnification, small-scale features such as shape of body bends cannot be reliably quantified; these require a high-magnification single-worm tracker.

We are using the MWT to quantify in detail the tap habituation behavior of *C. elegans*. For instance, we have recorded from tens of thousands of wild-type worms to generate highly reliable statistics, and carefully sampled inter-stimulus intervals from seconds through minutes. We are also preparing to screen for tap habituation mutants, which would be highly impractical without an automated system. We demonstrate that we can detect and cluster existing mutants both for baseline behavior and tap habituation, and use analysis of wild-type variability to design an effective protocol for conducting a large-scale screen. (With luck, we will present preliminary results from a pilot screen.)

Although the MWT was designed with tap habituation in mind, the system is generally useful for rapid quantification of behavior. We therefore have taken a number of steps to enable other labs to set up similar systems. The MWT software is open source (but requires commercial run-time libraries) and after the meeting will be available at http://sourceforge.net/projects/mwt; parts lists and installation instructions are also available. We also provide post-acquisition analysis software that plots or saves summary data with more sophistication than is possible online, and allows browsing of the data in 2D map format for easy manual validation of the results. We have conducted proof-of-principle experiments for foraging and chemotaxis; we hope that other labs will further develop these assays using the MWT or a similar system.

552C

Whole brain calcium imaging with plane illumination. Wafa Amir, Nicholas Swierczek, **Rex A. Kerr**. HHMI Janelia Farm Research Campus, Asburn, VA, U.S.A.

Calcium imaging provides a non-invasive means of monitoring the activity of individual neurons in the worm. Ideally, hypotheses about the function of neural circuits could be verified by simultaneous observation of the activity of the neurons involved in the circuit. However, moving beyond single-neuron imaging has been challenging.

The primary source of the difficulty is out-of-focus fluorescence; this is doubly-bad, as neurons out of the focal plane wash out the neurons of interest in the focal plane, and the out-of-focus calcium indicator molecules still photobleach as badly as always despite providing no useful information. To solve this problem, we are using plane illumination: the light source for fluorescent imaging is a laser brought in from the side and focused down to a sheet that is coincident with the focal plane of the microscope. This provides optical sectioning to approximately 6 microns, a 5-fold improvement over illuminating the entire nerve ring.

We have built a microscope for plane illumination in C. elegans (PICE). This system couples the laser input to the objective through a bracket, and includes a piezo collar for rapid z-focusing. Using high speed EMCCD cameras, we can capture the nerve ring at a rate of ten volumes per second.

We have demonstrated that the PICE system allows up to about two dozen neurons to be distinguished; full neuronal labeling still gives images which are difficult to analyze. We also show that the PICE method results in improved image quality and decreased photobleaching when performing volume scans as compared to traditional epi-illumination, and has minimal drawbacks when used for single-plane calcium imaging.

The PICE system was originally designed for imaging where worms are glued on top of an agarose pad. Microfluidic devices have been used to provide greatly enhanced control of the worm's spatial and chemical environment, but forming a light sheet inside such a device is nontrivial. We have devised several strategies to couple PICE with microfluidic devices and will describe the advantages of each and our progress towards implementing them.

553A

Improving optogenetic methods in *Caenorhabditis elegans*. **Christian Schultheis**[1], Georg Nagel[2], Alexander Gottschalk[1]. 1) Goethe University Frankfurt, D-60438 Frankfurt, Germany; 2) University Würzburg, D-97082 Würzburg, Germany.

The optogenetic approach uses exogenous, light-sensitive proteins for *in-vivo* light-dependent depolarization (Channelrhodopsin-2; ChR2) and hyperpolarization (Halorhodopsin; NpHR) of neurons or muscles, respectively. Optogenetic tools are becoming widely used for functional characterization of synaptic transmission and neuronal networks (Zhang et al.; Nature 2007; Liewald et al.; Nat. Meth. 2008). However, some issues prevent optogenetic tools to be used to their full potential: Two of those issues are challenged in the following experiments. To make use of intersecting promoters for a more cell-specific expression, Channelrhodopsin-2 was fragmented genetically in different loops between the TM domains. Reconstitution of two fragments after coexpression in body wall muscle cells was then monitored by fluorescence and by contraction effects resulting from the photoactivation of ChR2. Significant contraction effects confirm a functional reconstitution of two complementary fragments, though these effects were strongly reduced compared to the positive control. In a similar approach, Halorhodopsin was fragmented and analyzed for functional reconstitution. In a second approach for cell-specific expression of ChR2, we apply the FLP-recombinase (Davis et al., PloS Genetics, 2008). Expression of ChR2 from a first promoter is prevented by a transcriptional stop flanked by FLP recombination target (FRT) sites (FRT-block). Following FLP-recombinase expression with a second promoter, the FRT-block is excised and ChR2 is hence expressed in cells at the intersection of the two promoter expression patterns. In our experiments we adjusted this system for use in cholinergic motorneurons and the AVA-neurons. To allow a prolonged depolarization of excitable cells, e.g. to influence cellular events during development, various mutants of ChR2 were analyzed for use in *C. elegans*. Mutation of C128 results in accumulation of M, N and O intermediates during the photocycle of ChR2, and hence the channel stays much longer in the open state (Berndt et al., Nat Neurosci 2009). Upon expression of various ChR2 C128-mutants in cholinergic motorneurons we found prolonged contraction effects in comparison to full-length ChR2 after photoactivation with blue light. In addition to the directed inactivation of ChR2 C128 mutants with green light this allows a temporally very precise regulation of ChR2-dependent depolarization of excitable cells using only a short lightpulse of reduced intensity. The variants of ChR2 investigated here hence complement optogenetic tools for a precise light-driven *in-vivo* stimulation of neurons in *C. elegans*.

554B

C. elegans behavior in dynamic microfluidic environments. **Dirk R Albrecht**, Cori Bargmann. Laboratory of Neural Circuits and Behavior, The Rockefeller University, New York, NY.

Neuronal circuits that govern goal-directed behaviors such as chemotaxis are highly interconnected, suggesting that their emergent functions may not be evident from the properties of individual neurons or connections. To study the underlying neural computation and dynamics, an engineering approach would systematically quantify output responses to many precise inputs under many circuit perturbations. While *C. elegans* locomotory behaviors are easily measured, and genetic tools enable precise circuit modification, the presentation of input stimuli in typical agar-plate assays is often poorly controlled. To address this limitation, we developed microfluidic liquid-filled arenas that enable the study of freely-moving animals in highly precise and dynamic microenvironments.

We first optimized arena geometry to mimic *C. elegans* crawling motion, speed, and behavioral responses on agar surfaces. Microfluidic features create controlled liquid gradients that span several cm for population behavior or change sharply across the animal (<50 micron), and remain stable for hours or change rapidly within seconds. The transparent arenas are compatible with light-based neural control (via genetically-encoded rhodopsins) and fluorescent readouts of neural activity.

We are characterizing wildtype *C. elegans* responses to complex spatial and temporal odorant patterns (steps and ramps) to understand how modulation of specific behaviors (e.g., speed, types of turns) influences chemotaxis strategy. Similar studies of genetic mutants with disrupted neurons or neuronal connections are revealing the role of perturbed information flow in directing these behaviors. For example, we found new behaviors (gradient-directed turning), new circuit pathways (glutamate-independent speed regulation), and strong phenotypes in subtle neuromodulatory mutants. Overall, the vast improvement in stimulus control in these microfluidic arenas enables new studies to understand the flow of information in neural circuits governing behavior.

555C

Regulation of *C. elegans* male mate-searching behavior by hermaphrodite cuticular cues. **Arantza Barrios**, Scott Emmons. Dept Molec Gen, Albert Einstein Col Med, New York, NY.

Male mate-searching behavior in *C. elegans* provides a model to understand how animals integrate internal physiological needs and drives with external environmental stimuli to produce adaptive behavior. Unlike the self-fertile *C. elegans* hermaphrodite, the *C. elegans* male needs to mate in order to reproduce. The male has therefore evolved strategies to locate and remain within a source of both food and hermaphrodite mating partners. *C. elegans* males explore their environment in search of mates and will leave food if hermaphrodite mating partners are absent [1]. However, when mates and food coincide, male exploratory behavior is suppressed and males are retained on the food source [1].

What is the neural circuit that regulates male mate-searching? We have identified the male-specific ray neurons in the tail as part of the circuit that stimulates mate-searching behavior. In the absence of mates, ray neurons (mainly RnB with contribution from RnA) stimulate exploration away from food by promoting exits from the food lawn and inhibiting high angle turns upon exiting the lawn. In the presence of mates, periodic contact with a hermaphrodite, detected through the rays, changes the male's behavior during periods of no contact, preventing the male from leaving the lawn. The hermaphrodite signal is conveyed by the male-specific EF interneurons, which are post-synaptic to the rays and send processes to the nerve ring in the head [2].

What is the nature of the hermaphrodite signal? Male retention by hermaphrodites is independent of copulation since neither the hermaphrodite vulva nor the male spicules are required for suppression of mate-searching behavior. Furthermore, males are fully retained by dead PFA-fixed hermaphrodites but not PFA-fixed males and retention is lost if the hermaphrodites are washed with an organic solvent such as hexane. These results suggest that sex-specific lipids on the hermaphrodite cuticle are important for the suppression of male exploratory behavior. Indeed, males are retained by males with feminized hypodermis (by expression of *dpy-7::tra-2 IC* -construct provided by W. Mowrey and D. Portman) indicating that the *C. elegans* cuticle is composed of sexualized chemicals that produce an effect in the behavior of other males.

1. Lipton, J. *et al.* (2004). J Neurosci 24, 7427-34.
2. Barrios, A. *et al.* (2008). Curr Biol 18, 1865-71.

556A

PQN-21, a prion-like protein is involved in learning and memory in *C. elegans*. **Daphne Bazopoulou**, Nektarios Tavernarakis. IMBB, FORTH, Heraklion, Crete, Greece.

We are characterizing PQN-21, a *C. elegans* protein bearing a Q/N rich-'prion'domain. Prions are infectious agents implicated in a variety of neurodegenerative diseases in mammals, generally referred to as transmissible spongiform encephalopathies. The glutamine/asparagine (Q/N)-rich domain of prions appears to be responsible for their unusual capacity to fold into structurally and functionally distinct conformations, one of which is self-perpetuating. Usually the self-perpetuating-'prion' form is organized in self-seeding polymers and can induce other proteins with similar sequences to acquire the 'prion' state, creating a chain reaction. Prion-like proteins are widespread (found in mammals, fungi, yeast), conserved and serve diverse functions. In *Aplysia*, the prion-like neuronal protein CPEB functions as a positive regulator of mRNA translation in stimulated synapses and helps to maintain long-term synaptic changes associated with memory acquisition and storage. We find that *pqn-21* is expressed in *C. elegans* neuronal and glial cells and that PQN-21 localizes in the nucleus. Deletion of the *pqn-21* gene results in defective gonadal outgrowth and egg-laying. Interestingly, while PQN-21-depleted animals display normal chemotaxis to several soluble and volatile compounds, they show strongly impaired conditioning to chemicals, indicating a shortfall in associative learning and memory, as well as impaired olfactory adaptation. In addition, we find that mutants with defective glial cells show a similar phenotype. Relative to this, we showed that expression of PQN-21 in glial cells is necessary for olfactory adaptation. Based on these observations, we hypothesize that prion-like PQN-21 protein functions in glial cells to facilitate learning and memory. Given the conserved function of prion-like proteins among diverse species, *C. elegans* offers an attractive and versatile platform in which to dissect the relevant mechanisms. To this end, we are characterizing the function of PQN-21 and the involvement of glial cells in various paradigms of learning and memory in *C. elegans*.

557B

Agar groove masks locomotion related phenotypes. **Stefano Berri**[1], Jordan H. Boyle[1], Manlio Tassieri[2], Ian A. Hope[3], Netta Cohen[1,4]. 1) School of Computing, University of Leeds, Leeds LS2 9JT, UK; 2) School of Physics, University of Leeds, Leeds LS2 9JT, UK; 3) Institute of Integrative and Comparative Biology, University of Leeds, Leeds LS2 9JT, UK; 4) Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK.

When *C. elegans* crawls on agar, it carves a groove in the surface and leaves a track behind it. It has been postulated that this groove is integral to the mechanism of crawling, allowing the sinusoidal trajectory of the head to dictate the shape on the rest of the body. Indeed, a stiff groove would result in a strong asymmetry in the environment's resistance to motion in the normal (sideways) and longitudinal (along the body) directions, constraining the movement of the body. However, our recent findings show that the groove is not essential to generate a crawling shape in the wild type worm, as it can produce the typical crawling waveform even on a flat non-deformable surface. These results are consistent with the fact that swimming and crawling actually represent a single behavior[1], produced by a single neural control system. Any mechanism that fails without a groove could not account for locomotion in water.

We next asked whether the physical forces the groove applies could have greater significance when the worm is defective. We recorded various locomotion-related mutants moving in a range of media with increasing visco-elasticity. We found that the movement of unc-8 (e49) and vab-7 (e1562) mutants on agar is very similar to that of wild type worms. When observed in water, however, it is clear that they are highly defective in the posterior half of their body which usually fails to show dorso-ventral oscillations at the same frequency of the head. When observed in media with increasing stiffness, the phenotype gradually disappears as the properties of the environment progressively mask the defect in locomotion.We conclude that while the groove has minimal importance to wild type locomotion, it can have a strong masking effect when the worm is defective. We suggest that, when screening for locomotion related phenotypes, water may be a more appropriate medium than agar. A screen for mutants defective in swimming may actually be a screen for mutants affected in their single locomotion gait, but for which the phenotype is masked by the groove on the agar surface.

[1] Berri S, Boyle JH, Tassieri M, Hope IA and Cohen N. 2009. "Forward locomotion of the nematode *C. elegans* is achieved through modulation of a single gait." HFSP Journal, In press.

558C

Investigating C. elegans Learning as a Possible Approach to the Study of Consciousness. **Nikhil Bhatla**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

To study the mechanisms of consciousness, it would be useful to identify a highly manipulable organism that has perception, a key element of consciousness. I define perception to mean the ability to have an inner, qualitative experience of one's environment, beyond simply sensing and responding. Perception is the subjective experience of what something feels like, such as sugar tasting sweet, snow feeling cold, and 700 nm light looking red. One way to determine whether an organism is capable of perception is to determine whether it can be aware of its surroundings. In people, awareness can be measured indirectly by a trace conditioning experiment. In this experiment, a neutral stimulus is presented and removed, and after an appropriate period of time a different, aversive stimulus is presented and removed. Each trial is repeated many times. A person is said to have learned if she starts responding to the neutral stimulus in an aversive way. On average, those who successfully learn are aware, meaning that they verbally report that the neutral stimulus (e.g. sound) predicts the aversive stimulus (e.g. an air puff to the eye or an electric shock). Note that if there is no temporal delay between the stimuli, learning no longer correlates with awareness (Clark & Squire, Science 1998; Carter et al., PNAS 2003).

Since trace conditioning correlates with awareness, it might be possible to use this type of learning to test whether non-verbal organisms have the ability to perceive. I plan to investigate whether C. elegans can perform trace conditioning. Specifically, I plan to pair a neutral odor with carbon dioxide, which causes locomotion reversals (Hallem & Sternberg, PNAS 2008). If the presentation of the neutral odor alone is sufficient to induce reversals after the training period, I plan to screen for worms that cannot perform this task. In this way, I hope to identify genes and cells required for trace conditioning in worms. Perhaps these same genes will also be involved in perception and consciousness in worms and other animals.

559A

Looking for ALA-independent Sleep. **Julie Cho**, Paul Sternberg. Department of Biology, California Institute of Technology, Pasadena, CA.

The conservation of sleep across animal species and the impact of disordered sleep on health point to a critical function and highlight a need for greater understanding of sleep regulation. Previous work by Van Buskirk and Sternberg has shown that the ALA neuron in Caenorhabditis elegans is involved in inducing a quiescent state in the worm via the EGF/LET-23 signaling pathway. Ablation of the ALA neuron results in hyperactivity during lethargus, the normally quiescent period that precedes each larval molt. In parallel work by Raizen and colleagues, lethargus was shown to satisfy the behavioral criteria of sleep. Furthermore, the quiescence during lethargus was shown to be dependent on the activity of EGL-4, a cGMP-dependent protein kinase (PKG). An egl-4 loss-of-function mutation results in disruption of sleep-like behavior.

Despite the fact that EGFR activation in the ALA neuron is sufficient to induce a strikingly quiescent state, ALA ablation in wild type animals leads to a relatively mild loss of quiescence during lethargus. Thus it is likely that other pathways contribute to the sleep-like state. We are interested in characterizing the EGF-independent signaling pathways regulating sleep in C. elegans. In order to determine whether EGL-4 activity contributes to EGF-dependent or EGF-independent sleep, we have studied the interaction between the EGL-4 and ALA-ablated hyperactive phenotypes. We have found that the absence of the ALA did not significantly alter locomotion in the egl-4(lf) mutants during lethargus. These results are consistent with egl-4 and ALA acting in a common pathway. We aim to look for alternative pathways that may be involved in regulating sleep.

560B

Genetic and molecular dissection of nictation. **Myung-gyu Choi**, Harksun Lee, Junho Lee. Dept Biological Sci, Kwanak-gu, Seoul National Univ, Seoul, Korea.

Dauer is an alternative form during C. elegans development. C. elegans enters the dauer stage in adverse environment for long-term survival. Dauers have many unusual features which are not shown in other developmental stages. For example, pharyngeal pumping is almost arrested and fat storage is increased. Behavior pattern is also different. Chemotaxis and thermotaxis do not occur in the dauer stage. In plates, they are lethargic but actively respond to mechanical stimulation. Among many characters of dauers, nictation is a very notable behavior. Nictation is the dauer specific behavior that is observed in three-dimensional space. They can climb up onto any projection and wave their body on the top. Although C. elegans is known as the free-living organism in the soil, many Caenorhabditis species associate with other species such as snails and insects. Nictation would be beneficial behavior for their dispersal to other ecological niche. It was also reported that most of naturally isolated C. elegans were dauer larvae. To identify the genes and neural circuits for nictation, we established nictation assay system and used genetic approach. By screening of pre-existing mutants, we found that the function and development of specific neurons are important for nictation. Further characterization of candidate genes will elucidate the mechanism which underlies this specific behavior.

Corresponding author: elegans@snu.ac.kr (J. Lee).

561C

Analysis of Electrotaxis Behavior in C. elegans. **Steven D. Chrisman**, Lucinda Carnell. Biological Sciences, Central Washington University, Ellensburg, WA.

C. elegans sense both polarity and amplitude of an electric field by uninterrupted crawling toward the negative pole, a behavior referred to as electrotaxis (Sukul and Croll, 1978). Gabel et al. (2007) have identified specific amphid neurons and the neural circuitry important for this behavioral response by tracking the animal's movement in a rotating electric field. We are interested in studying the sensory response to an electric field at varying electric field strength in a non-rotating field. We observed that as previously described animal taxis toward the negative pole (cathode). Interestingly, we also observed that decreasing the field strength results in the animals' responding with an immediate reversal. To examine the sensory mechanism responsible for electrotaxis, we have tested known chemosensory-defective mutants and performed a genetic screen to isolate animals defective in the sensing of the electric field. We have isolated seven candidate mutants. The mutants fall into two general classes: those that taxis to lower field strengths (hypersensitive) and those that respond to higher field strengths (resistant). We are in the process of characterizing these mutants further by recording their response and measuring velocity using the automated tracking software, WormTracker (Ramot et al. 2008), which allows us to observe the behavior of a population of animals simultaneously. Initial experiments indicate the first observable sensory response of animals at low field strengths is to increase velocity, which is followed by a sensing of polarity at increasing field strengths. To determine and quantify the animals' sensitivity to an electric field, we are measuring velocity at different field strengths over time. Images will be captured at 15 second intervals every 30 seconds as the worms move across the agar plate to the negative pole. The velocities for each group of animals will be measured at field strengths ranging from 0.5 V/cm to 14V/cm.

Gabel et al. 2007 Journal of Neuroscience 27(28):7586-7596
Sukul, N.C. and Croll, N.A. 1978 J. Nematology 10, 314-315
Ramot et al. 2008 PLoS ONE 3(5): e2208. Doi:10.1371/journal.pone.0002208.

562A

Serotonin and dopamine as negative regulators of male mating behavior. **Paola Correa**[2], L. Rene Garcia[1,2]. 1) Howard Hughes Medical Institute; 2) Department of Biology, Texas A&M University.

We want to address how catecholamines and acetylcholine (Ach), work together to regulate male mating behavior in C. elegans. A step involved in male mating behavior is spicule insertion. Two events that occur during spicule insertion attempts are rhythmic sex muscle contractions, which causes the spicules to prod the vulva, and tonic spicule contraction, which sustains full insertion of the spicules. These two muscle contractile behaviors can be mimiced with Ach agonist levamisole (LEV), arecoline (ARE) and nicotine (NIC). These agonists induce spicule protraction by cholinergic activation of cation channels. Findings from others have shown that serotonin and dopamine are upstream modulators of egg laying, fat metabolism, feeding rates, and locomotion. Secretion of either of these neurotransmitters can result in multiple downstream effects including the increase/ decrease of Ach release. To test the modulatory effect of catecholamine in the spicule protraction circuit, we exposed virgin males to exogenous serotonin or dopamine and either LEV, ARE or NIC. We assessed the percentage of males that protracted their spicules when exposed simultaneously to catecholamine and acetylcholine agonist or when exposed sequentially (i. e. catecholamine exposure first, acetylcholine second). Our preliminary data suggested that exogenous activation of dopamine and serotonin signaling negatively regulates cholinergic signaling in the male spicule circuit. To test for positive effects in regulation of catecholamine, tph-1 and cat-2, serotonin and dopamine deficient mutants respectively, were analyzed for their ability to protract their spicules under Ach agonists. The spicule muscles of both of these mutants were resistant to ARE, but not to LEV, when exposed to high agonist concentrations. This indicates that endogenous serotonin and dopamine contribute to ARE induced protraction. Spicule protraction is also negatively regulated by UNC-103, UNC-43, and LEV-11, directly or indirectly. We have assessed spicule protraction after exposure to catecholamine and acetylcholine simultaneously in the loss of function mutants of these negative regulators. None of the negative regulators are involved with serotonin or dopamine inhibition of cholinergic signaling. We are also currently identifying the receptors that transmit the dopamine and serotonin signals by determining expression of dop-1, dop-2, dop-3, dop-4, ser-1, ser-2, ser-3, ser-4, ser-7 and mod-1 in the neurons and muscles of the spicule protraction circuit.

563B

Dissecting the role of serotonin and acetylcholine on egg-laying motor synapses by calcium imaging. **James F. Cregg**[1], Trushal V. Chokshi[3], Nikos Chronis[3], William R. Schafer[2]. 1) Department of Biology, University of California San Diego, La Jolla, CA, USA; 2) Laboratory of Molecular Biology, MRC, Cambridge, UK; 3) Department of Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA.

Egg-laying is a simple motor program whose regulation integrates responses to diverse sensory cues (food, touch and osmolarity). The principal egg-laying motorneurons (the HSNs) use at least two neurotransmitters, acetylcholine (ACh) and serotonin (5-HT), both of which have been shown to have stimulatory and inhibitory effects on egg-laying. We attempt to tease apart the contributions of these molecules at egg-laying neuromuscular synapses by observing of the effects of exogenous pharmacological agents on both intact and dissected worm preparations with calcium imaging. We have designed a microfluidic chip that allows us to rapidly apply neurotransmitters and other pharmacological agents to intact and dissected worms. Using this approach, we have observed rapid activation of vulval muscle (VM2s) calcium transients by acetylcholine and cholinergic agonists. Indicating that acetylcholine is indeed a potent excitatory neurotransmitter at vulval muscle synapses. We have also observed more sustained activation of muscle activity by serotonin, both in intact and dissected worms. Utilizing the previously characterized serotonin receptor mutants, mod-1, ser-1, ser-4, ser-7, and the newly discovered ser-5 (generously provided by the Komuniecki lab), we have created a collection of strains containing only a single functional receptor subtype, allowing us to assess the contribution of each receptor to vulval muscle modulation by serotonin. Each serotonin receptor confers a unique response in the VM2s to different concentration of 5-HT. One, ser-4, previously characterized as inhibitory, stimulates calcium responses at concentrations of 5-HT three orders of magnitude less that which is commonly used in egg-laying assays. Other receptors display activity at significantly higher concentrations of 5-HT, closer to conditions in previous assays. Loss of any one of the five receptors causes significant loss of sensitivity to 5-HT. Further analysis of receptor phenotypes under varied conditions and in different combinations will be presented.

564C

How do *egl-4* and *pde-4* interact in regulating lethargus? **Nooreen S Dabbish**, David M Raizen. University of Pennsylvania, Philadelphia, PA.

Both the cGMP-dependent kinase *egl-4* and the cAMP phosphodiesterase *pde-4* have been shown to regulate worm lethargus behavior [Raizen 2008]. A gain of function mutation in *egl-4* promotes quiescence during larval development and in adult worms. *egl-4(gf)* animals additionally show a prolonged response latency to dilute octanol, whereas *egl-4(lf)* animals have decreased quiescence and decreased octanol response latencies during lethargus. *pde-4(lf)* mutants, which have increased cAMP signaling, demonstrate decreased octanol response latencies during lethargus. We are analyzing the phenotypes of double mutants to determine whether *egl-4* and *pde-4* act in series or in parallel. Preliminary observations indicate that *pde-4(ce268); egl-4(n479)* double mutant animals grow slower, have a smaller brood size, and show defects in locomotion in comparison to single mutants. This synthetic phenotype is consistent with the idea that these genes are acting in parallel. We will report results of our analysis of other double mutant combinations.

565A

Analysis of a novel type of thermotaxis mutant *nj24*. **Taishi Emmei**[1], Nana Nishio[1], Hiroyuki Sasakura[1], Mari Akasaka[1,2], Atsushi Kuhara[1], Ikue Mori[1,3]. 1) Group of Molecular Neurobiology, Nagoya University; 2) Present Address: Group of Developmental Biochemistry, Nagoya University; 3) CREST-JST.

After cultivated with food, animals migrate to the cultivation temperature on a thermal gradient, whereas after cultivated without food, animals do not (Hedgecock and Russell, 1975; Mori and Ohshima, 1995). This behavior is called thermotaxis. Many thermotaxis-defective mutants were isolated and classified mainly into three classes; cryophilic mutant that migrates to a lower temperature than the cultivation temperature, thermophilic mutant that migrates to a higher temperature than the cultivation temperature and athermotactic mutant that migrates almost at random.

The *nj24* mutant isolated in behavioral screening (Mohri *et al.*, 2005) always migrates to a slightly higher temperature area than the cultivation temperature after cultivated with food at a certain temperature. After cultivated at 23°C or 20°C, respectively, the mutant migrated to about 1°C higher area than 23°C or 20°C area, respectively. Most thermophilic mutants previously reported migrate to higher temperature independently of their cultivation temperature. The thermotactic phenotype of the *nj24* mutant is thus novel. For proper thermotactic behavior, animals would sense two kinds of temperatures, cultivation temperature and assay plate temperature, and compare them on a temperature gradient. The *nj24* mutant might sense and memorize cultivation temperature as higher than the actual temperature, thereby exhibiting thermophilic phenotype on a thermal gradient. Another possibility is that the mutant would sense and memorize cultivation temperature correctly but could not compare it with assay plate temperature.

At starved condition where animals were cultivated without food for a few hours, the thermophilic phenotype of the *nj24* mutant was enhanced, suggesting starvation signal caused this enhanced phenotype. After starved at 23°C, N2 animals distributed between 20°C and 26°C areas uniformly, whereas the fraction of the mutants that migrated to higher than 23°C increased as compared with the mutants at fed condition. Similarly, after starved at 20°C, N2 animals distributed between 17°C and 23°C areas uniformly, whereas the fraction of the mutants that migrated to higher than 20°C increased. After starved at 17°C, N2 animals migrated to lower temperature than 17°C, whereas the mutants distributed between 14°C and 20°C areas uniformly. We are now mapping *nj24* to elucidate how the responsible gene is involved in thermotaxis at molecular, cellular and circuit level.

566B

Modulation of the neural requirement for sex pheromone perception in *Caenorhabditis elegans* by two conserved signaling pathways. **Kei C. Fan**, Lan Fu, King L. Chow. Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

Previous studies of *C. remanei* and *C. elegans* sexual behavior revealed that a sex attractant is produced in females of the dioecious *C. remanei*. However, males from both species can respond to this *remanei* sex pheromone. By genetic analyses and cell-specific ablation studies, the male-specific response towards sex pheromone in *C. elegans* was shown to be coordinated by the three neuronal cells: AWAs, AIZs and the male-specific CEMs. Males with one of these neurons defective failed to respond to the attractant in our chemo-attraction assays.

We showed that this sex pheromone-mediated behavior displayed by males is regulated by the IGF and TGF-beta signaling pathways. Through genetic analysis, male mutants of genes encoding various receptor components and signaling molecules in these two pathways are not responsive to the attractant, indicating the necessity of these pathways in the perception machinery. In *C. elegans*, the IGF and TGF-beta signaling pathways coordinate multiple biological processes such as mediating dauer formation, modulating the rate of aging and searching for food source. These activities are often elicited by various inputs, e.g., pheromone, chemicals and bacteria, picked up by the chemo-sensory neurons. In recent studies, we found that the IGF and TGF-beta pathways control the pheromone response through regulation of the nervous system. In this report, we will present preliminary results on the characterization of these various components acting in specific cell type through cell-specific knockdown and cell specific rescue experiments. Expression profiles of these molecules in dedicated neurons were documented. The effect of selected mutants in the IGF and TGF-beta signaling pathways in the neural patterning and connection was examined by con-focal microscopy. Ultimately, the potential implication of the interplay of paracrine factors and rapid chemosensory processing will be discussed. (This study is funded by the Research Grants Council, Hong Kong.).

567C

Measuring Effects of Serotonin on Locomotory Behavior. **Eric P. Foss**, Lucinda Carnell. Biological Sciences, Central Washington Univ., Ellensburg, WA.

Serotonin (5-HT) is a neuromodulator that regulates food-associated behaviors in both vertebrates and invertebrates. In *C. elegans* exposure to exogenous 5-HT slows locomotion. Several 5-HT receptors, *mod-1*, *ser-1*, and *ser-4* have been implicated in mediating this 5-HT-dependent decrease of locomotion rates. We are interested in studying the acute and long-term effects of 5-HT on locomotory behavior using the automated tracking software WormTracker (Ramot et al. 2008) to determine average velocities of a population of animals over time. From initial studies, examining the effects of increasing 5-HT concentration, we found that without food wild-type (N2) animals were slowed to 16% of control speeds at 4 mg/ml (10.3mM) 5-HT after 30 minutes. Control wild-type animals without 5-HT also display an increase of speed during the first 30 minutes, which is followed by a decrease after 90 minutes. We measured the speed of three 5-HT receptor mutants, *mod-1*, *ser-1*, and *ser-4* to assess their contribution to this slowing response with increasing concentrations of 5-HT. After 30 minutes at 4 mg/mL 5-HT, these mutant animals slowed to 28%, 32%, and 36% of control rates, respectively. However, these mutants all displayed greater resistance to the effect of 5-HT at lower concentrations. The larger goal of this project is to identify an adaptation response where the animals recover after time from the inhibitory effects of 5-HT, which has been previously observed. Because none of the animals displayed recovery over the 90 minute period, most likely due to the lack of bacteria, we have begun to measure speed over longer time periods in the presence of food.

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568A

Chemotactic control by a germline signal in *C. elegans*. **Manabi Fujiwara**, Noriko Satou, Shinich Maruyama, Taku Akamine, Takeshi Ishihara. Dept Biol, Grad Sch Sci, Kyushu Univ, Fukuoka, Japan.

Animals adequately change their behavioral patterns according to their internal states such as the extent of sexual maturation. To examine whether some signal(s) from gonad affect on worm's behavior, first we analyzed *glp-1(ts)* hermaphrodite animals which were raised at the restrictive temperature (25°C) during L2 stage to induce a germline proliferation defect. We found that the germline-defective *glp-1*(both *or178ts* and *e2141ts*) animals show a mild chemotaxis defect to diacetyl, an AWA-sensed volatile attractant. Chemotaxis to another AWA-sensed attractant, pyrazine, and AWC sensed attractants, benzaldehyde and isoamylalcohol, were not affected in these animals, suggesting that germline signal(s) modify the diacetyl specific pathway of chemotaxis. To confirm the germline effect, we ablated the germline precursor cells (Z2, Z3) in wild-type animals with a laser microbeam and found that the chemotactic response to diacetyl but not to pyrazine was reduced in these animals. It is possible that the gonad signal(s) affect the diacetyl sensitivity by regulating the expression of the diacetyl receptor ODR-10 at AWA cilia. We, however, could not observe any changes in the expression pattern and level of ODR-10 tagged gfp in the germline-defective *glp-1* animals.

To examine directly whether signal transduction in the AWA sensory neurons is affected in the germline-defective *glp-1* mutant, we observed the diacetyl-evoked calcium transients by expressing a calcium indicator, Cameleon under the AWA-specific promoter. Utilizing the PDMS microfulidic device (olfactory chip), we found that the AWA neurons, in the control animals with normally developed gonads, responded to diacetyl (1/1000 solution) presentation with sharp increases in calcium levels. The sharp increases were followed by relatively low increases which were sustained during the diacetyl application. The AWA neurons in germline-defective *glp-1* animals also responded to diacetyl (1/1000) similarly to control animals. We are currently analyzing with diacetyl in lower concentrations to see possible changes in the sensitivity in the germline-defective animals.

Finally, we are attempting to isolate suppressors of the chemotaxis defect to diacetyl of the germline-defective *glp-1* mutant. By clonal mutant screening with ~1800 haploid genomes, we have isolated several candidate mutants in which germline defect did not affect diacetyl chemotactic response. Further analyses including the identification of responsible genes will reveal the molecular and cellular mechanism by which gonad signals control the olfactory sensitivity to a specific attractant.

569B

Isolation of suppressors for the thermophilic defect of nPKC mutant. **Tomoyuki Furuta**[1], Atsushi Kuhara[1], Ikue Mori[1,2]. 1) Division of Biological Science, Graduate School of Science, Nagoya Unisversity, Nagoya, Japan; 2) CREST JST.

All animals can respond to surround environment by sensing stimuli and subsequently calculating sensory signals in the neural network. Proper regulations of sensory signals are required for accurate behavioral responses to environmental stimuli. For clarifying the mechanisms on regulations of sensory signals we focused on the thermotaxis behavior in *C. elegans*. After cultivation at a certain temperature with foods, wild-type animals migrate to the cultivation temperature on a thermal gradient. Previous study reported that *ttx-4* mutant animals lacking novel Protein Kinase C (nPKC) migrate to higher temperature than the cultivation temperature (thermophilic abnormality) (Okochi et al., 2005). nPKC(TTX-4) is thought to act as a modulator of temperature signaling in AFD thermosensory neuron, yet how nPKC regulates thermosensory signals is unknown. To elucidate the mechanism regulating thermosensory signals, we isolated suppressors for the thermophilic defect of *ttx-4* mutant. Through screening about 15,000 genomes, two putative *Mos1*-induced suppressor strains (IK806 and IK807) were isolated. On a thermal gradient, IK806 and IK807 migrate to the region of around 20 degrees after cultivation at 17 degrees, although they had no longer shown the similar thermophilic abnormality to that of *ttx-4* mutants. PCR analysis confirmed that *Mos1* transposon was inserted in the genome of suppressor strains. We are currently trying to determine the insertion site of Mos1 transposon and to observe their thermotaxis behavior in detail. We hope that analyzing suppressors for *ttx-4* mutants will give novel molecular insights involved in sensory signaling.

570C

Characterization of dopamine neuromodulation of *Caenorhabditis elegans* chemosensory behavior. **Marx P. Genovez**, Michael Y. Chao. Dept Biol, CSUSB, San Bernardino, CA.

Dopamine (DA) is an important neuromodulator in both vertebrates and invertebrates. Our lab is interested in the role of DA in regulating behavior in the roundworm *Caenorhabditis elegans*. DA has been shown to have a modulatory effect on mechanosensation and general locomotion in *C. elegans*, but its role in chemosensory behaviors is less well characterized. Some studies suggest that DA acts as a dampener of chemosensory signaling (Ferkey et al. 2007; Wragg et al. 2007). In our studies, the DA-deficient mutant *cat-2* had a decreased avoidance response to the toxic chemical octanol. Exogenous DA fully restores normal response to these mutants. These experiments suggest that DA positively regulates aversive chemosensory behavior in *C. elegans*. We have found that mutants lacking *C. elegans* AMPA/kainate-like receptors *glr-1* or *glr-2* had decreased response to octanol, similar to *cat-2*. Exogenous DA only partially restored response to octanol. *glr-1* and *glr-2* mutant animals were very similar to each other in most regards, as were the *glr-2 glr-1* double mutants. We are currently determining what effect exogenous DA has on *cat-2;glr-1* and *cat-2;glr-2* double mutants; results will be presented at the poster.

571A

Identification of EGL-4 downstream effectors. **Yan Hao**, Andrew Box, Laura Schaefer, Ho Yi Mak. Stowers Institute for Medical Research, Kansas City, MO.

We isolated a gain-of-function allele of *egl-4*/cGMP-dependent protein kinase in a genetic screen for mutants with abnormally high fat storage when fatty acid beta-oxidation is attenuated simultaneously. Besides the fat storage defect, the *egl-4(gf)* allele also confers pleiotropic phenotypes such as reduced foraging, reduced pharyngeal pumping rate and small body size.

We conducted a genetic screen for mutant alleles that restored normal feeding behavior and body size in *egl-4(gf)* animals. Molecular cloning of such suppressors of activated *egl-4* (*saeg*) genes revealed a novel transcriptional regulatory complex that mediates EGL-4 activity. Quantitative behavioral analysis indicates that *saeg-2* loss-of-function mutations do not confer altered feeding behavior but specifically suppress the lack of foraging in *egl-4(gf)* animals. SAEG-1 and SAEG-2 physically interacts with each other and we are currently identifying additional complex components using a proteomic approach.

It has been shown that EGL-4 acts upstream of DBL-1, a TGFβ ligand that regulates body size (Hirose et al., 2003). We found that the body size of *saeg-2; dbl-1* mutant animals is similar to that of *dbl-1*. This suggests that EGL-4 regulates body size by modulating the TGFβ pathway via SAEG-2.

Our results demonstrate how EGL-4 controls feeding behavior and body size via transcriptional regulation.

572B

Multiple monoamine receptors modulate nose touch in *C. elegans*. **Vera Hapiak**[1], Gareth Harris[1], Marios Chatzigeorgiou[2], Rachel Wragg[1], William Schafer[2], Richard Komuniecki[1]. 1) Department of Biological Sciences, University of Toledo, Toledo, OH 43606; 2) Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, CB2 0QH, UK.

The ASH sensory neuron in *C. elegans* is polymodal, responding to both noxious (high osmolarity/volatile repellents) and mechanical (nose touch) stimuli to initiate backward locomotion. Examination of one ASH-mediated locomotory behavior, avoidance to octanol, has revealed that modulation of the ASH neural circuit is complex and involves multiple monoamines and distinct amine receptors (Wragg *et al*., 2007; Harris *et al*., 2009). In the present study, we have identified the monoamine receptors involved in another 5-HT stimulated ASH-mediated aversive response, nose touch. Like responses to octanol, multiple 5-HT receptors also have been identified in the food/5-HT sensitization of nose touch. For example, the expression of *ser-5* in the ASHs appears to be essential for 5-HT dependent increases in aversive responses to nose touch. In contrast, both TA and OA inhibit nose touch and two monoamine receptors (F14D12.6 and SER-3) appear to be involved in octopaminergic inhibition. These receptors are currently being localized in the ASH locomotory circuit. Using the calcium indicator cameleon, we analyzed ASH Ca^{2+} responses to nose touch in *ser-5* null animals. ASH Ca^{2+} transients were independent of exogenous 5-HT and more robust in *ser-5* null animals than in wild-type or *ser-4; mod-1; ser-7 ser-1* quadruple null animals that presumably only express *ser-5*. Since we predict that SER-5 stimulates neurotransmitter release at the ASH synapse, the increased Ca^{2+} signaling in the ASH soma was surprising. We are currently exploring whether SER-5 signaling has other effects on the ASH or whether SER-5 also modulates the release of other ligands, perhaps peptides, that inhibit ASH Ca^{2+} dynamics.

573C

Modular connectivity among the motorneurons controlling locomotion. **G. Haspel**, M. J. O'Donovan. National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD, USA.

Seventy five motorneurons of eight classes innervate the body musculature that propels Caenorhabditis elegans forwards or backwards. These motorneurons receive input from five pairs of interneurons and are synaptically interconnected to create a "motorneuronal network". To date, only the anterior half of the motorneuronal network of one animal, spanning 42 motorneurons, has been reconstructed at the level of the electron microscope (White et al., 1986). This reconstruction was recently reexamined and annotated by Chen and colleagues (2006), has given us priceless knowledge, unavailable with any other animal model. We use a new approach to these data to find recurring patterns of connectivity based on the functional position and relative connections of each motorneuron within the network. For this purpose, every motorneuron was re-indexed based on the muscles it innervates. All the connections made by each motorneuron were than expressed according to their relative position along the body. Using cluster analysis, we found that VB and VA (but not AS and VD) exhibited two distinct sub-classes with slight differences in connectivity. We found that most of the connections made by each motorneuron recur within its class or sub-class. This enabled us to describe a single repeating unit that contains 11 motorneurons (DA, DB, DD and two of each AS, VA, VB, VD). Iterating this unit six times along the body of a nematode gives a modular representation of the motorneuronal network. We are using this modular network to give context to activity recorded in motorneurons during locomotion, to design experiments and to model network activity. We have recorded motorneuron activity with a genetically encoded calcium indicator while simultaneously monitoring locomotion behavior. We used a high magnification objective for epifluorescence imaging and an opposing low magnification objective for synchronized imaging of the whole animal. Hence, we are able to record from several motorneurons simultaneously alongside the behavior. We recently described the activity of A and B class motorneurons during tethered locomotion. While B motorneurons are active during forward locomotion, A motorneurons are active during backward locomotion. We next turn to record the activity of the inhibitory motorneurons (VD and DD) and the other excitatory motorneurons (AS and VC) and relate their activity to locomotion. The concurrent pattern of activity of all classes of motorneurons and its relation to the behavior describes the neural basis for sinusoidal locomotion.

574A

Sensory processing deficits associated with neuroligin deficient (*nlg-1*) mutants. **Jessica Heatherly**[1,2], Greg Mullen[1], Jerrod Hunter[1,3], John McManus[1], Angie Duke[1], Jim Rand[1,2,3]. 1) Genetic Models of Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104; 2) Oklahoma Center for Neuroscience, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; 3) Department of Cell Biology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104.

Neuroligins are postsynaptic cell adhesion molecules, and mutations in two of the four human neuroligin genes are associated with a subset of cases of autism spectrum disorders (Jamain et al., 2003; Laumonnier et al., 2004; Yan et al., 2005). *C. elegans* has a single neuroligin gene (*nlg-1*), and *nlg-1* null mutants display superficially normal growth, locomotion, and nervous system structure. Nevertheless, we have identified a number of specific behavioral phenotypes associated with *nlg-1* mutants. For example, when confronted with a thermal gradient, *nlg-1* mutants do not accumulate at their growth temperature, but instead move independently of temperature. This atactic behavior is independent of the temperature at which the animals were grown and their feeding state. In addition, *nlg-1* mutants have selective chemosensory deficits: they respond normally to many chemical attractants and repellants (both soluble and volatile) and to food, but they do not respond to the repellant n-octanol. Furthermore, although *nlg-1* mutants are similar to wild type in their attraction to diacetyl by itself and their avoidance of cupric acetate by itself, there is a dramatic difference in sensory processing when the two compounds are presented simultaneously in an "approach/avoidance" paradigm (Ishihara et al., 2002): *nlg-1* mutants are significantly more likely than wild type to cross a cupric acetate barrier placed between them and the attractant diacetyl. This response is different from responses of *hen-1* and *casy-1* mutants in the same paradigm: *hen-1* and *casy-1* mutants are significantly less likely than wild type to cross the cupric acetate barrier (Ishihara et al., 2002; Ikeda et al., 2008). In addition, *hen-1* and *casy-1* mutants also display deficits in behavioral plasticity mediated by paired stimuli (Ishihara et al., 2002; Ikeda et al., 2008), while *nlg-1* mutants have wild-type responses using such paradigms. Thus, *hen-1* and *casy-1* mutants have a quite different type of sensory processing deficit than *nlg-1* mutants. Finally, for each of these *nlg-1* mutant phenotypes, we have demonstrated rescue by transgenic expression of an NLG-1::YFP fusion protein. (Supported by a grant from Autism Speaks).

575B

Specific tubulins in sensory neurons optimize behavior and cilia structure. **Daryl D. Hurd**[1,2], Renee M. Miller[2], Douglas S. Portman[2]. 1) Biology Department, St John Fisher College, Rochester, NY; 2) Center for Neural Development and Disease, University of Rochester Medical Center, Rochester, NY.

Cytoskeletal microtubules support many critical cellular processes including mitosis, organelle transport and support for cellular extensions. Because the C. elegans genome encodes nine alpha-tubulin and six beta-tubulin isoforms, the largely conserved structure among paralogous tubulins raises the question of functional specialization. Are all tubulins equivalent, or do they have unique roles in specific cellular and developmental contexts? We have addressed this question by analyzing axonemal tubulins. Using data from recent genomic approaches, we identified two alpha-tubulins, tba-6 and tba-9, and one beta-tubulin, tbb-4, as likely components of the microtubule cytoskeleton in ciliated C. elegans neurons. Using transcriptional and translational reporter genes, we have found that these tubulins are expressed in specific subsets of sensory neurons in both sexes, localizing to the ciliated endings of dendrites. Because combinations of these tubulins are expressed in ciliated ray sensory neurons in the male tail, we assessed ray neuron function in males lacking tba-6, tbb-4, and/or tba-9. All single, pairwise double, and triple mutant animals were defective in the response to hermaphrodite contact, indicating that specific tubulins are necessary for optimal sensory function. We have also found that certain tubulins are important for other functions, consistent with their expression in a variety of sensory neurons. For example, single tba-9 mutant animals exhibited abnormalities in many parameters of locomotion and increased foraging, while triple mutants exhibited reduced exploratory behavior compared to wild type in dwelling assays. We have also examined the localization of signaling molecules, the structure of cilia, and components of intraflagellar transport (IFT) in order to better understand the roles of specific tubulins in sensory neurons. We observed that PKD-2, a TRP channel expressed in ray neurons, is mislocalized in mutants lacking tba-6 or tbb-4. However, preliminary data indicate that the localization of the IFT component OSM-6 does not appear to be disrupted in these single mutants. In addition, we examined the structure of the distal dendrite and cilia in the CEM neurons with KLP-6::GFP, a kinesin family member. We observed gross abnormalities in single tba-6 mutants that are enhanced by mutations in tbb-4. We are in the process of using additional markers to further analyze axoneme and transition zone structure. Together, our studies support a model in which specific tubulins build specialized axonemes that optimize sensory function.

576C

A minimal neural network model of klinotaxis behavior in *C. elegans*. **Eduardo Izquierdo**, Shawn Lockery. Institute of Neuroscience, University of Oregon, Eugene, OR.

Previous experiments have shown that *C. elegans* chemotaxis is based in part on a biased random walk. Recently, however, Iino and colleagues have described a complementary strategy, called klinotaxis, in which the direction of locomotion is continuously aligned to the direction of the chemical gradient. Here we combined neural network modeling and mathematical analysis to identify simple circuit motifs for klinotaxis and to understand how they function. The model involves an idealized representation of *C. elegans* sensory neurons, motor neurons, and neck musculature. The direction of locomotion is determined by the angle of the head with respect to the body, which is set by the difference between the activation levels of dorsal and ventral neck muscles. The circuit has two sensory neurons, representing the chemosensory neurons ASEL and ASER, and two motor neurons collectively representing the dorsal and ventral neck muscle motor neurons. In keeping with functional differences between ASE neurons in the biological network, the sensory neurons in the model respond to the derivative of the attractant concentration such that the model's ASEL neuron acts like an ON cell, whereas the model's ASER neuron acts like an OFF cell. The motor neurons are modeled as simple nonlinear dynamical neurons. In addition to input from the sensory neurons, the motor neurons also receive out-of-phase sinusoidal inputs from a central pattern generator, which is not modeled explicitly; however, connections between motor neurons were not included. We used an evolutionary algorithm to optimize synaptic strengths and intrinsic neuronal properties to generate klinotaxis behavior in simulated radial gradients. Connections were constrained to be symmetric across the dorsal-ventral midline. We found that it was possible to generate realistic klinotaxis behavior within the constraints described above. Importantly, the effects of simulated ablations of sensory neurons in the model were consistent with the same ablations in real animals. In particular, ablation of ASER produced a strong chemotaxis deficit whereas ablation of ASEL produced little or no deficit, unless combined with ablation of ASER. Further analysis of the simulated ablations showed that ASEL contributes mainly to the time it takes the worm to reach the gradient peak (efficiency), whereas ASER contributes mainly to the likelihood of reaching the peak (reliability). Finally, a dynamical systems analysis of the motor neurons suggested a phase-dependent sensitivity to input. It is this mechanism that enables the state-dependence necessary for klinotaxis. These findings provide novel hypotheses that can now be tested on real worms.

577A

Network Graph Analysis of the C. elegans Male Posterior Connectome. **Travis Jarrell**[1], Yi Wang[1], Meng Xu[1], David H. Hall[2], Scott W. Emmons[1]. 1) Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Complex networks of all kinds, from social networks to the internet to power grids, have been investigated for a number of years to understand how their properties emerge from their structure. The *C.* elegans male posterior connectome presents a complex network of cellular interactions in which each cell is connected to an average of 21 other cells. Artificial neural networks in which nodes are "neurons" and edges are "synapses" have been attractive models of the brain because they can store "memories" as particular stable and recoverable patterns of activity. The results of these studies serve as guides for investigating how the male neural network functions to generate multi-step copulatory behavior. We have analyzed the male network using available software packages, including VisANT (http://visant.bu.edu). The posterior connectome can be represented as a weighted, directed graph with 229 nodes (all neurons and muscles) and 3222 edges (chemical synapses, gap junctions and neuromuscular junctions). Both chemical and electrical synapses are identified as morphological structures and can be assigned weights based on the number of 50 nm serial sections over which they extend. The graph structure has a number of features found in many natural networks, including the hermaphrodite nervous system. These features have important implications for its function. It is a small world graph, meaning the number of edges traversed when going between any pair of randomly chosen nodes is small, here about 3. Small world graphs are robust and capable of high speed information processing. The graph has a high cluster coefficient, meaning two neurons connected to a given neuron are likely to be connected to each other. The degree distribution, that is the distribution of the number of edges per node, is characterized by presence of a small number of nodes with very high degree. The most highly connected neurons in the male tail are PVV, PVX, PVZ and PDB, PVV being the highest with 65 different synaptic partners. These neurons form hubs and result in the network having a single giant component that includes essentially every cell. There is repeated recurrence of a feed-forward loop network motif involving the shared neurons LUAL and LUAR. By displaying the graph with grouping of cells by cell type or connectivity, pathways of information flow through the network can be visualized. We are interested to learn the extent to which the separate sub-behaviors of the copulatory sequence are subserved by distinct circuits or instead emerge as functional modes of the network as a whole.

578B

Insights into the molecular mechanisms of *Caenorhabditis elegans* memory. **Paola Jurado**, Fuyuki Goto, Ikue Mori. Graduate Sch Sci, Nagoya Univ, Nagoya, Japan.

Caenorhabditis elegans responds to a variety of environmental signals (volatile and water soluble chemicals, temperature, etc). The animals memorize these cues and are able to associate them with other existent conditions. Thermotaxis is a well characterized behavior in which *C. elegans* associates its cultivation temperature with food (*Escherichia coli*). This association drives the worms to seek their memorized temperature even in the absence of bacteria (1,2). This presents an ideal situation to investigate the molecular and cellular bases of sensory integration leading to complex processes such as memory and learning. We are investigating how the assimilation of thermo-sensory cues leads to memory, and how this thermal memory is modified over time. We have performed two different screens to look for mutants with an abnormal memory or an altered decission making balance over thermal conditioning. On the first screen we searched for animals conditioned to find *E. coli* at a certain temperature that take longer to leave it, in the absence of food, than wild type animals. These animals display a longer lasting memory or a slower decission making when thermal memory is confronted with hunger or the absence of food. On the second screen we isolated several animals with a shorter memory or a faster decission making by selecting those that leave their memorized temperaure faster than the wild type animals. All these putative mutants are currently under characterization. 1. I. Mori and Y. Ohshima (1995). "Neural regulation of thermotaxis in *C. elegans*." Nature 376(6538): 344-8. 2. I. Mori, H. Sasakura and A. Kuhara (2008). "Worm thermotaxis: a model system for analyzing thermosensation and neural plasticity." Curr Opin Neubiol. 2007 Dec 17(6):712-9.

579C

CEP-sheath glia are required for normal locomotion in *C. elegans*. **Menachem Katz**, Yun Lu, Shai Shaham. Lab of Developmental Genetics, The Rockefeller Univ, New York, NY.

C. elegans glial cells share morphological, functional and genetic resemblance to their vertebrate counterparts. However, in contrast to vertebrate glia, *C. elegans* glia are not essential for neuronal survival, offering a unique opportunity to study the involvement of glia in the development and function of the nervous system *in vivo*. *C. elegans* CEP sheath glia (CEPsh) are bipolar cells that ensheath the dendrites of CEP neurons, and also envelope the nerve ring (the animal's brain) extending processes that abut synapses. During development, these cells are involved in regulating axon guidance, dendrite extension, formation of the nerve ring (Yoshimura et al., 2008), and synaptogenesis (Colón-Ramos et al., 2007). However, post-developmental roles for CEPsh glia have not been studied. Here we demonstrate that animals in which the CEPsh glia were genetically ablated during the first larva stage display various motor defects including reduced speed of locomotion and intermittent periods of extended paralysis. Ablated animals also display high levels of small-angle turning, resulting in a spiral pattern of movement that restricts their movement to small areas on the plate. EM analyses of CEPsh glia-ablated animals reveal disorganization of the nerve ring, and swelling of neurons in the ventral ganglion. Thus CEPsh glia may play key homeostatic roles in the neuropil.

To understand the molecular basis of CEPsh glia function we have begun to identify genes expressed in these cells using an mRNA-tagging method, with the aim of examining the roles of enriched genes in *C. elegans* locomotory behavior.

580A

Enhancement of 2-nonanone avoidance is regulated by *dop-3*-dependent dopamine signaling. **Kotaro Kimura**[1,2,3], Isao Katsura[1]. 1) Structural Biology Center, National Institute of Genetics, Mishima, JAPAN; 2) PRESTO, JST, Tokyo, JAPAN; 3) Present address: Department of Biological Sciences, Osaka University, Toyonaka, Osaka, Japan.

Reductions in sensory response after prior exposure to a stimulus, such as adaptation or habituation, have been extensively studied in various animals. In contrast, the *enhancement* of animals' sensory response after preexposure has been poorly studied, except in a few cases such as sensitization of pain in mammals and of gill-withdrawal reflex in *Aplysia*. We have reported that preexposure to a repulsive odor of 2-nonanone causes enhancement of avoidance behavior, rather than adaptation, to the odor in a food information-independent manner (Kimura and Katsura, the previous IWM and CeNeuro abstracts). Genetic analysis has revealed that mutations in the dopamine biosynthetic pathway, such as *cat-2, cat-1, cat-4* and *bas-1*, but not *tph-1*, significantly affected the enhancement 2-nonanone avoidance, suggesting the requirement of dopamine signaling for the enhancement.

Here, we report that the D2-like dopamine receptor *dop-3* is required for the enhancement of 2-nonanone avoidance. First, we conducted a pharmacological analysis with dopamine receptor antagonists. In mammals, dopamine receptors are classified into D1- and D2-subtypes, which regulate intracellular signaling positively or negatively, respectively. We found that the D2-specific antagonist haloperidol and the D1/D2 antagonist loxapine, but not the D1-specific SCH23390, specifically suppressed the enhancement of preexposed animals, suggesting that D2-like dopamine receptor(s) are involved in the process. Further, we found that deletion mutations in D2-like receptor *dop-3* significantly affected the enhancement. Mutations in another D2-like receptor *dop-2* did not affect the phenotype, and the phenotype of the double mutants *dop-2;dop-3* were similar to that of the *dop-3* single mutants. Taken together, these results suggest that the *dop-3* D2-like dopamine receptor plays a critical role in the enhancement of 2-nonanone avoidance. *dop-3* has been known to regulate the basal slowing response in the ventral cord motor neurons (Chase et al., Nat Neurosci, 2004). In the regulation of basal slowing response (Sawin, Neuron, 2000), as well as area-restricted search (Hills et al., J. Neurosci., 2004) and tap habituation (Kindt et al., Neuron, 2007), dopamine signals "the presence of food" in *C. elegans*. In contrast, the enhancement of 2-nonanone avoidance is independent of food information, suggesting that a *dop-3*-dependent novel dopamine signaling regulates the behavioral plasticity of the animals.

581B

Analysis of thermotactic-defective mutants isolated by GFP marker that detects abnormality of thermosensory signal transduction in AFD. **Kyogo Kobayashi**, Hiroyuki Sasakura, Keita Suzuki, Ikue Mori. Group of Molecular Neurobiology, Nagoya University, Japan.

When the animals were cultivated with food (bacteria) at a certain temperature, ranging from 15°C to 25°C, and placed on a temperature gradient, they migrated to the previous cultivation temperature and moved isothermally (Hedgecock and Russell, 1975). The thermosensory neuron AFD senses the environmental temperature and memorizes that thermal information. AFD-ablated animals cannot show Isothermal Tracking (IT), which is thought to be a sophisticated behavior that requires exact thermosensation and precise thermal memory. In the current model, cGMP produced by guanylyl cyclases (GCY-8, GCY-18, and GCY-23) is used as a second messenger and cGMP-dependent cation channel TAX-2 and TAX-4 are crucial for the activation of AFD. Ca^{2+}/calmodulin-dependent protein kinase 1 (CMK-1) also regulates thermosensory signal transduction in the downstream of TAX-4 (Satterlee *et al.,* 2004; Mori *et al.,* 2007). However, the molecular mechanism needed for thermosensation and thermal memory is still largely unknown. To isolate new genes involved in AFD thermosensation and/or memory, a forward genetic screening that did not depend on behavior was performed. The gene expression of *nhr-38* is AFD specific and regulated by temperature stimulus. For example, the mutations in *tax-4* or *cmk-1* gene induce the downregulation of *nhr-38::GFP* (Satterlee *et al.,* 2004; Sasakura *et al.,* 2007 INWM). Thus, *nhr-38::GFP* can be used as a GFP marker that detects abnormality of thermosensory signal transduction in AFD. Utilizing this marker, one strain that promotes as well as eleven strains that recover the downregulation of *nhr-38::GFP* gene expression in *cmk-1* mutants were isolated. We analyzed the behavior of thermotaxis in these mutants. Nearly all *cmk-1* mutant animals could not show IT clearly, while about 20% of both *nj78;cmk-1* and *nj73;cmk-1* mutants showed IT. These results indicate that *nj78* and *nj73* mutations are involved in thermosensation and/or thermal memory. We so far mapped *nj73* mutation to the region between +26.61 and +27.96 on the chromosome I. We are now conducting rescue experiments by injecting the cosmids covering that region. We thank T. Ishihara for *nhr-38::GFP*(*H13::GFP*), and CGC for *cmk-1* mutants.

582C

Exploring the neural code in the neural circuit for thermotaxis behavior. **Atsushi Kuhara**[1], Tomoyasu Shimowada[1], Noriyuki Ohnishi[1], Ikue Mori[1,2]. 1) Nagoya University; 2) CREST-JST.

Behavior is an ultimate consequence of orchestrated neural calculation. Thermotaxis of *C. elegans* is an ideal system for comprehensively understanding how a neural circuit encodes a behavioral output (1). In thermotaxis neural circuit, temperature is mainly sensed by AFD neuron, and its information is conveyed to AIY interneuron (1, 2). To elucidate neural calculation, we aimed to inactivate neuronal activity by employing light-activated chloride pump halorhodopsin (HR) (3). Millisecond scale pulsed-light system, which allows Hz-controllable deliveries of light, was developed and equipped in both calcium imaging microscope and *C. elegans* auto-tracking microscope. Calcium imaging analysis demonstrated that pulsed-excitation of HR in AFD partially reduced calcium influx in AFD for thermal stimuli. Since AFD-ablated animals move randomly or migrate to lower temperature than the cultivation temperature on a thermal gradient (1), we speculated that exciting HR in AFD induces cryophilic or athermotactic abnormalities. We unexpectedly found that pulsed-excitation of HR in AFD induced thermophlic abnormality. Similar abnormality was observed in the mutant exhibiting an abnormal glutamate synaptic transmission in AFD due to AFD-specific defect in EAT-4 (vesicular glutamate transporter) (N. O., A. K. and I. M., this meeting). Previous report (4) and our calcium imaging analysis revealed that calcium influx in AFD for thermal stimuli induced calcium influx in AIY. This suggests that temperature information of AFD is conveyed to AIY through “excitatory” connection. We however found that pulsed-excitation of HR in AFD notably enhanced calcium influx of AIY for thermal stimuli, despite AFD activity itself was partially reduced. Similar abnormal thermal responses of both AFD and AIY were observed in thermophilic mutant *tax-6* (calcineurin) that is defective in AFD thermosensory signaling (5, 6), implicating “inhibitory” connection from AFD to AIY. Altogether, these physiological and behavioral results are consistent with the notion that temperature signal in AFD dynamically affects AIY activity through both “inhibitory” and “excitatory” transmissions, which as a consequence likely generates opposite thermotactic behaviors, thermophilic and cryophlic migration on a temperature gradient.

(1) Mori and Ohshima, Nature, 1995 (2) Kuhara, Okumura, et al., Science, 2008 (3) Zhang et al., Nature, 2007 (4) Biron et al., Nature Neuroscience, 2006 (5) Kuhara et al., Neuron, 2002 (6) Kuhara and Mori, J. Neurosci, 2006.

583A

Mechanisms of dauer pheromone signal transduction. **Danna Zeiger**, Kyuhyung Kim, Piali Sengupta. Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA.

High concentrations of dauer pheromone trigger entry of *C. elegans* larvae into the alternate dauer developmental stage. Dauer pheromone has been shown to be a complex mixture of structurally related ascarosides, and regulates both dauer formation and adult behaviors (Butcher, et. al., 2007, 2008;Srinivasan et. al., 2008;). Pheromone signals are sensed by chemosensory neurons, and are transduced to downregulate TGF-β and IGF/Insulin signaling, leading to inhibition of reproductive growth and ultimately, dauer arrest. We recently showed that the SRBC-64 and SRBC-66 serpentine receptors mediate dauer formation in response to a subset of ascarosides (see abtract by Kim et al). Both receptors are expressed in, and localized to, the sensory endings of the ASK chemosensory neuron type, and genetic ablation of the ASK neurons abolishes dauer formation in response to some, but not all identified ascarosides. The *srbc* subfamily is predicted to encode a total of 73 chemoreceptors (Thomas & Robertson, 2008), suggesting that one or more of these receptors may play a role in the responses to additional ascarosides. We are currently determining the expression patterns of all *srbc* chemoreceptor genes, and genetically ablating amphid neurons in order to identify additonal neurons and receptors required for pheromone sensation.

Pheromone signals sensed by the ASK neurons must be transduced to the ASI neurons to downregulate TGF-β expression and promote dauer formation. This interneuronal communication is hypothesized to be mediated via neuroendocrine/peptide signaling. Previously, we and others had shown that pheromone also downregulates expression of the str-3 chemoreceptor gene in the ASI neurons, and that this downregulation requires srbc-64 and -66 (Nolan, et. al., 2002; Peckol, et. al., 1999; Kim, et al submitted). To identify genes required to transduce the pheromone signal within the ASK neurons, and from the ASK to the ASI neurons, we carried out a screen for mutants which fail to exhibit pheromone-mediated downregulation of *str-3*p::*gfp* expression. This screen identified several promising mutants, which also exhibit defects in pheromone-regulated dauer formation. We are currently further characterizing these mutants, and identifying the affected genes via whole genome re-sequencing (Sarin, et al 2008). We expect that identification of the intra- and inter-neuronal signaling mechanisms required for pheromone signal transduction will provide a better understanding of how animals respond to complex environmental signals, and how these signals are integrated to result in the appropriate developmental and behavioral responses.

584B

Chemosensory Context Conditioning in *Caenorhabditis elegans*: A Non-Food Related Form of Associative Learning. **H. L. Lau**, C. H. Rankin. Neuroscience Department, University of British Columbia–Brain Research Centre, Vancouver, BC, Canada.

We are studying context conditioning of mechanosensory habituation: in context conditioning of habituation, worms that are trained and tested in the presence of a chemosensory contextual cue show greater retention of a memory when compared to animals trained and tested in different contextual cues/environments. Previous results showed that retention of habituation to mechanical tap stimuli was greater if habituation training and testing occurred in the presence of the same chemosensory taste cue (soluble sodium acetate; Rankin, 2000); we have now also shown this for an olfactory chemosensory cue (volatile diacetyl). In the earlier study, we confirmed the associative nature of this learning by demonstrating that it showed both extinction and latent inhibition. In this study, we have dissociated the neural circuits for the taste and smell pathways underlying this form of learning. *odr-7* (encodes an olfactory-specific member of the nuclear receptor) worms, with non-functional AWA olfactory chemosensory neurons (that detects diacetyl), showed context conditioning to the sodium acetate taste but not to the diacetyl odor. Conversely, *osm-3* (encodes a homodimeric forming kinesin motor protein) worms, with non-functional taste chemosensory neurons (that detect sodium acetate), showed context conditioning to the diacetyl odor but not to the sodium acetate taste. This dissociation between taste and smell allows us to distinguish between non-associative (habituation) and associative (chemosensory context conditioning of habituation) learning genes. Additionally, these associative learning genes can be further discriminated from genes involved in the detection of taste or smell. A number of genes in *C. elegans*, such as *nmr-1* (encodes an NMDA-type ionotropic glutamate receptor subunit; Kano et al., 2008), have been suggested to be involved in associative learning. We found that *nmr-1* worms were not able to show context conditioning. nmr-1 is expressed in several interneurons in our associative learning neural circuit, so we are currently investigating in which interneurons *nmr-1* is essential for worms to show chemosensory context conditioning to mechanosensory habituation. This will help to elucidate the cellular mechanisms of this form of associative learning; providing insight into how interneurons integrate chemosensory context cues with mechanosensory taps to alter subsequent behavior.

585C

The DM gene *mab-3* generates a functional sex difference in the AWA neurons. **KyungHwa Lee**[1], Douglas Portman[2]. 1) Interdepartmental Graduate Program in Neuroscience, University of Rochester School of Medicine and Dentistry; 2) Center for Neural Development and Disease, University of Rochester Medical Center, Rochester, NY 14642.

To understand how the "sex" of the nervous system influences its function, we are studying sex differences in olfaction. Previously, we have observed significant sex differences in responses to single odorants and in an "olfactory preference" assay. These sex differences do not require gonadal signaling or the male-specific CEM sensory neurons. Instead, they arise from intrinsic sex differences in shared or "core" neurons. To directly address the role of neural sex, we manipulated sex determination genes in the nervous system. Interestingly, we have found that masculinization of the hermaphrodite nervous system through the pan-neural overexpression of the sex-determination gene *fem-3* is sufficient to generate male-like olfactory preference. Conversely, expression of activated *tra-2* in the male nervous system gives rise to hermaphrodite-like olfactory preference. To identify the neuronal foci that underlie these sex differences in behavior, we specifically sex-reversed the AWA olfactory neurons and examined behavioral responses in the diacetyl-pyrazine (da-py) preference assay. In wild-type animals, hermaphrodites tend to prefer da, while males prefer py. In contrast, sex-reversing AWA sex-reverses the behavioral phenotypes of both males and hermaphrodites, indicating that AWA itself harbors sexual dimorphism. To ask how AWA function may be regulated by sex, we examined the response to da, which in hermaphrodites is mediated predominantly by AWA. We find that male da odortaxis is significantly lower than that of hermaphrodites and is not mediated by AWA. Consistent with this, expression of the da receptor ODR-10 is almost undetectable in male AWA neurons. Feminizing the male AWA neuron restores this expression, indicating that *odr-10* is regulated by neural sex. Furthermore, the doublesex-like DM gene *mab-3* is necessary for this sex difference, as *mab-3* mutant males display significant ODR-10 expression in AWA. Together, these studies identify a mechanism by which cell-intrinsic sexual status regulates gene expression to control a sex difference in a shared sensory behavior.

586A

Multiple male-specific sensory neuron types function both additively and redundantly to control locomotion and tail posture during *C. elegans* mating. **Robyn Lints**, Pamela Koo, Meredith Bunkers, Fakhriddin Pirlepesov, Xuelin Bian. Dept Biol, Texas A & M Univ, College Station, TX.

In order to survive animals must be able to prioritize environmental stimuli and modify their behavior accordingly. *C. elegans* male contact response provides a striking example of sensory stimuli re-prioritization. Passing contact with a hermaphrodite causes the male to abruptly halt ongoing behaviors and attempt mating: he ceases forward locomotion, places his tail ventral side down against the hermaphrodite and begins backing along her surface in search of the vulva. Our objective is to understand how the hermaphrodite cue trumps all other sensory stimuli and how its detection dramatically alters the activity of locomotory and body posture circuits. Consistent with previous studies (1), we find that the male rays and hook are key sensors of hermaphrodite contact. The hook and rays function redundantly in promoting backing while the rays alone regulate tail posture. Each of the nine bilateral ray pairs has a distinct identity, defined in large part by the combination of neurotransmitters synthesized in their constituent neurons. However, despite these differences, we find that there is marked redundancy among rays in their ability to induce contact response: as few as three ray pairs, of any identity, are sufficient however the more rays present, the greater the likelihood of response and the longer contact is maintained. Using a combination of approaches (genetically-targeted-cell ablation (2), -light-inducible-depolarization and hyperpolarization (3, 4)), we find that functional redundancy extends even to the two sensory neuron types (A and B) within each ray. Channelrhodopsin-2(ChR2)-mediated activation of either ray neuron type can induce contact response-like behavior in solitary males. However, A neurons appear to be less effective transducers when activated by ChR2 or by hermaphrodite contact. While this extensive redundancy among rays and ray neuron types is unexpected, it is supported by the ray wiring data, which reveals that although different ray neurons synapse with different combinations of targets, there is considerable target overlap (5). Together these findings suggest that the *C. elegans* male has evolved a highly robust response system for sensing and maintaining contact with a mobile, non-compliant partner. 1. Liu and Sternberg (1995) Neuron 14: 79-89. 2. Hills et al. (2004). J Neurosci. 24:1217-25. 3. Nagel et al. (2005). Curr Biol 15: 2279-84. 4. Gruninger et al. (2008). PLos Genet. 4: e1000117. 5. Male Wiring Project, Albert Einstein College of Medicine: http://worms.aecom.yu.edu/pages/male_wiring_project.htm.

587B

Cholinergic signaling in spicule sensory-motor circuit coordinates spicules protraction during mating. **Yishi Liu**[2], Daisy Gualberto[1,2], L. Rene Garcia[1,2]. 1) Howard Hughes Medical Institute; 2) Dept Biol, Texas A&M Univ, College Station, TX.

In this study we ask how cholinergic signaling is used by *C. elegans* male spicule circuit to facilitate mating behavior. We focus on spicule prodding and insertion, behavioral steps that males use their copulatory organ to penetrate their mates' vulva. *C. elegans* male uses SPC and post-cloacal sensilla (p.c.s.) neurons and a group of sex muscles to execute these steps. Acetylcholine (ACh) is released from SPC and p.c.s. neurons to regulate behavior. We surveyed expression patterns of all nicotinic ACh receptor (nAChR) α subunits in spicule circuit, by driving YFP expression under different gene promoters. *acr-16*, *unc-38* and *unc-63* are expressed in spicule protractor and anal depressor muscles; *acr-12* and *unc-63* are expressed in spicule neurons. Protractor muscle contraction causes spicule prodding or protraction. To access the role of protractor nAChRs, we genetically removed functional nAChRs from protractor muscles. Males without nAChRs in spicule protractor muscles can still prod and insert spicules into hermaphrodite vulva. Gap junctions are found among protractor muscles and other muscles and neurons in the circuit. Our result indicates that these gap junctions might be recruited to initiate spicule muscle activity, and nAChRs on protractor muscles might be used to tune the switch from prodding to full protraction. Surprisingly, elimination of nAChRs in protractor muscles presumably causes muscle contraction defect, and this interferes with males' ability to stay at vulva. It suggests that muscle contraction can feedback to the spicule sensory-motor circuit to fine tune male's behavior. A muscarinic ACh receptor (mAChR), GAR-3, is also found in spicule circuit to facilitate mating. In previous study we found GAR-3 is coupled to Gq to enhance nAChRs mediated spicule activities. Deletion allele *gar-3(gk305)* reduces male mating efficiency by delaying spicule insertion. Deletion allele of an ERG potassium channel, *unc-103(n1213)*, can rescue *gar-3(gk305)* male mating defect. UNC-103 has been demonstrated to keep cell excitability low in spicule circuit before mating, and loss of UNC-103 function causes males to protract spicules constitutively in absence of their mates. The *gar-3(gk305)* allele could not rescue this phenotype in *unc-103(n1213)* males; this along with the previous result suggests that GAR-3 might regulate downstream UNC-103 activity in spicule circuit. Overnight exposure of wild type males to a mAChR agonist, oxotremorine M, causes them to protract spicules constitutively via GAR-3. *unc-103(n1213)* males display the same level of constitutive protraction as wild type under this condition, suggesting that oxo M activated GAR-3 downregulates UNC-103 activity in spicule circuit.

588C

Studies on neuronal function of a novel and conserved protein TTX-8. **Akiko Miyara**[1], Akane Ohta[1,2], Yoshifumi Okochi[1,3], Yuki Tsukada[1], Atsushi Kuhara[1], Ikue Mori[1]. 1) Lab of Mol Neurobiol, Nagoya Univ; 2) Present address: Dept of Virol, Nagoya Univ; 3) Present address: Lab of Integrative Physiol, Osaka Univ.

Animals can sense and respond properly to variable signals in surrounding environment. From receiving signals to behavioral outputs, many neurons contribute to transfer signal information. In principle, a particular stimulus is perceived by sensory neurons and then transmitted to downstream interneurons, and finally behavioral output is induced as a result of neural processing. During this processing, those neurons are needed to function accrately as might be expected. Here, we report that a novel protein TTX-8 plays a role in assisting basal neuronal function. TTX-8 is predicted to have transmembrane region in N-terminus and coiled coil region in C-terminus. The *ttx-8* mutants showed several abnormal behaviors including thermotaxis, chemotaxis to NaCl and odorants, and locomotion (Tsukada et. al., this meeting). We found that TTX-8 is expressed and functions in neurons. A human homologue of TTX-8 weakly but effectively rescued abnormal thermotaxis phenotype of the *ttx-8* mutant, suggesting that TTX-8 is conserved across species. When *ttx-8* cDNA was expressed simultaneously in major thermosensory neuron AFD, and downstream interneuron AIY and AIZ of *ttx-8* mutants, about 50% of transgenic animals showed normal thermotaxis phenotype. This result suggests that TTX-8 functions in AFD, AIY and AIZ neurons for thermotaxis. Consistently, calcium imaging analysis revealed that calcium influx in both AFD and AIY neurons of *ttx-8* mutant were notably decreased in response to thermal stimuli, indicating that TTX-8 is required for proper activation of AFD and AIY neurons. To determine which region of TTX-8 is necessary for its accurate function, we constructed two truncated TTX-8 proteins, one lacks transmembrane region and the other lacks coiled coil region, and did rescue experiments using each truncated protein. Both truncated forms could not rescue abnormal thermotaxis of *ttx-8*, suggesting that both regions are important to proper TTX-8 function. RIC-3, which resembles TTX-8 in protein structure, has been reported to be required for the maturation of acetylcoline receptor (Halevi et. al., 2002). In *ttx-8* mutant, a presynaptic marker SNB-1::GFP was mislocalized in motorneurons. Taken together, TTX-8 might be involved in the maturation of synaptic proteins. Consistent with this possibility, immunostaining experiments using HeLa cell showed that TTX-8 was localized to peri-nuclei region of cytoplasm, perhaps to ER and/or Golgi-body. Our data suggest that TTX-8 plays a role in supporting neuronal function probably through regulation of neural activation or maturation of synaptic proteins.

589A

Mathematical modeling of thermotactic behavior in C. elegans. **Kenichi Nakazato**, Atsushi Mochizuki. ASI, RIKEN, Wako, Saitama, Japan.

One of the adaptive behaviors of worms in their environment is thermotaxis, by which thery migrate toward a prefered temperature. The thermotactic behavior is accomplished by choosing thermophilic or cryophilic movement depending on the surrounding temperature. However, some experimental data are inconsistent especially for thermophilic movement, which is expected to be observed in lower than prefered temperatures. There are no experimental analyses which support thermophilic movement in the individual behavior of worms. Although mathematical modeling is used to study thermotactic behavior in C. elegans, no model provides a consistent explanation for this discrepancy. Here we develop a simple mathematical model based on biased random walk, which describes population behavior. We determined the parameters of the model according to the experimental results of individual movement assays. In addition to them, we examined hypothetical bias as thermophilic drive. Our model can not regenerate some behavioral patterns of population distribution without the hypothetical bias. However our model can regenerate all the population patterns reported in past studies without any contradiction if we use the hypothetical bias into our model. In addition, our model show that thermophilic movement can be observed only when the steepness of thermal gradient is sufficiently slight. If the gradient is too steep in contrast, our model do not show a sharp contrast between the results with thermophilic bias and the results without it. Our results therefore suggest that thermophilic movement would be observed, even in individual movements, when the thermal gradient is sufficiently slight. On the contrary, thermophilic movement dissapears when the thermal gradient is too steep. The steepness of thermal gradient is thus essential to understand the past experimental studies without discrepancy.

590B

THE GENETICS OF DEET RESISTANCE IN *Caenorhabditis elegans*. **Anh Quynh Nguyen**, Phil Hartman, Matt Freedman, Heather Copeland, Alfred Douglas, Julia Limes. Biology, Texas Christian University, Fort Worth, TX.

The molecular mechanism(s) by which DEET (N,N-diethyl-m-toluamide) acts as an insect “repellent” has not been resolved. Using forward genetic screens in *Caenorhabditis elegans*, we have isolated five DEET-resistant mutants (*der-1*) after EMS mutagenesis of DEET-sensitive N2. Two are allelic and have been mapped between 2.4 mu and 2.9 mu on linkage group (LG) IV. Specifically, 3F (factor) cross data between two markers *unc-5* (1.78 mu) and *egl-19* (3.34 mu) on LGIV showed that 11/22 (50%) Egl-19 recombinants were DEET-resistant and 11/22 (50%) were DEET sensitive for allele *hf175*. In case of allele *hf176*, the data were 19/32 (59%) and 13/32 (41%), respectively. In addition, all Lin-33 recombinants from the 3F cross between two references *unc-5* (1.78 mu) and *lin-33* (2.55 mu) showed DEET sensitivity in both *hf175* and *hf176*. Several additional 3F crosses are in various stages of completion. Interestingly, wild-type strains CB4856, AB1, and AB2 have been shown to be DEET resistant relative to the wild-type strains N2 and CB4852, which are both DEET sensitive. These findings suggest that DEET-resistance may be more common than we originally expected. Successful cloning of the *der-1* gene is an important step not only to elucidate the mechanism of DEET action but may prove useful in designing the next generation of chemicals that helps reducing insect-borne-diseases.

591C

Novel hydrolase AHO-3 has a role in the temperature-food associative learning behavior in *C. elegans*. **Nana Nishio**[1], Akiko Mohri[2], Eiji Kodama[3], Kotaro Kimura[4], Atsushi Kuhara[1], Ikue Mori[1,5]. 1) Nagoya Univ, Japan; 2) Present add: Natl Inst of Genet, Japan; 3) Present add: MRC, UK; 4) Present add: Osaka Univ, Japan; 5) CREST-JST.

Animals exhibit many types of behavioral plasticity. To elucidate the regulation mechanism of behavioral plasticity, we are using the temperature-food associative learning behavior in *C. elegans*. Well-fed animals migrate to their cultivation temperature, and starved animals disperse and do not migrate to their cultivation temperature on a linear temperature gradient. The *aho-3* mutant has abnormality in this learning behavior (1). After cultivated at starved condition, *aho-3* mutants migrated to higher temperature than their cultivation temperature, while they showed almost normal thermotaxis after cultivated with food. The *aho-3* gene encodes a novel hydrolase that is highly conserved among many species. However, the molecular properties have not been characterized. Interestingly, the abnormality in the learning behavior of *aho-3* mutant was almost fully rescued by expressing human homologue of AHO-3. This suggests that the molecular function of AHO-3 is conserved throughout human to *C. elegans*. GFP expression driven by *aho-3* promoter was observed in AFD, AWC and AIY neurons required for thermotaxis (2, 3) and ADF sensory neuron. The abnormal learning behavior of *aho-3* mutant was partially rescued by expressing *aho-3cDNA* in AWC neuron and not rescued by expressing in AFD, AIY or ADF. It was shown that Gα/ODR-3 acts in thermosensation in AWC (3). We found that the *odr-3* mutant has the same abnormality in the learning behavior as *aho-3* mutant. In addition, *egl-4* mutant defective in cGMP-dependent protein kinase required for olfactory adaptation in AWC (4) and *ins-1* mutant defective in the insulin-like peptide have a defect in the learning behavior (5); after cultivated at starved condition, these mutants migrated to their cultivation temperature, not to higher temperature like *aho-3* mutant. To analyze genetic epistasis between *aho-3* and these mutations, we made double mutants. *aho-3; odr-3* mutant showed almost the same abnormality in the learning behavior as that of each single mutant. The defect in the learning behavior caused by *egl-4* mutation was almost fully suppressed by *aho-3*, and the defect in the learning behavior caused by *ins-1* was partially suppressed by *aho-3*. These results imply that AHO-3, ODR-3 and EGL-4 can act in the same pathway in the temperature-food associative learning and that INS-1 can act parallel with AHO-3. Currently, we are analyzing the neural activity in *aho-3* mutant by Ca^{2+} imaging. (1) Mohri *et al*., 2004. (2) Mori and Ohshima, 1995. (3) Kuhara, Okumura *et al*., 2008. (4) L'Etoile *et al*., 2002. (5) Kodama *et al*., 2006.

592A

Genetic and Physiological Investigation of a TRPP Ion Channel Complex Needed for Male Mating. **Robert O'Hagan**, Maureen Barr. Dept. of Genetics, Rutgers University, Piscataway, N.J.

TRP ion channels are important in peripheral sensory neurons that detect environmental cues, central neurons of the mammalian brain where they associate with molecules needed for synaptic plasticity, and in non-neuronal tissues such as the kidney, where they function in sensation of the internal environment. A TRP polycystin (TRPP) ion channel composed of PKD1 and PKD2 localizes to cilia and is essential for normal human kidney function. This TRPP complex has been proposed to directly transduce the mechanical forces created by renal fluid flow, and mutations in the genes encoding these subunits cause polycystic kidney disease. However, we lack a thorough understanding of the molecular and physiological functions of TRP channels in these diverse tissues.

In an effort to understand the molecular and physiological functions of TRP ion channels, we are studying the TRPP ion channel complex subunits LOV-1 and PKD-2, the *C. elegans* homologs of PKD1 and PKD2. This complex, which is needed for proper male sex attraction and mating behaviors, localizes to cilia in the HOB hook neuron, the RnB ray neurons in the tail, and in the CEM neurons in the head. The sensory function and localization of this complex in cilia may be conserved in *C. elegans* male neurons and human renal epithelia.

Using *in situ* electrophysiology, we are examining the functions of male specific sensory neurons and the LOV-1/PKD-2 TRPP complex. Because these ciliated neurons are thought to perform sensory roles, we will observe responses to mechanical and/or chemical stimuli. Comparison of the responses of sensory neurons in wild-type and *lov-1* or *pkd-2* mutants should allow us to clarify the function of this TRPP complex and to determine if it is directly or indirectly activated by sensory stimuli.

The TRPP complex is localized to cilia and ER and may be performing separable roles in these locations. To determine the effect of mislocalization of this complex, we will test animals lacking the function of regulators of the abundance or localization of the LOV-1/PKD-2 complex, such as the kinesin KLP-6, the inositol 5-phosphatase CIL-1, and the calcineurin TAX-6. We are currently mapping and cloning another molecule needed for TRPP complex localization, called *cil-6*, which is mutated in *my12* and *my22* animals. These *cil-6* mutants have defects in PKD-2 localization and male mating behavior.

593B

Analysis of neuronal plasticity in salt chemotaxis learning of *C. elegans* by Ca^{2+} imaging. **Shigekazu Oda**, Masahiro Tomioka, Yuichi Iino. The University of Tokyo, Tokyo, Japan.

C. elegans raised under standard conditions shows an attractive behaviour to NaCl. However, after exposure to NaCl without food for 10-60 minutes, it shows less attractive or aversive behaviour toward NaCl. This behavioural change is not observed under the presence of food, suggesting that the integration of two stimuli, salt and starvation, is required for this behavioural plasticity called 'salt chemotaxis learning'. Addition of 5-HT (serotonin) during conditioning, which is known as a mediator of food signals in several behaviours, also abrogates salt chemotaxis learning in the absence of food. ASE neurons are major sensory neurons that sense NaCl and are essential for salt chemotaxis. For salt chemotaxis learning, ASER, the right-side member of ASE, and AIA interneurons are thought to be important. The action of the insulin/PI 3-kinase pathway and CASY-1, a homologue of mammalian Calsyntenin/Alcadein, in the ASER neuron is required for salt chemotaxis learning. However, it is still unclear how these neurons and molecules function dynamically and how input information is modulated in salt chemotaxis learning.

By using the genetically encoded Ca^{2+} indicator G-CaMP, we conducted time-lapse imaging to monitor the activity of neurons before and after salt chemotaxis learning. First, we monitored the activity of ASER. It has previously been reported that ASER responds to down-step of NaCl concentration based on measurements of Ca^{2+} dynamics in the soma. In this study, we performed calcium measurements in dendrites and neurites as well as soma to obtain more spatial information. All distinct parts of ASER showed a transient calcium response before exposure to NaCl. However, we found that ASER shows calcium responses with significantly longer duration after exposure to NaCl for 10 minutes compared to the response before the NaCl exposure. When 5-HT was added to the stimulus buffers, the increase in the duration of ASER responses was not observed any more. Furthermore, in the *ins-1 (nr2091)* mutant, which is defective in salt chemotaxis learning, the increase in peak duration was not observed, either. These results suggest that the input information of salt is modulated at the sensory level in salt chemotaxis learning, and the prolonged duration of ASER responses are important for this behavioural plasticity. We are currently studying how the observed change in the sensory response of ASER affects the response of downstream interneurons.

594C

Glutamate-mediated synaptic transmission in thermotaxis neural circuit of *C. elegans*. **Noriyuki Ohnishi**[1], Atsushi Kuhara[1], Masatoshi Okumura[1], Yoshifumi Okochi[1,2], Hitoshi Inada[1,3], Ikue Mori[1,4]. 1) Nagoya Univ, Japan; 2) (present address) Osaka Univ, Japan; 3) (present address) Harvard Univ, USA; 4) CREST-JST.

Glutamate is an essential neurotransmitter in the nervous system. Thermotaxis, a sensory behavior controlled by the simple neural circuit in *C. elegans*, is an ideal system to study regulation of glutamate-mediated synaptic transmission through neural circuit (1, 2). The loss-of-function mutations in VGLUT (Vesicular Glutamate Transporter) encoded by *eat-4* (3) led to thermotaxis defect appeared as a poor response to temperature. Expression of EAT-4 in the sensory neuron AFD and its downstream interneuron RIA of *eat-4* mutant induced migration to lower temperature than cultivation temperature, whereas expression of EAT-4 in the sensory neuron AWC and its downstream interneuron RIA of *eat-4* mutant induced migration to higher temperature than cultivation temperature. In addition, expression of EAT-4 in AFD, AWC and RIA restored partially the thermotaxis defect of *eat-4*. These results suggest that thermal signals from AFD, AWC or RIA to their postsynaptic neurons are transmitted by glutamate through EAT-4 VGLUT, and that glutamate signals from AFD and from AWC have opposite effect on thermotaxis each other. Interestingly, AIY interneuron, postsynaptic to both AFD and AWC neurons, could receive and distinguish glutamate signals from AFD and AWC. We found that mutation in chloride channel type inhibitory glutamate receptor GLC-3 (4) led to thermotaxis defect restored by expression of GLC-3 in AIY, implying that GLC-3 functions in AIY as receptor of glutamate signals from AFD or AWC. To investigate whether GLC-3 receives glutamate signals from AFD or from AWC, we examined the effect of several mutations on *glc-3* mutant. Defect of *gcy-23 gcy-8 gcy-18* triple mutant, which lacks thermal response of AFD, masked the defect of *glc-3*. Likewise, defect of the transgenic *eat-4* mutant expressing EAT-4 in AWC and RIA, which exhibits abnormal glutamate transmission of AFD, masked the defect of *glc-3*. On the other hand, defect of *odr-3* mutant, which lacks thermal response of AWC, influenced additively on the defect of *glc-3*. These results are consistent with a model that GLC-3 inactivates AIY with reception of EAT-4-mediated glutamate signals from AFD. To test this model, we are in the process of calcium imaging of AIY in *eat-4* mutant and *glc-3* mutant.

(1) Mori and Ohshima, 1995, Nature. (2) Kuhara, Okumura et al., 2008, Science. (3) Lee et al., 1999, J Neurosci. (4) Horoszok et al., 2001, Br J Pharmacol.

595A

Mapping suppressor of the *eat-16* mutant defective in G protein-coupled thermosensation. **A. Okazaki**, A. Kuhara, S. Tachikawa, I. Mori. Div Biological Sci, Nagoya Univ, Nagoya., IAR, CREST-JST, Japan.

Thermosensation is essential for animal metabolism and behavior. We focus on thermotaxis to understand molecular mechanism underlying thermosensation. Loss-of-function (lf) mutation in *eat-16* encoding RGS, a negative regulator of G protein, causes cryophilic phenotype due to hyper activation of thermosensory signaling in AWC sensory neuron, which acts as thermosensory neuron besides its role as an olfactory neuron (1). As we previously reported, the cryophilic defect of *eat-16* mutant is suppressed by known mutations in G protein-coupled thermosensory signaling in AWC.

To further identify new molecules involved in G protein-coupled thermosemsation, we isolated suppressors for cryophilic abnormality of *eat-16* by screening approximately 18000 genomes of *eat-16*(N2), a *eat-16* strain with N2 background. In an attempt to map *nj69,* one of the suppressor mutations, we used snip-SNPs with *eat-16*(HA), a Hawaiian CB4856 strain containing *eat-16* mutation. We found that *nj69* mutation mapped between -12.67 and -8.61 on the chromosome X, based on the snip-SNPs result that that region was all N2 type DNA in strains which suppressed *eat-16* mutation.

During the course of mapping, we found that *eat-16*(HA) strain tends to show stronger cryophilic phenotype than that of *eat-16*(N2) strain, implicating that this stronger cryophilicity may hide the suppression phenotype of *eat-16;nj69* mutant. In mapping *nj69* mutation, strains showing stronger cryophilic phenotype have CB4856 type DNA at least between -11.77 and -10.52 on the chromosome X. Also, stronger cryophilic phenotype is dominant. To confirm whether stronger cryophilic phenotype was derived from unidentified factors in the CB4856 background on X chromosome, we made *eat-16* strains, in which the chromosome X is chimeric between N2 and CB4856 DNA types but other chromosomes are mostly N2. Additionally, to determine stronger cryophilic phenotype depends on *eat-16* or not, we are making strains carrying only chimeric X chromosome by removing *eat-16* mutation. (1) Kuhara, A., Okumura, M., et al., Science. 320:803 (2008).

596B

Characterization of a Novel Conserved Neuronal Protein Possibly Involved in Synaptic Vesicle Exocytosis. **N. Paquin**, A. Froehlich, D. Omura, H. R. Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

Upon entering a bacterial lawn, well-fed wild-type *C. elegans* reduce their locomotion rate by about 30 percent. This response, known as the basal slowing response, requires dopaminergic modulation of the locomotory circuit. Worms that have been starved briefly prior to crawling onto a bacterial lawn slow by more than 75 percent. This behavior, dependent on the past feeding experience of the animal, is termed the enhanced slowing response and is modulated by serotonin. Animals lacking *mod-5*, a serotonin reuptake transporter, display a hyper-enhanced slowing response upon reaching a bacterial lawn, slowing by more than 90 percent. By mutagenizing *mod-5* animals, we identified mutations that cause well-fed worms to behave as if they had been deprived of food. These mutations might affect food-sensing or satiety mechanisms and make the enhanced slowing response independent of past feeding experience. We cloned a gene defined by one such mutation, *n4022*. This gene encodes a novel and highly conserved protein, C44B9.1. A rescuing *C44B9.1* translational GFP reporter that contains the endogenous *C44B9.1* promoter is expressed in the HSNs, multiple head and tail neurons and the ventral nerve cord. *C44B9.1* expression is observed mainly in cell bodies but also in processes as puncta. The locomotion defects of *n4022* worms are rescued by expressing *C44B9.1* from a pan-neuronal promoter (*unc-119*) but not from a body-wall muscle promoter (*myo-3*), indicating a neuronal function for *C44B9.1*. The *n4022* strain shows decreased sensitivity to aldicarb, an acetylcholinesterase inhibitor, and increased sensitivity to levamisole, an acetylcholine receptor agonist, suggesting a presynaptic role for *C44B9.1*. In addition to their locomotion defect, *n4022* mutants retain eggs in utero for an abnormally long period of time, which leads to eggs being laid at a later developmental stage. Furthermore, *C44B9.1* mutants display high temperature (27°C) induced dauer formation (Hid) and synthetic dauer formation at 25°C in combination with unc-31(e928) (Sdf). This phenotype and the drug sensitivity profile are similar to those of strains mutant in genes involved in the regulation of synaptic vesicle exocytosis, such as *unc-64*, *unc-31*, *hid-1* and *aex-3*. We are currently performing suppressor screens to place *C44B9.1* in a genetic pathway and are investigating the genetic interactions of *C44B9.1* with genes involved in neurotransmission. We plan to use a genetically-encoded calcium indicator and electrophysiology to assess the role of *C44B9.1* in synaptic vesicle exocytosis and cell excitability.

597C

The Dispersal Behavior of C. elegans. **Margherita Peliti**[1], John Chuang[2], Stanislas Leibler[2], Shai Shaham[1]. 1) Laboratory of Developmental Genetics, The Rockefeller University, New York, NY; 2) Laboratory of Living Matter, The Rockefeller University, New York, NY.

A major determinant of an organism's long-term success is its ability to locate and harvest resources, and to disperse once these are depleted. We seek to investigate the dispersal strategy of C. elegans, and to provide a characterization of its long-range spatial pattern of movement.

We have devised an imaging setup employing several flatbed scanners to visualize the movement of C. elegans over a large surface. Images of 1-day-old adult individual animals placed at the center of a 24 cm x 24 cm agar plate are collected for 90 minutes or until animals encounter the edge of the plate, and positional information is extracted with a tracking algorithm.

We utilize a geometrical criterion to quantify the directionality of paths at different length scales, and to sort trajectories into distinct behavioral classes. Our data shows that about 60% of wild-type trajectories are directional over length scales that are 50-100 times the animal's body-length. Furthermore, comparison of our data with simulated trajectories shows that directionality cannot be accounted for by a stochastic, random walk model of locomotion.

The overall direction of movement with respect to our imaging apparatus differs from animal to animal, suggesting that the observed directional bias is unlikely to result from a bias in the experimental setup. To gain insight into the basis of this large-scale directed movement we have begun to examine the tracks of animals with impaired sensory function. Concurrently, we are developing a camera imaging apparatus to validate these results in a different setup, as well as to investigate directionality at a finer time resolution.

598A

Dissecting the neural basis for locomotion in freely-behaving worms. **Beverly J. Piggott**[1,2,4], Zhaoyang Feng[1,2,3,4], Jie Liu[1,2], X.Z. Shawn Xu[1,2]. 1) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI 48109; 2) Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109; 3) Department of Pharmacology, Case Western Reserve University, Cleveland, OH 44106; 4) These authors contributed equally to this work.

Locomotion represents one of the most basic motor programs in the *C. elegans* behavioral repertoire. The command interneurons are believed to be the primary drivers of forward and backward locomotion. To better understand the role of these neurons in both sensory and spontaneous behavior, we have developed an automated calcium imaging system that permits simultaneous imaging of neural activity and behavior in freely-moving worms. We have named it the CARIBN system (CAlcium Ratiometric Imaging of Behaving Nematodes). Previous studies have been primarily conducted on restrained or semi-restrained worms that do not exhibit natural behavior. Our system provides a means to temporally examine how neural activity correlates to behavior under standard laboratory conditions where worms freely move on the surface of an NGM plate in an open environment. By using standard laboratory conditions, we can compare our work to the majority of behavioral studies performed by other groups over the past 40 years. We currently focus on the neuron AVA, as it has been implicated as a primary driver of backward locomotion in both spontaneous and sensory induced behaviors. Consistent with other reports, we found that AVA is activated in response to nose touch, osmotic shock, and in spontaneous long reversals. Surprisingly, we do not see a significant AVA activity in spontaneous short reversals. The CARIBN system provides a powerful tool to dissect how genes and neural circuits generate behavior in *C. elegans*.

599B

Role of neurotransmitters and neuropeptides in *C. elegans* nicotine dependent behavior. **Manish Rauthan**[1], Zhaoyang Feng[2], X. Z. Shawn Xu[1]. 1) Life Sciences Institute and Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Department of Pharmacology, School of Medicine, Case Western Reserve University, Cleveland, OH.

Nicotine is the main addictive drug in tobacco. In mammals, nicotine exerts its effects by binding to nicotinic acetylcholine receptors (nAChRs), which are ligand-gated ion channels and allow both mono- and divalent cations to pass through them. Nicotine acts by increasing the dopamine levels in mesolimbic dopamine system, thereby modulating the brain reward centre. Besides dopamine, other neurotrasmitters such as norepinephrine, serotonin and glutamate are involved in different aspects of nicotine dependence. Previous studies in our laboratory established *C. elegans* as a genetic model for the study of nicotine dependence (Feng, Z. *et al*. Cell 2006;127(3):621-33). Nicotine elicits various behavior responses in *C. elegans* such as acute response, adaptation, withdrawal and sensitization. We have found that neuropeptides are involved in nicotine-dependent behavior in worms. We have also found that various neurotransmitters such as dopamine, octopamine and tyramine are involved in modulating nicotine-dependent behavior in worms. In addition, we are identifying novel genes involved in regulating nicotine responses.

600C

The BAG Sensory Neurons are Activated by Environmental Carbon Dioxide. **Niels Ringstad**[1], Elissa Hallem[2], Bob Horvitz[1], Paul Sternberg[2]. 1) Dept. Biology, MIT, Cambridge, MA 02139; 2) Division of Biology, Cal Tech, Pasadena, CA 91125.

The BAG neurons are ciliated sensory neurons that recently have been shown to mediate avoidance behavior to carbon dioxide (CO_2) and to inhibit egg-laying behavior. To test whether the BAG neurons are CO_2 sensors, we generated transgenic animals expressing the high-affinity genetically encoded calcium sensor cameleon YC3.60 in the BAG neurons. We observed increases in BAG neuron calcium in response to application of as little as 0.1% CO_2 with half maximal increases evoked by application of 0.9% CO_2. The BAG neurons require the TAX-2 / TAX-4 cyclic nucleotide-gated cation channel to respond to environmental CO_2. The BAG neurons of *rgs-3* mutants, which lack a negative regulator of heterotrimeric G proteins, are defective for CO_2-evoked calcium responses, indicating that the BAG neurons are negatively regulated by a G protein signaling pathway. Using *in vivo* calcium imaging, we are seeking neurons that function downstream of the BAG sensory neurons in neuronal circuits that mediate CO_2 avoidance and the regulation of egg laying.

601A

Inward Rectifier Potassium Channels Inhibit C. elegans Egg Laying and Locomotion. **Niels Ringstad**, Bob Horvitz. Dept Biology, MIT, Cambridge, MA 02139.

Inward-rectifier K^+ (IRK) channels function to regulate membrane excitability in neurons and neuroendocrine cells. Three *C. elegans* genes, *irk-1*, *irk-2* and *irk-3*, are predicted to encode potassium channel subunits similar to mammalian Kir1-7, which form tetrameric inward-rectifier potassium channels that are activated by diverse intracellular signals, including G proteins, ATP and phosphoinositides. To study the function of *C. elegans* inward rectifier K^+ channels, we isolated deletion alleles of *irk-1*, *irk-2* and *irk-3*. *irk-1* mutants move faster than the wild type and lay early-stage eggs. *irk-1* deletion also suppresses the egg-laying defect caused by a gain-of-function mutation in the EGL-6 G protein-coupled receptor for FMRFamides that activate inhibitory G protein signaling in the HSN egg-laying motor neurons. We did not observe gross behavioral defects of *irk-2* and *irk-3* mutants.

An *irk-1::gfp* reporter transgene was expressed in a small number of neurons, including the HSNs. Expression of ChannelRhodopsin using the *irk-1* promoter caused animals to lay eggs and accelerate in response to a blue-light stimulus, indicating that *irk-1* functions to inhibit neurons that promote egg laying and locomotion. *Xenopus laevis* oocytes expressing *irk-1* showed inward-rectifying currents in high-potassium saline. We are currently testing whether signaling pathways known to modulate IRK channels in other organisms function to modulate IRK-1 *in vitro* and *in vivo*.

602B

Why do dauer and non-dauer *C. elegans* behave differently? **Nathan E Schroeder**, Maureen M Barr. Dept. of Genetics, Rutgers University, Piscataway, NJ.

The *C. elegans* dauer larva is an alternative juvenile stage that is formed under conditions of low food and high population density. Dauers differ from well-fed larvae in both altered sensory neuroanatomy and behavioral repertoire. For example, dauers often lie motionless in a quiescent state. However, dauers are also capable of nictation behavior, in which the animal climbs onto projections and stands on its tail. To analyze the causes of dauer-specific behaviors, we are comparing the sensory anatomy and behavior of dauers to well-fed larval and adult stages.

To establish a baseline comparison, we are performing a battery of behavioral assays on dauers versus non-dauers. We have found differences in chemotaxis behavior between dauers and well-fed adults in standard chemotaxis assays. Nictation behavior on exhausted culture plates was observed, and may be separated into four components: climbing, where nematodes move up a projection; waving, where the nematode stands on its tail while moving in three-dimensional loops and spirals; standing, where the animal maintains a rigid posture for extended periods of time while standing on its tail; and swarming, in which tens of nematodes aggregate together on top of a single projection. To study the molecular genetics of nictation behavior, we are starting with a candidate gene approach. To examine anatomy, we are using dye-filling. Amphid and phasmid neurons in dauers stained with DiI, DiO, and DiD in a time and compound-dependent manner. IL2 neurons in dauers never filled, confirming previous results that these neurons are less accessible to the environment in the dauer stage (1). Future work includes the continued collection of baseline behavioral data on dauers, an analysis of dauer quiescence, the use of microfluidic substrates to analyze nictation behavior, and a comparison of reporter gene expression in dauer and non-dauer animals.

Given the known changes that occur in behavior and anatomy between dauers and well-fed *C. elegans*, this work may lead to new discoveries in neuroplasticity. Additionally, the homology shown between dauers and the infective stage of many parasitic nematodes enables the use of the well-established tools of *C. elegans* to better understand the infective process of parasites that are estimated to afflict one-third of the world's population.

1.Albert and Riddle. 1983. J. Comp. Neur. 219:461-481.

603C

Integration of thermosensation and chemosensation during simultaneous presentation assay in *C. elegans*. **Ryuzo Shingai**, Ryota Adachi, Tokumitsu Wakabayashi. Dept Engineering, Iwate Univ, Morioka, Iwate, Japan.

Multi-sensory integration of neural function is important for organisms to discriminate environmental stimuli and to decide behavior. To address this function, we studied *C. elegans* behavior during simultaneous presentation of cultivation temperature and chemoattractant. *C. elegans* shows thermotaxis behavior that attracts to their cultivation temperature on a thermal gradient. Thermotaxis and chemotaxis behaviors are important for *C. elegans* to search food. It is possible that the integration of thermal and chemical information is occurred in animal's nervous system. At first, we examined thermotaxis behavior in the radial temperature gradient, and chemotaxis behavior toward sodium chloride or isoamyl alcohol. Then, assays for simultaneous presentation of 15°C (colder temperature than 20°C room temperature) and chemoattractant were performed using 15°C-cultivated wild-type worms because of evident thermotaxis behavior in these worms. Simultaneous presentation resulted in a biased migration to cold regions in the first 10 min for the assay, and sodium chloride-regions in the last 40 min. However, when sodium chloride was replaced with isoamyl alcohol in the simultaneous presentation, the behavioral index was very similar to the sum of separate single presentation indices. We then recorded tracks of single worms and analyzed their behavior. Migration toward 15°C in simultaneous presentation was faster than in 15°C-single presentation, while migration speed toward the chemoattractant was not affected by the presence of 15°C spot. For behavior toward sodium chloride, frequencies of forward and backward movements in simultaneous presentation were significantly different from those in single presentation. Angle formed between a worm, the sodium chloride spot, and 15°C spot in simultaneous presentation was larger than that for sodium chloride in single presentation . These results suggest that multi-sensory integration occurred between cultivation temperature and sodium chloride in the *C. elegans* nervous system.

604A

Molecular analysis of the integration of two sensory signals in *C. elegans*. **Yoichi Shinkai**[1], Makoto Tsunozaki[2,3], Cori Bargmann[2], Takeshi Ishihara[1]. 1) Kyushu University, Japan; 2) Howard Hughes Medical Institute, Laboratory of Neural Circuits and Behavior, Rockefeller University; 3) Herbert W. Boyer Program in Biological Science, The University of California.

Environmental information is received by sensory neurons as sensory signals and subsequently those signals are integrated in the central nervous system. The sensory integration is a process of an adaptive response to the environment. If the sensory information is improperly integrated, the resulting abnormal outputs caused behavioral defects and learning impairments. Despite its importance, little is known about the mechanisms at the molecular level.

To provide insights into the molecular mechanisms of the sensory integration, we performed a genetic screen to identify genes required for the sensory integration. In the mutant screening, an attractant diacetyl and a repellent copper ion were used. When a copper ion barrier exists between diacetyl and worms, worms must cross the copper ion barrier to reach the diacetyl spot. Mutants deficient in *gcy-28*, which encodes a receptor-type guanylate cyclase, less frequently crossed the barrier than wild type animals. In addition, *gcy-28* mutants show the defect in the salt chemotaxis learning, which is the form of behavioral plasticity induced by paired stimuli, starvation and NaCl. To investigate the genetic interaction between *gcy-28* and other genes involved in the sensory processing, we employed double mutant analyses in the sensory integration and in the salt chemotaxis learning. HEN-1/SCD-2 pathway regulates the sensory integration and the salt chemotaxis learning, whereas the insulin pathway regulates the salt chemotaxis learning. *gcy-28;scd-2* double mutant and *gcy-28;ins-1* double mutant showed stronger defects than each single mutant in both the salt chemotaxis learning and the sensory integration and in the salt chemotaxis learning, respectively. These results suggest that multiple pathways are involved even in a simple kind of sensory information processing.

gcy-28 encodes four isoforms (*gcy-28.a-d*) (Tsunozaki et al., 2008). GFP reporter analyses suggested that *gcy-28.d* isoform was expressed mainly in AIA interneurons. The expression of GCY-28.d by a promoter that drives the expression only in AIA interneurons was sufficient to restore the phenotype of the *gcy-28.d* mutant *tm3028* and partially rescued the defect of the mutant devoid of all *gcy-28* isoforms. Our results suggest that *gcy-28* regulates the sensory integration in multiple neurons including AIA interneurons, and provide insights into how sensory information processing is regulated in the nervous system.

605B

Notch signaling regulates adult behavior. **Gerard Somers**[1,2], Mark Corkins[1,2], Ed Anderson[1,2], Michael Y. Chao[3], Jonah Larkins-Ford[1], Tim Tucey[1], Hidetoshi Komatsu[1], Anne Hart[1,2]. 1) Massachusetts General Hospital Center for Cancer Research, Charlestown, MA; 2) Department of Pathology, Harvard Medical School, Boston, MA; 3) Department of Biology, California State University San Bernardino, San Bernardino, CA.

The Notch signaling pathway is best known for its role in cell fate specification during development. In a screen to identify genes required for octanol avoidance, we identified *osm-11*, which encodes a secreted Notch ligand; we recently demonstrated that *osm-11* acts in *C. elegans* vulval development (WBPaper00032102). Here, we focus on the non-developmental role of *osm-11* in *C. elegans* behavior. The direct transcriptional targets of Notch signaling in neurons are unclear. By elucidating the mechanisms and targets of the Notch signaling pathway in the adult nervous system, we hope to provide insight into the complex regulation of adult behavior in animals.

Here, we demonstrate that two behaviors are regulated by *osm-11* and Notch signaling: chemosensory response to octanol and quiescence. First, loss of *osm-11* results in a defective response to octanol. The two *C. elegans*' Notch receptors, LIN-12 and GLP-1, act redundantly to regulate octanol response. Receptor function is required in adult animals in non-overlapping subsets of neurons. Additional components of the Notch signaling pathway are also required including *lst-1*, a gene of unknown function that is a likely Notch target during development (WBPaper00006348).

Overexpression of OSM-11 results in inappropriate quiescence of adult animals. This *osm-11* induced quiescence is reminiscent of that seen in satiety or molting, but *osm-11* induced quiescence is independent of feeding status. *osm-11* acts via Notch receptors in this behavior as well; perturbing either the LIN-12 or GLP-1 Notch receptor prevents quiescence. *osm-11* induced quiescence was also dependent on *egl-4*, a cyclic GMP-dependent kinase, and the EGF receptor *let-23*, two genes previously implicated in *C. elegans* behavioral quiescence (WBPaper00031383 and WBPaper00031030). Our genetic analysis places *egl-4* downstream of Notch, thus *egl-4* may be a direct Notch target. A previous bioinformatic analysis found numerous consensus binding sites for LAG-1 (a critical Notch pathway transcription factor) in *egl-4* intronic sequences. We are currently working to validate these and additional downstream targets of Notch. Notch function in the adult nervous system may be conserved across species.

606C

Regulation of Motor Neuron Activity by Neuropeptide Signaling. **Tamara Stawicki**[1], Yishi Jin[1,2]. 1) Div of Bio Sci, UCSD, La Jolla, CA; 2) HHMI.

The anatomical basis of the C. elegans locomotor circuit is well known. Movement is initiated by command interneurons in the head and tail of the worm. These neurons activate the cholinergic motor neurons, which generate excitatory inputs to the muscle while simultaneously activating GABA motor neurons to inhibit the contralateral muscle. However, several open questions remain, such as which kind of neurotransmission occurs between the interneurons and the cholinergic motor neurons? How do the motor neurons of the locomotor circuit communicate with one another to coordinate the propagation of the sinusoidal wave?

We have recently reported the identification of a neuronal acetylcholine receptor that is expressed and functions in the cholinergic motor neurons to maintain their excitability (Stawicki et al. & Qi et al. abstracts in C. elegans Neural Topic Meeting 2008, and Jospin et al. unpublished). This AChR is composed of three α-subunits, UNC-38, UNC-63, ACR-12, and one non-α subunit, ACR-2. A gain of function mutant, *acr-2(n2420),* exhibits spontaneous contraction, partially resembling the classical "Shrinker" behavior caused by the loss of GABA motor neuron function. Because the command interneurons of the locomotor circuit do not release acetylcholine, we propose that the ACR-2 receptor may act to coordinate the communication between the motor neurons themselves.

To test this idea and define the signaling pathway of the ACR-2 receptor in the locomotor circuit, we have examined the genetic interactions between genes regulating synaptic transmission and *acr-2(n2420)*. We find that blocking neuropeptide release by a loss of function mutation in *unc-31* significantly suppresses the behavior of *acr-2(n2420)* worms, whereas a mutation leading to a reduction in classical fast neurotransmission, or in gap junction function, was not able to suppress *acr-2(n2420)*. Using cell-type specific expression studies, we find that this effect of neuropeptide signaling involves the action of *unc-31* in neurons, but not in the cholinergic motor neurons. Restoring *unc-31* function in motor neurons only can further suppress *acr-2(n2420)* behavior. Furthermore, we observed opposing effects on *acr-2(n2420)* behavior by the peptide processing pathway proteins *egl-3* and *egl-21*. These observations suggest the modulation of ACR-2 receptor activity likely involves both excitatory and inhibitory peptide action. We are in the process of testing candidate peptides and receptors. Lastly, we have identified a novel suppressor of *acr-2(n2420)* that appears to define a potentially new calcium pathway in the motor neurons.

607A

Genome-wide identification of a systemic thermosensory mechanism controlling a memory-based behavior in *Caenorhabditis elegans*. **T. Sugi**[1], Y. Nishida[1], I. Mori[1,2]. 1) Div Biological Sci, Nagoya Univ, Nagoya, AIchi, Japan; 2) CREST-JST.

For ensuring survival and reproduction, animals cope with environmental changes by altering behavioral strategy. However, molecular basis of thermosensation closely associated with animal behavior is still largely unknown, partly due to the lack of the molecular identity of a temperature sensor. The nematode *Caenorhabditis elegans* memorizes cultivation temperature and modifies behavioral responses. We show here that heat-shock transcription factor (HSF) acts as a body thermal sensor, thereby modifying this memory-based behavior. Genome-wide microarray analysis suggested that, despite the well-known responsiveness of heat-shock transcription factor to transient heat-shock stimuli, HSF-1 could also sense changes in a range of environmental cultivation temperatures throughout the *C. elegans* body. We showed that this thermosensation induces expression changes of previously unidentified *non-hsp* genes. These results further led us to find that HSF-1 and its downstream genes regulate the thermotactic neural circuit to modulate behavioral responses in accordance with cultivation temperature changes. We thus present a novel thermosensory mechanism involved in behavioral control, whereby systemic thermosensation by heat-shock factor is coupled to transcriptional changes of various genes, which in turn affects neural circuit to modify a temperature memory-regulated behavior.

608B

The video-based quantitative evaluation of IR-induced effects on locomotory behavior in *Caenorhabditis elegans*. **M. Suzuki**[1], T. Sakashita[1], Y. Hattori[1,2], S. Yanase[3], M. Kikuchi[1], T. Funayama[1], Y. Yokota[1], T. Tsuji[2], Y. Kobayashi[1]. 1) Japan Atomic Energy Agency, Takasaki; 2) Graduate School of Engineering, Hiroshima University, Higashi-Hiroshima; 3) Daito Bunka University School of Sports and Health Science, Higashi-Matsuyama.

Background and purpose: Locomotory behavior (motility) is a vital importance in animals. We examined the effects of ionizing radiation (IR) on locomotory behavior using *Caenorhabditis elegans*. We reported an IR-induced reduction of locomotory rate in the absence of food[1],[2]. In the previous experiments, wild-type animals were irradiated with γ-rays in the whole body, and measured the motility using "body bends" (the number of bends in the anterior body region at 20-s intervals)[3]. However, the IR-induced effects in the central and posterior body region were not evaluated by the body bends. In the present study, to investigate the IR-induced effects in more detail, we try to evaluate the motility of the whole body by using the video-based analysis. **Strains and culture:** The *C. elegans* wild-type Bristol N2[4] and the *Escherichia coli* HB101 strain, were obtained from the Caenorhabditis Genetics Center. Using standard methods[4], animals were grown at 20°C on 6-cm plates containing nematode growth medium (NGM) agar spread with *E. coli*. Well-fed adults were used in all experiments. **Irradiation and video-based analysis:** Animals were collected from culture plates and washed, and transferred to the 3.5-cm NGM plate without a bacterial lawn. The movements of animals were video-recorded for 1 min. Subsequently, animals were collected and placed on a plate with a bacterial lawn for 1h to avoid starvation, and irradiated with graded doses (< 1 kGy) of ^{60}Co γ-rays. Immediately after irradiation, animals were again transferred to the 3.5-cm NGM plate without a bacterial lawn, and the movements were video-recorded. To measure the whole-body motility, we are now constructing a worm-tracking system based on "the parallel worm tracker"[5]. Using this system, we analyze the trajectories and bends at several points of the body in multiple animals before and after irradiation. **Results and conclusion:** We propose a novel standard by using the video-based analysis for evaluation of IR-induced effects on motility in *C. elegans*. In the presentation, we will show preliminary results of the analyses, and would like to discuss about the effectiveness of the analysis for evaluation of IR-induced effects. **References:** [1] Sakashita, T., et al. (2008) *J. Radiat. Res.* 49: 285-291. [2] Suzuki, M., et al. (2009) *J. Radiat. Res.* 50 (in press). [3] Sawin, E.R., et al. (2000) *Neuron* 26: 619-631. [4] Brenner, S. (1974) *Genetics* 77: 71-94. [5] Ramot, D., et al. (2008) *PLoS ONE*,3: 5: e2208.

609C

Quantitative behavioral analysis of freely moving *C. elegans*. **Yuki Tsukada**[1,2], Akiko Miyara[1], Tomoyasu Shimowada[1], Noriyuki Ohnishi[1], Atsushi kuhara[1,2], Ikue Mori[1,2]. 1) Graduate School of Science, Nagoya University, Nagoya, Aichi, Japan; 2) CREST-JST, Japan.

C. elegans is an ideal system to study information processing mechanism by neural network because the patterns of connectivity for all 302 neurons had been mapped out. In addition, input stimuli and output behavioral responses are robust for qualitative measurement. Although previous studies have identified several neurons and molecules related to particular types of information processing, little is known about the mechanism of information processing in *C. elegans*. This is because the lack of dynamic information for stimuli and behaviors as input and output functions, respectively. We are approaching this problem by quantitatively measuring input stimulus and output behavior. To begin our analysis, we took advantage of our live imaging and tracking system that pictured freely moving single animals; the time-lapse images were recorded up to several hours at video rate (about 30 frames/sec). Then, curvature maps of worm body along head-to-tail axis were constructed by using homemade matlab programs, and thus locomotory states were represented by the patterns in the map. The map identified bending frequencies at each position along the body and speed of curvature wave from head to tail. As previously reported (Croll NA, 1975), curvature wave along head-to-tail axis showed various patterns. For example, reverse wave (tail-to-head) of curvature was observed when the animals moved backward. We compared wild-type (N2) animals with *ttx-8 (nj34)* mutants that showed defects in thermotaxis by circular thermal gradient assay and calcium imaging of thermosensory neurons (Miyara *et al.* unpublished). Our quantitative behavioral map analysis distinguished behavioral patterns of wild-type animals and the mutants. Since the behavioral patterns of the *ttx-8* mutant include normal migratory patterns, we assume that the mutants are normal in motor control but sensory and/or information processing of the neural network is defective. More speculatively, we suppose that judgmental process and/or maintenance of action planning in the mutants should be defective. We are currently working on the quantitative analysis for temperature input stimuli and attempting to establish mathematical model for information processing during thermotaxis.

610A

Development and function of the sleep-inducing ALA neuron of *C. elegans* . **Cheryl L. Van Buskirk**, Paul W. Sternberg. Div Biol, Caltech, Pasadena, CA.

The impact of sleep disorders on human health has underlined the need for a greater understanding of the regulation of sleep. Such studies have largely focused on mammalian systems, but recently sleep-like states have been described in invertebrates, including *C. elegans* (Raizen et al., 2008). Importantly, some of the factors regulating sleep behavior appear to be highly conserved, such as signaling through the Epidermal Growth Factor Receptor (EGFR) (Van Buskirk and Sternberg, 2007). In *C. elegans*, EGF-induced sleep is marked by a cessation of feeding and locomotion and is normally restricted to a short period preceding each larval molt. However, this sleep-like state can be induced at any time during development or adulthood through conditional overexpression of LIN-3/EGF. The effects of EGF expression in *C. elegans* are strikingly similar to those seen in response to EGF administration in mammals, pointing to an evolutionarily conserved role for EGF signaling in the regulation of behavior.

We have found that EGF-induced sleep is triggered by activation of the EGF receptor (LET-23) within a single neuron called ALA, and we have identified several EGF-resistant mutants that define components of a signal transduction pathway mediating this effect. In addition, we have identified a class of mutations that render animals EGF-resistant due to defects in ALA-specific gene expression, including impaired let-23 transcription. Lastly, using a combination of genetic analyses and laser ablations, we have investigated the mechanism by which the ALA neuron signals to effect sleep behavior. We will present the results of these analyses on the development and function of the ALA neuron.

Raizen, D.M. et al. (2008) Lethargus is a *C. elegans* sleep-like state. Nature 451, 569-72.

Van Buskirk, C. and Sternberg, P.W. (2007) Epidermal Growth Factor signaling induces behavioral quiescence in *C. elegans*. Nat. Neurosci. 10, 1300-07.

611B

Identification of targets of KIN-29 SIK signaling in the regulation of food-related behaviors and development. **Alexander M. van der Linden**[1,2], Piali Sengupta[1]. 1) Dept Biol. and National Center for Behavioral Genomics, Brandeis Univ, Waltham, MA (slinden@brandeis.edu); 2) Dept Biol., University of Nevada, Reno, NV (from 9/09).

The availability and quality of food fluctuates over time in an animal's environment. When food is scarce, an animal must undergo changes in its behavior, metabolism and physiology to promote survival. An animal must also regulate its feeding habits depending on nutritional status in order to maintain physiological homeostasis. Salt-inducing kinases (SIKs) are important regulators of energy homeostasis, and feeding and fasting behaviors in mammals (1). We previously found that KIN-29, the *C. elegans* homolog of SIK, regulates the expression of a subset of chemoreceptor (CR) genes in response to changes in external and internal conditions, and that this regulation likely underlies the ability of animals to correctly transduce food-derived signals to modulate behaviors such as quiescence and foraging as well as body-size (2).To further dissect the KIN-29 signaling pathways by which food signals are integrated to modulate behavior and development, we are taking three parallel strategies to define new targets of KIN-29. In the first, we are determining the gene expression profiles of specific neurons in wild-type and *kin-29* mutants using microarrays coupled with a mRNA-tagging strategy. Preliminary results indicate that a large subset of genes predicted to function in transcriptional regulation and phosphorylation events are up- and down-regulated, respectively, in wild-type and *kin-29* mutants in chemosensory neurons. Secondly, we are examining a KIN-29-dependent CR gene whose expression in the ADL chemosensory neurons is acutely altered by the presence and absence of food in adults. We will take advantage of these observations to identify the components and neurons by which food signals alter CR gene expression using genetic screens. Finally, we are examining the function of *Y20F4.2*, which encodes the single *C. elegans* homolog of TORC/CRTC. TORC is phosphorylated by SIK members, and is an important regulator of metabolism in mammals and flies (1). *Y20F4.2* is widely expressed, including in most neurons. As in mammals and flies, we found that subcellular localization of Y20F4.2 is regulated by phosphorylation and oxidative stress. Interestingly, expression of the *daf-7* TGF-β gene is reduced in *Y20F4.2* mutants, indicating that Y20F4.2 regulates *daf-7* TGFβ expression, and therefore may control metabolism and feeding behavior (3). Together these experiments will provide a detailed understanding of the remarkably complex effects of food on animal development and behavior. 1) Hietakangas, 2008; 2) Lanjuin and Sengupta, 2002; Van der Linden et al. 2007, 2008; 3) Greer et al. 2008.

612C

The C. elegans male posterior connectome matures during early adulthood. **Yi Wang**[1], Max Bernstein[1], Travis Jarrell[1], Meng Xu[1], Donna G. Albertson[2], Nicole Thomson[2], David H. Hall[3], Scott W. Emmons[1]. 1) Dept.of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) MRC Laboratory of Molecular Biology, Cambridge, UK; 3) Dept of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

We are carrying out reconstructions of the C. elegans male nervous system from serial section electron micrographs created at the MRC in Cambridge, UK, during the 1970's. At that time, several adult animals of different ages were sectioned and photographed through critical regions of the male tail containing the neural circuits that underlie copulatory behavior. Our most complete reconstruction is that of an animal known as N2Y, annotated as an "old adult." We have compared selected neurons in the pre-anal ganglion of N2Y to corresponding neurons in a second worm, JSI, annotated as a "young adult." The neural network of N2Y is more complex than that of JSI. The number of synapses and the number of synaptic partners of individual neurons increases more than fourfold. Comparison of individual neuron maps revealed that the reason appears to be that neuron processes in JSI are not fully grown out. The absence in JSI of many apparently major synaptic interactions present in N2Y made it appear doubtful whether JSI could have mated properly. Accordingly, we tested the mating ability of young males immediately after their molt to adulthood. When presented with five paralyzed hermaphrodites, only one of 6 just-matured males mated during the first 3 hr, whereas males matured overnight mate within the first 20 min. Mating began thereafter, suggesting 3-5 hr are necessary for maturation of the male nervous system. Maturation of the connectivity during adulthood raised the possibility that experience could influence the wiring process and might improve performance. In order to generate mature males that had never experienced sensory inputs associated with mating or mating-type behaviors, we allowed L4 males to mature overnight in liquid. When placed with hermaphrodites on plates, such males mated immediately and performed as well as males matured on plates with other animals. Conversely, the performance of several day old, experienced males was not improved over that of inexperienced males. Therefore we found no evidence that mating competence either requires or is improved by experience. It appears that the pattern of synaptic interactions necessary for efficient mating is fully established a few hours after the L4/adult molt and is sufficiently well-specified genetically to support mating behavior.

613A

Genetic analysis of dopamine signaling in *C. elegans*. **Khursheed A. Wani**, Daniel L. Chase. University of Massachusetts at Amherst, Amherst, MA.

Dopamine acts through G protein-coupled receptors to modulate neural activity in the brain. Defects in dopamine signaling underlie neurological disorders including Parkinson's disease, schizophrenia and drug addiction. Despite its clinical relevance, detailed understanding of how dopamine exerts its functions remains largely unknown. To identify the molecular mechanisms of dopamine signaling, we have taken a genetic approach using *C. elegans* as a model system. In *C. elegans*, dopamine acts through the D2-like receptor DOP-3 and the G-alpha (Gα) protein GOA-1 to inhibit locomotion in response to environmental cues. This inhibition of locomotion behavior is also observed when animals are exposed to exogenous dopamine with high concentrations causing paralysis. The paralytic effect of exogenous dopamine also acts through the DOP-3 receptor and allows us to perform robust genetic screens to identify novel components of dopamine signaling. Interestingly, null mutants lacking the DOP-3 receptor are partially resistant to paralysis, while null mutations in the Gα protein (GOA-1) coupled to this receptor cause complete resistance to exogenous dopamine. This suggests that there is another dopamine receptor in *C. elegans* coupled to GOA-1 that acts to inhibit locomotion. In order to identify this unknown receptor and its downstream effectors, we conducted an enhancer screen in which we mutagenized the *dop-3* receptor mutant and identified second-site mutations that cause complete resistance to the paralytic effects of exogenous dopamine (similar to that observed in *goa-1* null mutants). We screened 92,000 mutagenized haploid genomes and isolated 16 mutants. Mapping of these mutants using single-nucleotide polymorphisms followed by complementation analysis resulted in the identification of one previously-known dopamine signaling component as well as seven novel genes involved in dopamine response. We are characterizing these novel genes at the genetic, behavioral and molecular levels.

614B

Sensory regulation of male-specific motor behaviors of *C. elegans*. **Allyson J. Whittaker**, Paul W. Sternberg. Biology Division, HHMI, Caltech, Pasadena, CA.

To survive and reproduce, animals must modify their motor behavior in response to changes in their environment. To understand how animals modify motor behavior in response to sensory input, we are studying male mating behavior in *C. elegans*. This is a particularly powerful model because, as the hermaphrodite does not play a cooperative role in mating and continues moving, the male must modify his movement and body posture in order to maintain contact and copulate with the hermaphrodite. This requires the coordination of the activity of multiple muscle groups in response to both external passive cues from the hermaphrodite as well as internal proprioceptive cues. During mating, the most dramatic changes in male body posture are seen in its tail, which, as it contains almost all of the sensory structures required for copulation, must remain in contact with the hermaphrodite. Thus, a focus of our work is to understand the motor circuits regulating male tail posture. Previous studies have shown that serotonin regulates ventral curling of the male tail, and this requires male specific muscles. Using genetic and laser ablation analysis, in conjunction with behavioral assays, we have expanded our understanding of neurotransmitters, receptors, neurons and muscles required for the regulation of male tail posture. We find that proper tail posture is maintained by coordination of sex-specific and core muscle groups that bend the tail ventrally and dorsally, a model that fits with the male tail wiring data. We demonstrate that cholinergic neurons, acting, at least in part, independently of serotonin, regulate both dorsal and ventral curling of the male tail and this requires dorsal body wall muscles, and male specific muscles respectively. Cholinergic regulation of tail posture requires GABA. Males with mutations in the GABA receptor *unc-49* over curl their tails during mating suggesting that cross inhibition of muscle groups is important to maintain proper tail posture. We are also interested in the sensory pathways that regulate male motor behaviors. Our results show that the proprioceptive mechanosensory TRPN channel, *trp-4*, is required for males to remain at the vulva, and for efficient turning. We find that *trp-4* is expressed in the postclocal sensilla and hook neurons and male specific muscles and we are currently determining the site of action.

615C

Refinement of the concentration-dependent effects of ethanol on the behaviour of the C. elegans pharynx. **James C Dillon**[1], Ioannis Andrianakis[2], Richard Mould[1], Christopher James[2], Vincent O'Connor[1], Lindy Holden-Dye[1]. 1) School of Biological Sciences, University of Southampton, Southampton, United Kingdom; 2) Institute of Sound and Vibration Research, University of Southampton, Southampton, UK.

The effects of ethanol upon the mammalian brain are broad ranging and concentration-dependent. Low concentrations (17mM) intoxicate while greater than 65mM anaesthetize. The responses to ethanol involves multiple substrates, which include receptors, ion channels, intracellular signalling molecules and changes to the properties of membranes. The concentration-dependent effect of ethanol on C. elegans remains unclear due to conflicting reports of ethanol's equilibration across the worm cuticle. This compromises our understanding of C. elegans as a relevant model for human ethanol intoxication and dependence and for the identification of the underlying neuronal substrates. To further clarify the concentration-dependent effects of ethanol upon the C. elegans nervous system we have made electrophysiological recordings from the pharynx (electropharyngeograms (EPG)) in the presence of a range of ethanol concentrations (10-300mM). The EPG provides a readout of muscle activity driven by the pharyngeal nervous system. Recordings were made from a semi-intact preparation of the pharynx which allows ethanol direct access to the pharyngeal muscle and nervous system, thus the concentration acting on this tissue can be clearly defined. We have designed software that facilitates the semi-automated statistical analysis of EPG recordings, enabling the identification and comparison of discrete pharyngeal phenotypes. Using this software we have identified ethanol concentration-dependent effects on the behaviour of the pharynx. At 10mM, a concentration close to human intoxication ethanol caused a subtle change in the pattern of pharyngeal activity stimulating worms to pump in larger 'bursts'. 50mM ethanol caused a change in the shape of the EPG recording corresponding to muscle depolarization. We have identified the threshold for this effect is close to 50mM and that it becomes more pronounced as the concentration of ethanol increases. At 300mM, ethanol caused a rapid, transient excitation of pharyngeal pumping followed by a rapid inhibition. We are further investigating the effects of ethanol on the pharynx by performing intracellular recordings and video analysis of muscle behaviour to elucidate the nature of the electrophysiological changes observed at 50mM. It is intended that this will provide further insight into the molecular substrates and cellular mechanisms underpinning the acute and chronic effects of ethanol in the mammalian system.

616A

Identifying circuits that respond to changing conditions: CaMKII interacts with EAG K+ channels to reduce *C. elegans* male sex muscle excitability when food is scarce. **Brigitte L. LeBoeuf**, L. Rene Garcia. Howard Hughes Medical Institute Dept Biol, Texas A&M Univ, College Station, TX.

Organisms respond to conditions of food scarcity by up-regulating behaviors that help locate new food sources and down-regulate non-essential behaviors. Behaviors are down-regulated through circuits that reduce the organisms' response to stimuli, and include the down-regulation of neurons and muscles that produce a behavior. Reducing the activity of excitable cells lessens seizure susceptibility, but the molecular circuits that accomplish this are not well understood. When the *ether-a-go-go* related gene K+ channel/*unc-103* is mutated in *C. elegans*, male sex muscles seize, resulting in premature protraction of the copulatory spicules. Our genetic analysis demonstrates that CaMKII/*unc-43* and *unc-103*'s paralog, *egl-2*, function in the sex muscles to reduce excitability when males are food deprived.

We show using Yeast Two-Hybrid and *in vitro* binding assays that UNC-43 and EGL-2 directly interact. This interaction depends on a serine at amino acid 567, located in a potential CaMKII binding site on the EGL-2 c-terminus. Mutating S567 to F results in an active EGL-2 channel that can suppress *unc-103(0)*-induced muscle seizures, a function that the canonical gain-of-function allele (*n693*) of *egl-2* does not possess. Since an *egl-2(0)* allele does not disrupt mating behavior, we hypothesize that under well-fed conditions EGL-2 is inactive, while when food is scarce, phosphorylation by UNC-43 activates the channel, reducing excitability.

Two types of Ca^{2+} channels produce the muscle contractions that control spicule protraction, and under conditions where food is scarce, the effects of Ca^{2+} influx from both these channels needs to be attenuated. The channels are the cell-membrane located L-type voltage-gated Ca^{2+} channel (L-VGCC) and the sarcoplasmic-reticulum-membrane located ryanodine receptor (RyR). L-VGCCs induce a tonic sex muscle contraction that causes spicule protraction. RyRs are responsible for rhythmic contractions that enable the male to penetrate the tightly closed hermaphrodite vulva. We used acetylcholine agonists arecoline and levamisole that induce calcium influx via L-VGCCs and RyRs, respectively, to determine what UNC-43 and EGL-2 regulate. *egl-2(gf)* males display reduced response to arecoline but not levamisole. *unc-43(n468gf)* males display a reduced response to both agonists. We hypothesize that UNC-43 attenuates the effects of L-VGCCs through activation of EGL-2 and RyRs in a separate pathway.

617B

CNG channels and TRPV channel proteins are involved in the thermal avoidance response in *Caenorhabditis elegans*. **S. Liu**[1,2,3], R. Baumeister[1,2,3,4]. 1) Bioinformatics and Molecular Genetics (Faculty of Biology); 2) Center for Biochemistry and Molecular Cell Research (Faculty of Medicine); 3) Center for Systems Biology (ZBSA); 4) FRIAS Freiburg Institute of Advanced Studies, School of Life Sciences (LIFENET) Albert-Ludwigs-University Freiburg, Schänzlestr. 1, D79104 Freiburg i. Brsg., Germany.

Upon exposure to noxious temperature, *Caenorhabditis elegans* executes an escape reflex (Tav response) similar to the response to body touch. We had previously shown that sensory neurons in the head and tail, but not in the midbody region, are involved in the perception of heat (Wittenburg 1999 PNAS). For finding candidate sensory neurons acting as thermonociceptors, we developed a modified neuron ablation method using a 2-photon laser. For this purpose, we labeled individual sets of neurons with GFP for ablation, and identified two pairs of head sensory neurons and one pair of tail sensory neurons as thermonociceptors. In order to characterize the moleculars of the thermal avoidance response in these sensory cells at the molecular level, we focused on a functional assessment of channel proteins that are candidates for heat receptors involved in the Tav behavior. In vertebrates, the capsaicin-sensitive vanilloid receptor VR1, a channel protein of the TRP family, has been shown to be involved in the perception of heat, capsaicin, and low pH. Our data indicate that the double mutants of the two VR1 homologous in *C. elegans ocr-2(vs29)osm-9(ky10)* have reduced TAV response from 95.1% to 73.5% in the head and from 68.1% to 14.5% in the tail. The results suggest that OSM-9 and OCR-2 are required, but are not sufficient, for the perception of noxious heat and that these two VR1 channels have conserved functions across different species. In vertebrates, the cyclic nucleotide-gated channels play a significant role in sensory neurons in visual and olfactory systems. Indeed, the mutants of the two homologous cGMP-gated channels subunits, *tax-2(p671)* and *tax-4(p678),* display reduced head Tav response (95.1% to 70.7% and 68.8%, respectively). These defects can be rescued by transgenic expression of the tax-2 or tax-4 cDNA in only two head thermonociceptors we had identified in this study. This indicates that TAX-2 and TAX-4 are required cell autonomously for thermonociception in at least two pair of sensory cells. All together, our data suggest that thermal avoidance response in *C. elegans* is mediated by a cGMP signaling pathway that includes the cGMP-gated heteromeric channel TAX-2/TAX-4 and also require the TRPV channel proteins.

618C

Intraspecific variation in responses to drugs that target the neuro-muscular system in *C.elegans*. **Rajarshi Ghosh**[1,2], Anya Levinson[1], Leonid Kruglyak[1,2,3]. 1) Department of Ecology and Evolutionary Biology, Princeton University, Princeton, NJ; 2) Lewis Sigler Institute for Integrative genomics; 3) Howard Hughes Medical Institute.

Individuals within species often exhibit heritable variation in behavior that results primarily from differences in the properties of neural circuits. What genetic changes allow intraspecific variations in behavior? Does the genetic change alter the connectivity or computational properties of a neural circuit or both? We use advanced intercross recombinant inbred lines (RILs), which contain different fractions of N2 and CB4856 genomes, dervived from the laboratory strain N2 and a genetically divergent wild strain CB4856, to identify the genotypic variation between these two strains that causes differences in the nervous system.

To investigate the effect(s) of the genetic divergence between the N2 and CB4856 on the nervous system, we have generated dose response curves for several drugs that target the neuromuscular system. We found striking differences in drug sensitivity to levamisole (cholinergic agonist), praziquantel (putative adenosine receptor agonist) and avermectins (glutamate gated chloride channel agonist) in a liquid swimming assay, suggesting differences in several aspects of neuro-muscular properties .

N2 was more sensitive to the antihelmintic avermectin than CB4856, and an opposite effect was observed with praziquantel. Using 137 RILs, we have found the avermectin resistance to be significantly linked to a region on Chromosome V. We narrowed the region down to 60 genes by analysis of RILs with breakpoints in this region. We will present the progress in identifying the molecular variant, giving rise to avermectin resistance in CB4856. Additionally, using 70 RILs we have mapped praziquantel sensitivity to Chromosome IV. We are currently refining the interval on Chromosome IV to understand molecular basis of praziquantel resistance.

619A

Role of Rho GTPase signaling in olfactory adaptation. **Yoshiyasu Ohara**[1], Yutaro Sassa[1], Satoshi Itakura[1], Kotaro Motoshige[1], Sadaaki Tanaka[1], Masayuki Gosho[1], Hiroyuki Arai[2], Takao Inoue[2], Kazunori Kume[1], Hiroyuki Kobuna[2], Kohji Miyahara[3], Dai Hirata[1]. 1) Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Japan; 2) Graduate School of Pharmaceutical Sciences, University of Tokyo; 3) Department of applied Life Science, Sojo University.

We are interested in olfactory adaptation, a component of behavioral plasticity. To investigate the gene(s) involved in olfactory adaptation, we screened for the mutants defective in adaptation to odorants. From out of 4,000 EMS-mutagenized animals, we isolated 11 mutants exhibiting defects in adaptation to the AWC-sensed odorants. One of the mutants turned out to contain a mutation in gei-1, that encodes Rho-GAP (GTPase-activated protein). To investigate whether Rho-GTPase(s) acts in this behavior, we examined olfactory adaptation of transgenic animals expressing dominant active/negative forms of Rho-GTPase family. The dominant active RHO-1 inhibited normal olfactory adaptation as the gei-1 mutant. Furthermore, the genetic analyses indicate that RHO-1 acts downstream of GEI-1 and UNC-13 acts downstream of RHO-1 in olfactory adaptation. Next, to investigate whether the neurotransmitter Acetylcholine is important for the olfactory adaptation, we examined the effect of levamisole (activating Acetylcholine receptor) on the olfactory adaptation of wild-type strain. We found that the levamisole-treatment perturbs normal olfactory adaptation.

620B

CO_2 response and host seeking in free-living and parasitic nematodes. **Elissa A. Hallem**, Paul W. Sternberg. Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA.

Parasitic nematodes are a major health concern worldwide, and current strategies for preventing or eliminating nematode infections are insufficient. One possible control strategy is to interfere with the ability of nematodes to locate hosts. Carbon dioxide is an important host-seeking cue for many parasitic nematodes, yet little is known about the mechanism of CO_2 response in nematodes. We found that *C. elegans* displays acute CO_2 avoidance: exposure to CO_2 results in the cessation of forward movement and the initiation of backward movement. We found that multiple signaling molecules affect CO_2 avoidance, including TAX-2/TAX-4, RGS-3, TAX-6, and NPR-1. Nutritional status also modulates CO_2 responsiveness via the insulin and TGF-β pathways. Acute CO_2 avoidance is mediated primarily by the BAG neurons, and TAX-2/TAX-4 are required in the BAG neurons for CO_2 response. Additional ciliated sensory neurons also contribute to CO_2 response. We are now extending this analysis to other nematodes. We have found that the BAG neurons are required for CO_2 avoidance in the necromenic worm *Pristionchus pacificus,* and for CO_2 attraction in the insect-parasitic nematode *Heterorhabditis bacteriophora.* We are now investigating the mechanism by which analogous neurons mediate attraction in parasitic nematodes and repulsion in free-living nematodes. We are also investigating how CO_2 response changes depending on the life stage of the worm. Finally, we are examining behavioral responses to other potential host-seeking odorants in *H. bacteriophora* and a different insect-parasitic nematode, *Steinernema carpocapsae.* We have found that the two species show very different odor response spectra, raising the possibility that some of the tested odorants contribute to host specificity.

621C

PKC2 and a Downstream Effector, PRDX-2, are Essential for Thermotaxis. **M Land[1,2]**, C.S Rubin[1]. 1) Dept. Mol . Pharm., Albert Einstein Col., Bronx, NY; 2) Dept. Life Sciences, New York Institute of Technology, Wesstbury,NY.

Mammals express up to four diacylglycerol (DAG)/Ca^{2+} activated protein kinase C (PKC) isoforms in a broad range of tissues. However, functions and regulation of mammalian DAG/Ca^{2+}-controlled PKCs in normal, differentiated cells are poorly understood. Consequently, we are studying in vivo regulation, substrate-effectors and physiological functions of *C. elegans* PKC-2, a prototypical (DAG)/Ca^{2+}-activated PKC, to gain novel insights into the properties and biological significance of this family of highly conserved regulatory enzymes. PKC-2, the only DAG/Ca^{2+}-regulated PKC in *C. elegans*, is expressed in the AFD thermosensory neuron and other neurons that constitute the circuitry required for thermotaxis. Animals lacking PKC-2 (*pkc-2(ok328)* null) lose the ability to detect a previously-established cultivation temperature (athermotactic phenotype); they migrate randomly when placed in a temperature gradient. Elevated expression of PKC-2 also disrupts normal thermotaxis and elicits a cryophilic phenotype. Epistasis analysis placed PKC-2 downstream from a cGMP gated Ca^{2+} channel TAX-4/TAX-2. Channel mutants are athermotactic. Incubation with TPA, a DAG mimetic, elicited cryophilic behavior in WT worms and TAX-2 defective mutants (*tax-2(p671)*. Athermotactic behavior was not altered when PKC-2 depleted animals were exposed to TPA. The results suggest that cGMP, TAX-2/TAX-4, Ca^{2+} and PKC-2 are successive components in a signaling cascade that governs thermotaxis. Phosphorylation of PKC substrates is markedly reduced in *tax-2, tax-4 and pkc-2* mutants. Proteins from *pkc-2(ok328)* null worms and transgenic C. elegans expressing PKC-2 at 40X the WT level *(pkc-2(40x)*) were analyzed by 2-D difference gel electrophoresis. The mobility of several proteins shifted markedly, indicating alterations in phosphorylation or other post-translational modifications. A 25 kDa protein carried increased negative charge (consistent with increased phosphorylation) in *pkc-2(40x)* worms. Mass spectrometry revealed that the protein is PRDX-2, a 2-Cys peroxiredoxin that catalyzes peroxide reduction in the presence of thioredoxin, thioredoxin reductase and NADPH. 2-Cys PRDX's detoxify reactive oxygen species and diminish peroxide generated by metabolism and actions of growth factors and cytokines. A PRDX-2 deletion mutant, *prdx-2(gk169)*, was athermotactic and suppressed the cryophilic phenotype of *pkc-2(40x)* animals. This suppressor of elevated PKC-2 activity could be a substrate. Purified recombinant PRDX-2 was phosphorylated to high stoichiometry by PKC *in vitro*. Phosphorylated Ser/Thr in PRDX-2 will be identified; expression of phospho-mimetic and non-phosphorylatable PRDX-2 mutants in vivo will reveal their physiological relevance.

622A

Notch signaling plays a pivotal role in chemosensation, quiescence and osmotic stress adaptation. Michael Y. Chao[1], Mark Corkins[2], Gerard Somers[2], Jonah Larkins-Ford[2], Tim Tucey[2], Ed Anderson[2], Hidetoshi Komatsu[2], **Anne C. Hart[2]**. 1) Dept of Biology, California State Univ. San Bernardino, 5500 University Pkwy, San Bernardino, CA 92407; 2) Mass. General Hosp Ctr for Cancer Research & Dept of Pathology, Harvard Med School, Charlestown, MA 02129.

The Notch signaling pathway is essential for development. Yet, few direct transcriptional targets of Notch receptors have been described beyond the basic HLH transcription factors. In *C. elegans, lst-1* is a novel target gene which may play a role in Notch pathway inhibition of MAP kinase signaling (WBPaper00006348). Here, we focus on the non-developmental roles of Notch and on direct targets in neurons.

We identified *osm-11* as a gene is required for octanol avoidance. OSM-11 is diffusible Notch co-ligand that works with DSL ligands to activate LIN-12 Notch during development (WBPaper00032102). We find that loss of *osm-11*, *lst-1*, or other Notch pathway genes prevents octanol avoidance. The *C. elegans* Notch receptors, LIN-12 and GLP-1, act redundantly in non-overlapping subsets of neurons in adult animals in octanol avoidance. Previous studies established that *osm-11* loss results in inappropriate physiological adaptation to environmental osmotic stress (WBPaper00028499, WBPaper00028383). We also find that chronic osmotic stress results in adaptive behavioral changes including octanol response defects. Our results suggest that, although Notch signaling may regulate both behavioral and physiological osmotic stress response, these responses are genetically separable. In the simplest scenario, OSM-11 acts as a diffusible regulator of Notch receptors that act in distinct tissues to regulate behavioral and physiological osmotic stress response.

Over-expression of OSM-11 causes inappropriate quiescence reminiscent of the quiescence observed in satiety and molting. This quiescence is dependent on the LIN-12 and GLP-1 Notch receptors and on genes previously implicated in quiescence including the cGMP dependent kinase *egl-4*. (WBPaper00031383 & WBPaper00031030). A previous analysis found numerous consensus binding sites for LAG-1, a Notch pathway transcription factor in *egl-4* regulatory sequences (WBPaper00006348). *egl-4* may be a direct target of Notch in neurons. Combined with published data, our results thus far suggest that diminished Notch signaling results in physiological and behavioral adaptation to osmotic stress, while increased Notch signaling induces quiescence. The Notch pathway may play a role in stress response, quiescence, and chemosensation across animal species.

623B

The BAG neurons respond to temperature, and are necessary for cryophilic behavior. **Matthew H. Beverly**, Piali Sengupta. Department of Biology and National Center for Behavioral Genomics , Brandeis University, Waltham, MA.

C. elegans exhibits complex behavioral responses to thermal stimuli. When placed on a spatial thermal gradient at temperatures above its cultivation temperature (T_c), worms will move down the gradient towards colder temperatures (cryophilic behavior), whereas at temperatures below the T_c, worms are atactic. At temperatures around the T_c, worms track isotherms. The underlying circuit required for these behaviors includes the AFD sensory neurons, as well as the AIY and AIZ interneurons. A current model of circuit function in thermosensory navigation behavior suggests that there is an underlying default cryophilic drive regulated by the AIZ interneurons and as yet unknown sensory neurons. Exhibition of the cryophilic drive is restricted to temperatures above the T_c via activity of the AFD and AIY neurons, which are also essential for isothermal tracking behavior. The goal of my project is to identify additional components of the thermosensory circuit, and in particular, to identify the sensory neuron(s) mediating cryophilic behavior.

We found that animals with genetically ablated BAG neurons exhibit strong defects in cryophilic behavior. Although the BAG neurons are not directly presynaptic to the AIZ interneurons required for cryophilic behavior, this neuron type is presynaptic to the RIR and RIG interneurons which in turn synapse directly onto AIZ. The BAG neurons have previously been implicated in oxygen and carbon dioxide sensation; however, our experiments suggest that temperature responses are independent of gas sensation in this neuron type. BAG expresses known genes involved in sensory pathways including the *tax-2/4* cyclic nucleotide gated ion channels as well as the soluble guanylyl cyclases *gcy-31* and *-33*, *gcy-31* and *-33* mutants also show defects in cryophilic behavior on a thermal gradient. Preliminary calcium imaging data indicate that BAG responds to both small increases and decreases in temperature (0.2ºC) above and below the animal's cultivation temperature. I am further characterizing the responses of BAG to temperature by examining changes in intracellular calcium and cGMP levels using genetically encoded sensors. I will also determine whether communication between the AFD and BAG neurons play a role in mediating temperature responses, and identify additional BAG-expressed genes required for thermosensory signal transduction.

624C

Integrin Signaling is Required for Mechanosensation in the Touch Receptor Neurons. **Xiaoyin Chen**, Martin Chalfie. Biological Sciences, Columbia University, New York, NY.

The touch receptor neurons are attached to the body wall through components that resemble the dense bodies of the muscles, and many dense body proteins are expressed in both types of cells. These proteins, many of which are associated with integrin signaling may be ultimately linked with extracellular matrix proteins, which are required for touch sensitivity. The role of the integrin signaling proteins in the touch receptor neurons is unknown, however, because elimination of the genes for these proteins usually results in a *pat* phenotype (paralyzed at two fold stage). Using a strain with enhanced RNAi in neurons and reduced or absent RNAi in non-neuronal tissues, we tested for effects of the loss of these genes on touch sensitivity. RNAi for genes encoding components of the dense bodies in body wall muscles (*pat-2* encoding an α-integrin, *pat-4* encoding integrin-linked kinase, *pat-6* encoding actopaxin, *unc-97* encoding PINCH, and *unc-112* encoding a Mig-2-like gene) reduced the response of the animals to gentle touch. In accordance with the RNAi result, *pat-2* and *unc-112* promoter GFP fusions showed expression in the touch receptor neurons, while *pat-4*, *pat-6* and *unc-97* have previously been reported to be expressed in these cells. Interestingly, RNAi for *unc-97* also altered the number and distribution of MEC-2 puncta in the touch receptor neurons, a phenotype we also observed for the weak allele *unc-97(su110)*. These results suggest that integrin signaling may have roles both in the development and organization of the transduction channels in the touch receptor neurons and in their function. We are currently characterizing the roles that these components and other components of integrin signaling play in touch receptor neuron function.

625A

MEC-5 collagen expressed from muscle may link the extracellular matrix to the mechanotransduction channel complex responsible for gentle touch. **Brian Coblitz**, Irini Topalidou, Martin Chalfie. Biological Sciences, Columbia University, New York, NY.

Previous genetic screening identified components of a mechanotransduction channel complex responsible for the gentle touch sensation by touch receptor neurons (TRNs). Expressed in TRNs, such components include pore-forming subunits (MEC-4 and MEC-10) and accessory membrane-localized subunits (MEC-2 and MEC-6). Genetics also identified extracellular matrix (ECM) proteins (MEC-1, MEC-5, MEC-9) necessary for touch sensation. These matrix proteins appear to organize the channel complex into discrete puncta along the TRN processes. While TRNs express *mec-1* and *mec-9*, the location of *mec-5* expression has been unclear. Contradictory evidence comes from a previous study where *lacZ* and GFP promoter (1.3kb) fusions were expressed in hypodermis, and our unpublished microarray result demonstrating an 8.25 fold enrichment of *mec-5* transcript in TRNs. We then observed that a new YFP promoter fusion with the full 4.7kb upstream region demonstrated body wall muscle expression. To determine what cells normally express *mec-5*, we used two approaches. First, we expressed translational fusions under control of hypodermal (P_{dpy-5}), TRN (P_{mec-3}), and muscle (P_{myo-3}) promoters to check for proper localization. Only P_{myo-3}*mec-5::YFP* was able to reproduce the pattern of P_{mec-5}*mec-5::YFP*. Surprisingly, P_{mec-3}*mec-5::YFP* only localized to a small region in the TRNs, perhaps indicating degradation as a result of abnormal processing. Our second approach used the same hypodermal, TRN, and muscle promoters to express untagged *mec-5* to rescue touch sensation in *mec-5* animals. Only *mec-5* expression in muscle was sufficient to restore touch sensation.

Previous studies indicated strong genetic interactions of *mec-5* with *mec-6* and *mec-9*. These genetic interactions may suggest physical interactions between the encoded proteins. To investigate potential binding we transiently cotransfected the genes of interest in HEK293T cells and performed coimmunoprecipitation followed by western blot. MEC-5 bound to MEC-6. Conversely, no interactions were identified between MEC-5 and MEC-9, nor between MEC-6 and MEC-9. Further demonstrating specificity, MEC-5 did not bind to MEC-4. As a collagen, MEC-5 likely contributes structural rigidity to the ECM, which translates the mechanical forces of touch from the hypodermis to the TRNs. If MEC-5 binds MEC-6 in vivo, this could be a critical junction between mechanical forces applied to the ECM and the mechanotransduction channel complex.

626B

The PHB-domain protein STO-1 is required for sensitive chemotaxis to diacetyl. **John E. Kratz**, Martin Chalfie. Dept. Biological Sciences, Columbia University, New York, NY.

The Prohibitin homology domain is found in a large number (currently 2700 in the SMART database) of membrane proteins spanning all taxa. A subset of these proteins embed in the membrane via a non-spanning hydrophobic 'hook' and are primarily neuronal. For example, three of the four such mammalian proteins (Stomatin, Podocin, and Stomatin-Like Proteins 1 and 3) are expressed in neurons (Podocin is in the kidney). All ten Stomatin-like proteins in *C. elegans* are expressed in subsets of neurons. Mutations in three of the worm genes (*mec-2*, *unc-1* and *unc-24*) lead to relatively well-characterized sensory and behavioral defects. MEC-2 and UNC-24 are components of the mechanotransduction channel that detects gentle body touch. Deletion alleles of the remaining six worm Stomatin-like genes (*sto-1-6*) are superficially wild type, perhaps because they are redundant or exert subtle effects.

We are characterizing the expression of the *C. elegans* genes using transcriptional and translational GFP fusions. The *sto-1* promoter drove expression in ADL, ASH and PHA, but the translational construct was more widely expressed; we saw additional expression in the lining of the gut, PHB and more amphid neurons. In the head and tail, STO-1::GFP is most strongly localized to the amphid and phasmid sensory cilia. This suggested that STO-1 could have a role in chemosensation. We tested *sto-1(tm1503)* animals for defects in chemotaxis and found diminished chemotaxis to low to moderate concentrations of the volatile attractant diacetyl. Mutant animals behaved similarly to wild type at high concentrations of attractant, so STO-1 is only needed for high sensitivity perception of diacetyl. This defect is not general to all volatile odorants, because chemotaxis to benzaldehyde and isoamyl alcohol, is unaffected.

627C

Analysis of GPCR that is specifically expressed in thermosensory neurons. **Hiroyuki Sasakura**, Keita Suzuki, Hiroko Itoh, Ikue Mori. Group of Molecular Neurobiology, Nagoya University, Japan.

C. elegans senses the environmental temperature by AFD and AWC sensory neurons. Genetic analysis suggested that signal transduction of temperature in AFD and AWC is similar to that of olfactory and visual system in *C. elegans* and other animals (Mori et al., 2007). Although thermoreceptor itself is not identified, it is plausible that GPCRs sense the environmental temperature based on analogy to olfactory and visual system. It has been reported that *srtx-1* encodes GPCR and expressed in sensory ending of both AFD and AWC (Colosimo et al., 2004, Biron et al., 2008). To examine the more detail of *srtx-1* gene expression, we analyzed the promoter::GFP fusion gene. We newly found that *srtx-1p::GFP* was expressed strongly expressed in AFD and very weakly only in AWC OFF neuron. To examine *srtx-1* gene function, we isolated two deletion mutants, *nj62* and *nj63*. From deletion sites, both alleles are likely to be null. When they are cultivated in 20 or 23 degree, they migrated to the lower temperature than wild type. On the other hand, when they are cultivated at 17 degree, they migrated to the higher temperature than wild type. These results suggest that *srtx-1* mutants can not response to the wide range of temperature from 17 to 23 degree, but only sense the smaller range of temperature.Next, we asked the cellular site of action. We expressed *strx-1cDNA* under the AFD or AWC specific promoter in *nj62* mutants and examined the thermotaxis after the cultivation at 23 degree. The expression in AFD almost completely restored cryophilic phenotype of *nj62* mutants, however the expression in AWC did not. These results suggest that STRX-1/GPCR function in AFD, not in AWC OFF is sufficient to thermotaxis at 23 degree, consistent with the expression pattern that the strong expression in AFD and the faint in AWC OFF. We are now undergoing the ectopic and overexpression experiments to further know the function of STRX-1/GPCR. The starvation is known to affect the thermotaxis (Hedgecook and Russell, 1975; Mori and Ohshima, 1995). When animals are cultivated at 23 degree with no food, wild type cancel the migration to 23 degree and instead migrate to both higher and lower temperature region than 23 degree. We found that *nj62* mutants always migrate only to lower temperature region after starvation at 23 degree. These results suggest that thermal preference to low temperature in *nj62* is crucial for thermotaxis after starvation. In sum, STRX-1/GPCR is key player for proper migration to cultivation temperature both fed and starved conditions.

We thank T. Ishihara for *nhr-38:(H13)*promoter, H. Oliver for *ceh-36* partial promoter, H. Inada for TMV-UV library.

628A

Right Way to Have Sex. **Bilge Birsoy**, Joanna C. Downes, Shin Sik Choi, Tim Bloss, Joel H. Rothman. Dept MCDB, NRI, Univ California, Santa Barbara, Santa Barbara, CA 93106.

While the mechanism that breaks left-right (L-R) anatomical asymmetry (handedness) has been described in *C. elegans*, we have found that at least one additional mechanism must exist to establish other handedness cues that generates L-R differences in the deployment of alternative cell death pathways (see abstract from our lab by Choi et al.). To assess whether such a system might affect a behavior in the worm, we investigated whether male mating behavior exhibits L-R handedness. Upon recognizing a hermaphrodite, males initiate backward locomotion and continuously scan along the hermaphrodite's body. When their tails reach either the head or tail of the hermaphrodite, males turn to maintain contact and then continue backward locomotion on the opposite side of the hermaphrodite. We found that this turning behavior shows a distinct right-handed bias: when individual males were allowed to mate with *lin-2(e1309)* vulvaless hermaphrodites, >70% of the turns when made over the hermaphrodites' body (away from the agar surface) and >55% of turns when made under the hermaphrodites' body (toward the agar surface) are right-handed. To determine whether this bias in turning direction correlated with L-R asymmetry in the male mating structure, which results from stochastic EGL-1-dependent loss of sensory rays (Choi et al. abstract), we examined the turning bias in *egl-1(n1084n3082)* mutants. We were surprised to find that, although wild-type males lack rays more frequently on the right, the right-hand turning bias is actually increased in the *egl-1* mutant, in which no rays are lost and therefore rays are always symmetrically arranged. Thus, the intrinsic turning bias is even more apparent in males with symmetric mating structures. To assess whether this L-R behavioral bias is dependent on anatomical handedness, we analyzed *gpa-16(it143)* mutants in which the L-R asymmetry of the internal organs is reversed as a result of the reversal in the early embryonic symmetry break. Males with reversed anatomical asymmetry showed a virtually identical right hand bias in turning, demonstrating that a handedness-determining system that is independent of the previously described L-R symmetry breaking event must control this behavior. These findings raise the possibility that, analogous to brain laterality in humans, functionally L-R asymmetry in the *C. elegans* motor nervous system may be independent of anatomical handedness.

629B

Worm Tracker 2.0: Generating a phenome database. **Victoria Butler**[1], Eviatar Yemini[1], Tadas Jucikas[1], Chris Cronin[2], Paul Sternberg[2], William Schafer[1]. 1) Cell Biology, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom; 2) HHMI and Division of Biology, CalTech, Pasadena, CA.

The nematode Caenorhabditis elegans is widely used for the genetic analysis of nervous system function. This analysis relies on the precise description of behavioural phenotypes but standard methods for classifying the behavioural patterns of mutants are qualitative and imprecise. Together with the Sternberg lab, we have developed the Worm Tracker 2.0 system; a low cost, feature-rich single worm tracker that allows the rapid and consistent quantification of the effects of mutations on behaviour. This system allows us to obtain measurements of a wide range of morphological and behavioural features, including velocity, flex, bending frequency, track amplitude and track wavelength, and we will present data collected from crawling and swimming assays. A standardized phenotyping system makes it possible to compare behavioural data collected by different researchers in different labs and we are using this system to start the generation of a comprehensive C. elegans phenotypic database that could be used to explore the clustering and relative similarities of mutant phenotypes.

630C

Concentration dependent differential activity of signalling molecules in *Caenorhabditis elegans*. **Fatma Kaplan**[1], Hans Alborn[1], Jagan Srinivasan[2], Ramadan Ajredini[3], Omer Durak[2], Parag Mahanti[4], Frank Schroeder[4], Paul Sternberg[2], Peter Teal[1], Arthur Edison[3]. 1) USDA-ARS CMAVE, Gainesville, FL; 2) California Institute of Technology, Pasadena, CA; 3) University of Florida, Gainesville, FL; 4) Boyce Thompson Institute and Cornell University, Ithaca, NY.

Caenorhabditis elegans employs specific glycosides of the dideoxysugar ascarylose (the 'ascarosides') for monitoring population density/ dauer formation and finding mates. A synergistic blend of three ascarosides, called ascr#2, ascr#3 and ascr#4 acts as a dauer pheromone at a high concentration (nM-μM) but the same ascarosides attracts males at a lower concentration (pM) and thus functions as a mating signal (1). The ascaroside blends produced by adult and young adult worms were similar but differed significantly from that of the larval stages. Furthermore, all four larval stages (L1-L4) produce similar ascaroside mixtures. We also found that the absolute amounts of ascarosides produced and their relative ratios depended on whether nematode secretions were collected in the presence or absence of bacteria. Possible explanations for the observed differences will be discussed. References 1. Srinivasan J, et al. (2008) A blend of small molecules regulates both mating and development in. Caenorhabditis elegans. Nature 454:1115-1118.

631A

A role for the germline in the regulation of hermaphrodite quiescence? **David M. Raizen**. Dept Neurology and Ctr Sleep, Univ Pennsylvania, Philadelphia, PA.

ad450, a gain of function mutant of the cGMP dependent kinase gene *egl-4*, stops movement as an adult and therefore forms few tracks on a lawn of bacteria. With the hope of identifying down stream signaling components of EGL-4/PKG, I screened 1200 F2 progeny of mutagenized *egl-4(ad450)* worms for mutants that form increased tracks on a lawn of bacteria. Surprisingly, most of the mutants I found, 24 mutants of a total of 32 isolated, were sterile. To test the possibility that there is an effect of the germline on behavioral quiescence, I compared behavior of *glp-4(ts); egl-4(ad450)* double mutants and of *glp-4(ts)* single mutants raised at the restrictive temperature to the same strains grown at the permissive temperature. Worms that lacked the germline due to the *glp-4* mutation formed significantly more tracks than control worms of the same genotype, suggesting a role for the germline in the regulation of hermaphrodite behavioral quiescence. I will report the results of analyzing other *ad450* double mutants. I will also reports the consequence of laser ablation of the germline precursors.

632B

Neurophysiological alterations and oxidative stress induced by Cypermethrin in Caenorhabditis elegans and its amelioration by Alpha- Tocopherol (Vitamin-E). **Shashikumar Shivaiah**, Padbhanabhan Sharda Rajini. Food protectants and infestation control, Central food technological research institute, Mysore, Karnataka, India.

Synthetic pyrethroids (SP), are derived from pyrethrins are being used as insecticides and potently toxic to insect species,offer the advantage of low mammalian toxicity. Extensive use of SP raises concerns of toxicity to non-target organisms. SP produce symptoms viz: behavioral changes, tremors and hyperexcitation in animals. SP mainly act on nervous system by delaying closing of voltage dependent sodium channels. Their secondary effects involve oxidative stress leading to macromolecular damages. Reports are available on the toxicological effects of SP in rats, mice, Drosophila melanogaster and other model organisms. There is paucity of data on effects of SP on Caenorhabditis elegans, which is rapidly gaining popularity as a model for toxicological evaluations. In this study, we investigated the effects of cypermethrin (CYP) a potent type-Π SP on physiological endpoints (brood size, egg laying and feeding), indices of neurotoxicity (acetylcholinesterase and acetyl choline) and markers of oxidative imbalances in C. elegans. Exposure of worms to maximum soluble concentration of CYP (20mM) failed to induce mortality in worms. Hence experiments were conducted with sublethal concentrations of CYP (1, 5, and 15mM). Exposure of worms to the above concentrations of CYP for 4h was associated with concentration-dependent decrease in brood size (18-54%), feeding rate (28-54%) and egg laying (55-67%) increase in the activities of acetylcholinesterase (9-59%) & carboxylesterase (26-86%) & acetylcholine (25-47%) levels. Alterations in oxidative balance as a consequence of CYP treatment were evident as elevated levels of ROS (20-56%). Exposure to CYP initiated at the egg stage and continued for 24, 36, 48 and 72h produced qualitative changes in fatty acid profile of worms and decrease in body length. Further studies were conducted with α-tocopherol to explore the possibility of ameliorating oxidative imbalances caused by CYP. Co-treatment of worms with α-tocopherol abrogated CYP-induced oxidative imbalance as reflected by lower levels of ROS (30 vs 46%) and protein carbonyls (14 vs 28%) compared to worms exposed to CYP alone. α-tocopherol also protected worms from CYP-induced decrease in reduced glutathione and alterations in the activities of antioxidant enzymes. In conclusion, our data suggest that at sublethal concentration CYP induces neurophysiolical alterations and oxidative stress, the latter of which was amenable for amelioration by α-tocopherol. Hence C. elegans could be reliably employed for understanding the role of oxidative stress in toxic outcomes of xenobiotics.

633C

Genetics of food choice behavior in *Caenorhabditis elegans*. **Hyun Sung**[1], Emad A Abada[2], Meenakshi Dwivedi[1], Joon-Hyung Chang[1], Joohong Ahnn[1]. 1) Department of Life Science, Hanyang University, Seoul, South Korea; 2) Botany and Microbiology Department, Faculty of Science, Helwan University, Cairo, Egypt.

Caenorhabditis elegans is a free living soil nematode and thus in its natural habitat, *C. elegans* interacts with many different soil bacteria. Some of the natural bacterial food may be good food sources, while some of them may be pathogenic for worms. Thus, we undertook a study to locate some soil bacteria as a natural food source for worms in comparison to laboratory bacteria *E. coli*. We also suggested the possible sensory neurons by which *C. elegans* senses and responds to the food. In this study, the food preference by binary choice assay was determined for the wild type worms and mutants defective in chemosensory receptors genes. The bacterial strains preferred (choice shown for test bacteria) by wild type worms were then scored for pharyngeal pumping and the resultant lifespan was recorded. Our results suggested that the odorant receptor mutants are defective in preference assay compared to wild type *C. elegans*. We conclude that the odorant receptor genes are involved in the bacterial choice preference in the habitat of *C. elegans*.

634A

Arousal regulation by sensory stimulation and neuromodulators in adult *C. elegans*. **Yoshinori Tanizawa**, William Schafer. Cell Biology Division, MRC Labolatory of Molecular Biology, Cambridge, United Kingdom.

Changing behavioral strategy according to environmental and internal context is essential for animals to survive. *C. elegans* can respond to a wide variety of sensory cues, and modify their behavior from their experience. Another parameter influencing behavior is arousal, which is defined as a state of increased motor activity, enhanced sensory responsiveness and emotional changeability in the case of higher animals. In *C. elegans*, the best characterized example of arousal regulation is lethargus, a sleep-like state which accompanies every moult during development. In this project, we characterize a new aspect of arousal regulation, evoked by sensory stimulation, in adult *C. elegans*.

To characterize arousal regulation by external cues, we applied sensory stimulation to adult animals and observed their behavior. We are currently using two types of stimuli in different modalities: tapping the agar plate using a mechanical tapper, and artificial activation of ASH nociceptive neurons by specifically-expressed channelrhodopsin-2, a light-gated cation channel. Both of these stimuli induced transient upregulation of motor activity lasting for one to two minutes depending on the stimulus strength. They also caused crossmodal sensitization, in which one stimulus enhanced behavioral response to another stimulus presented later. Arousal is likely to be affected and regulated by neuromodulators such as monoamines and neuropeptides. We examined the available mutants for these molecules, and found that signaling through tyramine and a neuropeptide receptor candidate (ZC412.1) inhibits and promotes arousal change caused by sensory stimulation, respectively. We are now in search for the signaling partners for these molecules by candidate gene approach and ligand screening in heterologous expression system (collaboration with Peter Evans group in Babraham institute, Cambridge). These results suggest that arousal is regulated by sensory cues and neuromodulators in adult animals, and prove that *C. elegans* is a good model to study arousal regulation in its simplest form.

635B

Cocaine modulates locomotion behavior in *C. elegans*. **Alex Ward**[1,3], Vyvyca Jones[4], Zhaoyang Feng[1,5], X. Z. Shawn Xu[1,2,3,4]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI; 3) Neuroscience Graduate Program, University of Michigan, Ann Arbor, MI; 4) Program in Biomedical Sciences, University of Michigan, Ann Arbor, Michigan; 5) Department of Pharmacology, Case Western Reserve University, Cleveland, OH.

Cocaine, a potent addictive substance, is an inhibitor of monoamine transporters, including DAT (dopamine transporter), SERT (serotonin transporter) and NET (norepinephrine transporter). Cocaine administration induces complex behavioral alterations in mammals, but the underlying mechanisms are not well understood. Here, we tested the effect of cocaine on *C. elegans* behavior. We show for the first time that acute cocaine treatment evokes changes in *C. elegans* locomotor activity. Interestingly, the neurotransmitter serotonin, rather than dopamine, is required for cocaine response in *C. elegans*. The *C. elegans* SERT MOD-5 is essential for the effect of cocaine, consistent with the role of cocaine in targeting monoamine transporters. We further show that the behavioral response to cocaine is primarily mediated by the ionotropic serotonin receptor MOD-1. Our data reveal a critical role for serotonin in cocaine sensitivity in *C. elegans*, suggesting that cocaine impinges on serotonin signaling in worms.

636C

cdk-1(ne2257) suppressor screen and the developmental function of CDK-1/CYB-3 complex in polarity generation during early embryogenesis. **Takao Ishidate**[1], Soyoung Kim[1], Masaki Shirayama[1], Rita Sharma[1,2], Craig Mello[1,2]. 1) Program in Molec Med, Univ Massachusetts, Worcester, MA; 2) Howard Hughes Medical Institute.

We have isolated a rare temperature-sensitive allele of *cdk-1*(ne2257) that does not affect cell cycle progression but instead causes very specific developmental defects that appear to stem from a failure to degrade the CCCH-type zinc finger proteins OMA-1 and OMA-2 prior to the onset of the first mitosis. Persistence of the OMA-1/2 proteins leads to defects in polarity generation and in the expression of other developmental regulators. OMA-1/2 degradation depends on direct phosphorylations by a set of conserved kinases, including MBK-2, KIN-19 and GSK-3. However, although *cdk-1* and cyclin B3 lesions both lead to a dramatic stabilization of OMA-1/2 proteins, their role in promoting OMA-1/2 degradation is not clear since unlike the other kinases, CDK-1/CYB-3 does not phosphorylate the OMA-1/2 proteins directly. In order to study the developmental function of the CDK-1/CYB-3 complex in generating embryonic polarity, we have undertaken a suppressor screen, looking for mutants that rescue *cdk-1*(ne2257) embryonic lethality at restrictive temperature. From this powerful screen we have isolated multiple rare suppressing mutations including reverting mutations in *cdk-1* that restore the altered amino acid to wild-type, compensatory mutations in *cyb-3* that accommodate the altered *cdk-1*-mutant interface, as well apparently three novel suppressors that map to LGI (2 alleles), LGII (6 alleles) and LGIV (1 allele). As expected, the *cdk-1* and *cyb-3* suppressor mutations rescue the OMA-1 degradation defect seen in *cdk-1*(ne2257). However, in contrast the novel suppressors on LGI, II and IV all continue to exhibit persistence of OMA-1, suggesting that they function downstream of the OMA proteins. We are currently attempting to clone these suppressors using a deep sequencing approach. Characterizing these suppressors will likely shed light on the role of the cell cycle machinery in coupling the onset of mitosis to polarity generation during C.elegans early embryogenesis.

637A

The PAM-1 aminopeptidase regulates microtubule dynamics during meiosis and polarity establishment in the early *C. elegans* embryo. Pauline Greene[1], Sara Marshall[1], Lauren Brady[1], Christopher Reeves[1], Darren Brooks[2], Elwyn Isaac[3], **Rebecca Lyczak**[1]. 1) Biology Dept, Ursinus Col, Collegeville, PA; 2) Biomedical Sciences Research Institute, University of Salford, Salford, UK; 3) University of Leeds, Leeds, UK.

The PAM-1 aminopeptidase is required for numerous processes in the early embryo including meiotic exit, axis polarization, and chromosome segregation. As these developmental processes require distinct microtubule movements, we sought to examine the role of PAM-1 in microtubule dynamics. In localization studies we found that PAM-1 is largely cytoplasmic; however specific localization during meiosis and early polarity establishment was also observed around microtubules. During meiosis in *pam-1* mutants, we have found that the meiotic spindle wanders along and away from the cortex during meiosis II much more than in wild-type embryos. Additionally, during polarity establishment, the centrosomes move dynamically and prematurely away from the posterior cortex. We hypothesized that this centrosome movement may cause the polarity defect in *pam-1* mutants. To test this, we inactivated the microtubule motor dynein, DHC-1, and its regulator LIS-1, in an attempt to prevent centrosome movement from the cortex and restore anterior-posterior polarity. This was indeed what we observed, with *pam-1; dhc-1*(RNAi) embryos exhibiting normal pseudocleavage and localization of the P granules and PAR proteins, signs of polarity absent in *pam-1* mutants alone. We conclude that DHC-1 and LIS-1 are required for the abnormal centrosome movements in *pam-1* embryos. Additionally, we show that PAM-1's role in axis polarization is to prevent premature movement of the centrosome from the posterior cortex, ensuring proper axis establishment in the embryo. This coupled with the defects in meiotic spindle movement implicate PAM-1 in numerous microtubule dependent processes in the early embryo.

638B

Wnt dependent and independent cell polarization during asymmetric divisions in *C. elegans*. **Yuko Yamamoto**[1,2], Hisako Takeshita[1], Hitoshi Sawa[1,2]. 1) Dept Cell Fate Decision, CDB, RIKEN, Kobe, Japan; 2) Dept of Biol., Grad. Sci., Kobe University.

Various signal molecules function in cell-cell communication. In many cases, a single cell receives multiple signal molecules that belong to the same family such as Wnt family. It is important to understand how cells can properly respond to multiple signals. During development of *C. elegans*, a Wnt pathway called Wnt β-catenin asymmetry pathway controls polarities of most cells undergoing asymmetric cell divisions. But it is not well understood how five *wnt* (l*in-44, cwn-1, egl-20, cwn-2* and *mom-2*) genes in *C. elegans* genome regulate polarities of many cells. In these cells, the Wnt pathway components, POP-1/TCF and WRM-1/β-catenin localize asymmetrically during cell divisions. Despite the functions of these components in most cell divisions, only a small number of cells have been reported to be regulated by Wnt proteins. For example, Wnt proteins regulating divisions of the somatic gonadal precursors that produce distal tip cells (DTCs) have not been identified, even though these divisions are regulated by the Wnt signaling components including Frizzled receptors. These observations suggest that cells can be polarized either in the absence of Wnts or by multiple Wnts that function redundantly. To examine these possibilities, we analyzed strains (quintuple *wnt* mutants) with mutations in all *wnt* genes. We found that DTCs were normally produced in quintuple *wnt* mutants, indicating that cells can be polarized in a Wnt independent manner. In contrast, polarities of all seam cells (epidermal stem cells) were defective in triple *wnt* mutants, indicating three Wnts coordinately regulate polarity of these cells. Now we are investigating where three Wnts are expressed and how three Wnts regulate all seam cells.

639C

Segmenting Early Embryogenesis in *Caenorhabditis elegans*. **Kurt Warnhoff**[1], Alexandra Wehrman[2], Scott Thatcher[2], Timothy Walston[1]. 1) Biology, Truman State University, Kirksville, MO; 2) Mathematics and Computer Science, Truman State University, Kirksville, MO.

An understanding of the forces acting on cells in the early embryo can provide important information for how cells interact to determine their shapes, movements and fates. These forces can be physical and genetic, as well as intracellular or extracellular. The early *Caenorhabditis elegans* embryo provides an excellent environment to explore the forces acting during embryogenesis and to develop techniques and models that can be applied later to more advanced biological events. We report the continued development of a four-dimensional GGH (Glazier-Granier-Hogeweg) model to simulate the four-cell stage of embryogenesis. In addition, a semi-automated procedure for the segmentation of four-dimensional differential interference contrast (DIC) microscopic data has been developed. A precise and accurate segmentation scheme offers many opportunities for the quantitative analysis of early embryogenesis and other cellular systems. Our segmentation technique incorporates a combination of level set and watershed methods to properly delineate cells of the embryo in DIC micrographs. Segmented data serves as an input into the current GGH code and data from many embryos are needed as a source of calibration and validation for the model. This segmentation technique for DIC images of the *C. elegans* embryo should also be applicable to DIC images of other embryos or biological samples.

640A

Post-transcriptional regulation of early development by multiple RNA-binding proteins. **Brian M Farley**, Sean P Ryder. Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA.

Early development is governed in part by a post-transcriptional regulatory network that acts through the 3' untranslated regions (3' UTR) of targeted transcripts. This network requires RNA-binding proteins with weak sequence specificity, confounding attempts to identify regulatory targets. To date, few direct regulatory targets of these proteins have been identified. *glp-1*, which encodes a Notch-like receptor critical for development, is directly regulated by at least two RNA-binding proteins, POS-1 (1) and GLD-1 (2). Both are co-expressed in the posterior of the early embryo, while *glp-1* mRNA is present throughout the early embryo. Embryos lacking either protein exhibit ectopic GLP-1 expression (1,2). Previously, we determined the sequence specificity of both POS-1 (3) and GLD-1 (4) and predicted binding sites for each protein in 3' UTRs. The region of the *glp-1* 3' UTR required for translational repression in embryos contains a GLD-1 binding site flanked by adjacent POS-1 binding sites. The close proximity of these sites and the similar GLP-1 expression patterns in embryos lacking POS-1 or GLD-1 suggest that both proteins co-repress *glp-1* translation.

Using *in vitro* methods, we show that POS-1 does not cooperate with GLD-1, but instead antagonizes GLD-1 binding to the *glp-1* 3' UTR. In addition, we show that POS-1 is homodimeric, suggesting that specific recognition of *glp-1* mRNA by POS-1 is driven by self-association. Other mRNA-protein complexes may also play a role in regulating *glp-1*, as the *glp-1* 3' UTR contains two MEX-3 binding sites that surround the POS-1 and GLD-1 sites. This cluster of sites is conserved across nematode species, and is essential for proper *glp-1* expression (2). Thus, we hypothesize that conserved clusters of binding sites provide the basis for specific mRNA recognition. To identify other clusters of conserved binding sites, we have built a genome-wide database of predicted RNA-binding protein sites, 3' UTRs, and conservation scores. Well-conserved binding sites tend to cluster in 3' UTRs and may be more predictive of regulatory elements than individual binding sites alone.

1. Ogura K, et al. Development 2003:2495-503
2. Marin VA, Evans TC. Development 2003:2623-32
3. Farley BM, et al. RNA 2008:2685-97
4. Ryder SP, et al. Nat Struct Mol Bio 2004:20-8.

641B

Regulation of male gonadal cell fates. **Andrea K. Kalis**, Mary B. Kroetz, Kathleen M. Larson, David Zarkower. Dept Genetics, Cell Biol, Dev, Univ Minnesota, Minneapolis, MN.

Male and hermaphrodite *C. elegans* gonads develop from apparently identical primordia, but are very different organs as a result of sex specific differences in axis formation, cell lineages, leader cell migrations and terminal differentiation. Axis formation in both sexes requires a Wnt pathway, and this is overlaid with sex-specific regulation involving the global sex determination pathway, acting through *tra-1*, and the organ-specific sexual regulator *fkh-6*. We performed a genome-wide RNAi screen using sex-specific gonadal GFP reporters as sensitive indicators of disrupted gonadogenesis in animals treated with RNAi as larvae. This screen identified genes required for gonadal differentiation in both sexes. Strikingly, although we identified a number of genes whose RNAi feminizes the male gonad, no genes were identified whose RNAi masculinizes the hermaphrodite gonad. This suggests that a shared developmental program is present in both sexes and must be modified in XO animals to permit male gonadal differentiation. Genes whose RNAi feminizes the male gonad include *fkh-6*, the Wnt pathway members *pop-1*, *lit-1*, *sys-1*, and *bar-1*, the cell-cycle regulators *cdk-1* and *cyb-3*, the matrix metalloprotease *gon-1*, and the Hox gene *egl-5*. For some of these genes early gonadal development and cell fate specification appeared normal but terminal differentiation was feminized, indicating that disruption of the mid-larval male gonadal program can cause adoption of a more hermaphrodite-like program. However, most knockdowns with male gonadal defects did not cause feminization. It is notable that during the L3 proliferative stage male gonadal cells undergo a number of asymmetric divisions while the hermaphrodite divisions are mostly symmetrical. The prevalence of Wnt pathway genes and cell cycle regulators among those causing feminization during this period suggests that disruption of cell division asymmetry can transform male cell fates to more female-like fates. *egl-5*, the Abdominal B homolog, is among several transcription factors identified in the screen, and gonads of *egl-5* mutant males are extensively feminized, consistent with previous phenotypic analysis by Andrew Chisholm (Development, 1991). A small regulatory element ~15kb upstream of the *egl-5* start codon is sufficient for male-specific gonadal expression of reporter genes and is regulated positively by *fkh-6* and negatively by *tra-1*. Ectopic expression of *egl-5* in hermaphrodites masculinizes gonadal tissue, indicating that EGL-5 plays an instructive role in male gonadal fate determination.

642C

The role of the posterior Hox genes, *php-3* and *nob-1*, in male tail tip morphogenesis. **Matthew D. Nelson**, David H. Fitch. Dept Biol, New York Univ, New York, NY.

In C. elegans, the posterior Hox genes *php-3* and *nob-1*, both abdominal-B (abd-B) homologs, play crucial patterning roles in both sexes during embryogenesis (Van Auken et al., 2000). An ongoing RNAi screen in our lab has also identified a specific role for these genes in patterning the development of the male tail tip during larval development.

The male tail tip undergoes an interesting morphogenesis when the tail tip cells fuse, detach from the overlying cuticle, change shape and move anteriorly (Nguyen et al., 1999). A null allele of *php-3 (ok919)* results in a failure of tail tip morphogenesis. Similar phenotypes result from a new allele, *ny7*, which changes a highly conserved aspartic acid to a tyrosine located within the homeodomain. A translational GFP fusion of PHP-3 constructed from the WT allele, but not from the *ny7* allele, partially rescues the tail tip phenotype of the *ok919* allele. This indicates that *ny7* is most likely a null. Both transgenes express variably in the tail tip and more broadly in the male tail. To test if PHP-3 functions cell autonomously to promote tail tip retraction we expressed *php-3* under the tail tip specific promoter from *lin-44*. We find that PHP-3 is required in all four tail tip cells.

Both *php-3* and *nob-1* are upstream of the transcription factor *dmd-3*, as they positively regulate appropriate *dmd-3* expression levels. A conserved abd-B consensus site is present in a 1.1 kb region of the promoter of *dmd-3*, termed the *E(ht)* enhancer. The *E(ht)* has previously been shown to be both sufficient and necessary for tail tip expression (Mason et al., 2008). We are currently investigating the possibility that PHP-3 and NOB-1 function by directly binding to these conserved domains within the *E(ht)* and enhance the expression of *dmd-3* during morphogenesis.

Mason, D. A., Rabinowitz, J. S. and Portman, D. S. (2008). *dmd-3*, a doublesex-related gene regulated by *tra-1*, governs sex-specific morphogenesis in C. elegans. Development 135, 2373-82.

Nguyen, C. Q., Hall, D. H., Yang, Y. and Fitch, D. H. (1999). Morphogenesis of the Caenorhabditis elegans male tail tip. Dev Biol 207, 86-106.

Van Auken, K., Weaver, D. C., Edgar, L. G. and Wood, W. B. (2000). Caenorhabditis elegans embryonic axial patterning requires two recently discovered posterior-group Hox genes. Proc Natl Acad Sci U S A 97, 4499-503.

643A

E01A2.4 is a conserved negative regulator for Notch in C. elegans. **Ron Chen**, Julie Ahringer. Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

We previously identified E01A2.4 in a screen for new synthetic multivulval genes, a group of genetically interacting genes that encode chromatin and transcription regulators required to prevent inappropriate vulval development. E01A2.4 is an essential gene that encodes an uncharacterised protein conserved in Drosophila, mouse and human. The Drosophila ortholog of E01A2.4 can bind CIR-1 (CBF-1 Interacting coRepressor; CG6843) in a yeast two hybrid assay. Human CIR is a transcriptional co-repressor that binds to CBF-1, (LAG-1 in C. elegans), the Notch regulated transcription factor activated by binding the Notch intracellular domain. These results suggest that E01A2.4/NKAP and CIR-1 may function in Notch dependent transcription regulation. Of possible relevance to E01A2.4 function, negative regulators produced by the Notch pathway antagonise Ras signalling to ensure normal vulval development. Our preliminary studies suggest that E01A2.4/NKAP may function to inhibit Notch dependent transcription activation. We find that E01A24 encodes a widely expressed nuclear protein. RNAi of E01A2.4 or cir-1 leads to phenotypes similar to those of Notch pathway mutants and also enhances the germ-line tumour phenotype of glp-1/Notch gain of function mutants. We further found that NKAP (the human ortholog of E01A2.4) and CIR-1 can downregulate the activation of a Notch reporter in cell culture. We are currently characterizing the repressive role of E01A2.4/NKAP on Notch dependent transcription, its genome distribution, and its possible effect on chromatin.

644B

MLS-2 functions with Ras to promote excretory duct development. **Ishmail Abdus-Saboor**, Craig Stone, Meera Sundaram. Genetics, University of Pennsylvania, Philadelphia, PA.

The Ras-stimulated Raf-Mek-Erk kinase cascade is used repeatedly during development to control the specification of multiple cell fates. In C.elegans, Ras signaling specifies multiple cell types, such as the excretory duct cell fate versus the pore cell fate. Identifying additional targets of Ras signaling will provide insight into the specificity achieved by Ras signaling in promoting distinct cell types. While the core constituents of the Ras pathway have been defined, their role in eliciting cell-specific responses is unclear. The lab identified the homeodomain transcription factor MLS-2 in a screen for mutants defective in excretory system function. Approximately 20% of *mls-2* mutants have a lethal excretory system phenotype and *mls-2* enhances the lethality of various Ras pathway mutants. Expression data I have generated from a rescuing MLS-2::GFP translational reporter and an MLS-2 antibody suggest that MLS-2 is expressed in the duct and pore cells prior to specification and subsequently only expressed in the duct cell after specification and during differentiation.

To make a distinction between MLS-2 functioning in duct specification or differentiation I assessed *mls-2* mutants with two excretory system markers. *lin-48*::GFP is a late duct differentiation marker. AJM-1::GFP is a marker for the pore cell and another junction in the excretory system. Ras pathway loss-of-function mutants have two pore cells due to lack of duct specification. I found that *mls-2* mutants lack the late duct differentiation marker and do not have a duct-to-pore fate transformation. These data suggest that MLS-2 is involved in differentiation of the duct cell, rather than specification of this cell type.

MLS-2 and Ras appear to function together to promote a differentiation step of duct development. We have made use of a temperature sensitive Ras pathway loss of function mutant, *sos-1 ts*, with excretory system markers in the background. By shifting this mutant at various time-points, we have witnessed numerous phenotypes demonstrating that Ras has a role in not only specification of the duct cell but likely several other roles in differentiation of this cell type. *mls-2* and Ras pathway double mutants have a synergistic duct differentiation phenotype. Because MLS-2 is expressed in the duct lineage before Ras signaling initiates, we favor a model in which MLS-2 acts in parallel to the Ras pathway. However, since MLS-2 expression is maintained in the duct but not the pore cell, there may be some Ras dependent up-regulation of MLS-2 to promote duct development.

645C

The *C. elegans* nuclear receptor NHR-25 controls T cell differentiation. M. Hajduskova[1], M. Jindra[1], M. A. Herman[2], **M. Asahina[1]**. 1) University of South Bohemia and Biology Centre ASCR, Budweis, Czech Republic; 2) Kansas State University, Manhattan, KS, USA.

Asymmetric cell divisions produce new cell types during animal development. Studies in *C. elegans* have identified major signal transduction pathways that determine the polarity of cell divisions. How these relatively few conserved pathways interact and what modulates them to ensure the diversity of multiple tissue types is an open question. Our previous work (Asahina et al. 2006; Dev Cell 11, 203-) has shown that a nuclear receptor NHR-25 antagonizes a Wnt/β-catenin-dependent pathway during differentiation of the *C. elegans* somatic gonad. Here, we focus on the epidermal T cell, whose asymmetric division is necessary to establish the neural cell fate and to form male-specific sensory rays in the tail. We show that reduced NHR-25 function disturbs ray differentiation. Lineage analyses and dye filling experiments in *nhr-25* mutant hermaphrodites reveal altered T cell polarity. We further demonstrate by means of genetic interactions that NHR-25 cooperates with Wnt/β-catenin asymmetry signaling pathway to ensure the asymmetry of the T cell division. While loss of NHR-25 enhances the impact of the mutated nuclear effector of the Wnt/β-catenin asymmetry signaling pathway POP-1 on T cell polarity, it suppresses the effect of the same mutation in the gonad. Therefore, NHR-25 can influence Wnt/β-catenin signaling either positively or negatively depending on the tissue context. Our findings implicate this nuclear receptor as a versatile modulator in Wnt/β-catenin-dependent cell fate decisions. Supported by projects 204/07/0948 and 204/09/H058 from the Czech Science Foundation, 2B06129 from the Czech Ministry of Education, and GM56339 and P20RR016475 from the NIH (NCRR).

646A

Identification and analysis of a conserved non-coding element required for correct *bro-1* expression. **Charles Brabin**, Peter Appleford, Alison Woollard. Department of Biochemistry, University of Oxford, Oxford, United Kingdom.

Comparative sequence analysis between *C. elegans* and *C. briggsae* has proved to be an immensely useful tool in genome annotation. We have used comparative genomics to identify a small (122bp), conserved non-coding element (CNE) in *bro-1*, a gene which functions with the RUNX gene *rnt-1* to regulate cell proliferation and differentiation in the stem cell-like seam cells of the worm. Deletion of the CNE from a *bro-1::dsred* reporter construct significantly changes the expression pattern of the construct, and abolishes its ability to rescue *bro-1* mutants. Furthermore, the *bro-1* CNE can drive GFP expression in the seam cells and has been shown to be able to rescue the *bro-1* mutant phenotype when used to drive a *bro-1* cDNA::GFP reporter construct. Therefore this CNE is both necessary and sufficient for correct *bro-1* expression. Current work involves the dissection of this non-coding region as well as a reverse genetic screening approach, based on predictions of transcription factor binding sites in order to build up our knowledge of the regulatory pathways in which *rnt-1* and *bro-1* operate.

Yeast one-hybrid screens are being used as the basis of complementary screens for novel *bro-1* and *rnt-1* regulators. The CNE from *bro-1* and a region of the *rnt-1* promoter have been used as 'baits' with which a transcription factor cDNA library has been screened, with the aim of identifying genes that interact with *bro-1* and *rnt-1*. So far, supporting our predictions based on binding site analysis, the *bro-1* yeast one-hybrid screen has identified the GATA transcription factor *elt-1* as a major regulator of this gene.

647B

The Role of *glp-1* in Restricting the Pluripotency in the AB Lineage. **Nareg J-V Djabrayan**, Joel H Rothman. NRI, UCSB, Santa Barbara, CA.

Cells in early embryos undergo a transition from pluripotency to committed differentiation, as evident by reduced susceptibility to developmental reprogramming as embryogenesis proceeds. For example, while the endoderm-specifying transcription factor END-3 is capable of activating endoderm development in virtually all somatic cells in the early embryo, cells become refractory to such reprogramming beyond the ~100 cell stage. To characterize the regulatory mechanisms controlling this pluripotency → commitment transition, we have identified a number of genes that when debilitated by RNAi allow cells to remain responsive to reprogramming into endoderm after the 100 cell stage. Among the factors identified is GLP-1/Notch, which regulates several sequential inductive interactions in the AB lineage of early embryos. We found that in *glp-1(e2144)* embryos grown at restrictive temperature, non-endodermal cells remain competent to become reprogrammed into endoderm at least 6.5 hours after division of the zygote, a stage at which virtually all cell types have already been specified. Isolation of blastomeres in early *glp-1(RNAi)* embryos revealed that only descendants of AB were competent to be reprogrammed late in development, consistent with the known role for GLP-1 in reception of inductive signals in the AB lineage. Temperature-shift experiments showed that the temperature-sensitive period (tsp) for restricting late reprogramming correlates with the known tsp for early embryonic inductions by GLP-1 (i.e., before the 28-cell stage). This observation suggests that *glp-1* acts during early embryogenesis to set a state of restricted potency many cell divisions later. This effect appears to be mediated through the normal GLP-1 signaling pathway, as knockdown of *lag-1*, which transduces the GLP-1 signal, produces the same result. However, *ref-1*, a key player in *glp-1*-mediated inductions in the embryo is not required to restrict reprogramming, suggesting that only a subset of factors in *glp-1* inductions is involved in this process. To further investigate *glp-1* function in restricting pluripotency through cell-cell communication, we have established an *in vitro* cell culture system. Using this system, we found that young descendants of isolated blastomeres are capable of being reprogrammed into endoderm, thereby allowing us to assess the effect of intercellular interactions on the pluripotency → commitment transition.

648C

Identification of cell-type specific Wnt pathway targets in *C. elegans*. **Lakshmi Gorrepati**, David Eisenmann. Biological Sciences, UMBC, Baltimore, MD.

The Wnt signaling pathway is one of the key extracellular pathways involved in many developmental processes in both vertebrates and invertebrates. In the nematode *C. elegans*, there exists a BAR-1/β-catenin mediated Wnt signaling pathway and a divergent Wnt/β-catenin asymmetry pathway. Both are involved in cell specification, polarity and migration. We and others have shown that the BAR-1 mediated Wnt signaling pathway plays a crucial role in the specification of the vulval precursor cells (VPCs) that form the hermaphrodite vulva. Work from our lab has also implicated Wnt signaling in the specification of seam cells (Julie Gleason unpublished data). Seam cells are specialized epithelial cells that lie along the apical midline of the worm hypodermis. We propose to identify the targets of the Wnt pathway functioning in the VPCs and seam cells using Affymetrix microarray analysis. We will study the alterations in gene expression due to over-activation and under-activation of the Wnt pathway by utilizing worms expressing a heat shock inducible stable variant of BAR-1/β-catenin (over-activation), or a dominant negative variant of POP-1/TCF (under-activation). The transcript pools from the VPCs and seam cells will be specifically isolated by mRNA-tagging method. In this method, transcripts will be co-immunoprecipitated with a FLAG-tagged variant of the poly-A tail binding protein PAB-1 that is expressed only in the cell-types of interest (Roy et al., 2002 and Von Stetina et al., 2007). The specificity of the transcript pool obtained by mRNA-tagging was verified by qRT-PCR. The experimental strains showed 2 fold or greater increase in expression than control strain for seam cell and VPC specific transcripts tested. Microarray analysis will be performed on the experimental and control strains. Transcripts showing 2 fold or greater change in expression will be identified as potential Wnt targets and verified by quantitative Real-Time PCR (qRT-PCR). The targets that pass this verification screen will be further analyzed for regulatory sequences, spatial and temporal expression patterns, and mutant phenotypes by RNAi.

649A

Analysis of Successive Protein Expression Profiles during Early Embryogenesis in *Caenorhabditis elegans* using 2-D Difference Gel Electrophoresis. **Shizuka Hino**, Dai Sasahara, Ayako Terasawa, Masahiro Ito. Bioinfomatics, Ritsumeikan University, Kusatsu, Shiga, Japan.

Protein expression profiles of the developmental stages of *Caenorhabditis elegans* were determined using matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and liquid chromatography-mass spectrometry (LC-MS). The protein expression profiles of the following 6 developmental stages were analyzed: the embryo, 4 larvae, and adult stages. However, these profiles were found to be discrete and not successive. Thus, the elucidation of life systems using systems biology requires quantitative and successive data.

In this study, we focused on the protein expression profiles during early embryogenesis in *C. elegans* because it is known that the cell fate of this organism is determined by the maternal genes expressed up to the 12-cell stage. Worms were synchronized thrice using the alkali-bleach method, and early embryos were collected. The obtained embryos were allowed to develop for 40 minutes, and samples were collected every 10 minutes. The successive samples thus obtained were analyzed using 2-D difference gel electrophoresis (2D-DIGE). As a result, successive data for 521 spots (proteins) were obtained. Among the spots, we assumed that those which showed decreased expression levels were related to cell fate determination. The successive protein expression profiles of the 521 spots were grouped by cluster analysis. Furthermore, several spots were identified using MALDI-TOF MS, and the remaining spots are been identifying using MALDI-TOF MS and LC-MS.

650B

Distinct protein domains regulate stability and patterning of MEX-3 in the *C. elegans* embryo. **Nancy N Huang**[1], Craig P Hunter[2]. 1) Biology Department, The Colorado College, Colorado Springs, CO 80903, USA; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA.

The KH domain protein MEX-3 is central to the temporal and spatial control of PAL-1 expression in the *C. elegans* early embryo. PAL-1 is a caudal-like homeodomain protein required to specify the fate of posterior blastomeres. While *pal-1* mRNA is present throughout the oocyte and early embryo, PAL-1 protein is expressed only in posterior blastomeres starting at the 4-cell stage. MEX-3 protein is present uniformly throughout the newly fertilized embryo but is enriched in the anterior relative to the posterior by the 4-cell stage. This anterior enrichment is consistent with its role in repressing PAL-1 translation and requires the CCCH Zn-finger protein MEX-5, and the RNA Recognition Motif protein SPN-4, which were identified as MEX-3 binding proteins. Genetic evidence indicates that *mex-5* is required for MEX-3 stability in the anterior, while *spn-4* is required for MEX-3 degradation. In *mex-5(-)* embryos, MEX-3 is rapidly degraded and PAL-1 translation is no longer restricted to the posterior. In *spn-4(-)* embryos, MEX-3 is present at unusually high levels throughout the embryo, but is largely unable to repress PAL-1 translation. MEX-5 protein is present in the anterior, while SPN-4 protein is present throughout the early embryo. Intriguingly, when *par-4* is depleted, the abundant MEX-3 in *spn-4(-)* embryos retains the ability to repress PAL-1 translation throughout the embryo. PAR-4 is a serine/threonine kinase enriched in the cell cortex, but not asymmetrically localized along the anterior-posterior axis. These data suggest the following model: MEX-5 binds to MEX-3 in the anterior and protects it from degradation, allowing MEX-3 to bind the *pal-1* 3'UTR and repress translation. MEX-3 that is not bound to MEX-5 becomes inactivated in a *par-4* dependent fashion, possibly through phosphorylation, then targeted for degradation through binding to SPN-4. In *spn-4(-)* embryos, inactivated MEX-3 binds to and interferes with active MEX-3.

To determine which regions of the MEX-3 protein are required for stability and which for degradation, different regions of the protein were fused to GFP and introduced into the *C. elegans* embryo. The N-terminus of MEX-3, which contains the two KH domain RNA binding domains, confers unusual *mex-5*-dependent stability to GFP. In contrast, the C-terminus of MEX-3, which contains potential phosphorylation sites, confers unusual *spn-4*-dependent instability to GFP. Experiments are in progress to determine which phosphorylation sites, if any, are required for MEX-3 degradation.

651C

Wnt Pathway Regulation Is Implicated In The Expression Of *C. elegans* Cuticular Collagen Genes. **Belinda M. Jackson**, David M. Eisenmann. Dept Biological Sciences, Univ of Maryland Baltimore County, Baltimore, MD.

The Wnt singlaing pathway is highly conserved, playing an integral part in development and homeostasis of metazoan organisms. This pathway regulates cell polarity, migration and fate determination and its mis-activation has been implicated in assorted birth defects and cancers. In *C. elegans*, Wnt signaling is required for embryonic and larval development. Homologs have been identified for most of the core pathway components, but only a handful of target genes have been found. The Eisenmann lab is interested in Wnt pathway regulation of cell fate determination, and has shown that Wnt/beta-catenin signaling is involved in fate specification of certain epithelial and neuronal cells. My research interest is to identify *C. elegans* Wnt pathway gene targets.

Using microarray analysis I identified 117 genes that are differentially expressed upon *beta-catenin/bar-1* over-expression. qRT-PCR analysis of these putative Wnt targets showed that 23 are *bona fide* Beta-catenin/BAR-1 responsive genes. Of these, five are cuticular collagens, three are dauer pathway related, five show DAF-16 dependence and eight are novel.

Currently I am characterizing the collagen targets for temporal and spatial expression patterns. Under wild-type conditions, these genes are primarily transcribed during the L4 larval stage in hypodermal cells. Preliminary results suggest that Wnt pathway hyper- or hypo-activation is sufficient to alter the timing and/or intensity of select collagen gene expression. Promoter analysis to identify the Wnt responsive regulatory elements and phenotypical analysis via RNAi are also underway. Wnt signal regulation of collagen expression has been reported in vertebrates, but these are novel findings in *C. elegans*.

652A

The forkhead transcription factor LET-381 functions to pattern the *C. elegans* postembryonic mesoderm. Nirav Amin, Herong Shi, **Jun Liu**. Dept Molec Biol & Gen, Cornell Univ, Ithaca, NY.

The *C. elegans* postembryonic mesodermal lineage, the M lineage, is a powerful system in which to study mesodermal development at single cell resolution. This lineage produces 14 striated bodywall muscles (BWMs), 2 non-muscle coelomocytes (CCs), and 2 multipotent progenitor cells of non-striated muscles called the sex myoblasts (SMs). We have previously found that the MyoD homolog HLH-1, the Hox factor MAB-5 and a C2H2 zinc finger protein FOZI-1 function within the M lineage to specify both M-derived BWMs and CCs, and that the absence of all three factors cause almost all M lineage cells to become SMs. The distinction between the myogenic BWM and the non-myogenic CC fates requires the Six homeodomain protein CEH-34 and its cofactor EYA-1 to act as CC-promoting factors. However, additional factors must also exist in this process as CEH-34 and EYA-1 alone are not sufficient to convert all myogenic BWM precursors to CCs. Here, we have identified a role for the conserved forkhead transcription factor LET-381 in patterning the M lineage. *let-381* is expressed in progenitors of the M-derived CCs and their sister BWM cells. Knockdown of *let-381* caused fate transformation of CCs to SMs. Additionally, *let-381* is required for *ceh-34* expression. Our studies lead to a model for how LET-381 functions as a competence factor to pattern the *C. elegans* postembryonic mesoderm in order to produce both myogenic and non-myogenic cells.

653B

Mechanism and role of HLH-2 protein downregulation in the AC/VU cell fate specification during somatic gonad development in C. elegans. **J. T. Ohlmeyer**, I. Greenwald. HHMI/Dept. of Biochemistry and Molecular Biophysics. Columbia University. New York, NY.

HLH-2 is a type I bHLH protein that is orthologous to Drosophila Daughterless and mammalian E proteins (Krause et al). Type I bHLH proteins can form active homodimers or heterodimers with other HLH proteins. HLH-2 has been implicated in several cell fate specification events during somatic gonad development in C. elegans (Karp & Greenwald 2003 and 2004). We are particularly interested in studying properties of HLH-2 in the anchor cell (AC)/ventral uterine precursor cell (VU) fate decision. During the AC/VU decision, LIN-12 and its ligand LAG-2 ensure that only one of two equivalent cells becomes the AC while the other becomes a VU. Both cells initially express lag-2 and lin-12; feedback mechanisms that assess the relative levels of lin-12 activity in the two cells cause lag-2 transcription to become restricted to the presumptive AC and lin-12 transcription to become restricted to the presumptive VU cell (Seydoux, Wilkinson). Post-transcriptional downregulation of HLH-2 appears to be part of a negative feedback mechanism that leads to cessation of lag-2 transcription when LIN-12 is activated (Karp 2003). This model was based on three key observations. (1) hlh-2(RNAi) can result in the failure of VU specification. (2) HLH-2 promotes lag-2 transcription through E-boxes, canonical bHLH binding sites. (3) hlh-2 is transcribed in both equivalent cells, but accumulates only in the presumptive AC, suggesting that HLH-2 is post-transcriptionally downregulated in the presumptive VU. We are investigating the mechanism and role of HLH-2 downregulation. We are currently investigating the contribution of several motifs in the HLH-2 protein for their potential contributions to HLH-2 stability and activity through the course of the AC/VU decision: a predicted destruction box, putative lysine residues that could be used as sites of ubiquitination, and a highly conserved cysteine that might mediate homodimerization via a thio-ester bond. In addition to illuminating the mechanism, the identification of a mutation that stabilizes HLH-2 in the presumptive VU without compromising its function will enable us to investigate the role of HLH-2 downregulation in the AC/VU decision. We will report on our progress at the meeting.

654C

Deciphering the cellular steps required for *in vivo* reprogramming. **Jai Prakash RICHARD**[1], Nadine FISCHER[1], Valeria PAVET[1], Nadège VAUCAMPS[1], Yannick SCHWAB[2], Sophie JARRIAULT[1]. 1) IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Department of Cell and Developmental Biology; 2) IGBMC, Electronmicroscopy Plateform ; INSERM U964, CNRS UMR 7104, Université de Strasbourg, 67404 Illkirch-CU Strasbourg.

We are interested in the mechanisms endowing a cell with the competence to adopt a new identity. Understanding the molecular events underlying cell plasticity has important implications from organ regeneration to cancer. Various examples of cellular reprogramming have been described, including the reprogramming of a nucleus (dedifferentiation), the reprogramming of a committed cell (transdetermination) or of a differentiated cell (aka transdifferentiation). However, the exact mechanisms by which a cell of a particular type can change its identity are poorly understood. We have characterised an epithelial-to-neuron reprogramming that occurs during *C. elegans* development. We use this unique in vivo system to understand the mechanisms underlying the reprogramming of a cell. We have performed an initial characterisation of this process and explored factors pertaining to competence, lineage and local environment. We found that this event does not depend on fusion with a neighbouring cell; and that competence to be reprogrammed is restricted and requires 2 transcription factors and the LIN-12/Notch signalling. To further identify the genetic cascades involved, we have performed a genetic screen for mutants unable to reprogram. Our characterisation of one mutant retrieved suggests that reprogramming proceeds through intermediary cellular steps, rather than through concommitant loss and gain of the initial and final identities, even in absence of cell division. We are currently testing if transient reversion to a blastic state is involved. This study will shed light on the cellular steps that are used in a physiological reprogramming event and will likely contribute to improve reprogramming strategies in regenerative medicine.

655A

POPping the Patterning Question. **Casey Roehrig**, Craig Hunter. Dept Molec & Cell Biol, Harvard Univ, Cambridge, MA.

In *C. elegans*, many cell fate decisions rely on a variant of the canonical Wnt signaling pathway in which post-translational modifications of the TCF transcription factor, POP-1, modulate its interactions with cofactors that regulate its activity. These interactions allow POP-1 to both activate and repress a number of different genes to determine the fates of cells throughout the animal. For example, in the EMS lineage, POP-1 has been implicated in both the activation and repression of endodermal genes including *end-3* (Maduro et al. 2005).

In the C lineage, POP-1 has been shown to play a role in distinguishing between skin and muscle cell fates. Loss of *pop-1* causes the C lineage to generate only muscle, whereas loss of its regulator, the kinase *lit-1*, causes C to produce hypodermis (Fukushige and Krause 2005). The role that POP-1 plays in this process, however, remains unclear. Does it bind to and repress muscle gene expression, or promote the transcription of epidermal genes, or both? To better understand POP-1's role in this decision, we are investigating the binding of POP-1 to the promoters of transcription factors required for skin and muscle cell fates. We have performed chromatin immunoprecipitation of embryos carrying a GFP-POP-1 fusion protein that is expressed either in the EMS lineage or the C and D lineages. Semi-quantitative PCR analysis suggests that POP-1 binds to the promoters of transcription factors involved in skin and muscle specification in a lineage-specific fashion to coordinate cell fate determination. Further analysis and identification of binding sites are underway to clarify POP-1's function in cells destined to take on different fates and to determine how POP-1 interacts with other factors required for these cell fate decisions.

656B

Genome wide ChIP-chip analysis for identification of POP-1/TCF target genes. **Kenneth Thompson**, David Eisenmann. Dept Biological Sci, Univ Maryland, Baltimore City, Baltimore, MD.

In *C. elegans*, Wnt signaling functions in many processes, including endoderm induction, Q neuroblast migration, anteroposterior axon guidance, T cell and somatic gonad precursor cell polarity, and P12, seam cell, and vulva precursor cell fate specification. Key components of this pathway are members of the β-catenin (BAR-1 and SYS-1) and TCF/LEF (POP-1) families of proteins. β-catenin and TCF/LEF family members form a bipartite transcription factor in which β-catenin supplies the activation domain and TCF/LEF proteins provide the DNA binding domain. Two of the *C. elegans* β-catenin homologs, BAR-1 and SYS-1, interact with POP-1 to activate gene expression. POP-1 has been shown to bind DNA via a high mobility group DNA binding domain in a sequence specific manner. To date, only a few POP-1 target genes have been identified. Thus, the identification of POP-1 binding sites within the *C. elegans* genome will potentially aid in the identification of POP-1 target genes. In order to identify the cis-regulatory elements bound by POP-1 I have used chromatin immunoprecipitation coupled to microarray analysis (ChIP-chip). ChIP-chip has become a standard technique used for the identification of cis-regulatory elements bound by transcription factors of interest. ChIP involves the chemical crosslinking of protein to DNA, followed by immunoprecipitation of the complex with an antibody specific to the transcription factor. Following isolation of the bound DNA fragments, a whole genome tiling array can be used to determine the genomic locations of transcription factor interactions. Initial attempts to immunoprecipitate endogenous POP-1 with available antibodies has been unsuccessful. To circumvent this, I have created a transgenic line of *C. elegans* with an epitope-tagged variation of POP-1, which is under the control of a heat shock inducible promoter. Western analysis demonstrates that this epitope-tagged POP-1 is expressed upon heat shock and can be immunoprecipitated. Preliminary qRT-PCR analysis of known POP-1 binding sites suggests enrichment of known POP-1 bound fragments. Microarray experiments are underway to identify genomic POP-1 binding sites.

657C

SEM-2 is an SRY-box transcription factor required for egg laying in *C. elegans*. **C. Tian**[1], C. Colledge[2], H. Shi[1], M. Stern[3], R. Waterston[2], J. Liu[1]. 1) Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY; 2) Department of Genome Sciences, University of Washington School of Medicine, 1705 N.E. Pacific Street, HSC, Rm357, Health Sciences K-357B, Box 357730, Seattle, WA 98195-7730; 3) Department of Biology, University of Central Florida, 4000 Central Florida Blvd., Orlando, FL 32816-2368.

C. elegans egg laying system includes the vulva, the gonad, the hermaphrodite specific neurons and the egg-laying muscles. *sem-2(n1343)* mutant animals lack all the egg-laying vulval and uterine muscles (collectively called sex muscles) and are therefore egg-laying defective. The sex muscles are derived from two sex myoblasts (SMs), which are descendants of the M cell, the progenitor of the postembryonic mesodermal lineage. The absence of the sex muscles in *n1343* mutants is due to a fate transformation of the SMs to their sister cells, the bodywall muscles (BWMs). We have found that *sem-2(n1343)* is a mutation in *sox-1*(C32E12.5) based on results from cosmid rescue and RNAi knockdown experiments. Furthermore, the *n1343* allele contains a TC1 transposon insertion in the first 4.5kb intron of *sem-2*. In order to determine how *sem-2* functions in regulating the SM/BWM fate decision, we examined *sem-2* expression using a functional GFP::SEM-2 fusion construct. SEM-2::GFP is present in the nuclei of the SM mother cells (M. vlpa and M. vrpa), SM cells and their descendants as well as a variety of other cell types, including hypodermal cells and gut cells. By performing genetic and molecular epistasis experiments, we found that *sem-2* expression in the M lineage is under the control of both dorsal-ventral and anterior-posterior patterning mechanisms that include the LIN-12/Notch pathway, the SMA-9/TGF-beta pathway and the Wnt/beta-catenin asymmetry pathway. We further found that the expression of *sem-2* in the M lineage requires a conserved Hox/PBC binding site in its first intron and that this site is disrupted by the TC1 insertion in *n1343* mutants. Previous studies have shown that the Hox factors MAB-5 and LIN-39 and the PBC Hox cofactor CEH-20 all function in the M lineage. We are currently testing the hypothesis that *sem-2* is a direct target of Hox/PBC in the M lineage.

658A

Unique Roles for the GATA Transcription Factors *end-1* and *end-3* During *C. elegans* E-lineage Development. **Max E. Boeck**, Thomas Boyle, Robert Waterston. Dept Genome Sci, Univ Washington, Seattle, WA.

end-1 and *end-3* are GATA transcription factors that are important for determining endoderm cell fate in *C. elegans*. Deletion of both factors causes embryonic lethality and a fate change in the endoderm-specifying E-lineage. Deletion of either factor individually leads to apparent wild-type development, which would indicate they are redundant to each other. Despite appearing redundant these two factors have been preserved between *C. elegans* and *C. briggsae*, a split of more than 40 million years. If they were truly redundant such a long period of time should have lead to one of the genes becoming non functional. We sought to understand why these two apparently redundant transcription factors have been preserved. Utilizing our automated cell-lineaging platform we were able to assay phenotypes in cell movement, division times and downstream reporter gene expression. Defects in cell movement and division time were found to be associated only with the *end-3* deletion, while downstream reporter gene expression is perturbed in both single deletions. Using a maximum likelihood phylogenetic approach we show that *end-1* and *end-3* have split to form two distinct clades in Caenorhabditis species. These results would indicate that these two genes have evolved into unique gene with specific functions during development. Our findings highlight the ability of our automated lineaging platform to describe subtle developmental phenotypes.

659B

The tailless ortholog *nhr-67* functions in ventral uterus development. **Brittany Sanford**[1], Eliana Verghese[1,2], John Schocken[1], Jessica Nesmith[1], Sheila Clever[1], Bruce Wightman[1]. 1) Biology Dept, Muhlenberg College, Allentown, PA; 2) Drexel University School of Medicine, Philadelphia, PA.

The *tailless* family of nuclear receptors is highly conserved among animals. The *C. elegans tailless* ortholog, *nhr-67*, is expressed in a dynamic pattern in pre-uterine cells. *nhr-67* is initially expressed in the 4 pre-VU cells during the L2 stage, and subsequently expressed at higher levels in the anchor cell (AC) and lower levels in the VU cells, apparently in response to the *lin-12/lag-2* reciprocal signaling system. During the L3 stage, *nhr-67* expression is maintained at high levels in the AC and briefly at low levels in the six π cells whose twelve progeny form the UTSE and UV1 cells of the adult ventral uterus. Development of the π cells also depends on a *lin-12*–based signal from the AC. From the mid L4 stage through adulthood, *nhr-67* is expressed at low levels in the nuclei of the UTSE syncytium.

In mutants homozygous for hypomorphic *nhr-67* promoter mutations that were identified by Bernard Lakowski's laboratory, the development of the π cell descendants is defective by multiple criteria. In contrast, the AC appears normal during the L3 and early L4 stages, although it fails to fuse with UTSE in late L4 (likely due to the defective UTSE). The expression of *nhr-67* is downstream of *lin-12(RNAi)* knockdown and *lin-12(gf)* mutations in the AC and the π cells, and *nhr-67(lf)* mutations are epistatic to *lin12(gf)* mutations in the π cells. In *lag-1 (RNAi)* L2 animals *nhr-67* is expressed at higher levels in both the presumptive AC and VU cells of animals. In *nhr-67(RNAi)* L2 animals, *egl-43* is expressed at higher levels in both the presumptive AC and VU cells. Taken together, these data indicate that *nhr-67* is a component of the regulatory response to *lin-12* signaling in the VU and π cells.

Possible null alleles that delete most of the *nhr-67* ligand-binding domain cause L1 arrest after hatching. Rare escapers are Pvl, Egl and Ste. The arrested L1 larvae display tail defects in the hyp10 epithelial cell similar to those caused by mutations in the cadherin gene *cdh 3*, although expression of *cdh-3::gfp* in tail hypodermal cells is not obviously altered in *nhr-67* mutants.

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660C

Genetic Dissection of Wnt Signaling in *C. briggsae* Vulva Development. **Philip Cumbo**. Biology, McMaster University, Hamilton, Ontario, Canada.

The *C. elegans* vulva is an established model system to understand how genes and pathways function to control organ formation. The study of vulval development has been crucial to identifying the components of many major conserved signaling pathways, such as Wnt, and dissecting their mechanism of function. The canonical Wnt signaling pathway has been extensively studied in *C. elegans* and the components that play a pivotal role in vulval development have been identified. We are interested in understanding the role of Wnt signaling in other nematode species and its evolutionary differences from *C. elegans*. To facilitate this we are focusing on Wnt pathway genes in *C. briggsae*, a sister species of *C. elegans*. We have isolated *pry-1/axin* mutants in *C. briggsae* and studied its genetic properties and differences from the *C. elegans* counterpart. Our results show that, unlike *C. elegans* where *pry-1* mutants exhibit a multivulval phenotype, *C. briggsae pry-1* mutants exhibit simultaneous multivulva and vulvaless phenotypes. These phenotypes result because the VPCs anterior to P6.p are induced to adopt 2° cell fates whereas VPCs posterior to P6.p adopt non-induced fates. This may be due to a diversification of *pry-1* regulation and/or altered activities of the downstream targets of Wnt signaling. Among the *pry-1* interacting genes in *C. briggsae*, we have identified *bar-1, pop-1, lin-39,* and *mab-5* as crucial regulators of vulval cell proliferation. These results demonstrate the presence of a canonical Wnt signaling pathway in *C. briggsae* vulval development. We are taking various experimental approaches to understand the regulation of *pry-1* and other Wnt pathway components in *C. briggsae* and their differences from *C. elegans*. Current experiments focus on the dissection of the *pry-1* regulatory region and genome-wide analysis of Wnt targets. Since ectopically induced VPCs in *pry-1* mutants adopt a 2° cell fate we are also examining the role of the LIN-12/Notch pathway in Wnt-mediated vulval development. These studies will reveal the mechanism of Wnt signaling function and diversification in vulva formation.

661A

ERM-1 Regulates LET-23 EGFR Localization During Vulval Development. **David Kradolfer**, Peter Gutierrez, Erika Froehli, Alex Hajnal. Institute of Zoology, University of Zürich, Switzerland.

The *C. elegans* hermaphrodite vulva is formed during larval development by the descendants of three out of six vulval precursor cells (VPCs, P3.p-P8.p). Vulval development is organized by the gonadal anchor cell (AC), which produces the EGF-like growth factor LIN-3. P6.p lies closest to the AC and receives the highest amount of the inductive LIN-3 signal. LIN-3 binds to the EGFR homolog LET-23 and activates the conserved RAS/MAPK signaling pathway, resulting in the induction of the primary cell fate in P6.p. In order to receive the LIN-3 signal, LET-23 has to be localized to the basolateral surface of P6.p facing the AC. LIN-2, LIN-7 and LIN-10 form a complex that binds to LET-23 and is required for its basolateral localization. In *lin-2*, *lin-7* and *lin-10* mutants, LET-23 is mislocalized to the apical compartment of the VPCs. As a consequence, the EGF signal is only weakly transduced and most mutants are vulvaless. Our focus lies on the identification of proteins that modulate EGF signaling by regulating LET-23 localization.

ERM-1, which is a *C. elegans* ortholog of the Ezrin/Radixin/Moesin (ERM) protein family, was identified in a screen for regulators of LET-23 localization during vulval induction. ERM proteins form a link between the cytoskeleton and transmembrane proteins by their C-terminal F-actin binding site and their N-terminal FERM domain. ERM proteins have diverse functions, including membrane organization and the modulation of intracellular signaling pathways. In *erm-1(tm677)* mutants, we observed reduced basolateral localization of LET-23 in the primary vulval cells and an accumulation of the receptor in intracellular punctae. Moreover, *erm-1* interacts genetically with mutations in components of the inductive EGFR/RAS/MAPK pathway. We thus propose that ERM-1 regulates vulval induction by sequestering LET-23 in a distinct plasma membrane compartment.

662B

Analysis of the C. elegans Transcription Factor, LIN-31, Using the Yeast Two-Hybrid. Scott Montgomery, Elico Teixeira, Adam Smith, **Leilani Miller**. Dept Biol, Santa Clara Univ, Santa Clara, CA.

Cell fate, or how a cell decides to differentiate into a certain cell type, depends largely on which genes are expressed. Transcription factors are proteins responsible for such regulation, although the biochemical characterization of such systems is incomplete. For example, very few gene targets have been identified. In addition, transcription factors often heterodimerize with other factors in order to regulate their activity. The winged-helix transcription factor LIN-31 is involved in cell fate decisions during development of the vulva, an egg laying structure, in the model organism *Caenorhabditis elegans*. This protein contains a DNA-binding domain and several other regions of unknown function. The current model for LIN-31 function is that it plays two roles in vulval development: 1) it heterodimerizes with another transcription factor, LIN-1, to promote non-vulval cell fates in some cells and 2) when the dimer is disrupted due to cell-cell signaling events, it promotes a vulval cell fate in other cells (Miller et al, 1993 and Tan et al, 1998).

In *C. elegans*, the LIN-31 protein is expressed in very few cells, making direct biochemical analysis difficult. Attempts to express and purify this protein in bacterial and insect cells have been impeded by low expression levels and insolubility. To learn more about the role of this protein without purifying it, we are using a yeast-based system for two-hybrid (Y2H) analysis. Prior to conducting a full-scale screen, we are directly testing interactions between LIN-31 and its interaction partner, LIN-1. First, we used the Y2H system to confirm LIN-31's interaction with LIN-1. Previous Y2H experiments in other labs had failed to see this interaction, but removal of LIN-31's DNA binding domain allowed interaction with LIN-1 in our Y2H system. Second, we showed that LIN-31's small acidic domain is required for this interaction. Elimination of this domain by non-conservative amino acid substitutions resulted in disruption of the LIN-31/LIN-1 interaction. This is consistent with previous site-directed mutagenesis experiments indicating that this acidic region is required for proper LIN-31 function in non-vulval cells (Morris et al, in prep).

663C

Wnt and FGF signaling control vulval secondary lineage polarity. **Paul Minor**[1], Anand Asthagiri[2], Paul Sternberg[1]. 1) HHMI/Biology Division, Caltech, Pasadena, CA; 2) Chemistry and Chemical Engineering Division, Caltech, Pasadena, CA.

The C. elegans vulva is formed from divisions of three vulval precursor cells (VPCs): P5.p, P6.p, and P7.p. P5.p and P7.p are induced and divide to form a 2° lineage pattern in which the daughter cells of P5.p and those of P7.p form a mirror. The orientation of these cells is established by the interaction of multiple Wnt signals: Wnts LIN-44 and MOM-2 act through Fz/LIN-17 and Ryk/LIN-18, respectively, to promote the wild-type, anterior-facing P7.p, vulval lineage, termed refined polarity, whereas Wnt EGL-20 acts through VANG-1 and Ror/CAM-1 to promote posterior-reversed vulval lineage (P-Rvl) of the P7.p daughter cells, termed ground polarity. Here we show that additional components of the planar cell polarity (PCP) pathway as well as the FGF pathway control vulval orientation. Our data suggest that DSH-1 and DSH-2 (two homologs of the PCP and canonical Wnt pathway protein Dishevelled) work downstream of EGL-20 orienting P7.p to face the posterior. In contrast, the third Dishevelled homolog, MIG-5, as well as FMI-1, the homolog of the PCP protein Flamingo, act in the pathway leading to refined polarity. Finally, preliminary data suggests that the FGF pathway also controls polarity in the vulva. FGFR/EGL-15 and its adaptor protein, SEM-5, both act to orient P7.p to the anterior, based on both single mutant phenotypes as well as genetic interactions with a lin-18 mutation. Thus we implicate a fourth signaling pathway in the orientation of VPC lineages, and raise the question of how Wnt and FGF signaling are integrated in VPC lineages.

664A

Cross-talk between the RAS/MAPK and PI3K/PTEN signaling pathways during vulval development. **Itay Nakdimon**, Alex Hajnal. Dept Zoology, Zurich Univ, Zurich, Switzerland.

The development of the Caenorhabditis elegans vulva, the egg-laying organ of the hermaphrodite, serves as an excellent model to study how inter- and intracellular signals control cell fate specification and pattern formation during organogenesis. We are interested in the crosstalk between the different signaling pathways controlling vulval fate specification. One well-characterized example for signaling crosstalk is the antagonism between the RAS/MAPK and Notch pathways. Here, we examined a possible connection between the phosphoinositide-3 kinase (PI-3K)/PTEN and the RAS/MAPK signaling pathways during vulval development. It has been previously shown in mammalian cells that RAS can directly activate PI3K, while AKT, a downstream target of PI3K, phosphorylates and thereby inhibits the activity of RAF, the downstream target of RAS. To examine a possible role of the Insulin/PI3K pathway during vulval development, we combined mutations that either decrease or increase the activity of the canonical insulin pathway with mutations that alter the RAS/MAPK signaling intensity. daf-18 , the homologue of the mammalian tumor suppressor PTEN, encodes a lipid phosphatase that catalyzes the dephosphorylation of phophatydylinositol-3,-4,-5-trisphosphate (PIP3), thereby antagonizing the role of the AGE-1 PI-3 Kinase. A loss-of-function (lf) mutation in daf-18 blocks entry into the dauer stage and reduces life span due to hyperactivation of the PI-3K pathway. Our results suggest that DAF-18 also inhibits LET-60 RAS signaling during vulval development. Loss of daf-18 function increases the number of Vulva Precursor Cells (VPCs) induced in a let-60(gf) background and suppresses the vulvaless phenotype of different RAS pathway mutants. Our results also indicate that this effect is, at least in part, independent of the insulin pathway. Loss-of-function mutations in age-1 or daf-16, which are core components of the insulin pathway, do not change the number of VPCs induced in a let-60(gf) background. Furthermore, daf-18(lf) not only affects the number of VPCs induced, but also their fates, since an increase in the expression level of the primary cell fate marker egl-17::cfp was observed in a daf-18(lf) mutant. Mammalian PTEN is reported to have both lipid as well as protein phosphatase activities. The fact that part of the effect of daf-18 on vulval development is independent of the Insulin pathway suggests that a protein phosphatase activity of DAF-18 might play a role in the negative regulation of RAS/MAPK signaling. Experiments that distinguish between the two DAF-18 activities will be presented at the meeting.

665B

A role for the putative LIN-12/Notch target gene *ttr-11* during the specification of the secondary vulval cell fate. **Stefanie Nusser**, Ivo Rimann, Sarfarazhussain Farooqui, Alex Hajnal. University of Zurich, Zurich, Switzerland.

The development of the hermaphrodite vulva is an excellent model system to study cell signaling and pattern formation. Several highly conserved signaling pathways control specification of the vulval cell fates. In wild-type animals, P6.p, one of the six equivalent vulva precursor cells (VPCs), adopts the primary (1°) fate in response to the inductive EGFR/RAS/MAPK signaling pathway. The two neighboring VPCs, P5.p and P7.p, express secondary (2°) vulval fates in response to the lateral LIN-12/Notch signal. LIN-12/Notch signaling inhibits the 1° and promotes the 2° vulval cell fate in P5.p and P7.p. The currently known target genes of the LIN-12/Notch lateral signaling pathway all function by inhibiting the EGFR/RAS/MAPK pathway in the 2° cells. However, genes that promote the execution of the 2° cell fate are still unknown. We have identified *ttr-11* as a putative LIN-12/Notch target gene in an RNAi screen. By investigating the expression pattern of *ttr-11* in wild-type animals and in *lin-12* gain- and loss-of-function mutants, we find that LIN-12/Notch signaling regulates *ttr-11* expression in 2° VPCs. The *ttr-11* deletion allele (*tm3381*) partially suppresses the vulvaless phenotype of the weak *lin-12(n302)* gain-of-function allele. Moreover, in *ttr-11(tm3381);lin-15(n309)* double mutants, more VPCs adopt the 1° cell fate than in *lin-15(n309)* single mutants, suggesting that TTR-11 promotes 2° fate specification. Additional experiments to investigate the role of *ttr-11* during vulval development will be presented.

666C

Natural variation of signaling pathways in *C. elegans*. **Tobias Schmid**, Juan M. Escobar Restrepo, Alex Hajnal. Institute of Zoology, University of Zurich, Zurich, Switzerland.

Complex diseases such as cancer are responsible for the death of more than 50% of the adult human population. They arise from interactions between genes involved in different signaling networks and with the environment. Previous studies were mainly focused on the identification of genes involved in the development of rare monogenic diseases. Nevertheless, investigations of the more common complex, multigenic diseases are required. It has been shown that the genetic background has a considerable effect on the formation and outcome of many complex diseases, but further investigation is needed to decipher the underlying genetic factors.

Cancer-related signaling networks can be investigated in the *C. elegans* vulva with the aim to enlarge the knowledge about the signaling pathways in complex diseases. The development of the *C. elegans* vulva depends mainly on the coordinated action of three key signaling pathways: The EGFR/RAS/MAPK, Notch and Wnt pathways. These conserved pathways are involved in the formation of various types of cancer in humans.

To address the question of how the genetic background influences these signaling networks, we crossed mutants of the EGFR/RAS/MAPK, Notch and Wnt pathways generated in the reference N2 Bristol background into different genetic wild isolates of *C. elegans* and selected for suppression or enhancement of the mutant phenotypes. Thanks to the complete sequence of each wild isolate, mapping of possible modifiers is achievable without big effort.

These results can be further used in predicting the susceptibility of individuals to certain complex diseases and thereby help to find possible drug targets.

667A

T08D10.1, a novel mediator of Ras signaling during vulval development. **Michelle Stokes**, Segen Aklilu, Sarah Edwards, Douglas Fantz. Chemistry, Agnes Scott College, Decatur, GA.

The Ras/MAP kinase signaling pathway promotes cell divisions during the development of many multicellular organisms. Study of this signaling pathway is significant since mutation of the *ras* gene in humans is an important step in the generation of cancerous tumors. Many proteins are involved in the regulation and transmission of the Ras/MAP kinase signal, and we have initiated a study of a novel target of this signaling pathway during *Caenorhabditis elegans* development. The *C. elegans* predicted open-reading-frame, T08D10.1/*nfya-1*, codes for member of the CCAAT-box family of DNA-binding proteins with homology to the nuclear factor Y family. T08D10.1 is also codes for multiple potential phosphorylation sites and putative docking sites for extracellular regulated kinase (ERK) MAP kinase, suggesting that it may function as a transcription factor that is regulated by Ras signaling during development. Preliminary data indicate that T08D10.1 acts as a high affinity substrate for ERK MAP kinase and functions to inhibit Ras signaling during *C. elegans* vulval development through transcriptional repression. We have characterized the function and expression of this novel component of the Ras signaling pathway through biochemical and genetic methods. T08D10.1 appears to function as a high affinity substrate of ERK MAP kinase *in vitro*, and this activity is reduced upon mutation of an ERK MAP kinase docking site sequence within the protein. Km values of wild-type T08D10.1 are similar to other characterized ERK substrates such as myelin basic protein and LIN-1. Loss of *nfya-1* function through RNAi experiments and deletion mutant analysis has suggested that this gene plays a role in regulating Ras-mediated developmental processes such as vulval development and the formation of the male tail. The temporal and spatial expression pattern of *nfya-1* have been investigated through analysis of transgenic animals expressing transcriptional GFP reporter constructs and has confirmed previously reported results indicating a role in vulval and male tail development. The function of T08D10.1 as a transcription factor has been tested using transactivation potential assays conducted in cultured cells. These assays suggest that T08D10.1 functions to inhibit promoter activity, and the induction of the Ras signaling pathway can relieve this inhibitory function. This research has provided evidence that T08D10.1 functions as a transcription factor that is directly regulated by Ras/MAP kinase mediated signaling to mediate vulval and male tail developmental fates.

668B

A Mass Spectrometry-Based Approach to Identify New Interaction Partners of the Tyrosine Phosphatase DEP-1. **Michael Walser**, Alex Hajnal. Institute of Zoology, University of Zurich, Switzerland.

During hermaphrodite vulval development six vulval precursor cells (VPCs, P3.p–P8.p) are competent to generate vulval tissue and adopt an invariant pattern of three cell fates. The anchor cell secretes the LIN-3 EGF growth factor that activates in P6.p the EGFR/RAS/MAPK pathway to specify the primary (1°) cell fate. Subsequently, a lateral signal from P6.p inhibits the 1° and induces the secondary (2°) cell fate in the neighboring VPCs P5.p and P7.p via the LIN-12 Notch pathway. Several negative regulators of the EGFR/RAS/MAPK signaling pathway, such as LIP-1 and the density enhanced phosphatase DEP-1 are up-regulated in P5.p and P7.p to inhibit the primary fate specification in these VPCs.

DEP-1, which is a member of the class III receptor protein tyrosine phosphatase family (den Hertog 1999), negatively regulates LET-23 EGFR signaling (Berset et al. 2005). Human Dep-1 is often mutated in colon, breast, skin, and lung carcinomas (Ruivenkamp et al. 2002). Despite its importance as a tumor suppressor in various epithelial tissues, the physiological functions of mammalian Dep-1 are still poorly understood. Furthermore, the available data are based almost exclusively on *in vitro* experiments. Therefore the role of Dep-1 in cell fate specification, pattern formation during normal development, tumorigenesis, as well as its physiological substrates have still to be elucidated.

In order to identify new interaction partners of DEP-1 in *C. elegans*, we are performing mass spectrometry based approaches. For the co-purification of DEP-1 together with its physiological binding partners we are using HA-Strep-III- and GST-tagged versions of wild-type DEP-1 and a substrate trapping mutant in the catalytic phosphatase domain. After affinity purification of the tagged fusion proteins, the interacting partners can then be identified by mass spectrometry. Progress of these approaches will be presented at the meeting.

669C

Identification of *dsh-2* Genetic Interactors that regulate Asymmetric Neuroblast Division through a β-catenin Independent Pathway. **Kyla Hingwing**[1], Sam Lee[2], Tim Walston[3], Jeff Hardin[4], Nancy Hawkins[1]. 1) Dept Molecular Biol, Simon Fraser Univ, Burnaby, BC, Canada; 2) Dept. of Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 3) Dept. of Science, Truman State University, Kirksville, MO, USA; 4) Dept. of Zoology, University of Wisconsin-Madison, Madison, WI, USA.

Asymmetric neuroblast division is essential to generate neuronal diversity during nervous system development. In Caenorhabditis elegans, Wnt signaling regulates many asymmetric cell divisions. During embryogenesis, the C. elegans Dishevelled (Dsh) homolog, DSH-2, regulates asymmetric neuroblast division in the lineage that generates the PHA sensory neuron. MOM-5, a Frizzled homolog, and CWN-1, a Wnt homolog, are also required for asymmetric division in this lineage. Dsh is a key intracellular component of both β-catenin dependent and β-catenin independent Wnt pathways. Through a domain analysis of DSH-2, we have determined that a β-catenin independent Wnt pathway regulates asymmetric division in the PHA lineage. To identify additional components involved in this division, we performed a large-scale genetic screen to isolate suppressors of *dsh-2* asymmetric neuroblast division defects. Over 60 suppressors were obtained and all were dominant. These suppressors may be activating mutations in downstream pathway components or in a parallel pathway that regulates the same division. Two of the strongest suppressors have been chosen for further characterization. One suppressor, *Sup305*, maps to the middle of the X chromosome while the second suppressor, *Sup245*, maps to the right arm of chromosome I. Further mapping experiments are ongoing to clone the corresponding genes. We are also undertaking a further phenotypic and genetic characterization of the two suppressors to determine the specificity of their function. In addition to PHA, loss of *dsh-2* function affects the production of many additional neurons. We are determining if the suppressors are specific for the PHA lineage or suppress other *dsh-2* defects. Also, we are testing if these suppressors are specific for DSH-2 or if they also affect processes regulated by the two other Dsh homologs in C. elegans, MIG-5 and DSH-1. We are also investigating whether these suppressors display genetic interactions with other Wnt signaling mutants. Thus far, we have shown that *Sup245* also suppresses *lin-17*/Frizzled asymmetric division defects of the somatic gonadal precursor cells, Z1 and Z4. These studies will provide insight into the role of β-catenin independent Wnt signaling in asymmetric neuroblast division.

670A

Semi-automatic system for the creation of cell shape models in *C. elegans* embryogenesis. **Hideaki Hiraki**, Yumiko Ueta, Yuji Kohara. Genome Biology Lab. National Institute of Genetics, Mishima, Japan.

Cell to cell interactions play critical roles in early embryogenesis, therefore, it is very important to have information about the arrangements of cells, cell shapes and the contact among them. We have been developing a computer system to create cell shape models from a time series of confocal microscopic images of the embryo whose plasma membrane is stained with a vital fluorescent dye or is marked with a fluorescent protein. This system consists of two subsystems. In the first step, cell shapes are automatically calculated by a seeded region growing algorithm from a 3D image and a set of seed point coordinates. Manual editing of the seed coordinates is required and is assisted by its graphical user interface. The second step is an active balloon model under gradient vector flow executed on a PC cluster system. This step removes the bumps of cell shapes derived from image noises. Consequently, cell-to-cell contacting areas can be quantified.

We recorded images of OD58, the strain expressing GFP targeted to the plasma membrane, with a multiphoton confocal microscope. We used glass bottom dishes to avoid variable pressure from coverslips. We could record for 1.5 hours from one cell stage on the condition that the embryos continued the development and hatched successfully after the recording. Ten normal embryos were recorded and their cell shape models were built. We analysed the time course of each cell-to-cell contacting area by fitting a line to its graph and found that some of them increased or decreased consistently from embryo to embryo and others were variable in this respect. This suggested that some part of cellular arrangement was intrinsically variable and that the consistency of cell-to-cell contacts was not necessary to be complete for normal embryogenesis.

We are improving the system more, and plan to apply this system to compare the cellular arrangements and the cell-to-cell contacts among mutant embryos and the embryos from other species closely related to *C. elegans*.

671B

Regulation of WRM-1/β-catenin by the cell-cycle regulator CDK-1 during asymmetric cell division in *C.elegans*. **Takao Ishidate**[1], Soyoung Kim[1], Masaki Shirayama[1], Rita Sharma[1,2], Craig Mello[1,2]. 1) Program in Molec Med, Univ Massachusetts, Worcester, MA; 2) Howard Hughes Medical Institute.

In the 4-cell stage *C.elegans* embryo, the β-catenin related protein WRM-1 responds to a polarizing signal that induces the EMS cell to divide asymmetrically and accumulates in the nucleus of posterior daughter E. WRM-1 localizes uniformly to the cell cortex in EMS during the interphase, however, at the onset of M-phase, it dissociates from the cortex proximal to the signaling cell P2 in a Wnt-signaling dependent manner. We noticed a conserved region in WRM-1 that contains potential phosphorylation sites for CDK-1 and GSK-3. Through in vitro studies we found that CDK-1 can phosphorylate WRM-1 directly at this site priming WRM-1 for subsequent phosphorylation by GSK-3. WRM-1 proteins with mutations at either the CDK-1 and GSK-3 sites failed to be released from the cortex and its downstream asymmetric nuclear accumulation was also diminished. In a rare temperature-sensitive mutant of *cdk-1*(ne2257) that exhibits a partial defect in Wnt signaling, WRM-1 dissociation from the cortex was found to be defective. Consistent with these findings, mutant CDK-1 isolated from the embryonic extract showed reduced kinase activity toward WRM-1 recombinant protein. To investigate the physiological significance of these observations in vivo, we have used mosSCI to introduce single-copy insertions of wild-type and mutant WRM-1 constructs. While our interpretations of these preliminary studies are still complicated by a lack of full rescue from the wild-type construct, we were encouraged to find that for each different construct, Western-blot analysis revealed that independent transgene lines exhibited very reproducible levels of expression. We were surprised, however, to find that the mutation of the CDK-1 site resulted in a significantly stronger rescue of the *wrm-1* mutant strain, and also resulted in much higher levels of protein expression in each of the 2 lines examined. Similar evidence for increased activity from this mutant WRM-1(ΔCDK) construct was observed from a bombardment derived transgenic line. The GSK-3-site deletion construct did not exhibit this enhanced level of rescue and expression. These findings support the notion that WRM-1 is a direct regulatory target of CDK-1, and call into question the importance of its cortical localization during endoderm induction. We are currently trying to improve the transgene rescue utilizing different WRM-1 constructs. Through these studies, we would like to shed more light on the potential involvement of cell cycle regulator in developmental decisions.

672C

Study on localization mechanisms of maternal *mex-3* mRNA in *C. elegans*. **Hiroyuki Konno**[1], Koki Noguchi[2], Yuji Kohara[1,2]. 1) Department of Genetics, The Graduate University for Advanced Studies (SOKENDAI), Japan; 2) Genome Biol Lab, Natl Inst Genetics, Mishima, Japan.

In *C. elegans* embryos, several maternal mRNAs are localized to a subset of blastomeres, yet little is known about the mechanisms. We have been studying on localization mechanisms of maternal *mex-3* mRNA, which is localized to anterior blastomeres in embryos. After fertilization the *mex-3* mRNA is gradually localized to the anterior half at the one-cell stage, and is predominantly localized in the anterior AB cell at the two-cell stage. To identify the cis-element for *mex-3* mRNA localization, we firstly produced a reporter plasmid construct consisting of the *mex-3* coding sequence fused in-frame to the VENUS sequence and the *mex-3* 3' untranslated region (3'UTR) , which is driven by the *pos-1* promoter. The plasmid construct was introduced into *C. elegans* by bombardment to generate transgenic lines. mRNAs transcribed from the construct was detected by *in situ* hybridization using the venus antisense sequence as a probe. The localization of the mRNA was the same as that of the endogenous *mex-3* mRNA, suggesting that the mRNA transcribed from the construct is under control of *mex-3* mRNA localization machinery. Secondary, we generated transgenic lines of reporter plasmid constructs that contained various parts of the *mex-3* gene by bombardment. As a result, the mRNA containing the 3'UTR of the *mex-3* gene was localized to the anterior blastomere, suggests *mex-3* 3'UTR has major activity for mRNA localization. On the other hand, the 3'UTR dependent mRNA localization was disrupted by deletion of a 35-nucleotide sequence in the 3'UTR, indicating that the 35-nucleotide sequence is important for the *mex-3* mRNA localization. We are currently testing whether the 35-nucleotide is sufficient for mRNA localization.

673A

A screen for temperature-sensitive embryonic lethal mutations affecting cell focussing in *Caenorhabditis elegans*. **Nadin Memar**, Katharina Martin, Anne Wiekenberg, Ralf Schnabel. Institut für Genetik, TU Braunschweig, Braunschweig, Germany.

Schnabel et al. (2006) proposed cell focussing as mechanism for global cell sorting by cell identity in the *C. elegans* embryo. It should be helpful to find mutants to define the mechanism of this process. Interestingly a very large collection of nonconditional embryonic lethal mutants did not contain a mutant affecting cell focussing. We supposed that genes affecting this process might be highly pleiotropic, since it should also keep larvae and adults in shape and/or maternal rescue may occur in the embryo. Therefore, ts mutants may be the tool of choice to identify genes. We designed a new screen for ts mutants using the worm sorter, since a very large number of mutants may be required but the yield is less than 1%. Identification of specific mutants is very cumbersome since candidates have to be subjected to a careful 4D analysis. Only mutants in which fates are wild-type–but the positioning of subsets of cells is wrong–qualify as potential cell focussing mutants. A first screen yielded two candidates in approx. 1000 ts mutants. In the next round we isolated again 1000 mutants, which now await analysis. We expect that up 10000 ts mutants may be required to define a cell focussing pathway. However, the mutant collection may be also a valuable resource for the worm community.

674B

A partial E to MS restoration in *pop-1 end-1 end-3* triple mutant embryos. **Melissa Owraghi**, Maduro Morris. Biology Dept, UCR, Riverside, CA.

The E and MS blastomeres are sister cells born at the 7-cell stage of embryonic development. E makes the entire endoderm (gut), while MS makes many mesodermal cell types, including pharynx, body muscles and the four embryonically-derived coelomocytes. The E cell is specified by the combined activities of two similar genes, *end-1* and *end-3,* while MS is specified by the T-box gene *tbx-35* and another regulator, *ceh-51* (see abstract by Broitman-Maduro et al. this meeting). The Wnt nuclear effector POP-1 is critical to making MS and E different from one another. The MS-to-E transformation that results from loss of *pop-1* function is well documented (e.g. Lin et al., 1995), and shows that the main requirement for POP-1 in *C. elegans* is in repression of endoderm fate in MS. Recently, our laboratory has reported that E adopts an MS-like fate in *C. briggsae* embryos depleted for *Cb-pop-1* function. This and other observations prompted us to look for the requirements of POP-1 in specification of MS in *C. elegans*. Because of the ectopic expression of *end-1* and *end-3* in *pop-1(-)* embryos, however, we cannot observe effects on MS fate. As well, the only way to genetically eliminate *end-1,3* has been to use large deficiencies. Instead, to eliminate *end-1,3* activity we have constructed double mutants for *end-1* and *end-3* using the *ok558* deletion in *end-1* and either the *ok1448* or *zu247* mutations in *end-3*. In both cases, specification of endoderm was found to be abolished in *end-1,3* double mutants, although the phenotype is surprisingly mild: Most embryos elongate, and many hatch into (inviable) larvae.

We examined *pop-1; end-1,3* mutant embryos and found that although such embryos lack endoderm (as expected), they generate additional tissues that are normally made by MS, specifically pharynx muscle, body wall muscle, and coelomocytes. Using a laser microbeam to isolate MS or E, we have found that both cells make these tissues in *pop-1; end-1,3* mutants. However, E isolations in the triple mutant embryos also express the hypodermal marker *nhr-25*::YFP, suggesting that E generates some C-like tissues in the absence of *end-1,3*. Collectively, these results demonstrate that E is only partially restored to an MS-like fate. The conclusion is that POP-1 is dispensable for some aspects of MS specification in *C. elegans end-1,3* mutants, and that there may be a role for POP-1 in blocking MS fate in E. We will present the details of these studies and further genetic evidence that support a model in which POP-1 contributes to normal E specification in part by blocking MS specification in E.

675C

The cytoplasmic cell fate regulator MEX-5 is required for the establishment of cortical polarity. **Silke Reiter**, Carrie R. Cowan. IMP, Vienna, Austria.

MEX-5 is a cytoplasmic regulator of cell fate in C. elegans. MEX-5 is thought to act downstream of PAR polarity to restrict maternally supplied germline proteins to the germline (P) lineage. However recent reports (Cuenca et al. 2003) suggest that MEX-5 may have an earlier role in polarity establishment. To investigate the role of MEX-5 in polarity establishment, we are using quantitative time-lapse microscopy to analyze MEX-5 depleted one-cell embryos. Depletion of MEX-5 results in a failure to establish PAR polarity at the onset of the first cell cycle. Loss of MEX-5 does not affect overall acto-myosin contractility, centrosome maturation, or centrosome-cortex proximity, known prerequisites for polarity establishment. Thus MEX-5 defines a novel regulatory point in polarity establishment. MEX-5 is a zinc finger protein with RNA binding capability. We are currently investigating whether RNA binding is important for MEX-5's role in polarity establishment and identifying MEX-5 regulated RNAs required for polarization.

676A

ELT-7 strongly synergizes with ELT-2 to regulate intestinal differentiation. **Erica M. Sommermann**[1], Keith R. Strohmaier[1], Morris F. Maduro[1,2], Joel H. Rothman[1]. 1) Department of Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, CA; 2) Current Address: Department of Biology, University of California, Riverside, CA.

Specification of the *C. elegans* endoderm (E lineage) is directed by a transcriptional regulatory cascade that includes maternally provided SKN-1, its targets, the genes encoding the MED-1 and -2 GATA-like transcription factors, and their targets, the genes encoding the END-1 and -3 GATA factors. The transition from endoderm specification to intestinal differentiation is mediated in part by the ELT-2 GATA transcription factor. Based on recent comprehensive SAGE analyses, McGhee and colleagues proposed that ELT-2 is the dominant regulator of intestinal differentiation, and that other GATA transcription factors expressed in the late developing intestine, ELT-7 and ELT-4, play at most a subsidiary role. However, the intestines of *elt-2(0)* animals, while defective in function, are well-differentiated, and contain a well-developed brush border and rhabditin granules throughout. The most striking phenotypes of *elt-2(0)* animals are larval lethality and an obstructed gut lumen that is otherwise clearly evident; in short, these mutants appear to undergo largely normal gut differentiation, implying that ELT-2 functions with other factors to direct gut differentiation. A candidate for such a factor is ELT-7, which we found shows gut-specific expression at the appropriate time in development and whose expression is sufficient to activate gut differentiation when expressed outside of the E lineage. Indeed, while *elt-7(tm840)V* mutants are essentially wildtype, we found that intestinal differentiation is dramatically impaired in an *elt-7(tm840)V;elt-2(ca15) X* double mutant. ~80% of *elt-7(0); elt-2(0)* worms (n = 41) contain no conspicuous lumen and only periodic patches of gut granules, and 17% show only sporadic differentiation of brush border at the anterior and posterior termini of the gut. We observe apoptotic corpses in the anterior third of the intestinal region in about half of the arrested double mutant worms, suggesting that a failure to properly regulate intestinal differentiation may lead to derepression of apoptosis. Thus, as is the case with the END factors in endoderm specification, ELT-7 and ELT-2 strongly synergize to achieve intestinal differentiation.

677B

Asymmetric regulation of the homeobox gene *ceh-5* in early embryogenesis of *C. elegans*. **Lois H Y Tang**[1], Konstantin Cesnulevicius[1], Jurgen Hench[1], Akram M Abou-Zied[2], Thomas Burglin[1]. 1) Dept. of Biosciences and Nutrition, Karolinska Institutet and Södertörn University, Stockholm, Huddinge, Sweden; 2) King Abdull-Aziz University, Faculty of Medical Sciences, Saudi Arabia.

Bilateral symmetry of an animal's body plan is one of the unique features of higher animals. For instance, the nervous system is largely bilaterally symmetric on a morphological level, yet often displays striking degrees of functional left/right (L/R) asymmetry. It remains unclear how functional asymmetries of morphologically bilaterally symmetric structures are established within the nervous system and how they relate to visceral asymmetries. The homeobox genes provide an interesting entry point to study how left/right asymmetries are generated in the nervous system (Chang et al., 2003; Johnston et al., 2006), given that little is understood about cell fate specification after the beginning of gastrulation.

Homeobox genes are highly conserved transcription factors that play key roles in the development of humans and animals (Burglin, 2005). *ceh-5*, an ortholog to the human VAX genes, shows a unique expression pattern during early embryogenesis. It is strongly expressed in two distinct groups of cells that later would become neurons: one laterally and one anteriorly. Both expressions are chronologically and spatially separated. In addition, a third group of cells also expresses CEH-5 but at a much lower level. This group is in mirror-symmetry to the first expression pattern that is expressed in the lateral group. Therefore, we believe that *ceh-5* displays left/right asymmetry in this manner.

We are interested to dissect the promoter region of *ceh-5* to understand how the distinct spatio-temporal expression is generated. We created a series of deletions in the promoter region of *ceh-5* fused to GFP that were used to make transgenic animals, which were monitored using 4D-imaging in live animals lab (please see the two abstracts from J. Hench et al. and J. Henriksson et al. at this meeting). This investigation revealed a 21bp in the promoter region is essential for the regulation of *ceh-5*. This motif is highly conserved between different *Caenorhabditis* species. In order to look for transcription factors involved in regulating the expression of *ceh-5*, we screen via RNAi knockdown searching for candidates that would create an altered *ceh-5* expression pattern. Although the screen is far from saturation, a putative transcriptional regulator of *ceh-5* has been identified.

678C

Branched-chain fatty acid C17ISO is involved in a novel mechanism that regulates post-embryonic growth and development in C. elegans. Marina Kniazeva, Emylie Seamen, Jennifer Blanchette, Tanya Euler, Rencheng Wang, Max Cohen, Spencer Watson, **Min Han**. Dept of MCDB, HHMI and Univ Colorado, Boulder, CO.

Growth and development of animals are regulated by signaling systems that sense the availability of nutrients and metabolic status. In C. elegans, the DAF-2(InR)/DAF-16(FOXO) pathway are known to regulate post-embryonic development in response to changes in food availability. We have shown that a monomethyl branched-chain fatty acid (mmBCFA), C17ISO, a product of leucine catabolism, is an essential factor for postembryonic development. Worms depleted of C17ISO arrest uniformly in L1 prior to the 1st M cell division; this phenotype can be reversed by feeding animals with chemically synthesized C17ISO. Our work also suggests that C17ISO acts in a novel mechanism in parallel to DAF-2/DAF-16, and that the two pathways converge on the expression of cki-1 that represses cell cycle. We also show that C17ISO homeostasis is regulated by an SREBP-1c-mediated specific feedback mechanism. We hypothesize that C17ISO acts as a nutritional factor in a mechanism that regulates post-embryonic growth and development. To identify factors acting in the C17ISO-involved pathway, we have carried out screens for mutations that suppress the L1 arrest phenotype caused by C17ISO depletion (elo-5(-) mutation). After screening more than 10,000 haploid genomes, we obtained 9 suppressors that may be divided into three classes: (1) two suppressors recovered the ability of the elo-5(-) strain to synthesize C17ISO; (2) three suppressors, all alleles of one single gene, permited the C17ISO-depleted animals to pass L1 arrest, but did not support development in subsequent generations without mmBCFA supplement; (3) four suppressors permited C17ISO-depleted animals not only to bypass L1 arrest, but also support continuous growth in following generations without mmBCFA supplement. Class (1) suppressors likely alter the activity an enzyme to compensate for the loss of ELO-5. The gene defined by the class (2) suppressors acts to repress the C17ISO function; Loss-of-function mutations may increase the activity of C17ISO mediated function, but not completely bypass the requirement of mmBCFAs. This gene encodes a membrane bound protein likely involved in lipid translocation. To understand its role, we have also isolated two enhancer mutations that caused a lethal or sterile phenotype only in combination with a class (2) suppressor allele. Genetic mapping indicated that class (3) mutations are alleles of at least three different genes that may potentially define negative factors acting downstream of C17ISO to regulate L1 growth. Position cloning of several genes defined by suppressor/enhancers is underway.

679A

Robust Cell Detection for Automated Lineaging in Time Lapse Confocal Microscopy. **Anthony Santella**, Zhirong Bao. Developmental Biology, Sloan Kettering Institute, NY, NY.

Reliable, automated cell detection is a critical component of automated biological image analysis. Accuracy is particularly important for efficient, high-throughput cell lineaging. When tracing a C. elegans lineage overall detection error rates as low as one or two percent result in lineages that can require hours of hand editing to correct. To achieve more reliable automation we are investigating image processing approaches tailored to the appearance of nuclei in confocal fluorescence images. Our approach uses a Difference of Gaussians blob detector to guide an efficient extraction of the nuclear boundary as a set of disks. Preliminary results show a significant improvement in detection rates over Starrynite, our existing detection and lineaging system. Total detection error during the 9th stage of cell division drops from 4.5 to 1.1 %, overall error through the 9th round is reduced from 1.5 to .78%. The method is both accurate and fast enough to be used in real time imaging applications. Results suggest that with further development on extraction and tracking, editing will cease to be a bottleneck. This will enable automated lineaging through the previously daunting 10th and final round of cell divisions and make possible uniquely detailed, large-scale studies of embryonic development.

680B

A view on the embryogenesis of the *Caenorhabditis* Family. Nadin Memar, **Katharina Martin**, Ralf Schnabel. Institut fuer Genetik, TU Braunschweig, Braunschweig, Germany.

The molecular differences of the four *Caenorhabditis* species *C. elegans, C. briggsae, C. remanei* and *C. brenneri* are currently of great interest, however little is known about development. Zhao *et al.* (2008) reported an automatic lineage of *C. briggsae* and came–based mostly on the cleavage pattern and cell positions–to the conclusion that the embryogenesis of the two species is very similar. We now present detailed 4D analyses of the species including the terminal differentiation patterns. All analyses including bioinformatical quantifications of cell behaviour show a huge similarity between those species. Immunochemical analyses of the tissue distributions only reveal a difference in the intestinal differentiation of *C. brenneri*. Interestingly hybrid embryos always appear to fail in different ways in embryogenesis.

681C

Novel regulators of RNT-1/BRO-1 induced hyperplasia. **Samantha L. Hughes**, Sara Maxwell, Alison Woollard. Dept Biochemistry, Oxford University, Oxford, United Kingdom.

The balance between cell proliferation and differentiation is controlled, in part, by a complex of runx and CBFβ. In *C. elegans rnt-1* and *bro-1* (homologs of mammalian runx and CBFβ) are expressed in seam cells and control their divisions in a stem-like pattern. During development, seam cells undergo asymmetric divisions to produce daughter cells which fuse with the hypodermal syncytium. Sensory rays in male tails are formed from precursors which arise as a consequence of additional seam cell divisions. Nematodes containing an integrated array of *rnt-1* and *bro-1*, AW257, have an excessive number of seam cells; approximately 40 compared to a wild type number of 16. Seam cell hyperplasia laterally expands the seam giving the nematode a dumpy phenotype. Suppressors of the hyperplasia are relatively easy to isolate as they would arise as a return to wild type appearance. Here we present the results of a full genome RNAi screen to identify suppressors of hyperplasia.

682A

Examining the interaction of the T-box factor MLS-1 with UNC-37/Groucho in determination of uterine muscle cell fate. **Raymond R Miller**, Tanya Crum, Peter G Okkema. Department of Biological Sciences and the Laboratory for Molecular Biology, University of Illinois at Chicago.

T-box family transcription factors play important developmental roles in all multi-cellular animals, including in the development of the limb, heart, and pituitary. However, the mechanism by which these factors function is largely unknown. In many cases, the study of T-box factor function is difficult because of the complex phenotypes T-box factor mutants display, and the difficulty in rescuing loss of function. In *Caenorhabditis elegans*, the T-box factor muscle lineage specification-1 (MLS-1) is responsible for the decision of precursor cells between vulval and uterine muscle fate. The *mls-1* loss of function phenotype is easily examined, and is readily rescued using transgenes. We are using *mls-1* to explore the mechanism of T-box factor function. We have found that MLS-1 interacts with the *C. elegans* Groucho family co-repressor UNC-37 in yeast 2-hybrid experiments, and this interaction is dependent on an MLS-1 engrailed homology 1 (eh1) motif. At low penetrance, *unc-37(RNAi)* phenocopies the *mls-1* loss of function phenotype producing extra vulval muscles, suggesting MLS-1 requires UNC-37. We are currently performing *in vivo* studies to determine if MLS-1 functions as an UNC-37/Groucho dependent transcriptional repressor. We have found that UNC-37 also interacts with the T-box factor TBX-2 in yeast 2-hybrid assays via a degenerate eh1 motif. eh1 motifs are found in several T-box factors in different species, and we hypothesize interaction with Groucho family co-repressors is a conserved feature of T-box factor function.

683B

PUF family translational regulators and their roles in cell fate specification. **C. J. Herrmann**, A. Hajnal. University of Zurich, Zurich, Switzerland.

Members of the conserved PUF (Pumilio and FBF) protein family selectively bind the 3'UTR of their target mRNAs to regulate translation and thereby exert spatiotemporal control on various developmental processes, such as proliferation and self-renewal of stem cells. In the *C. elegans* germline, the PUF proteins FBF-1 and FBF-2 maintain germline stem cells by restricting the activity of the differentiation- and apoptosis promoting MAP-Kinase (MAPK). FBF-1, FBF-2 and PUF-8 also act in the soma to influence cell fate decisions in the *C. elegans* vulva.

During vulval development, cell fates are specified by differential activation of the RAS/MAPK pathway in adjacent vulval precursor cells (VPCs). The cell with the strongest RAS/MAPK activation adopts the primary cell fate and induces the lateral inhibition signal (LIN-12/Notch) in the neighbouring VPCs to lower MAPK signalling and promote the secondary cell fate. PUF-8 was shown to restrict the competence of VPCs to respond to inductive signals, whereas the FBFs restrain the RAS/MAPK signal to ensure that only one cell adopts the primary cell fate (Walser et al., 2006).

Although the consensus binding sites for PUF proteins have been determined, the physiological mRNA targets of the FBFs and PUF-8 in distinct cells are to date unknown. In order to elucidate the different mechanisms by which individual PUF proteins influence cell fate decisions, we are employing an RNA binding protein immunopurification protocol by expressing PUF proteins fused to an HA-Strep-tag III in worms. mRNA targets will then be determined by microarray analysis of co-purified RNA (RIP-Chip), and interaction partners of the PUF proteins will be identified by mass spectrometric analysis. Finally, putative targets and interaction partners will be extensively analysed by genetic, biochemical and bioinformatics means.

684C

Phenotypic characterization of cell fusion in *eff-1* alleles. **Ksenia Smurova**, Tamar Gattegno, Nirit Assaf-Reizel, Benjamin Podbilewicz. Technion- Israel Institute of Techology, Haifa, 32000, Israel.

Developmental cell fusion is an important process in eukaryotes. The *eff-1* (epithelial fusion failure) gene encodes membrane proteins required for epithelial cell fusion in *C. elegans*. *eff-1* encodes two single-pass integral membrane proteins and two secreted polypeptides by alternative splicing. The transmembrane proteins contain a long extracellular portion, with a TGF-β receptor type 1 domain and 8 pairs of cyteines, a transmembrane domain, and a short intracellular tail. Comparison between the phenotypes caused by different alleles of *eff-1* enables us to create an allelic series and deduce the importance of the mutated domains to the function of the proteins. We isolated six viable cell fusion mutants with a broad cell fusion blockage through all developmental stages (*hy32*, *hy40*, *hy41*, *hy42*, *hy43* and *hy44*). All the new alleles failed to complement *eff-1(hy21)* and we compared them with *eff-1* alleles that were previously characterized (*hy21*, *np29*, *ok1021* and *oj55*). Cell junctions in embryos and larvae were visualized using immunofluorescense with MH27 antibodies. We analyzed the projections of 3D stacks that were obtained by confocal microscopy. In all *eff-1* mutants, except *oj55*, *hy43* and *hy44*, all the embryonic hypodermal cells precursors to hyp6 and hyp7 failed to fuse. Surprisingly, we found partial embryonic hypodermal fusion in *oj55*, *hy43* and *hy44* embryos. *hy40* displayed the most severe Eff-1 phenotype that included Dumpy-Unc animals, deformed tail tip and a complete cell fusion blockage through all stages of development. *hy40* resulted in a stop codon at amino acid 224; this short protein represents about half of the extra cellular part of the EFF-1 protein. *hy41* gave rise to a missense mutation of the extracellular part of the EFF-1 protein. *hy41* also showed rather severe Eff-1 phenotype. *hy32* mutation is a cystein 135 change at the TGF-β receptor type 1 like domain of EFF-1. *hy32* shows a more severe phenotype than *hy21* allele and a complete loss of embryonic and larval cell fusion revealing the importance of the C135 in the structure and function of the fusogenic activity of the extracellular domain and in particular the domain that may fold as a TGF-β receptor type 1 like domain. *hy42*, *hy43* and *hy44* showed less severe phenotypes and have variable fusion blockage during development. We are working on the molecular characterization of these mutations.

685A

Drug discovery towards preclinical molecules that halt the gluatamatergic neuron degeneration caused by human Aβ42 in C. elegans. **Y. shu**, S. Cao, S. Parker, K. Caldwell, G. Caldwell. Department of Biological Sciences, University of Alabama, Tuscaloosa, AL.

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease. The cost associated with the treatment and the care of AD patients represents a huge economic burden on our society. Current treatments are only symptomatic and do not halt the progression of the disease over time. Thus, there is a dire need to expedite the discovery of preclinical drug candidates that can treat the disease at its root, neurodegeneration. One of the pathological hallmarks of AD is the loss of the gluatamatergic neurons in the brain. Taking advantage of the small number of glutamatergic neurons in C. elegans, we established a transgenic model overexpressing a secreted form of human amyloid-beta fragment (Aβ42) specifically in the glutamatergic neurons. By labeling these neurons with green fluorescent protein, we were able to observe the age-dependent neurodegeneration caused by Aβ42. Subsequently we utilized this model to screen through 210 compounds that were previously selected for their structural diversity and low toxicity in worms. Notably, the majority of these molecules are FDA-approved, making them more likely to produce candidates that will succeed in clinical trials. After the primary screen and two rounds of confirmation screen, we identified three compounds that suppressed neurodegeneration when applied to worm growth media. In order to generate the best candidates for clinical trials, we are planning to test more compounds within each class of chemical structure. By structure-activity relationship (SAR) analysis, we will be able to generate information that can help us better design the chemical structure of potential candidates. Furthermore, any promising candidate will also be tested in a cell-culture-based Aβ42 model to confirm its cross-species activity. It is our hope that by combining the cost-effectiveness and the speed of cultivating worms, we worm researchers as a community can lend a hand to easing the bottleneck of AD drug discovery.

686B

Dopamine Signal Transduction Components Alter Neurodegeneration in a Parkinson's Disease Model. **Laura A. Berkowitz**, Shu Hamamichi, Kim A. Caldwell, Guy A. Caldwell. Dept Biological Sci, University of Alabama, Tuscaloosa, AL.

Improvements to the diagnosis and treatment of Parkinson's disease (PD) are contingent upon knowledge about susceptibility factors that render populations at risk. Our work has focused on a functional analysis that exploits the experimental attributes of *C. elegans* to accelerate the translational path toward identification of effectors of dopamine (DA) neuron degeneration *in vivo*. *C. elegans* has only 8 DA neurons, providing a simple system in which to study PD-like neurodegeneration with unparalleled accuracy in quantitation of neuron survival in statistically substantial numbers of test animals. Our prior application of *C. elegans* in this directed manner has been successful in rapidly predicting genes that have significant consequences for neuronal survival in mammalian systems (Gitler et al., 2008; PNAS; Gitler et al., 2009, Nat. Genetics)

Human α-synuclein, a protein found in the PD-associated aggregates called Lewy bodies, when overexpressed in *C. elegans* DA neurons, causes them to degenerate in an age- and dose-dependent manner. GAIP Interacting Protein C-terminus (GIPC) was identified in a large-scale RNAi screen for genes that alter both α-synuclein aggregation and DA neuron degeneration (Hamamichi et al., 2008, PNAS). GIPC interacts with the RGS-GAP (GTPase Activating Protein) GAIP, which attenuates G-protein signals. Additionally, GIPC interacts with a number of G-coupled receptors including the dopamine receptor, where it has been proposed to play multiple roles in DA signal transduction such as: 1) membrane recruitment of GAIP (which down-regulates DA signaling); 2) DA receptor internalization; and 3) DA receptor recycling. Here we report that genetic loss of GIPC enhances neurodegeneration, whereas overexpression (OEx) is neuroprotective. To determine what GIPC-dependent function(s) and possible interactor(s) is involved in its neuroprotective capacity, proteins that act in the above pathways are being analyzed via genetic knockouts and DA neuron-specific overexpression. This analysis has revealed that loss of presynaptic dopamine D2 receptors (*dop-2*) increases degeneration. Moreover, the neuroprotective capacity of GIPC OEx is eliminated in a *dop-2* mutant background, suggesting that GIPC protects through the DA receptor, as opposed to acting via any number of other potential receptors. Loss of the *C. elegans* homolog of GAIP, *rgs-2*, is also protective while overexpression of this GIPC interactor enhances DA neuron loss. Taken together, these results suggest that components of the DA signaling cascade contribute to neuron survival. Further analysis of GIPC activity may provide mechanistic insights into PD.

687C

SUT-2 potentiates tau-induced neurotoxicity in C. elegans. **Chris R. Guthrie**[1,2], Brian C. Kraemer[1,2]. 1) Geriatrics Research Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, WA 98108; 2) Division of Gerontology and Geriatric Medicine, Department of Medicine, University of Washington, Seattle, WA 98104.

We have used our transgenic C. elegans model of human tauopathy diseases by expressing human tau in worm neurons to explore pathways that contribute to tau-induced neurodegeneration. Expression of mutated human tau causes uncoordinated locomotion (Unc) in C. elegans. This animal recapitulates produces several hallmarks of human tauopathies including accumulation of detergent insoluble phosphorylated tau protein and neurodegeneration. We carried out a forward genetic screen for mutations that prevent the tau-induced Unc phenotype in order to identify genes that control tau neurotoxicity. Recessive loss of function mutations in the sut-2 locus suppresses the Unc phenotype, tau aggregation, and neurodegenerative changes caused by human tau. We cloned the sut-2 gene and found it encodes a novel sub-type of CCCH zinc finger protein conserved across animal phyla. SUT-2 shares significant identity with the mammalian SUT-2 (MSUT-2). To identify SUT-2 interacting proteins, we conducted a yeast two hybrid screen and found SUT-2 binds to ZYG-12, the sole C. elegans HOOK protein family member. Likewise, SUT-2 binds ZYG-12 in in vitro protein binding assays. Furthermore, loss of ZYG-12 leads to a marked upregulation of SUT-2 protein supporting the connection between SUT-2 and ZYG-12. Of the three human orthologs of ZYG-12, MSUT-2 binds only to HOOK2. This suggests the interaction between SUT-2 and HOOK family proteins is conserved across animal phyla. Immunofluorescent microscopy of endogenous MSUT-2 revealed that MSUT-2 localizes nuclear domains known as nuclear speckles or interchromatin granule clusters. These nuclear sub domains are known to contain components of the pre-mRNA splicing machinery. The identification of sut-2 as a gene required for tau neurotoxicity in C. elegans may suggest new neuroprotective strategies capable of arresting tau pathogenesis in human tauopathy disease.

688A

Genetic dissection of the molecular functions of the dystrophin complex in *C. elegans*. **Hongkyun Kim**, Hyun Oh, Linu Abraham. Cell Biology & Anatomy, Chicago Medical School, Rosalind Franklin University, North Chicago, IL.

Duchenne muscular dystrophy is one of the most common neuromuscular diseases in humans with an incidence of one in 3,500 live male birth. Currently there is no cure for this disease. Mutations in dystrophin are responsible for this disease. Several genetic and biochemical studies have established that dystrophin forms a complex with several other proteins in the muscle membrane. This complex is collectively called the dystrophin associated protein complex, DAPC. Interestingly, components of the DAPC are responsible for many different forms of muscular dystrophies, including limb girdle muscular dystrophies. We previously established a genetic screen for identifying mutants that show the same locomotory phenotype as *dys-1* (a dystrophin homolog). Interestingly, the identified genes in this screen encode either known components of the DAPC or novel proteins. This fact implies that novel genes identified in our screen may be previously unidentified components of the DAPC and/or functions in the same pathway as the DAPC genes. One novel gene we identified in our study encodes a putative potassium channel. We show that this gene is expressed in body wall muscle. Furthermore, muscular expression of this gene rescues the characteristic locomotory phenotype of the mutant. This screen yielded *snf-6* (an acetylcholine/choline transporter) and *slo-1* (the voltage- and calcium-dependent BK potassium channel), both of which regulate muscle excitation negatively. Based on this data, we postulates that the novel gene also negatively regulates muscle excitation. We will present our further findings and ongoing model in the meeting.

689B

Establishing a worm model of motor neuron diseases involving the TAR-binding protein, TDP-43. **Michelle L. Tucci**, Stacey A. Fox, Guy A. Caldwell, Kim A. Caldwell. Dept Biological Sci, Univ Alabama, Tuscaloosa, AL.

TAR binding protein, TDP-43, is an RNA-binding protein that has been recently identified as a key factor in several motor neuron diseases, resulting in a broad classification of TDP-43 proteinopathies. This protein has been recognized as a common link between frontotemporal lobar dementia with ubiquitin positive inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS). Considering its multifaceted functionality, which includes mRNA splicing and transport, as well as transcriptional and translational regulation, the role of TDP-43 in association with these neurodegenerative diseases is uncertain. The normal nuclear localization of TDP-43 is altered in disease patient neurons, where this protein is found to be fragmented, mislocalized to the cytoplasm, hyperphosphorylated, and polyubiquitinated. In some previous studies, TDP-43 also forms aggregates and is toxic. We are establishing a worm model to investigate TDP-43 related diseases using both the C. *elegans* homolog, TDP-1, as well as the human protein. To conduct a preliminary analysis of human TDP-43 and ALS-associated mutations, we generated transgenic nematodes with expression of these genes driven under control of the UNC-54 promoter to monitor cellular localization in body wall muscles. We are also determining if overexpression of normal or mutant TDP-43 variants in specific neuronal classes (dopamine and GABA) result in neurodegeneration. There is approximately 40% homology between the human and C. *elegans* gene products; these share similar domains important for normal function, with the exception of a C-terminal glycine-rich domain, essential for splicing, being absent in the worm protein. Using C. *elegans*, TDP-1, we are further characterizing its role of TDP-43 in the disease state. In order to recapitulate the phenotype of mislocalization and aggregate formation, we have mutated the nuclear localization sequence (NLS) and are determining if mislocalization results in neurodegeneration in the aforementioned neuronal classes. By establishing a C. *elegans* model of TDP-43-associated dysfunction and/or neurodegeneration, we hope to facilitate the identification of genetic and chemical modifiers that may attenuate effects.

690C

Analysis of CED-4-dependent cell size control. **Ling Chen**, Joel H. Rothman. Neuroscience Research Inst, Univ California, Santa Barbara, Santa Barbara, CA.

Cells, organs, and organisms reach a reproducible size appropriate for their functions through the interconnected and complementary action of cell size regulation, cell proliferation, and apoptosis. Coordination of these processes is critical for normal development and their dysregulation can lead to pathologies such as tumor formation. Though apoptosis and cell size regulation are controlled by largely distinct molecular regulatory events, we found that these two critical processes are linked by the action of several factors, including TFG-1, the homologue of the human proto-oncogene TFG (TRK-fused gene), and the pro-apoptotic regulator CED-4. We found that while TFG-1 is a suppressor of apoptosis, its removal leads to decreased cell and nuclear volume, resulting in small adults (Sma phenotype). Mutations in *ced-4* suppress this defect in cell and nuclear size. However, simultaneous inactivation of the CED-3 and CSP-1 caspases does not suppress the diminutive cell size of adult *tfg-1(RNAi)* worms, suggesting that CED-4 acts through a caspase-independent mechanism to suppress cell growth. We found that CED-4 acts broadly to antagonize the action of a number of cell size-regulating proteins, including cAMP response element-binding (CREB) protein, translation initiation factor eIF2B, and the nucleolar p53-interacting protein nucleostemin, but not those functioning in the TGF-beta signaling pathway. CED-4-regulated body and cell size control is highly correlated with total protein level, suggesting that CED-4 might regulate cell size through modulation of protein production and accumulation. To better understand the role of CED-4 in cell size regulation, we conducted functional genomics screens for genes required for CED-4-dependent body and cell size regulation. Consistent with our observed correlation between CED-4-dependent cell size and protein content, genes involved in protein synthesis, including ribosomal structure proteins, aminoacyl-tRNA synthetases, and translation initiation factors, are enriched in the screen. Moreover, genes functioning in metabolic processes, including lipid metabolism and glycolysis, also affect body size in a CED-4-dependent manner, suggesting that metabolic changes might also contribute to CED-4-dependent cell size regulation. Thus, in addition to its central role in apoptosis, CED-4 performs a critical role in limiting cellular growth.

691A

Conserved genes and cellular pathways modulate Survival of Motor Neuron (SMN) loss of function defects. **M. Dimitriadi**[1], J.N. Sleigh[1,2], A.K. Walker[1], J. Harris[1], T. Barsby[1,2], G. Kalloo[1], A. Sen[3], C-HC. Chang[3], J.S. Satterlee[1], D. van Vactor[3], S. Artavanis-Tsakonas[3,4], A.C. Hart[1]. 1) Center for Cancer Research, Massachusetts General Hospital & Department of Pathology, Harvard Medical School, Boston, MA, USA; 2) University of Bath, Bath, UK; 3) Department of Cell Biology, Harvard Medical School, Boston, MA, USA; 4) Collége de France, Paris, France.

Spinal Muscular Atrophy (SMA) is caused by diminished function of the Survival of Motor Neuron (SMN) protein, but the neuromuscular pathways critical for SMA pathology remain unclear. SMN functions in spliceosome assembly, but SMN likely also acts in additional cellular processes including axonal mRNA transport. There is only one genetic modifier of SMA in humans; in female patients, SMA severity is dependent on the level of Plastin 3 (PLS3), an actin-bundling protein. The mechanisms underlying this interaction remain unclear. Identifying the genetic and molecular pathways pertinent to SMA should help us to understand SMA pathological mechanisms and accelerate therapy development. Here, we have used invertebrate models to identify genetic modifiers of SMA. *Drosophila* and *C. elegans* each have one SMN gene; diminished SMN function in these invertebrate models caused lethality and neuromuscular defects. We found that RNAi knockdown of PLS3 orthologs enhanced SMN loss of function defects in *C. elegans* and *Drosophila* validating these models. To identify additional conserved genes that modulate SMN loss of function defects across species, we used two strategies. First, *C. elegans* orthologs of previously described, candidate *Drosophila* SMA modifier genes were tested using *C. elegans* assays for function as cross-species SMN modifiers. We found that 13 of the 40 genes crossed invertebrate species and modified larval lethality and/or neuromuscular defects caused by loss of *C. elegans smn-1* function. Among the cross-species modifiers were *atf-6* and *atn-1*, which have been implicated in Amyotrophic Lateral Sclerosis (ALS) and plastin-associated pathways, respectively. Second, we undertook an independent *C. elegans* genome-wide RNAi screen for SMA modifiers and recovered one known and four previously unknown modifier genes. Thus far, two of these new genes modulate neuromuscular defects in *Drosophila* and suggest roles for SMN in pathways not previously implicated in neurodegeneration. Our ongoing studies address the importance of these pathways in SMN loss of function pathology. Overall, our results demonstrate that conserved genes and cellular pathways act as genetic modifiers of SMN loss of function and suggest mechanisms and potential SMA therapeutic targets.

692B

Small heat shock proteins protect against necrotic cell death. **Nikos Kourtis**, Nektarios Tavernarakis. IMBB, FORTH, Heraklion, Greece.

Necrotic cell death contributes to severe pathological conditions in humans such as trauma, stroke and neurodegenerative diseases. The molecular mechanisms underlying necrosis are not fully understood. The heat shock response is a highly conserved gene expression program, which is engaged under conditions of stress and orchestrates the coordinated expression of specific genes that protect cells against various stressors. We are investigating the role of the heat shock response in necrotic cell death. We find that activation of the heat shock response pathway by means of a brief heat shock treatment strongly suppresses necrotic cell death in *C. elegans*. This protective effect is not due to delay of necrosis initiation or removal of the necrosis initiating insult. Elimination of heat shock factor 1 (HSF-1), the master transcription regulator which orchestrates the heat shock response in *C. elegans*, abolishes the protective effect of heat shock. By contrast, overexpression of HSF-1 suppresses necrosis. While screening for potential mediators of the protective effect of heat shock, we found that the genes encoding for the small heat shock proteins HSP-16.1 and HSP-16.48 are specifically required for the protective effect of heat shock on necrosis. Moreover, overexpression of HSP-16.1 provides protection against necrotic cell death and circumvents the requirement for heat shock response activation. Further characterization of the protective function of the heat shock response activation and HSP-16.1 in necrosis may facilitate the development of intervention strategies aiming to counter necrotic cell death.

693C

A C. elegans model of ALS and other TDP-43 proteinopathy disorders. Nicole Liachko[1,2], Chris Guthrie[1], **Brian Kraemer**[1,2]. 1) Geriatrics Research Education and Clincal Center, VA Puget Sound Health Care System, Seattle, WA; 2) Department of Medicine, Gerontology Division University of Washington Seattle, WA.

Aging related neurodegenerative disorders are frequently characterized by lesions containing deposits of insoluble protein aggregates. Recently, the lesions seen in the degenerating neurons of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitin positive inclusions (FTLD-U) were discovered to consist primarily of the TDP-43 protein. Likewise TDP-43 inclusions are also seen in many cases of other diseases leading to dementia including Alzheimer's disease (AD), Parkinson's disease (PD), dementia with Lewy bodies (DLB), and Guam amyotrophic lateral sclerosis/Parkinson's dementia complex (ALS/PDC). Disorders with deposits of TDP-43 are now referred to as TDP-43 proteinopathy disorders. Of these, ALS is of particular interest because there is a causal link between TDP-43 and ALS. TDP-43 mutations cause some forms of familial ALS proving that abnormal TDP-43 can be a cause of neurodegeneration, although the mechanism by which TDP-43 abnormalities lead to neurodegeneration is poorly understood. To model TDP-43 neurotoxicity, we have transgenically expressed human TDP-43 protein in the neurons of C. elegans. Neuronal expression of normal human TDP-43 causes a relatively mild Unc phenotype, while expression of ALS causing mutant forms of TDP-43 leads to a robust Unc phenotype. Consistent with the mild Unc phenotype, we see little if any neurodegeneration caused by the wild type TDP-43 transgene. However, ALS mutant TDP-43 expressing transgenic animals exhibit dramatic degeneration of motor neurons. As in authentic human TDP-43 proteinopathy disorders, we observe the accumulation of insoluble TDP-43 protein in our model, both for wild type and mutant TDP-43 transgenes. This is in keeping with the observation that wild type TDP-43 forms inclusions in most sporadic ALS cases, while mutant TDP-43 is known to form aggregates in familial ALS cases. Detailed characterization of TDP-43 associated phenotypes and analysis of the genes and pathways contributing to TDP-43 mediated neurodegeneration are underway. The long term goal of this work is to develop neuroprotective strategies for neurodegenerative disorders with TPD-43 protein deposits.

694A

Functional characterization of the *C. elegans* homologs of human neurodegenerative disease proteins TDP-43 and PGRN. **Nicole F. Liachko**[1,2], Brian C. Kraemer[1,2]. 1) University of Washington, Seattle, WA; 2) VA Puget Sound Health Care System, Seattle, WA.

Neurodegenerative diseases affect a growing percentage of the human population as average lifespan increases. It is of great importance to characterize the causative agents involved in these diseases, as these will provide clues for treatments or disease-delaying interventions. Recent studies have identified the TAR DNA binding protein-43 (TDP-43) to be the pathological protein involved in a large percentage of cases of frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS). Loss of function mutations in the progranulin (PGRN) gene have been linked to TDP-43-pathological FTLD, as PGRN mutants accumulate TDP-43 neuronal aggregates. In addition, cell culture studies have shown that PGRN may mediate caspase-dependent cleavage of TDP-43, but it remains to be determined the exact nature of the interaction between PGRN and TDP-43 as it relates to FTLD. It is essential to characterize the normal and pathological functions of PGRN and TDP-43 to identify possible interventions in FTLD and ALS. To this end, we are testing the *C. elegans* TDP-43 and PGRN homologs *tdp-1* and *pgrn-1*. These genes have 39% and 55% homology, respectively, to their human counterparts, and are the primary homologous genes identified following protein-protein BLAST using either the *C. elegans* or human sequences as queries. Since the protein sequences are highly conserved between the human and C. elegans TDP-43/*tdp-1* and PGRN/*pgrn-1*, it is possible that their functions are conserved as well, and observations made using *C. elegans* may complement research in mammalian systems. We are characterizing the behavior and physiology of mutant *tdp-1* and *pgrn-1* to provide clues into the normal functions of these genes, including observations of movement, responses to stimuli, and neuronal development. In addition, we are performing microarrays to determine which gene expression levels are affected by *tdp-1* or *pgrn-1* mutations, and are comparing differentially regulated genes between the two mutants for further insight into their normal functions and functional interactions. In the long term, this work will provide a *C. elegans* system to study evolutionarily conserved functions of the human disease genes TDP-43 and PGRN, providing tools for understanding the mechanisms of FTLD.

695B

Uncovering the mechanisms of axonal degeneration in *C. elegans*. **Brent Neumann**, Leonie Kirszenblat, Massimo Hilliard. Queensland Brain Institute, University of Queensland, Brisbane, QLD 4072 Australia.

Axonal degeneration is a common hallmark of both neurodegenerative disease and neuronal injury. Recently is has become evident that degeneration of the axon is distinct from, and often precedes, neuronal cell death. However, the molecular mechanisms that lead to axonal degeneration are largely unknown. We have used the *C. elegans* PLM and PQR neurons to discover the genes regulating axonal degeneration. Each PLM neuron (left and right) has its cell body in the tail and extends a long anterior process of mixed dendrite/axon identity. PQR also has its cell body in the tail, but has a defined dendrite (which extends posteriorly into the tail) and a long axon that extends anteriorly. We used the *pmec-4::GFP* and *pgcy-36::GFP* transgenes to visualise the PLM and PQR neurons respectively, and to identify mutant animals in which the integrity of their neuronal processes were disrupted. Following EMS screenings, we have identified four mutant strains with spontaneous axon breaks: *ky850*, *vd005*, *vd021* and *vd024*. The strain carrying the *ky850* mutation presented spontaneous breaks in the PLM process, a phenotype only evident in adult animals. The penetrance of the defect was 20% in hermaphrodites, while males were less affected. By following individual animals over time we have found that these breaks led to degeneration of the separated, distal fragment. These analyses have also revealed that the cell body remained intact throughout the lifetime of the animals, suggesting the mutated gene is specifically involved in degeneration of the process and not the cell body. Animals with the *ky850* mutation were somewhat insensitive to light mechanical touch. The other three mutant lines of interest (*vd005*, *vd021* and *vd024*), obtained from a second EMS mutagenesis screen, all presented with spontaneous breaks in the PQR axon. The dendrite of PQR was unaffected by the mutations in these strains, indicating that the mutated genes are specifically involved in axonal degeneration and not that of the dendrite. Penetrance of the phenotype ranged from eighteen to forty percent amongst these three strains. We are now in the process of further characterising these four strains and identifying the mutated genes using SNP mapping techniques.

696C

The non-canonical cell death program governing tail-spike cell death requires the F box protein DRE-1. **Michael Chiorazzi**, Carine Maurer, Shai Shaham. Rockefeller Univ, New York, NY.

The development of a multicellular organism requires precise control of cell births and deaths in spatial and temporal dimensions. To investigate this process, we are studying the mechanism of cell death in the tail-spike cell, a cell that is unique developmentally and genetically among cells fated to die in *C. elegans*. While the vast majority of dying cells are gone within 30 minutes of their birth, the tail-spike cell lives for 300 minutes and dies as a differentiated cell, making it more similar to dying cells in other organisms. Also, previous work uncovered a unique genetic program governing tail-spike cell death in which the BH3-only gene *egl-1* is dispensable for death while the required caspase, *ced-3*, must be transcriptionally upregulated immediately prior to death to kill the cell. This transcriptional regulation requires the homeodomain transcription factor *pal-1*. In a screen for inappropriate survival of the tail-spike cell, we isolated a mutation in the F box domain of *dre-1* (*daf-12-redundant-1*), which was previously described as a heterochronic gene. Three alleles tested show varying levels of inappropriate tail-spike cell survival, with the F box mutation being the strongest. Weak *dre-1* mutations enhance *ced-3*, *ced-4* and *egl-1* loss of function mutations for tail-spike cell survival. In addition, a *ced-9* null mutation partially suppresses the inappropriate survival phenotype of a *dre-1* allele. Finally, *pal-1* is epistatic to *dre-1* with respect to *ced-3* transcription in the tail-spike cell. These genetic results are consistent with *dre-1* acting upstream of *pal-1* and upstream or independently of *ced-9* to regulate tail-spike cell death. Importantly, a *dre-1* transcriptional reporter is expressed in the tail-spike cell, suggesting that *dre-1* acts within the tail-spike cell to regulate its death. What role *dre-1* plays in cell death remains to be determined. However, because it is predicted to be an E3 ubiquitin ligase, we speculate that it may be responsible for tagging for degradation an anti-apoptotic protein, thereby placing the tail-spike cell on the path to death. This model is especially intriguing considering the central role that ubiquitination of apoptotic proteins plays in other organisms to regulate cell death. We have undertaken a forward genetic screen for dominant mutations that prevent tail-spike cell death, in the hope that an allele that interrupts binding of the anti-apoptotic target to DRE-1 will be dominant because it cannot be ubiquitinated by DRE-1. Such an allele may help us understand how the tail-spike cell is specified for death, and how it executes the apoptotic program at the proper time.

697A

egl-1-Dependent and Independent Pathways Cooperate to Control M4 Sister Cell Death. **Takashi Hirose**, Brendan Galvin, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

In *C. elegans*, 131 somatic cells undergo programmed cell death during wild-type hermaphrodite development. While genes that cause programmed cell death have been well studied, less is known about how a particular cell is specified to survive or to die by programmed cell death. To identify pathways involved in the cell-type specific specification of programmed cell death, we screened for mutants defective in the programmed cell death of the sister of the pharyngeal M4 motor neuron. The M4 neuron is generated during embryonic development and survives to regulate muscle contraction in the pharynx, while the M4 sister undergoes programmed cell death.

A genetic screen and a subsequent candidate-gene approach identified five genes required for M4 sister cell death: *ceh-32*, *ceh-34*, *eya-1*, *pig-1* and *sptf-3*. *ceh-32* and *ceh-34* encode Six class homeobox proteins, and *eya-1* encodes a transcriptional cofactor homologous to *Drosophila* Eyes absent. We have shown that *ceh-34(n4796)*, *eya-1(ok654Δ)* or *egl-1(n4820)* causes defects in programmed cell deaths of the M4 sister cell and also of the pharyngeal I1 sister cells and that a complex consisting of CEH-34 and EYA-1 directly activates *egl-1* expression through an *egl-1* 5'*cis*-regulatory element surrounding the *egl-1(n4820)* mutation to promote M4 sister and I1 sister cell deaths. In our genetic screen we also isolated *n4827*, which causes a defect in M4 sister cell death. *n4827* complemented *ced-3* and *ced-4* for the defect in M4 sister cell death and mapped to LG III. Animals heterozygous for both *n4827* and an *egl-1* mutation (which maps to LG V) have a defect in M4 sister cell death, while for both mutations this phenotype is recessive. These results suggest that *ceh-34*, *eya-1* and the gene defined by *n4827* interact with *egl-1* to regulate M4 sister cell death.

pig-1 encodes a serine/threonine kinase and affects asymmetric neuroblast divisions (Cordes *et al*., *Develop*. 133, 2747, 2006). *pig-1(gm344Δ)* additively enhanced the defect in M4 sister cell death of *ceh-34(n4796)*, *eya-1(ok654Δ)* and *egl-1(n4820)* animals. *sptf-3* encodes an Sp1 family transcription factor and did not affect *egl-1* expression in the M4 sister cell. These results suggest that *pig-1* and *sptf-3* function independently of *egl-1* to regulate M4 sister cell death.

We are analyzing how these *egl-1*-dependent and independent pathways cooperate to control M4 sister cell death.

698B

PINK-1 Functions as an Activator of Programmed Cell Death during Embryogenesis. **Julia E. Palter**, Joel H. Rothman. MCDB/BMSE, Univ California, Santa Barbara, Santa Barbara, CA.

Programmed cell death (PCD) plays critical roles in development and its inappropriate activation can result in pathological conditions including neurodegenerative diseases. Autosomal recessive mutations in PINK1 (PTEN-induced kinase 1) are the second most common cause of early-onset Parkinson's disease (PD). PINK1 is a serine-threonine kinase that is partially localized to mitochondria. It appears that mutations in PINK1 cause PD at least in part through mitochondrial dysfunction. Increased cell death is observed in PINK1 knockout mutants of Drosophila and cultured cells, possibly accounting for the loss of dopaminergic neurons in PD patients. While these studies suggested that PINK1 functions as a PCD suppressor, we have found that it instead appears to activate PCD in *C. elegans*.

We identified *C. elegans* PINK-1 by computational data-mining designed to identify candidate genes that might function in PCD based on their likelihood of interacting functionally with core PCD regulators. To test for potential pro-apoptotic activity, candidates were assayed by RNAi for their ability to suppress the embryonic lethality of *ced-9(lf)*. *ced-9(lf)* results in excessive PCD and 100% lethality, nearly all of which occurs during early embryogenesis. Both excessive PCD and embryonic lethality are suppressed by *ced-3(lf)* and *ced-4(lf)*. We found that RNAi of *pink-1*, and the *pink-1(tm1779)* mutant significantly suppress the embryonic lethality of *ced-9(lf)*, resulting in an increase of hatching animals. In addition, *pink-1(tm1779); ced-9(lf)* embryos that do not hatch arrest later on average compared to *ced-9(lf)* alone. These phenotypes suggest that, in contrast to its proposed anti-apoptotic roles in humans and flies, PINK-1 may function positively in apoptosis in worms. Indeed, consistent with a possible pro-apoptotic role for PINK-1 in worms, we observed that *pink-1(tm1779)* animals show reduced PCD corpses during embryogenesis and an increase in the number of particular GFP-marked neurons, whose sisters normally undergo PCD. While *pink-1(tm1779)* animals are viable, the *pink-1(tm1779); ced-9(lf)* animals that hatch arrest as L1s, apparently owing to a fully penetrant detached pharynx (Pun) phenotype, suggesting either that PINK-1 performs a developmental function distinct from its role in apoptosis, or that it promotes PCD in only selected cell types that do not include cells involved in pharynx attachment. Our findings reveal that PINK1 may perform a more complex function in PD than strictly apoptotic suppression.

699C

An RNAi screen for enhancers of programmed cell death. **Robert H. Pollok**, Scott Cameron. Departments of Pediatrics and Molecular Biology, UTSouthwestern Medical Center, Dallas, TX 75390.

Defects in programmed cell death are common in cancer, and discovering how cell death is regulated during normal development has revealed new avenues for medical research. While critical regulators of cell death in *C. elegans* have been discovered through traditional genetic screens, genetically redundant pathways and cell death regulators with subtle functions may have been overlooked. Use of a sensitized genetic background with a partial defect in cell killing can identify genes that contribute to, but are not individually necessary for programmed cell death. We are using the P_{lin-11}*gfp* reporter, which is expressed in the VC motor neurons of the ventral nerve cord, in a weak *ced-3* mutant background to identify enhancers of the weak cell death defect; in wild type hermaphrodites six VC neurons survive, and five VC-like Pn.aap cells undergo programmed cell death and are easily scored. In animals with a strong defect in programmed cell death, all five of the VC-like cells survive and express P_{lin-11}*gfp*. With this assay, we used RNAi to screen the transcription and chromatin factor subset library to identify genes that lead to complete survival of the VC-like cells. We identified new candidate genes without previously described cell death defects including *zfp-1* and *set-9*/*set-26*. The human homologues of *zfp-1* and *set-9*/*set-26*, AF10 and MLL5 respectively, are leukemia-associated proteins. AF10 is a fusion partner in different leukemias, but an unambiguous death defect associated with it has not been described. I am now further characterizing cell death defects caused by *zfp-1*, *set-9*/*set-26* and other candidate genes identified in the screen. Elucidating a role for *zfp-1* and other hits from the screen may open new avenues in cancer research.

700A

The E3 ubiquitin ligase, *eel-1*, regulates DNA damage-induced germ cell apoptosis in *C. elegans*. **Ashley J. Ross**, Michelle Li, Brent Derry. Program in Developmental & Stem Cell Biology, The Hospital for Sick Children, Toronto, Ontario, Canada.

CEP-1, the sole p53 family member in *C. elegans*, is a key regulator of DNA damage-induced germline apoptosis. In response to DNA damage, CEP-1 activates the transcription of EGL-1, resulting in inhibition of the anti-apoptotic protein CED-9 and activation of the pro-apoptotic proteins CED-4 and CED-3. In mammalian cells, members of the E3 ubiquitin ligase family have been shown to target several cell death proteins for degradation, including the p53 and Bcl-2 family of proteins. To investigate the role of E3 ubiquitin ligases in *cep-1* mediated germline apoptosis, we systematically inhibited the expression of all predicted E3 ligases by RNAi and quantified DNA damage-induced apoptosis in the germline.

From this screen we identified the gene *eel-1* as a positive regulator of DNA damage-induced germ cell apoptosis. Animals depleted of *eel-1* by RNAi or mutation had significantly lower levels of germ cell apoptosis in response to ionizing radiation compared with controls. EEL-1 is a member of the HECT domain family of E3 ligases and is homologous to the human protein ARF-BP1. In human osteosarcoma cells, ARF-BP1 has been shown to negatively regulate both the tumour suppressor protein p53 and the anti-apoptotic protein Mcl-1, a CED-9 ortholog. We found that *egl-1* transcript levels were induced to similar levels in *eel-1*(lf) mutants as wild-type controls treated with the same dosage of ionizing radiation. This indicates that *eel-1* does not affect CEP-1 activity and therefore does not target CEP-1 for degradation. Epistasis experiments suggest that *eel-1* acts upstream of *ced-9*, and we are currently testing the hypothesis that EEL-1 targets CED-9 for degradation in response to DNA damage. Our results suggest that unlike the mammalian protein ARF-BP1, *eel-1* impinges on *ced-9* downstream of *cep-1* to regulate apoptosis in the *C. elegans* germline.

701B

Novel Roles of the Tumour Suppressor ing-3 in Caenorhabditis elegans. **Sitar Shah**[1], Jingjing Luo[1], Karl Riabowol[1,2], Paul E. Mains[1,3]. 1) Biochemistry and Molecular Biology; 2) Dept of Oncology; 3) Medical Genetics University of Calgary, Calgary, Canada.

The INhibitor of Growth (ING) family proteins are involved in multiple cellular processes such as growth regulation, DNA repair and apoptosis. Loss or downregulation of ING protein function frequently occurs in tumours, thus classifying them as type II tumour suppressors. ING proteins are activated by stress, such as ionizing radiation, which also leads to the activation of p53 and consequently apoptosis. ING proteins in mammals and yeast read the histone code in a methylation-sensitive manner, targeting histone acetyltransferase (HAT) and histone deacetylase (HDAC) complexes to chromatin, which subsequently regulates gene expression. Mammals have five ING genes and three are found in C. elegans. Mammalian ING proteins have overlapping functions, making the interpretation of individual roles challenging. Here we characterize C. elegans ing-3, the gene with the highest sequence identity to mammalian ING3. ING-3 co-localizes with chromatin in C. elegans embryos, germ line and somatic cells during all stages of the cell cycle. The embryonic death rate in the ing-3 mutant strain demonstrates that the gene likely functions in the same pathway as the C. elegans p53 homolog, cep-1, to induce germ cell apoptosis in response to ionizing radiation. Although we found that ing-3 does not influence the transcription of the cep-1 target egl-1 in the DNA damage apoptosis pathway. We have recently found other ing-3 phenotypes. ing-3 mutants have a weak kinker phenotype, which may suggest neuronal function. Although ing-3 mutants show a 10% reduced body length compared to wild type, they seem to develop at a slightly faster rate. Finally, the ing-3 lifespan is 10% longer than wild type, consistent with the effect of ing-3 loss in cultured mammalian cultured cells. In light of ING proteins being characterized as tumour suppressors, studying their functional role in C. elegans development will increase our knowledge about the role ING plays in cancers and cancer therapy.

702C

Genetic characterization of the CED-4 translocation event during apoptosis. **Chun-Ling Sun**, Huey-Jen Lai, Josh Friedman, Xiaochen Wang, Jay Parrish, Ding Xue. MCD Biology, University of Colorado, Boulder, CO.

Apoptosis plays an essential role in maintaining tissue homeostasis and animal development. In *C. elegans*, the BH3-only cell death initiator EGL-1 induces cell death by binding to CED-9 and causing release of the CED-4 dimer from the CED-4/CED-9 complex tethered on the surface of mitochondria (1, 2). CED-4 dimers then further oligomerize to result in activation of the CED-3 zymogen. Intriguingly, upon release from the surface of mitochondria, CED-4 translocates to the peri-nuclear membrane but the role of such translocation in regulating cell death activation is not understood (1). We have carried out a CED-4::GFP based genetic screen to look for mutations that block translocation of CED-4 to the peri-nuclear membrane and have isolated four mutations that result in *mi*s-localization of *C*ED-4 (*mic*). These four *mic* mutations define three new genes, *mic-1(sm158, sm160), mic-2(sm161),* and *mic-3(sm162).* Phenotypic analysis reveals that *mic-1* mutations cause stronger Mic phenotype than *mic-2* and *mic-3* mutations. Although these *mic* mutants alone do not display an obvious cell death defect, they can block cell death in sensitized genetic backgrounds. Our results suggest that translocation of CED-4 from mitochondria to the peri-nuclear membrane may play an important regulatory role in cell death activation. We have recently cloned the *mic-1* gene and are in the process of cloning *mic-2* and *mic-3*. Further molecular and biochemical characterization of MIC proteins should reveal how CED-4 translocates to the peri-nuclear membrane and what its role is in cell death activation in *C. elegans*.

1. Chen, F. et al., Translocation of *C. elegans* CED-4 to nuclear membranes during programmed cell death. *Science* 287 (5457), 1485-1489 (2000).

2. Yan, N. et al., Structure of the CED-4-CED-9 complex provides insights into programmed cell death in *Caenorhabditis elegans*. *Nature* 437 (7060), 831-837 (2005).

703A

Clathrin-mediated endocytosis and intracellular trafficking are required for necrotic cell death in *C. elegans*. **Kostoula Troulinaki**, Nektarios Tavernarakis. IMBB, FORTH, Heraklion, Crete, Greece.

Defects in endocytosis and trafficking of endocytic vesicles and organelles are apparent and have been implicated in many human neurodegenerative conditions such as Alzheimer's disease, Huntington's disease and ALS. One of the early events in the course of neuronal necrosis in *C. elegans* is the formation of small, tightly wrapped membrane whorls that at a later stage are internalized and coalesce into large, electro-dense membranous structures, implicating mechanisms of endocytosis and trafficking in necrotic cell death (Hall et al., J. Neurosci. 17: 1033). To gain insight into the molecular mechanisms underlying these cellular events, we examined the requirement for specific endocytosis and trafficking genes in necrosis. In addition, we monitored endocytosis during cell death. We find that neurodegeneration induced either by hyperactivated ion channels or by chemical treatment is suppressed in genetic backgrounds deficient for proteins that are required for clathrin mediated endocytosis and transport of vesicles along microtubules. In addition, we find that specific endocytotic proteins and two kinesins function together with calpain cytoplasmic proteases, and lysosomal cathepsin proteases to mediate necrosis. Furthermore, endocytotic components synergize with autophagy, a process which is induced and contributes to cellular destruction. Our observations indicate that clathrin-mediated endocytosis and intracellular trafficking are important for necrotic cell death in the nematode.

704B

Context-dependent regulation of the *egl-1* programmed cell death gene by a unique E2F family member. **Jennifer Winn**, Scott Cameron. Departments of Pediatrics and Molecular Biology, UT Southwestern, Dallas, TX.

Loss of a Hox protein complex that includes LIN-39, CEH-20 and UNC-62 results in programmed cell death of specific cells, the VC motor neurons. We sought to investigate the mechanism responsible for this context-specific pattern of cell death. Specifically, why does the VC neuron descendant of each Pn.a cell of *lin-39* mutants die, but the other five neurons generated by Pn.a survive? To address this question, we performed an RNAi screen in a *lin-39* background for repressors of programmed cell death, as visualized by a $P_{egl\text{-}1}$*gfp* reporter. In this strain, *egl-1* is expressed all of the cells of the ventral nerve cord that die in wild type animals and in the six VC neurons that die in *lin-39* mutants, but not in any other P cell descendants. From this screen, we identified the gene *F49E12.6*. In *lin-39; F49E12.6(RNAi)* animals, *egl-1* is expressed in the VA and VB motor neurons, in addition to the VC neurons. Remarkably, repression of *egl-1* by *F49E12.6* is redundant with *lin-39*. In *F49E12.6(RNAi)* animals, *egl-1* is expressed in the VA and VB neurons in the posterior P cell lineages outside the midbody region in which *lin-39* is normally active; *egl-1* is not expressed in the midbody, where *lin-39* is active. These data suggest that F49E12.6 and LIN-39 act redundantly to repress transcription of *egl-1* in the VA and VB motor neurons of the midbody, and may in part explain the context-dependent requirement for *lin-39* function in ensuring VC neuron survival.

F49E12.6 encodes a *C. elegans* homolog of the mammalian transcription factors E2F7 and E2F8. E2F7 and E2F8 are recently discovered proteins that are unique in having two E2F DNA-binding domains and in lacking domains present in other E2F family members, such as DP/DPL-1-binding or Rb/LIN-35 binding domains. Mice with knockouts of E2f7 or E2f8 are viable, but the double mutant is lethal with widespread apoptosis. The precise mechanisms through which loss of E2f7 and E2f8 result in apoptosis are as yet unclear, and our data suggest transcriptional derepression of programmed cell death pathway genes in the double mutants, perhaps in a context-dependent manner involving Hox genes, is one such mechanism.

705C

Systematic identification of steroids in *Caenorhabditis elegans*. **Chirag Pungaliya**[1], Joshua Wollam[2], Axel Bethke[2], Kristen Seim[1], Rabia Malik[1], Adam Antebi[2], Frank Schroeder[1]. 1) Boyce Thompson Institute and Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 2) Huffington Center on Aging, Molecular and Cellular Biology, Baylor College of Medicine, Houston, Tx.

Recently Motola et. al. identified the dafachronic acids, bile-acid like steroids that act as the endogenous ligands of the nuclear hormone receptor DAF-12. They directly bind to DAF-12 to influence interactions with its cofactors and thereby regulate transcriptional activity. It is possible that other endogenous steroids influence DAF-12 or act on other nuclear hormone receptors that modulate various *C. elegans* life history traits. We have begun a systematic analysis of *C. elegans* steroid metabolism based on chromatographic separation, bioassays and differential analysis by 2D NMR spectroscopy (DANS), comparing the wild-type metabolome to that of mutants with suspected defects in steroid metabolism. So far our investigations have revealed several steroids whose presence in *C. elegans* had not previously been recognized.

706A

A Steroid Biosynthetic Pathway that Modulates Dauer Formation and Lifespan in *C. elegans*. **Joshua Wollam**, Veerle Rottiers, Brittany E. Ford, Dongling Li, Adam Antebi. Huffington Center on Aging, Baylor College of Medicine, Houston, TX.

Aging can be described as a decline in biological function over time, and is associated with decreased resistance to stress and increased susceptibility to disease. Both environmental and genetic factors are known to modulate the processes of aging, and conserved signaling pathways have been discovered that act in this regulation of longevity. Among these pathways, the importance of endocrine systems has become increasingly apparent. In *Caenorhabditis elegans*, the nuclear hormone receptor DAF-12 regulates developmental timing and controls the decision to undergo normal reproductive development or enter dauer diapause, a long-lived state of arrested development. For this decision, the insulin/IGF-I and TGF-β signaling pathways converge to activate DAF-12 through the bile acid-like steroids Δ^4- and Δ^7-dafachronic acid (DA).

Although the DAs are derived from dietary sterols, the synthesis and regulation of these hormones by upstream inputs is not well understood. We previously identified DAF-36, a Rieske-like oxygenase, and DAF-9, a cytochrome P450, as acting in the first and last steps of this pathway, respectively. In order to identify additional components, we performed RNAi screens looking for enhancement of *daf-36* gonadal cell migration (Mig) and dauer formation-constitutive (Daf-c) phenotypes. We have identified several genes possibly acting in DA biosynthesis, including DAF-37, a cytochrome P450, DHS-16, a short chain dehydrogenase, and EMB-8, a cytochrome P450 reductase. Genetic experiments of synergy and epistasis place both *daf-37* and *dhs-16* within the pathway. We rescued *dhs-16* deletion mutants by providing lathosterone, a proposed precursor of the DAF-12 ligands, placing DHS-16 at a specific step in the pathway. In addition, we have successfully been able to express this enzyme and synthesize the rescuing product *in vitro*, confirming the enzymatic activity of DHS-16.

Furthermore, DAF-12 and DA also act to regulate lifespan in response to signals from the germline. Whereas laser ablation of the germline precursors leads to an increased lifespan in wild-type animals, lifespan extension is significantly reduced in both the *dhs-16* and *daf-37* mutant backgrounds, consistent with roles in DA biosynthesis. Through these studies, we have elucidated new components of DA biosynthesis and reveal how these genes influence the life history of *C. elegans*.

707B

Caenorhabditis elegans utilizes dauer pheromone biosynthesis to dispose of toxic peroxisomal fatty acids for cellular homeostasis. **Hyoe-Jin Joo**[1], Yong-Hyeon Yim[2], Pan-Young Jeong[1], You-Xun Jin[2], Jeong-Eui Lee[1], Heekyeong Kim[1], Seul-Ki Jeong[1], David J Chitwood[3], Young-Ki Paik[1]. 1) Dept Biochemistry, Yonsei Univ, Seoul, Korea; 2) Korea Research Institute of Standards and Science, Daejon, Korea; 3) Nematology Laboratory, USDA, ARS, Beltsville, MD 20705 USA.

C. elegans excretes dauer pheromones or daumones composed of ascarylose and a fatty acid side chain, perception of which enables worms to enter the dauer state for long-term survival. To elucidate both the daumone biosynthesis and its physiological impact, the origin of ascarylose and the role of two enzymes, DHS-28 and SCPx, involved in peroxisomal β-oxidation, were studied using *E. coli* mutants deficient in ascarylose biosynthesis and *C. elegans* mutants defective for these enzymes. Our results suggest that the ascarylose moiety is synthesized de novo and that peroxisomal β-oxidation of very long-chain fatty acid (VLCFA) is required for daumone biosynthesis. Dauer assays and chemical analyses revealed that two mutants, *dhs-28(tm2581)* and *scpx(ok693)*, lacked daumones and thus were dauer defective; this coincided with massive accumulation of fatty acyl-CoAs (up to 100-fold over N2) inside worm bodies. Deficiency in daumone biosynthesis caused severe developmental defects as well as reduced lifespan (up to 30%), suggesting that daumone biosynthesis appears to be an essential part of *C. elegans* homeostasis through which some toxic peroxisomal VLCFAs from the worm body are metabolized in the form of readily excretable daumones.

708C

Receptor trapping in *C. elegans*: identifying the targets of ascarosides and other endogenous small molecules. **Inish M O'Doherty**, Frank C Schroeder, Andreas H Ludewig. Chemistry and Chemical Biology, Cornell university, Ithaca, NY.

Recent studies have led to the identification of several families of small molecule signals in *C. elegans*[1]. These compounds affect key pathways regulating lifespan, development, and metabolism. Further knowledge of corresponding receptors will be essential for understanding their biological role. We are developing a chemical approach to identifying small-molecule receptors in *C. elegans*. This approach is based on designing probes that are structural analogs of the endogenous small-molecule ligands but feature additional structural elements. These chemical modifications of the ligand allow covalent tagging of the small molecule to its receptor; with this linkage established it may then be possible to pull down and isolate the specific receptor in question. Design of these probes takes advantage of recently developed methods for *in-vivo* photocrosslinking of ligands to their corresponding receptors[2,3] and further introduces a bioorthogonal handle for subsequent pull down of the ligand-receptor complex for mass spectrometric analysis[4,5]. Our current investigations focus on receptors of the ascarosides, ascr#1-ascr#8, a recently discovered family of small molecules that play important roles in male mating, and dauer formation. We have designed ascaroside-derived probes featuring a receptor-binding motif based on the pheromone component ascr#8, a photolabile receptor-tagging group, and a third functionality for subsequent pull down. Results from bioactivity studies of synthetic model compounds will be reported. 1. Srinivasan et al.(2008) Nature 454:1115-1118. 2. Gubbens et al. (2009) Chemistry & Biology 16, 3-14. 3. Wang et al.(2004) J. AM. CHEM. SOC. 126, 14435-14446. 4. Cravatt et al.(2007) QSAR Comb. Sci. 26, No. 11-12, 1229-1238 5. ACS Chem. Bio. 2006,Vol.1, No.10, 644-648.

709A

Identification of transcriptional regulators of the developmental timing gene *lin-42* . **Tracy James**, Diya Banerjee. Biological Sciences, Virginia Tech University, Blacksburg, VA.

The developmental timing gene *lin-42* functions in the heterochronic pathway to regulate the correct timing of division and differentiation of epidermal and vulval cells during *C. elegans* larval development. *lin-42* expression cycles with each larval stage, with expression peaking during the intermolts. It is not known how *lin-42* expression is transcriptionally regulated to occur in this specific temporal pattern, and our aim is to identify and characterize *lin-42* transcription factors. First, we used a candidate gene approach based on the fact that *lin-42* is the *C. elegans* homolog of the circadian timing gene *period*. Thus, we hypothesized that *lin-42* may be regulated by *C. elegans* homologs of circadian pathway transcription factors or by related basic helix-loop-helix (bHLH) transcription factors. We made or obtained RNAi constructs of candidate transcription factors and evaluated the effect of gene knockdown on the retarded heterochronic phenotype of *let-7(n2853)* loss-of-function mutant worms, and on *lin-42* expression levels in wild type worms. *let-7* microRNA negatively regulates *lin-42* expression, and *let-7* loss-of-function results in a retarded development phenotype partially due to the resulting elevated level of *lin-42* expression. Thus, we reasoned that expression knockdown of a *lin-42* transcription factor in a *let-7* loss-of-function genetic background would result in suppression of the retarded phenotype of *let-7(n2853*) mutant worms. Similarly, we reasoned that expression knockdown of a *lin-42* transcription factor would phenocopy the *lin-42* loss-of-function precocious developmental phenotype and would result in a decrease or loss of *lin-42* expression levels and cycling. The results of our candidate transcription factor screen indicate that none of the *C. elegans* homologs of known circadian pathway transcription factors regulate *lin-42*. We are now using two different protein-DNA interaction assays to identify *lin-42* transcriptional regulators: (1) Yeast-one-hybrid assay, and (2) transcription factor capture with *lin-42* promoter fragments and subsequent identification of the protein by Mass Spectroscopy. We will present validation of our assays by showing interactions between known *C. elegans* transcription factor and promoter sequence pairs, and we will present the results of our transcription factor-*lin-42* promoter interaction screens.

710B

Environmental Stress Modulates the *lin-42* Mutant Phenotype. **Katherine A McCulloch**, Jason M Tennessen, Karla Opperman, Ann E Rougvie. Department of Cell Biology, Development, and Genetics University of Minnesota, Minneapolis, MN.

Loss of function of the heterochronic gene *lin-42*, the C. elegans homologue of the Period family of circadian rhythm genes, causes animals to execute certain developmental events too early. This phenotype is sensitive to environmental conditions. *lin-42(ve11)* mutants raised at 20°C exhibit precocious development and synthesize adult cuticle at the L3 molt; however, those grown at 25°C are often suppressed for this timing defect. The insulin-like and TGFβ signaling pathways that mediate dauer formation in response to stress are not responsible for this suppression. Neither *daf-16(lf)*, nor Daf-d mutations in *daf-3* or *daf-5* alter the temperature-induced suppression of the *lin-42(lf)* phenotype. In contrast, mutations in the nuclear hormone receptor *daf-12(0)* eliminate the temperature sensitivity of *lin-42(lf)*. DAF-12 acts as a critical molecular switch that regulates stress response and developmental timing. Under favorable conditions, ligand bound DAF-12 activity is thought to negatively regulate the heterochronic gene *lin-28*. Since the equilibrium between ligand bound and ligand free DAF-12 is controlled by environmental conditions, LIN-28 levels may also fluctuate depending on external stress. We are testing a model wherein a temperature-induced rise in LIN-28 compensates for loss of *lin-42* activity. In support of this model, we find that *lin-42* and *lin-28* act in parallel to time development: *lin-28(lf)* mutants are precocious, with adult alae observed as early as the L2 molt, albeit at low frequency, and concomitant removal of *lin-42* activity greatly enhances this phenotype. Quantitative analysis of LIN-28 levels will provide a critical test of this model. Although the temperature suppression of *lin-42(lf)* is not dependent on TGFβ or insulin signaling pathways, these pathways can influence *lin-42* in other situations. For example, when *lin-42(ve11)* animals containing Daf-c mutations in either *daf-7* or *daf-2*, components of TGFβ and insulin signaling pathways respectively, are raised at the semi-permissive temperature of 20°C, the *lin-42* precocious alae phenotype is suppressed. This result shows that *lin-42(lf)* is sensitive to environmental stress generally and not just temperature. It is clear that *lin-42* is a key component of a complex regulatory network that integrates environmental signals with the developmental clock. In the future, it will be critical to determine how temporal and environmental cues interact to regulate *lin-42* and to identify other factors that modulate *lin-42* activity and expression. Reagents are currently being prepared to investigate these issues.

711C

Timing the Molting Cycle. **G.C. Monsalve**, J. Davie, A.R. Frand. Biological Chemistry, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA.

Nematodes develop through periodic molts but molecular mechanisms that set the pace of the molting cycle are not well understood. Molting involves detachment of the old exoskeleton from the underlying hypodermis and synthesis of the new exoskeleton, an extracellular matrix composed largely of collagens. Epidermal stem cells divide in sync with the first three molts but terminally differentiate at the final molt. A period of behavioral quiescence (lethargus) accompanies remodeling of the exoskeleton. Animals subsequently use specialized movements to escape the old skeleton (ecdyse). Improper coordination of these cell biological and behavioral events can be fatal.

We aim to identify genes and molecules that regulate the timing of the molting cycle. The best-characterized biological timers control circadian rhythms in the physiology and behavior of humans and other animals. In theory, worm counterparts of the core circadian clock proteins might also regulate the ultradian rhythm of the molting cycle. We are therefore investigating the role of clock gene homologs in the reiterative molecular and behavioral events characteristic of molting. As a complementary approach, we conducted a forward genetic screen for mutants that molt prematurely, using expression of the *mlt-10p::gfp-pest* reporter to indicate synthesis of a new cuticle. In a pilot screen of approximately 18,000 genomes, we indentified one candidate that expressed the *mlt-10* reporter too early and then partly shed the L1 exoskeleton. This particular animal perished, possibly due to the exposure of an incomplete L2 skeleton to the environment. In ongoing work, a clonal screen will allow the recovery of lethal mutations from siblings of affected animals. To further investigate the physiologic regulation of the molting cycle, we have cloned and characterized new mutations that cause supernumerary molts after puberty. These alleles emerged from a genetic screen conducted by Alison Frand and Sascha Russel in the laboratory of Gary Ruvkun. By studying the regulation of the molting cycle, we expect to uncover novel but conserved mechanisms for the endocrine control of development and behavior.

712A

The Tip60-p400 chromatin remodeling complex promotes maintenance of the undifferentiated state in *C. elegans* seam stem cells by inhibiting *let-7*-dependent terminal differentiation. **Rachael A. Nimmo**, Frank J. Slack. Dept of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06511.

The Tip60-p400 chromatin remodeling and histone acetyl-transferase complex is recruited to both active and inactive promoters in embryonic stem (ES) cells to maintain the undifferentiated stem cell fate. We have discovered that in *C. elegans*, *ssl-1* (p400)or *mys-1* (Tip60) knockdown causes the seam stem cells to partially differentiate early. In addition, *ssl-1* RNAi enhances the precocious terminal differentiation exhibited by *lin-14* mutants and partially suppresses retarded seam cell defects in *let-7* mutants, indicating that *ssl-1* may be regulating,or be regulated by, *let-7* family miRNAs. We are currently investigating how *ssl-1* and *mys-1* promote the undifferentiated state and regulate the timing of terminal differentiation of the seam cells. It is known that Tip60-p400 complexes are recruited to H3K4me3 marks in ES cells in a Nanog-dependent manner and regulate transcription of important stem cell genes. LIN-28 is an important stemness factor, and is highly expressed in undifferentiated ES cells and worm seam stem cells, but is downregulated prior to differentiation. LIN-28 inhibits *let-7* processing in ES cells and in *C. elegans lin-28* and *let-7* are important members of the heterochronic pathway regulating developmental timing. Terminal differentiation of the seam cells occurs precociously in *lin-28* mutants but is delayed in *let-7* mutants, therefore LIN-28 and *let-7* act in opposing ways to regulate the balance between proliferation and differentiation. In mammals, Myc recruits Tip60-p400 to promoters in order to promote cell proliferation and Myc inhibits *let-7* via transactivation of *lin-28*. Therefore we are investigating whether the Tip60-p400 HAT complex promotes maintenance of the undifferentiated fate by inhibiting *let-7* family miRNAs through upregulation of LIN-28, as well as how Tip60-p400 genes interact with other heterochronic genes such as *lin-41* and *hbl-1*. Interestingly, Tip60 is known to interact with both the Amyloid Precursor Protein (APP) Intracellular Domain (AICD) and the Notch Intracellular domain (NICD) and in *C. elegans* both *apl-1* (APP) and *lin-12*(Notch) have been found to regulate the timing of terminal differentiation of the seam stem cells, consistent with evidence from mammalian studies suggesting that the NICD disrupts AICD-Tip60 complex formation and that Tip60 is required for the intracellular signaling role of APP. We are therefore investigating whether MYS-1, LIN-12 and APL-1 interact in *C.elegans* and how chromatin remodeling may participate in Notch and APP signaling in the temporal regulation of terminal differentiation in seam stem cells.

713B

pqn-47/let-25/emb-23, a Novel Conserved Essential Gene Involved in Molting. **Sascha Russel**[1,2], Gary Ruvkun[1,2]. 1) Dept Mol. Biol, Mass Gen Hosp, and; 2) Dept. of Genetics, Harvard Medical School, Boston, MA.

Molting requires precise coordination of developmental and temporal events including not only synchronization amongst tissues but also the coordination of special behaviors, degradation and synthesis of the exoskeleton. Given the global changes of the molting cycle, it is expected that some type of neuroendocrine regulation will be found.

In order to discover genes that regulate molting in C. *elegans* we did a forward genetic screen (in collab. w/ Alison Frand) for genes that are required to cease the molting cycle in adults. We chose to focus on a mutant that, like other retarded heterochronic mutants, molts as an adult, but is unique because it does so out of an adult cuticle. We identified *mg412* as a weak allele of *pqn-47*(F59B10.1).

Our identification and characterization of additional *pqn-47* alleles have revealed that the gene is required for embryonic development and the earlier larval molts in addition to the cessation of molting in adults. A hint of an earlier function was that *pqn-47*RNAi causes larval arrest with a defective molt phenotype. We found that a deletion allele from the Mitani collection (thank you) *pqn-47(tm2707)* causes a similar phenotype. Surveying lethals in the region identified candidate loci including *let-25* (Herman RK, 1978) and *emb-23* (Cassada, R et al 1981) in which we found mutations in the *pqn-47* ORF. *emb-23* causes embryonic lethality at NPT, suggesting that maternal contribution of *pqn-47* allows embryonic development, whereas zygotic expression becomes required for the first larval molt. We are trying to understand why *mg412* causes an adult molt.

pqn-47 encodes a large uncharacterized protein of unknown function and an excess of glutamine residues, though it has strong conservation in fly and human. The conserved domain has no annotated function, and orthologues in other species have not yet been implicated in any processes, although the human orthologue C11orf9 is highly expressed in neuronal tissue and is upregulated in metastatic cancers and in EMT cancer models.

PQN-47 is expressed in neurons and other tissues in perinuclear dots that maybe ER. These studies have the potential to contribute to our understanding of the regulation of molting in C. *elegans* and elucidate the function of a new class of proteins involved in cell proliferation and differentiation.

714C

Identification of the circadian gene homologs *sur-6*/PP2A, *gsp-1*/PP1, *gsp-2*/PP1, *kin-2*/CKIIα and *kin-10*/CKIIβ as developmental timing regulators of cell fate during *C. elegans* post-embryonic development. **Autumn Timpano**, Xin Chen, Diya Banerjee. Biological Sciences, Virginia Tech University, Blacksburg, VA.

The development of multicellular animals is a complex process, which requires that molecular and cellular events occur in specific locations and in a specific sequence. Both spatial and temporal patterning of cell fate during animal development is genetically controlled. The most extensively investigated developmental timing pathway is the *C. elegans* heterochronic Pathway, which controls the timing of cell fate determination during post-embryonic, larval development. The heterochronic genes *lin-42*, *kin-20* and *tim-1* are the *C. elegans* homologs of the core circadian genes *period*, *casein kinase Iε*, and *timeless*, respectively. These three circadian genes are core components of the circadian clock, a molecular timing mechanism that regulates gene expression and behavior over the 24-hour day/night cycle. We have previously shown that the *C. elegans* circadian gene homologs regulate the timing of cell fate differentiation to occur during the larval to adult stage transition. These circadian gene homologs that have developmental timing function thus define a molecular and functional parallel between the circadian and developmental clocks, and suggest the hypothesis that the two types of timing pathways utilize a conserved mechanism of temporal regulation. To test this hypothesis, we have identified the *C. elegans* homologs of all known *Drosophila* and mammalian circadian timing genes and we are investigating the possible function of these homologs in control of developmental timing, using RNA interference (RNAi) to knock-down gene expression. We will present evidence that the *C. elegans* Casein Kinase II genes, *kin-3* and *kin-10*, the Protein Phosphatase regulatory subunit gene, *sur-6*, and two Protein Phosphatase 1 genes, *gsp-1* and *gsp-2*, interact genetically with the heterochronic pathway and thus may function as developmental timing genes.

715A

let-7 family microRNAs directly regulate the developmental timing gene *lin-42* and the circadian timing gene *period*. **Autumn Timpano**[1], Tracy James[1], Lena Chin[2], Frank Slack[2], Diya Banerjee[1]. 1) Biological Sciences, Virginia Tech University, Blacksburg, VA; 2) Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven CT 06520.

let-7 microRNA, *lin-42* and *kin-20* function in the *C. elegans* heterochronic pathway to regulate the correct timing of cellular differentiation during larval development. *lin-42* and *kin-20* are homologs of the core circadian timing genes *period* and *casein kinase I* epsilon, respectively. Genetic analysis places these two circadian gene homologs downstream of *let-7*, but it is not known whether they are directly regulated by *let-7*, or whether they constitute a parallel pathway that controls developmental timing. MicroRNAs (miRs) post-transcriptionally downregulate expression of their target genes by attaching to partially complementary sites in the 3' UTR of the message and preventing translation. We have identified potential *let-7* complementary sites (LCSs) in the 3'UTRs of *lin-42* and *kin-20*. In order to determine whether *lin-42* or *kin-20* is post-transcriptionally regulated by *let-7* miR, we used a *lacZ* reporter gene attached to *lin-42* or *kin-20* 3'UTRs. *kin-20* post-transcriptional expression does not appear to be regulated via its 3'UTR. However, we found that two of the three *lin-42* isoforms, *lin-42A* and *lin-42B*, are down-regulated via their 3'UTRs. This down-regulation during the larval to adult transition is likely to be mediated directly by *let-7* as the regulation is dependent both on the presence of the LCSs and the presence of *let-7* miR. Furthermore, the *let-7* paralogs, *mir-84*, *mir-48* and *mir-241*, also directly regulate *lin-42A* and *lin-42B*, but do so at earlier stages of larval development. By using quantitative real time PCR to measure *lin-42* mRNA levels, we found that levels of *lin-42A* and *lin-42B* are elevated in *let-7* loss-of-function genetic backgrounds. Interestingly, while loss of the *let-7* paralogs in the VT1066 strain resulted in an increase at the L2 stage, subsequent levels of *lin-42A* and *lin-42B* in L3 and L4 stages were normal, and lin-42 expression cycling was not disrupted. On the other hand, mRNA levels of lin-42C, which does not contain any LCSs in its 3'UTR, was unaffected in *let-7* loss-of-function genetic backgrounds compared to N2/wild type. Our results validate *lin-42* as a direct regulatory target of *let-7* family microRNAs, and raise the possibility that *let-7* microRNAs may regulate the circadian timing gene *period*. In humans, all three *period* genes have putative LCSs in their 3'UTRs. To test for regulation by *let-7* miRs, we utilized a luciferase reporter gene attached to period 3'UTRs and assayed for reporter expression in human cell lines in which *let-7* expression manipulated. We have found that *Hs period 3* is regulated by *let-7* miR.

716B

Biosynthesis of the *C. elegans* dauer pheromone. **Rebecca A. Butcher**[1], Justin R. Ragains[1], Weiqing Li[2], Gary Ruvkun[3], Jon Clardy[1], Ho Yi Mak[4,5]. 1) Dept Biol Chem & Molec Pharm, Harvard Medical Sch, Boston, MA; 2) Department of Biological Structure, University of Washington, Seattle, WA; 3) Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA; 4) Stowers Institute for Medical Research, Kansas City, MO; 5) Department of Molecular and Integrative Physiology, University of Kansas Medical Center, Kansas City, KS.

To sense its population density and to trigger entry into the stress-resistant dauer larval stage, *C. elegans* uses the dauer pheromone, which consists of ascaroside derivatives with short, fatty acid-like side chains. Although the dauer pheromone has been studied for twenty-five years, its biosynthesis has remained completely uncharacterized. The *daf-22* mutant is the only known mutant defective in dauer pheromone production. We have shown that *daf-22* encodes a homolog of human sterol carrier protein SCPx, which catalyzes the final step in peroxisomal fatty acid β-oxidation. We have also shown that *dhs-28*, which encodes a homolog of the human D-bifunctional protein that acts just upstream of SCPx, is also required for pheromone production. Long-term *daf-22* and *dhs-28* cultures develop dauer-inducing activity by accumulating less active, long-chain fatty acid ascaroside derivatives. Thus, *daf-22* and *dhs-28* are required for the biosynthesis of the short-chain fatty acid-derived side chains of the dauer pheromone and link dauer pheromone production to metabolic state.

717C

Nuclear receptors play subtle roles in dauer recovery in *Caenorhabditis elegans*. **Kirsten Crossgrove**, Brenda Garland, Kiah Green. Dept Biological Sci, Univ Wisconsin, Whitewater, Whitewater, WI.

Dauer recovery in *Caenorhabditis elegans* is an interesting model for understanding how organisms respond to changes in their environment. Dauers form in response to low food, high crowding and high temperature and recover when those conditions are reversed. A great deal is known about the genetic pathways involved in dauer formation, but dauer recovery is not as well characterized. Microarray analysis identified a group of genes whose expression patterns suggest roles in gene regulation during dauer recovery (Wang and Kim. 2003. Development. 130: 1621-1634). Specifically, these genes are transiently induced during dauer recovery, peaking approximately two hours after the introduction of food. The transiently expressed genes include ten nuclear receptor (NR) genes. NRs are transcription factors with known roles in coordinating transcriptional cascades during development. We are analyzing these genes to see whether they function in dauer recovery. As part of our analysis, we are using quantitative Real Time PCR to confirm the expression patterns shown by microarray analysis. We report some differences in the expression patterns compared to the microarray analysis, but all genes tested to date are induced during dauer recovery. To test the function of these genes in dauer recovery, we are using double stranded RNA interference in *daf-2(e1370)* and *daf-7(e1372)* worms. Interestingly, of the NR genes tested to date, none show defects in dauer recovery in a *daf-2(e1370)* background, while dsRNAi treatment does inhibit dauer recovery in *daf-7(e1372)* worms for at least a subset of these genes.

718A

The novel cilia protein DAF-25 is required for DAF-11 cilia localization. **Victor L. Jensen**[1], Nathan J. Bialas[2], Sharon Bishop-Hurley[3], Michel R. Leroux[2], Donald L. Riddle[1,4]. 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 3) CSIRO-Livestock Industries, Queensland Biosciences Precinct, Brisbane, QLD, Australia; 4) Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada.

Four alleles of *daf-25* were isolated from screens for new temperature-sensitive Daf-c (dauer-constitutive) mutants. At restrictive temperature, *daf-25* mutants form dauer larvae even when food is abundant. The Daf-c phenotype can be rescued by maternally supplied *daf-25(+)*. Compared to N2, *daf-25* adults failed to avoid sucrose and high concentrations of NaCl, indicating that they are defective in sensing osmotic gradients. There is also a reduction of brood size when worms are shifted to the restrictive temperature (25°C) at L4, despite normal levels of progeny at the permissive temperature (15°C). The Daf-c phenotype of *daf-25* is suppressed by *daf-10*/IFT122 but not *daf-6*/PTCHD3. This puts DAF-25 function downstream of DAF-6 but upstream of DAF-10. This implies that DAF-25 function requires cilia because DAF-10 is required for proper cilia development, whereas DAF-6 is required for proper formation of the receptor channel and exposure of the ciliated neurons to the environment. DAF-25 does not appear to be required for ciliogenesis, because there is a normal pattern of DiI staining in all stages, including the dauer. A rescuing DAF-25::GFP fusion protein is expressed in ciliated neurons, as well as some interneurons, and shows sub-cellular localization to the cilia. Because the phenotype, epistatic order and expression profile of *daf-25* are similar to *daf-11* (encoding a membrane-bound guanyl cyclase), we thought there may be an effect on DAF-11 in a *daf-25* mutant background. Indeed, DAF-25 is required for proper DAF-11::GFP localization to the cilia. This may be a specific interaction because *daf-25* mutations do not affect the cilia localization of GFP translational fusions for TAX-4/CNGA1 or OSM-9/TRPV4. We show that DAF-25 is a novel cilia protein. We are currently exploring the possible role of the mammalian ortholog of DAF-25 in human disease.

719B

RNAi screen of DAF-16/FOXO target genes links dauer formation and innate immunity. **Victor L. Jensen**[1], Yu-Hui Lee[2], Karina T. Simonsen[3], Donha Park[2], Donald L. Riddle[1,2]. 1) Dept Medical Genetics, Univ British Columbia, Vancouver, BC, Canada; 2) Michael Smith Laboratories, the University of British Columbia, Vancouver, BC, Canada; 3) Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark.

The DAF-16/FOXO transcription factor is the major downstream output of the insulin/IGF1R signaling pathway for dauer formation. To identify novel downstream genes, we screened previously identified candidate genes putatively regulated by DAF-16 using RNAi. We used a sensitized genetic background [*eri-1(mg366)*; *sdf-9(m708)*], which enhances both RNAi and constitutive dauer formation (Daf-c). Among 515 genes screened, 22 displayed a synthetic Daf-c (SynDaf) phenotype with *sdf-9*. Two of these genes, *srh-99* and *hpd-1*, were previously identified SynDaf genes, but twenty have not previously been associated with dauer formation. Two of the latter genes, *lys-1* and *cpr-1* are known to participate in innate immunity and six more are predicted to do so, suggesting that the immune response may contribute to the dauer decision. Indeed, knockdown of two of these genes, *lys-1* and *clc-1* causes *C. elegans* to be more susceptible to infection by the pathogen *Staphylococcus aureus*. *clc-1* may represent a novel defense mechanism in *C. elegans* as it has been shown to function in epithelial cohesion, and its expression is up-regulated upon infection. Dauer formation exhibited by *daf-8(m85)*, *sdf-9(m708)*, and wild-type N2 (at 27°C) were all enhanced by exposure to pathogenic bacteria. We conclude that knockdown of a gene required for innate immunity increases infection by pathogens, and this led to increased dauer formation in our screen. We propose that formation of the non-feeding dauer dispersal stage is a behavioral response to pathogens.

720C

CED-4-dependent regulation of germ cell proliferation in dauer larvae by protein phosphatase 2A. **Pan-Young Jeong**, Pradeep M. Joshi, Bilge Birsoy, Joel H. Rothman. NRI, UCLA, Santa Barbara, Santa Barbara, CA.

When *C. elegans* senses inappropriate growth conditions (high temperature, limited food supply and dauer pheromone), it enters the long-lived dauer larva state. It has been previously reported that the germ cells in dauers remain in G2 arrest until exit from the dauer state. To investigate the mechanisms that promote this arrest, we analyzed the function of genes that when inactivated by RNAi result in supernumerary germ cells (i.e., substantially more than the ~42 present in normal dauers) in dauer-constitutive *daf-2* and *daf-7* mutants. The gene set analyzed included those that are strongly upregulated during dauer development based on microarray data (P.-Y. Jeong et al., PlosOne 2009) and previously known cell cycle regulators. This screen identified *wee-1.3, cki-1, ngp-1, nst-1, paa-1 and let-92*, as suppressors of germ cell proliferation during the dauer stage. We found that RNAi of *paa-1*, which encodes a structural subunit of protein phosphatase 2A, and *let-92*, which encodes the catalytic subunit of this enzyme, results in an ~50% increase in germ cell number in dauer larvae. Prolonged culture of *daf-2; paa-1(RNAi)* and *daf-2; let-92(RNAi)* dauers did not result in a further increase in germ cell number, revealing that additional regulatory mechanisms must exist in dauers to inhibit germ cell proliferation. Many of the arrested germ cells showed a condensed nuclear morphology and stain positively for phospho-histone H3, suggesting that they are arrested at M-phase; in some animals, virtually all of the germ cells show such morphology. Thus, PP2A regulates G2 arrest in dauer larvae, and in its absence, other factors cause cells to accumulate in mitosis. Remarkably, we found that the increase in germ cell number is partially dependent on the pro-apoptotic regulator CED-4 but is independent of CED-3 and CED-9 function. Thus, in addition to other activities recently ascribed to CED-4, these findings implicate CED-4 in germ cell arrest during dauer diapause apparently independent of its role in regulating programmed cell death.

721A

Two chemoreceptors mediate responses to dauer-inducing pheromone in *C. elegans*. **Kyuhyung Kim**[1], Koji Sato[2], Mayumi Shibuya[1], Danna Zeiger[1], Rebecca Butcher[3], Justin Ragains[3], Helen Yeung[1], Jon Clardy[3], Kazushige Touhara[2], Piali Sengupta[1]. 1) Department of Biology, Brandeis University, Waltham, MA; 2) Department of Integrated Biosciences, University of Tokyo, Chiba, Japan; 3) Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA.

Species-specific chemical communication mediated by mixtures of released chemicals called pheromones regulates a broad range of social behaviors. Similar to other animal pheromones, *C. elegans* pheromone elicits both long-term 'primer' effects on development by regulating entry into the alternate dauer developmental stage, as well as immediate 'releaser' effects on adult sexual behavior (Golden and Riddle, 1982; Srinivasan et al., 2008). Dauer pheromone is a complex mixture of structurally related derivatives of the dideoxy sugar ascarylose (Jeong et al., 2005; Butcher et al., 2007, 2008). The signaling pathways required for dauer pheromone responses are unknown.

We identified two G protein-coupled receptors, *srbc-64* and *srbc-66*, that mediate the dauer regulatory effects of dauer pheromone. *srbc-64* and/or *-66* null mutants exhibit strong defects in dauer formation and fail to downregulate *daf-7* TGF-β and *str-3* chemoreceptor gene expression in the ASI chemosensory neurons in response to a subset of dauer pheromone components. However, adult responses to pheromone are unaffected. Both *srbc-64* and *-66* chemoreceptors are expressed exclusively in the ASK chemosensory neurons, and full-length GFP-tagged SRBC-64 and -66 proteins are localized to the sensory cilia. Animals lacking the ASK neurons exhibit strong defects in dauer formation, suggesting that the ASK neurons are the major sensory neuron type regulating pheromone-mediated dauer formation. Heterologous expression of both, but not each receptor alone, in the HEK293 cells are sufficient to confer pheromone-mediated responses, further indicating that SRBC-64 and -66 are receptors for dauer pheromone components in the dauer formation pathway. These chemoreceptors are members of a *Caenorhabditis*-specific subfamily that has undergone extensive expansion in *C. elegans* (Thomas and Robertson, 2008). We propose that expansion of the SRBC subfamily may contribute to the fidelity and diversity of the pheromone response in *C. elegans*. Current experiments are aimed at identifying the neurons and receptors that mediate dauer formation in response to additional pheromone components, and the signaling pathways that transmit pheromone information from the ASK to the ASI neurons to regulate dauer formation (see abstract by Zeiger et al).

722B

Role of autophagy in *daf-2*-mediated fat metabolism, dauer formation and longevity. **Lizbeth Núnez**[1], Marlon Jansen[2], Lana Tolen[1], Alicia Meléndez[1,2]. 1) Biology, Queens College of the City University of New York, Flushing, NY; 2) Biology, The Graduate Center, CUNY, New York, NY.

Age and obesity are two factors that predispose individuals to Type II diabetes. Although the connection between obesity, age and the onset of Type II diabetes is well recognized, the molecular basis for it remains elusive. A reduction in *daf-2*/insulin-like signaling is associated with entry into dauer and long lifespan. This is also associated with a change in fat storage. As animals enter dauer, they arrest feeding, and age very slowly. Our hypothesis is that autophagy may be a process by which aging and fat metabolism are commonly regulated. Autophagy, through the sequestration and delivery of cargo to the lysosomes, is the major route for degrading long-lived proteins and cytoplasmic organelles in eukaryotic cells. Using a loss of function mutation in the *daf-2*/insulin-like gene, we have shown that *bec-1*, the *C. elegans* ortholog of the yeast and mammalian autophagy gene *ATG6/VPS30/beclin 1* is essential for dauer morphogenesis and lifespan extension in *C. elegans*. Interestingly, we find that *bec-1* deficient animals display a reduction in fat storage. This lack of fat accumulation may be due to an increase in fat-hydrolyzing lipase activity. Reducing *bec-1* gene dosage results in a dramatic increase in lipase activity when compared to wild-type animals. Conversely, the RNAi mediated knock down of lipase activity appears to suppress the lethality associated with *bec-1* mutant animals. We will present our data and discuss how the process of autophagy may be essential for survival during dauer diapause and long-term fasting.

723C

Role of the nuclear hormone receptor DPR-1 in dauer development. **Taiga Suzuki**, Bilge Birsoy, Erin Newman-Smith, Gina Broitman-Maduro, Joel H. Rothman. Department MCD Biology and Neuroscience Research Institute, UC Santa Barbara.

The dauer larva is an alternative stage of *C. elegans* development induced by a combination of low food abundance and a high concentration of dauer pheromone, a mixture of small lipophilic compounds. The mechanism of reception of dauer pheromone and response to starvation are not fully understood. We previously isolated a gene encoding an apparent nuclear hormone receptor (NHR) *dpr-1* (*d*auer *p*heromone *r*esponsive) that modulates dauer larva formation. Immunoreactive DPR-1 is expressed in gut cells, and apparently some neurons, of embryos and L1 larvae. Surprisingly, DPR-1 protein is cytoplasmically localized in sparsely grown worms, but appears to relocalize to the plasma membrane of gut cells in worms exposed to high concentrations of dauer pheromone. Overexpression of *dpr-1* from the *elt-2* promoter leads to an increase in the number of dauer larvae when worms are grown at high density. This led us to hypothesize that DPR-1 may regulate dauer development and might be a component of the pheromone reception system, perhaps acting through a non-canonical NHR signaling system. Consistent with a possible role for DPR-1 in regulating dauer formation, we found that in both liquid and plate assays, the *dpr-1(tm898)* mutation partially suppresses the formation of dauer larvae induced by pheromone, indicating that *dpr-1* is a partial daf-d gene. Further, we found that the daf-c phenotypes of *daf-11(m47)* and *daf-7(e1372)* were partially suppressed by *dpr-1(tm898)* mutations, suggesting that *dpr-1* may function downstream of these genes in the dauer pathway. Further studies are focused on elucidating the function of DPR-1 and its unusual intracellular distribution during dauer signaling.

724A

Heterochronic genes regulating male tail tip morphogenesis. **R. Antonio Herrera**, Karin Kiontke, Samuel Ahn, Jamie B. Plevy, David H. A. Fitch. Dept of Biology, New York University, New York, NY.

In *C. elegans* the heterochronic pathway regulates the expression of stage-specific events, like molts and the development of adult alae, vulva and the male tail. The male tail tip is particularly sensitive to perturbations of the heterochronic pathway. During L4 male development, the four epidermal cells, hyp8-11, which compose the pointed tail tip of the larva, fuse and retract to form the rounded tail tip of the adult. Loss-of-function mutations in *let-7* and gain-of-function mutations in *lin-41* lead to a delay in male tail tip morphogenesis and the retention of a pointed tail tip in the adult [1]. In contrast, loss-of-function mutations of *lin-41* cause precocious tail tip morphogenesis in L3 males [1].

We have recently identified additional genetic loci involved in the timing of tail tip morphogenesis. A deletion screen for tail tip mutants uncovered *lep-4* on LG IV and *lep-5* on LG X. Array comparative genomic hybridization (aCGH) revealed deletions of 10kb for *lep-4(ny4)* and 80kb for *lep-5(ny10)*. Two additional mutants fail to complement *ny4 (ok900 and bx147)*, suggesting that the ORF corresponding to *lep-4* is Y55F3AM.6.

All of these mutations cause delayed tail tip retraction as in the case of some *let-7* and *lin-41* mutants. Additional phenotypes include protruding vulva, adult molts and adult tail tip retraction. Some of these mutants are defective in male mating behavior. Dauer suppresses the male tail phenotypes. Such phenotypes are caused by mutations in other heterochronic genes.

We are determining where these genes fit in the regulatory network of tail tip morphogenesis. So far, we know that this network includes Wnt [2], Hox (see M. D. Nelson abstract) and sex determination (see D. A. Mason abstract; [3]) pathways. Using expression epistasis, we have found that *lep-4* and *lep-5* are upstream of *dmd-3*, a central effector of male tail morphogenesis. Within the heterochronic pathway, *lep-4* is upstream of *lin-41*. Additional epistasis experiments are underway.

[1] Del Rio-Albrechtsen et al. 2006, Dev. Biol. 297:74.
[2] Zhao et al. 2002, Development 129:1497.
[3] Mason et al. 2008, Development 135:2373.

725B

Dysfunction of the novel MLT-10 family of repetitive, proline-rich secretory proteins blocks the molting cycle. **Alison Frand**[1], Gary Ruvkun[2]. 1) David Geffen School of Medicine, UCLA, Los Angeles, CA; 2) Department of Molecular Biology, Mass. General Hospital, Boston, MA.

The molting cycle involves complete remodeling of collagen-rich extracellular matrices, but the signaling and enzymatic cascades that trigger and execute this remodeling are not well understood. Here, we describe the *mlt-10* gene isolated in a forward genetic screen for mutants unable to molt. MLT-10 is the first reported member of a large family of annotated glycoproteins characterized by a domain of unknown function (#644) and a repetitive C-terminal region rich in serine/threonine and proline. The substitution mutation *mlt-10(mg364)*, which disrupts the repetitive region, or increased expression of *mlt-10(+)* renders larvae unable to shed old cuticles. The development of *mlt-10(mg364)* larvae also requires a continuous supply of cholesterol, suggesting a reduced capacity to utilize sterols. Loss of *mlt-10* has no obvious impact on development, possibly due to functional redundancy among the thirteen *C. elegans* homologs. Nevertheless, the conserved nuclear hormone receptors NHR-23 and NHR-25 promote expression of *mlt-10* in the hypodermis whenever the exoskeleton is remodeled, showing that *mlt-10* naturally relates to the molting cycle. Further, exposure of glycans on the surface of molting larvae corresponds with expression of *mlt-10*. We propose that MLT-10 is one of a suite of cuticular glycoproteins whose assembly and disassembly are highly regulated during the molting cycle.

726C

Identification of heterochronic genes that suppress over-expression of *mir-48*, a *let-7* family miRNA. Tamar D. Resnick, **Theresa L.B. Edelman**, Sarah J. Malmquist, Ann E. Rougvie. University of Minnesota, Minneapolis, MN.

The heterochronic genes of *C. elegans* regulate the timing of developmental events throughout postembryonic stages. Disruption of these genes leads to the skipping or reiteration of certain developmental events. Among these genes are the first-described miRNAs, *lin-4* and *let-7*. Loss of function for either of these genes causes a "retarded" phenotype in which developmental events are reiterated in subsequent stages, delaying differentiation of adult tissues. Several miRNAs, including miR-48, miR-241, and miR-84, share identity with the 5' end of *let-7* miRNA and may target an overlapping set of mRNAs. Disruption of these three *let-7* sisters together, but not individually, results in a pronounced retarded phenotype, indicating that the sisters function redundantly. This redundancy makes isolation of hypomorphic alleles of the *let-7* sisters from forward genetic screens unlikely. However, gain-of-function alleles of *mir-48* were recovered as suppressors of *lin-4* retarded phenotypes and on their own cause a precocious phenotype. Over-expression of *mir-48* from multicopy arrays leads to enhanced precocious defects, including aberrations in vulva precursor cell divisions, resulting in disruption of egg-laying. To identify additional players in the pathway, we screened for suppressors of the Egl phenotype in *mir-48* over-expressing animals. These screens are expected to identify new heterochronic mutants, miR-48 target genes, and genes involved in miRNA expression and function. We isolated 36 suppressed lines from 48,000 haploid genomes screened. Preliminary analyses have identified at least four complementation groups among the suppressors recovered, including alleles of the heterochronic gene *lin-66*, which validates this approach for identification of regulators of developmental timing. *lin-66* and the *let-7* family of miRNAs have been suggested to act in parallel to inhibit *lin-28* post-transcriptionally. We are taking advantage of these new *lin-66* alleles to perform a screen for suppressors of *lin-66* lethality and further expand our pool of interacting heterochronic genes.

727A

Identification and characterization of novel heterochronic genes involved in the *let-7* microRNA-dependent developmental timing pathway in *Caenorhabditis elegans*. **K. Hada**[1,2], H. Hasegawa[2,3], Y. Kanaho[3], F.J. Slack[4], R. Niwa[1,2]. 1) Graduate School of Life and Environmental Sciences, University of Tsukuba, Japan; 2) Initiative for the Promotion of Young Scientists' Independent Research, University of Tsukuba, Japan; 3) Graduate School of Comprehensive Human Sciences, University of Tsukuba, Japan; 4) Department of MCD Biology, Yale University, New Haven, CT, USA.

The development of multicellular organisms occurs in four dimensions; the three axes of space and a fourth axis of time. While much is known about the fundamental mechanisms of spatial pattern formation, the regulation of the timing of developmental events is less understood. During postembryonic development of the nematode *Caenorhabditis elegans*, the timing of cell fate determination is controlled by the heterochronic gene regulatory pathway. One of the essential components is *let-7*, a founder member of microRNAs (miRNAs). The *let-7* family miRNAs and their targets are evolutionally conserved in many animals, suggesting that similar genetic pathways control developmental timing across phylogeny. To further elucidate genes functioning in the *let-7*-dependent developmental timing pathway, we have conducted a loss-of-function screen using a GFP reporter construct fused with a regulatory promoter of *apl-1*, a homolog of human *amyloid precursor protein gene* known to be involved in Alzheimer's diseases. The *apl-1* expression is regulated by the *let-7* family microRNAs and their targets (Niwa et al. *Dev. Biol.* 315: 418-425, 2008). Therefore the *apl-1::gfp* construct can be used for monitoring the activity of the *let-7* pathway in the mutant screen. Here we report identification and characterization of novel genes functioning in the *let-7* heterochronic pathway. We will also discuss evolutionarily conservation of the downstream gene components of the *let-7*-dependent timing pathway.

728B

Daf-16-mediated fat storage was regulated to the developmental stage of *C. elegans*. **M. Horikawa**, K. Sakamoto. The University of Tsukuba, Tsukuba, Japan.

Caenorhabditis elegans has DAF-2/insulin like signaling in response to starvation. DAF-16 is localized to nuclei in fasting and regulates expressions of genes those were involved in several metabolisms. It is known that fat metabolism is regulated by insulin like signaling. It is suggested that regulation of fat metabolism is changed in development timing. Fatty acid synthesis is activated and fat storage is increased under the control of *daf-16* in dauer. On the other hand, starvation increases expression of *acs-2* that is involved in beta-oxidation in adult worms. It is unclear that these different regulations of fat metabolism in each development timing.

We reported that RNAi of fatty acid desaturase-2 (*fat*), *fat-6*, *fat-7* and elongase-2 (*elo*) reduced fat storage and activated *daf-16* in adult worms. We also observed nuclear localization of *daf-16* in *fat-2* RNAi worms. In *daf-16* mutant (*mgDf50*) or RNAi worms, RNAi of *fat-2, fat-6, fat-7* and *elo-2* did not reduce fat accumulation. These results suggest that *daf-16* has the function of fat reduction in adult worms.

Next, we used *daf-2* mutant (*e1370*) for analysis of *daf-16* function of fat accumulation in different development timing. We observed increase of fat storage by dauer induction in *daf-2* mutant. But 25°C heat treatment of L4 larvae reduced fat storage in adult *daf-2* mutant. Both of fat accumulation in dauer and fat reduction in adult worms were suppressed by RNAi of *daf-16*. And fat accumulations were as same as N2 (WT) in both of L2-L3 larvae and adult worms without 25°C heat treatment. These results suggested that *daf-16* function of fat storage was changed in development timing and a response mechanism of heat stress was also involved in fat regulation.

729C

Study of AMPK during the L1 diapause. **Julie Mantovani**, Richard Roy. Department of Biology, McGill University, Montreal, Quebec, Canada.

AMPK is a metabolic master switch which is activated in response to various nutritional and stress signals. In the *C. elegans* dauer larvae, this serine/threonine kinase is necessary to maintain the quiescence of germ cells and to promote long-term survival. When *C. elegans* eggs hatch in a medium without food, they enter a diapause stage and can survive for extended periods of time (20 days) as first larval stage animals or L1. Since these larvae are motile and appear healthy, the study of AMPK during the L1 diapause can provide different clues to better understand this pathway is regulated. *C. elegans* hatchlings can live about 18 to 20 days in the absence of nutrients and then resume postembryonic development without consequence. This diapause requires the kinase activity of aak-2, therefore we are performing a proteomic survey of AMPK targets that may be required to extend the duration of the L1 diapause. In parallel we have used bioinformatics to identify potential AMPK targets based on the consensus phosphorylation sequence throughout the *C. elegans* genome. Phosphorylation of these proteins likely affects the appropriate allocation of energy resources during this period of nutrient stress. Many of these targets appear to be conserved in mammalian cells and thus are likely downstream AMPK substrates. We are currently verifying expression and both biochemical and genetic interactions with aak-2 during the L1 stage.

730A

Screen of microRNA deletion alleles in sensitized genetic background reveals additional microRNAs may function in developmental timing. **J. L. Brenner**, A. L. Abbott. Dept Biological Sci, Marquette Univ, Milwaukee, WI.

microRNAs have critical roles in animal development and disease. Because the majority of microRNAs are not individually essential for C. elegans development, we hypothesize that microRNAs may function together to control common targets or pathways. To test this, we built a set of compound mutant strains with deletion alleles of microRNA genes and the gk214 allele of alg-1. alg-1 encodes one of two Argonaute proteins that are required for microRNA activity in C. elegans. alg-1 mutants have reduced levels of microRNAs and display weak developmental timing defects as determined by alae formation, a failure to exit the molting cycle, and adult lethality. In order to identify developmental defects, assays for embryonic lethality, gross morphology, egg laying, larval and adult lethality, and alae formation were performed. Five microRNA; alg-1 mutants display enhanced embryonic lethality compared to alg-1 single mutants. Four strains display increased gonad migration defects. Two strains display increased alae formation defects, and three strains display reduced alae formation defects. Of the three that display reduced alae formation, we found that mir-54/55/56 alg-1 and mir-238; alg-1 double mutants also display decreased adult stage lethality compared to alg-1 single mutants. To identify miR-54/55/56 and miR-238 targets whose upregulation results in decreased alae formation and adult lethality, we used RNAi to knockdown the activity of predicted targets. We have identified vhp-1 as a candidate target for miR-54/55/56. We propose that vhp-1 is regulated by miR-54/55/56 and may function specifically to regulate the developmental timing pathway or more generally to regulate microRNA activity.

731B

The overlapping roles of *lin-4* and *let-7* microRNA families during nematode development. **Kimberly A. Breving**, Kenya T. Madric, Aurora Esquela-Kerscher. Department of Microbiology and Molecular Cell Biology, Eastern Virginia Medical School, Norfolk, VA.

MicroRNAs (miRNAs) negatively control gene expression in a sequence specific manner and direct essential biological processes related to cellular growth and differentiation. The most thoroughly characterized miRNAs, *lin-4* and *let-7*, direct cell fate determination during the larval transitions in *C. elegans* and act as key regulators of temporal gene expression. *lin-4* and *let-7* are founding members of two distinct families of miRNA genes sharing strong sequence homology primarily in the 5' end of the mature miRNAs. Although little is known about the *lin-4* homologue *mir-237*, the *let-7* family members, *mir-84*, *mir-48*, and *mir-241* are shown to redundantly control the timing of the L2-to-L3 larval transition (Abbott, et. al. 2005). We previously found that *mir-237* and certain *let-7* homologues display overlapping expression patterns in the developing gonad and vulva, which suggest that combinations of miRNAs from across these families control common developmental events. To test this hypothesis, we have begun to analyze the single, double and triple mutant phenotypes for the *mir-237*, *mir-84* and *mir-48* genes. We find that the *mir-237; mir-84; mir-48* triple mutant animals exhibit distinct abnormalities not found in the single or double deletion combinations, which include significantly decreased brood sizes (WT: 250-300; Triple: ~50) as well as severely increased lethality rates (WT:~5%; Triple: ~60%). In an effort to identify miRNA targets mediating these effects, we have utilized miRGen bioinformatic analysis to compile a list of genes with binding sites for all three of our miRNAs of interest in their 3' UTRs. Many of these potential targets direct activities associated with gonad and larval development, cellular growth, cell cycle progression and meiosis. We have initiated an RNAi-by-feeding screen to determine if these genes can suppress the phenotypic abnormalities noted in our *mir-237; mir-84; mir-48* mutant animals, before embarking on a larger genome-wide suppressor screen. Our work is indicative of redundancy between the *lin-4* and *let-7* families and reveals the complexities of miRNA function during development. (This work is supported by a grant from the Thomas F. and Kate Miller Jeffress Memorial Trust.).

732C

Proteomic analysis of O-GlcNAcylation during dauer formation in *C. elegans*. **J. Lee[1,2]**, K. Kim[1,2], J. Lee[1], Y. Paik[1]. 1) Yonsei Proteome Research Center, Yonsei Univ, Seoul, Korea; 2) These authors contributed equally to this work.

Modification of proteins at serine or threonine residues with N acetylglucosamine (O-GlcNAcylation) plays a regulatory role in many model organisms. Here, we investigated the mechanism by which O-GlcNAcylation regulates entry into the stress-induced dormant state dauer in *Caenorhabditis elegans*. We confirmed that *ogt-1* (O-GlcNAc transferase) mutants exhibited a dauer-defective phenotype whereas *oga-1* (O-GlcNAcase, catalyzes O-GlcNAc removal) mutants exhibited a dauer-prone phenotype when treated with daumone. Consistent with these findings, treatment with low levels of daumone and the O-GlcNAcase inhibitor PUGNAc enhanced the frequency of dauer entry. Treatment of *daf-2* with PUGNAc increased the frequency of dauer entry, providing additional evidence that O-GlcNAcylation promotes dauer formation. Alterations to the proteome as a result of induction of O-GlcNAcylation were analyzed by two-dimensional electrophoresis (2DE) using lysates of N2 and *oga-1* worms. Seven differentially expressed protein spots were further analyzed using LC-MS/MS. The identities of these proteins suggest that O-GlcNAcylation influences stress resistance, protein folding, and mitochondrial function. To identify potential target proteins that are O-GlcNAcylated during dauer formation, we specifically labeled O-GlcNAc with fluorescent dye using O-GlcNAc labeling system containing TAMRA fluorescence dye targeting O-GlcNAcylated proteins. The fluorescently labeled samples were resolved by 2DE and 20 protein candidates were selected. Our data suggest that O-GlcNAcylation may regulate a shift in some proteins including cytoskeletal and protein turnover during dauer formation. Thus, these data may be valuable in identifying the mechanism by which high levels of O-GlcNAcylation enhance dauer formation. (This study was supported by a grant from the Korea Health 21 R&D project, Ministry of Health and Welfare of Republic of Korea [A030003 to YKP].).

733A

An RNAi Screen for Potential ASNA-1 Interactors Reveals Two New Modulators of Insulin Signaling. **Gautam Kao[1]**, Balasubramanian Natarajan[1], Ola Billing[1], Simon Tuck[2], Peter Naredi[1]. 1) Dept. of Surgery, Umea University, Umea, Sweden; 2) Umea Center for Molecular Medicine (UCMM), Umea University, Umea, Sweden.

asna-1 is a conserved ATPase that is found throughout the phylogenetic spectrum from bacteria to humans. Work in different model systems by several groups including ours indicates that ASNA-1 has many seemingly unconnected roles in development and physiology. These include insulin secretion, resistance to certain metals and to ER stress. Experiments with yeast and mammalian cell extracts have also indicated a role for ASNA-1 in the insertion of the ”TA” class of transmembrane proteins. To understand more about the mechanistic basis of the various functions of ASNA-1, we made a list of 143 potential interactors based on various criteria. Genes that upon RNAi showed phenotypes in common with those of *asna-1* mutants were selected for further study. The screening yielded two genes that when inactivated by RNAi showed an L1 arrest phenotype and lowered strength of insulin signaling; a phenotype that is also seen in *asna-1* mutants. The first gene is a protein translocase homolog and the other is a chaperone. However the overlap of phenotypes is not complete since neither one posseses the pale body phenotype of *asna-1* mutant adults, nor its characteristic set of germline defects. Further, the translocase homolog and ASNA-1 have a role in resistance to metal toxicity, while *asna-1* mutants and worms with inactivated chaperone function display ER stress. Thus each of these two genes possibly work with ASNA-1 on a subset of its functions and their analysis will lead to a more complete understanding of how ASNA-1 exerts its various effects.

734B

Nutritional signalling and *rnt-1* regulation of proliferation and differentiation. **Toby Braun[1]**, Kimberley Bryon[1], Nicole Saad[1], Rachael Nimmo[2], Alison Woollard[1]. 1) Department of Biochemistry, University of Oxford, Oxford, United Kingdom; 2) Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, USA.

RNT-1 is the only *C. elegans* member of the RUNX family of transcription factors, shown to be critical regulators of proliferation, differentiation and stem cell maintenance in mammals as well as invertebrate model systems. Mutations in RUNX genes have been implicated in a range of cancers in humans, acting both as oncogenes and tumour suppressors. Previous work in our lab has shown that RNT-1, together with its binding partner BRO-1, is a critical regulator of the development of seam cells with their stem cell-like lineage during larval development. We have several independent strands of evidence that nutritional status impinges on the *rnt-1*-dependent development of the seam cell lineage. In L1 larvae exiting starvation-induced diapause, asymmetric seam cell divisions fail in a *rnt-1* background, but not in wild type worms exiting L1 diapause or in well fed *rnt-1* mutants (not going through diapause) (Nimmo et al, 2005). Additionally we have seen a slight increase in total seam cell number if the worms are grown on non-uracil-deficient bacteria (OP50 are uracil-deficient), potentially representing a richer food source. In contrast, passage through starvation-induced L1 diapause partially suppresses the massive seam cell hyperplasia induced by combined over-expression of RNT-1 and BRO-1. We are currently investigating this link between nutritional status, potentially mediated through insulin signalling, and *rnt-1*-dependent regulation of seam cell proliferation and differentiation and will provide further evidence at the meeting.

735C

Does *lin-29* control AFF-1-dependent fusion of the seam cells? **Lilach Friedlander**, Benjamin Podbilewicz. Department of Biology, Technion-Israel Institute of Technology, Haifa, Israel.

During the larva to adult transition the seam cells undergo terminal differentiation. The seam cells stop dividing, fuse with each other and synthesize the adult cuticle. AFF-1 is a fusogen in *C. elegans* required for the fusion of the lateral seam cells at the L4-adult transition, generating the lateral epithelial syncytium on each side of the animal. The seam cells terminal differentiation is controlled by the heterochronic pathway. The zinc finger transcription factor LIN-29 is the most downstream regulator of the seam cells terminal differentiation. *lin-29* regulates the collagen gene *col-19* which is one of the genes involved in the seam cells terminal differentiation. *lin-29* is a good candidate to be a positive regulator of AFF-1 expression in the seam cells. To determine whether *lin-29* controls *aff-1* through transcriptional regulation we examined *lin-29(n482)* loss-of-function mutant worms which also express *aff-1p::GFP*. While wild-type worms express *aff-1* in the seam cells starting from the L4 stage, we found that some of the *lin-29* mutant worms had a reduced or no expression of *aff-1* in the seam cells at the L4 stage. These results suggest that *lin-29* may activate *aff*-1 transcriptionally. However, the mutant allele that was chosen has partial penetrance and may not be a complete null allele. Therefore, we chose different *lin-29* mutant alleles that are complete null alleles for further examination of *aff-1* regulation. We are currently generating strains of either *lin-29(n333)* or *lin-29(n546)* which also express *aff-1p::GFP*. Next, we will also test the influence of ectopic expression of *lin-29* on *aff-1* expression. It was shown that *lin-29* binds to specific sites of *col-19* promoter (1). In order to determine if *lin-29* regulates *aff-1* directly, we will examine whether similar sites exist in *aff-1* promoter using comparison of the *col-19* and *aff-1* promoter sequences. We will also examine whether *lin-29* binds to sites in *aff-1* promoter in vitro, and whether these sites are indeed necessary for in vivo activation of *aff-1*. In case our results indicate that *lin-29* activates *aff-1* indirectly we will search for other factors that are involved in this regulatory pathway. An alternative hypothesis is that *lin-29* regulation of *aff-1* activity is posttranscriptional. To investigate this possibility we have generated the translational reporter AFF-1::mCherry. We found that in Sf9 heterologous insect cells AFF-1::mCherry induces cell-cell fusion. We will show expression of AFF-1::mCherry in the seam cells and explore *lin-29* potential regulation of AFF-1 localization and activities in the terminal differentiation of the seam cells. (1) Rougvie, A. E. and Ambros, V. (1995) Development 121, 2491-2500.

736A

Histone H3 lysine 9 methylation in the *C. elegans* germ line. **Jessica B. Bessler**[1], Erik Andersen[2], Anne Villeneuve[1]. 1) Stanford University, Stanford, CA; 2) MIT, Cambridge, MA.

The spatial organization of the *C. elegans* germ line allows us to visualize changes in chromosome structure and covalent chromatin modifications as germ cells switch from mitosis to meiosis and progress through gamete differentiation. We are investigating how chromatin relates to the function and inheritance of chromosomes, focusing on the acquisition and function of methyl modifications on lysine 9 on histone H3. We find that H3K9me2 and H3K9me3 are differentially localized in adult germ lines. In agreement with previous data, we saw H3K9me2 accumulating on chromosomes in hermaphrodite germ lines during meiotic prophase progression. Further, H3K9me2 becomes concentrated on the single male X-chromosome, on unpaired hermaphrodite X-chromosomes in hermaphrodites and on a repetitive array containing a germline-competent promoter. In contrast, H3K9me3 has a broad chromosomal distribution in both hermaphrodite and male germ lines, and is not enriched on X-chromosomes. Strikingly, H3K9me3 is enriched on simple arrays carrying somatic-competent promoters. Although H3K9me3 is associated with weakly/non-expressed genes in *C. elegans*, H3K9me3-enriched arrays correspond to DAPI-light DNA domains, indicating that the arrays do not adopt a classical (DAPI-bright) heterochromatin organization. We also find that different methyltransferases account for the di- and tri-methyl marks in adult germ cells. MET-2 is required for all detectable H3K9me2 in the germ line. *met-2* mutants are fertile but exhibit a low-frequency meiotic defect, demonstrating that while H3K9me2 is not required for germ line formation and meiosis, MET-2 is required for meiosis to proceed with high fidelity. Despite the lack of H3K9me2 in a *met-2* mutant, a germline array was not desilenced indicating that H3K9me2 is not required to maintain array silencing. While H3K9me3 was unaffected in a *met-2* mutant background we discovered that MES-2, a known H3K27me2/3 histone methyltransferase, is required for H3K9me3. These and other data indicate that H3K9me3 is unlikely to be built upon H3K9me2 and underscore the independence of both acquisition and function of these chromatin modifications. A simple array integrated into an X chromosome has allowed us to investigate potential competition between H3K9me2 and H3K9me3. When a heavily trimethylated array is present on the dimethylated male X chromosome, we find the di- and tri-methyl marks remain disparate. In spite of the array being unpaired, this region is resistant to dimethylation. We are currently investigating if H3K9me3 is sufficient to prevent dimethylation of the array and if these distinct chromatin domains reflect the presence of chromatin boundaries.

737B

A novel role for *lin-61* in the DNA damage response. **Nicholas Michael Johnson**, Joris Pothof, Marcel Tijsterman. Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, Netherlands.

The *C. elegans* germline is an excellent model for studying cellular responses to DNA damage. In particular this is due to the spatial separation, in the germline, of the two DNA damage checkpoint responses: cell cycle arrest and apoptosis. We have identified a gene, *lin-61*, which is essential for both of these processes. Mitotic cell cycle arrest and meiotic apoptosis is defective in *lin-61* mutants following damage. In addition, these mutants are hypersensitive to ionizing radiation, indicating a role in protection from DNA damage.

Homologues of *lin-61* are implicated as tumor suppressors in other animals. Mutants of the *Drosophila* homologue, *l(3)mbt*, develop optic neuroblast tumors while the human homologue is frequently deleted in certain human malignancies. These proteins are members of a class of conserved transcriptional repressors. Their defining feature is the presence of so-called malignant brain tumor (MBT) domains, which have recently been shown to compact chromatin by directly interacting with methylated histones. Previously, Harrison et al (Genetics 2007) showed *lin-61* to be a class B synMuv gene, which acts to control vulval development. Here we reveal a novel role for *lin-61* in protecting the germline from DNA damage. At this meeting, we are particularly focusing on the apoptotic role of *lin-61*. Following damage, the pro-apoptotic proteins, *egl-1* and *ced-13* are up-regulated in a manner dependent on the p53 homologue, *cep-1*. Using qRT-PCR, we show that up-regulation of *egl-1* and *ced-13* is normal in *lin-61* mutants, indicating that *lin-61* acts in parallel to, or downstream of, *cep-1*. Further, the expression level of the anti-apoptotic gene *ced-9*/Bcl2 is also normal in *lin-61* mutants. We are now using genetic analysis to determine how *lin-61* mutants are defective for damage-induced apoptosis.

The *Drosophila* homologue of LIN-61 is part of a transcriptional repressor complex. To search for components of a putative LIN-61 complex and to find factors that act redundantly with *lin-61*, we performed a genome-wide RNAi screen for genes that are essential for viability in *lin-61* mutants but are dispensable in wild type worms. Intriguingly, this screen identified, in addition to several other synMuv genes, two components of the nuclear transcription factor Y (NF-Y). In mammalian cells, this conserved transcription factor works with p53 to modulate the expression of cell cycle genes and apoptotic genes such as the *ced-9* homologue, Bcl2. We are investigating the genetic connection between *lin-61* and NF-Y and their respective contributions to the DNA damage response.

738C

Exploring the biological roles of the histone lysine demethylase LSD1. **Amanda C. Nottke**[1,2], David J. Katz[3], William G. Kelly[3], Valerie Reinke[4], Yang Shi[1], Monica Colaiácovo[2]. 1) Dept of Pathology, Harvard Medical School, Boston, MA; 2) Dept of Genetics, Harvard Medical School, Boston, MA; 3) Biology Department, Emory University, Atlanta, GA; 4) Dept of Genetics, Yale University, New Haven, CT.

Disruptions affecting either the structural or regulatory functions of the protein:DNA complex referred to as chromatin can lead to dramatic effects on cellular homeostasis, for example by affecting global transcriptional processes. One way cells regulate the local chromatin environment is by post-translational modifications of the DNA-packaging histone proteins, including lysine methylation. The recent discovery of a lysine-specific histone demethylase (LSD1) confirmed that regulation of histone methylation involves the active removal of methyl groups. These newly discovered enzymes provide novel opportunities to understand how histone modifications function in chromatin-related processes, with model organisms providing a valuable *in vivo* approach. We are therefore using the nematode *C. elegans* to study the biological roles of the LSD1 homolog SPR-5. The mammalian LSD1 is a H3 lysine 4 demethylase, and SPR-5 shows *in vitro* activity against H3K4me2 as well, indicating conservation of enzymatic activity between worms and mammals.

spr-5 mutant germlines show an increase in H3K4me2, indicating SPR-5 acts *in vivo* in the germline to modulate H3K4me2. Further studies uncovered an unexpected role for SPR-5 in meiotic DNA double strand break repair (DSBR). *spr-5* mutants experience increased levels of p53-dependent germline apoptosis, indicating that a DNA damage checkpoint is triggered in the germline. Immunolocalization of RAD-51, a marker of DSBR, shows increased number of foci in *spr-5* mutants, suggesting either an increase in the levels of DSB formation or a delay in DSBR. *spr-5* mutants are also sensitive to DSB-inducing radiation, and in wild-type animals SPR-5 relocalizes within the nucleus upon radiation, suggesting SPR-5 may play an active role in responding to DNA damage in the germline. Perturbations of meiotic DSBR may be associated with alterations in chromatin state, so we are further evaluating whether additional histone modification patterns and progression of DNA damage repair are affected in the germline in *spr-5* mutants. An alternate (but not mutually exclusive) explanation is that loss of SPR-5 may lead to misregulation of DNA damage repair processes through the deregulation of target genes. We have therefore performed germline-specific microarrays to identify potential SPR-5 targets that are important for normal meiotic processes including DSBR.

739A

Numerous synMuv B genes show temperature sensitive early larval arrest and ectopic germline gene expression that is suppressible by germline chromatin modifiers. **Lisa N. Petrella**[1], Wenchao Wang[2], Caroline A. Spike[3], Andreas Rechsteiner[1], Susan Strome[1]. 1) Department of MCD Biology, University of California, Santa Cruz, Santa Cruz, CA. 95060; 2) Dana-Farber Cancer Institute, Boston, MA 02115; 3) Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN 55455.

In many animals the primordial germ cells are separated from the somatic lineages very early in development. In C. elegans this separation begins as early as the first embryonic cell division and is complete by the 24-cell stage. Newly hatched L1 larvae contain only two primordial germ cells, Z2 and Z3, which can be distinguished from somatic cells by the presence of P granules. Recent studies have revealed that mutants in several of the synMuv B class of chromatin regulators display phenotypes that suggest a partial transformation of soma to germ line. These phenotypes include ectopic expression of P-granule components in somatic cells of L1 larvae and hypersensitivity of worms to RNAi. In addition, the synMuv B gene *mep-1* shows an early larval arrest phenotype (Unhavaithaya et al., 2002; Wang et al., 2005). These phenotypes can be suppressed by mutations in the germline chromatin regulators *mes-2*, *mes-3*, *mes-6* and *mes-4*. Here we show that 9 of 15 synMuv genes tested arrest as L1 larvae at elevated temperature. The synMuv B mutants that arrest are those that express P-granule components in somatic cells. The high temperature L1 arrest phenotype is suppressed by mutations or RNAi of the mes genes and a subset of other known synMuv B suppressors. We determined that the synMuv B L1 arrest phenotype requires loss of both maternal and zygotic synMuv B gene function, is not dependent on the *daf-2* insulin signaling pathway, and is irreversible. Microarray analysis showed that, in addition to P-granule genes, several other classes of germline-specific genes are also over-expressed in *lin-35* and *lin-15B* arrested L1s. This over-expression is suppressed by RNAi of *mes-4*. Immunostaining for two over-expressed meiosis factors revealed ectopic somatic expression in arrested L1s, similar to that of P-granule components. We are currently performing a genome-wide RNAi screen to identify additional genes that can suppress the L1 arrest phenotype, with the goal of identifying chromatin regulators that participate along with the MES proteins in the crucial germ versus soma decision.

740B

The DM-domain gene *dmd-3* functions in multiple male-specific processes. **D Adam Mason**[1,2], Matthew D Nelson[3], David HA Fitch[3], Mark W Murphy[4], David Zarkower[4], Douglas S Portman[2]. 1) Siena College, Loudonville, NY 12211; 2) University of Rochester Medical Center, Rochester, NY 14624; 3) New York University, New York, NY 10003; 4) University of Minnesota, Minneapolis, MN 55455.

The majority of animal species generate two morphologically distinct sexes. In contrast to most developmental pathways, genetic pathways controlling sex differences are highly divergent between different taxa. The exception to this is the conserved use of DM domain transcription factors in determining sexual phenotypes in a variety of animals. Despite this conservation, the molecular mechanisms underlying DM domain gene function are still being elucidated. Therefore, characterizing the function of DM domain genes in *C. elegans* may inform our understanding of sex-specific development in all animals. *C. elegans* males and hermaphrodites are dimorphic for a number of traits including body size, tail morphology and gonad development. The *C. elegans* DM domain gene *dmd-3* is expressed in a number of tissues that undergo male-specific development. For example *dmd-3* is expressed strongly in the male, but not hermaphrodite L4 tail tip. The male tail tip undergoes a remodeling process that results in the male tail being blunt ended. Previous work has demonstrated that DMD-3 plays a critical role in regulating sex-specific male tail tip remodeling. Specifically, DMD-3 activity is both necessary and sufficient to induce the changes in cell shape, migration and fusion that drive retraction of the tail tip. Preliminary work suggests that DMD-3 activates a battery of genes that direct this morphogenetic process. Using a combination of biochemistry, genetics and bioinformatics we have identified a list of candidate genes that are potentially directly activated by DMD-3. These genes include the chondroitin sulfate synthase *sqv-5* and the rab GTPase *rab-6.2*. We are further characterizing these genes to determine if they function as downstream effectors of DMD-3 activity in the male tail tip. In addition, preliminary evidence suggests that *dmd-3* and *mab-3*, a similar DM domain gene, function together to control aspects of male gonad development. In male larvae, the linker cell guides the developing gonad to the hindgut. The linker cell is then engulfed by the hindgut cells and undergoes programmed cell death. Both *dmd-3* and *mab-3* are expressed in the migrating linker cell and hindgut. Furthermore, aspects of linker cell migration and programmed cell death are defective in *mab-3; dmd-3* double mutant males. These findings suggest that DMD-3 mediates multiple male-specific developmental processes.

741C

fs8 is a new mutant that disrupts the timing of male tail morphogenesis. **Edward Vuong**, Adam Mason, Douglas Portman. University of Rochester, Rochester, NY.

The morphogenesis of the male tail in *C. elegans* provides an excellent system to study the integration of sexual, spatial and temporal cues in the regulation of a developmental process. During the L4 stage, the male tail undergoes a dramatic transformation in which the the tail tip and anterior tail retract, resulting in the blunt-ended tail of the adult. As a master regulator of tail tip retraction, the DM-domain protein DMD-3 is both necessary and sufficient to specify this process. In WT males, *dmd-3* is expressed in hyp8-11 starting in early L4 and its expression is quickly extinguished as retraction completes during late L4. Temporal, sexual and spatial signals act to control male tail morphogenesis by regulating *dmd-3* expression. In order to better understand this process, we are studying *fs8*, a mutant in which males display a highly penetrant, severely unretracted Lep (Leptoderan) tail tip phenotype. In *fs8* males, we found a loss of *dmd-3* expression specifically in hyp10 during L4 and ectopic expression of *dmd-3* in the unretracted tail tip of adults. This suggests that *fs8* acts upstream of *dmd-3* to affect the timing of its expression. Other evidence that indicates *fs8* might be a developmental timing gene is that the Lep phenotype of *fs8* mutants is suppressed by post dauer development. To place *fs8* into the *lin-41* mediated heterochronic pathway, we constructed *lin-41(lf); fs8* double mutant males. These animals did not have Lep tails, suggesting that *fs8* functions upstream/in parallel to *lin-41*. We have found that *fs8* is on the X chromosome between +2 and +11. Further mapping is in progress. Characterization of this new mutant will provide greater insight into the complex process of male tail morphogenesis as well as the developmental timing pathways regulating it.

742A

Interaction of BMP facilitator, *crm-1*, with a downstream target *lon-1*. **Caleb K.H. Wong**, May G.Y. Mok, King L. Chow. Biology Department, HKUST, Hong Kong.

CRM-1 encoded by the worm homolog of vertebrate *Crim1*, has multiple cysteine-rich (CR) domains with potential bone morphogenetic proteins (BMP) binding activity. *crm-1* mutant displays small body phenotype with decreased ploidy in the hypodermis, a feature opposite to *lon* mutants. We have previously suggested that *crm-1* controls body size via modulation of *dbl-1*/*sma* activity in the hypodermis and thus endoreduplication, and it functions non-autonomously in facilitating *dbl-1* signaling event. Interestingly, data from the *C. elegans* Interactome Network showed that CRM-1 interacts with LON-1 in yeast. Would this LON-1 transmembrane protein regulated by BMP signal be able to act extracellularly to antagonize BMP signaling as a feedback circuit to sustain its own effect of blocking endoreduplication? As an alternative, CRM-1 may indirectly facilitate BMP signaling via association of LON-1 and blocks the *lon-1* repression on endoreduplication. To test these hypotheses, characterization of *crm-1* and its interaction with *lon-1* was initiated. Isoforms of *crm-1* were tested for their ability to interact with *lon-1* product. Body length rescue experiments by *crm-1* cDNA isoforms were also conducted to identify functional domains of the CRM-1 protein. At this moment, only the intracellular function of *lon-1* has been documented for repressing endoreduplication in the hypodermis. We will examine the extracellular function of LON-1 by ectopically expressing *lon-1* cDNA in tissues outside the hypodermis. Mutant rescue activities are monitored in these ectopic expression studies to evaluate *lon-1*'s potential non-autonomous and autonomous function. Furthermore, the double mutant of *crm-1* and *lon-1* has been constructed, the body length and hypodermal ploidy will be examined to elucidate their genetic relationship. The data from these experiments and the implication will be discussed in this poster. (The study is supported by Research Grants Council, Hong Kong.).

743B

Regulation of RNP Granule Assembly in Oocytes. **Ashley Alker**, Mariah Hanson, Cynthia Aguirre, Andrew Goike, Merrick Lincoln, Jennifer Schisa. Biology Department, Central Michigan University, Mount Pleasant, MI.

As *C. elegans* hermaphrodites age, sperm become depleted, ovulation arrests, and oocytes accumulate in the gonad arm. Large ribonucleoprotein (RNP) granules form in arrested oocytes that include several groups of RNA-binding proteins: P granule proteins, P body-associated proteins, and stress granule-associated proteins. Large RNP granules are also induced in non-arrested oocytes when worms are exposed to environmental stresses such as heat shock and anoxia (1). One hypothesis for the function of RNP granules in arrested or stressed oocytes is they maintain mRNA stability or prevent precocious translation until fertilization or a stress-free environment resumes. Previous work indicated one branch of the major sperm protein (MSP) pathway regulates the subcellular changes of at least some RNA-binding proteins in arrested or stressed oocytes (1). Our current goal is to better define the pathways regulating the assembly of the different classes of proteins that appear to aggregate into large RNP granules in oocytes. We have undertaken a functional RNAi screen to identify genes required for the RNA-binding protein, MEX-3, to assemble into granules when ovulation is arrested. We have identified 11 genes to date, and we are now determining whether these genes also regulate the assembly of P body and stress granule-associated proteins into granules. In parallel, we are asking if the genes that regulate the response to arrested ovulation are also necessary in effecting the oocyte response to environmental stress. In addition to elucidating the pathways that regulate the assembly of RNP granules in oocytes, we are also characterizing the effects on germline development and RNA metabolism when RNP granule assembly is defective to test our hypothesis for RNP granule function. 1) Jud et al., 2008. *Dev Biol* 318: 38-51.

744C

The role of VPR-1 in C. elegans germline development. **Pauline Cottee**, Sung Min Han, Michael Miller. Department of Cell Biology, The University of Alabama at Birmingham, Birmingham, AL.

The Major sperm protein (MSP) of C. elegans has an important role in sperm motility and oocyte maturation and ovulation. MSP secreted from sperm binds to the VAB-1 Eph receptor and other receptors on the surface of oocytes and sheath cells. The MSP domain structure is highly conserved throughout evolution. The human gene VAPB contains an MSP domain and a single point mutation (P56S) within this domain causes an inherited form of the neurodegenerative disease Amyotrophic Lateral Sclerosis (ALS) (Nishimura et al., 2004). VAPB is found at reduced levels in sporadic ALS patients and a mouse ALS model. Prior work has shown that VAPB MSP domains are cleaved, secreted, and act as ligands for Eph receptors (Tsuda et al., 2008). However, the P56S MSP domain in flies fails to be secreted, suggesting that this protein has a signaling function involved in ALS. VPR-1 is the C. elegans homologue of human VAPB. Loss of VPR-1 in the worm causes multiple metabolic defects that are reminiscent of defects seen in some ALS patients. Further, vpr-1 null mutants are sterile due to germline proliferation and differentiation defects. Neuronal-specific overexpression of VPR-1 in a null mutant background leads to partial rescue of the metabolic defects. In addition, neuronal overexpression partially rescues the germline proliferation and differentiation defects in some animals, while causing germline tumors in others. Binding studies suggest that MSP domain receptors are absent or at very low levels in proliferating germ cells, but expressed in the distal sheath and possibly the distal tip cell. The molecular basis of the VPR-1 germline development defects is unknown. My objective is to understand the biological function of VPR-1 in germline development. First, I am characterizing the germline defects of vpr-1 null mutants using molecular markers. Second, I am generating transgenic lines that express VPR-1 in the germ line to test for rescue of the sterility phenotype. Finally, I am performing an RNAi screen to identify suppressors of the VPR-1 sterile phenotype, as well as modifiers of the tumor phenotype. My preliminary results will be presented.

745A

RNAi-based identification of genes involved in germline proliferation. **Diana Dalfo**, E. Jane Albert Hubbard. Developmental Genetics Program, Skirball Institute and Department of Phatology, NYU School of Medicine.

Precise control of cell proliferation is necessary to create organs of the correct size and shape. We are interested in understanding the molecular basis for the control cell proliferation in vivo, and we are using the *C. elegans* germ line as a model. In *C. elegans*, as in other organisms, germline proliferation is a key step in the development of a reproductively competent individual. Previously, we isolated mutants that disrupt the pattern of germline development and ultimately produce a large ectopic mass of proliferating germ cells (a germline tumor) in the proximal part of the gonad arm (Pepper et al., 2003a,b; Killian and Hubbard, 2004; Voutev et al., 2006). This phenotype, proximal proliferation (Pro), is observed with certain mutant alleles of *glp-1* (Pepper et al., 2003). *glp-1* encodes a receptor of the LIN-12/Notch family, and its activity promotes the proliferative or undifferentiated state and/or inhibits differentiation (meiosis) in the germ line (Austin and Kimble, 1987; Yochem and Greenwald, 1989). We are performing a genome-wide RNAi screen for enhancers of the Pro phenotype to look for factors that affect early germline proliferation and initial meiotic entry.

746B

Analysis of Viruslike Particles in the *C. elegans* germline. **Shannon M Dennis**[1,2], James R Priess[1,2,3]. 1) Molecular & Cellular Biology Program, Univ. of Washington, Seattle, WA; 2) Div. of Basic Sciences, FHCRC, Seattle, WA; 3) HHMI, Seattle, WA.

Retroviral DNA is reverse-transcribed in the cytoplasm and forms a large nucleoprotein pre-integration complex (PIC) that is transported to the nucleus. In non-dividing cells, the PIC must be imported through nuclear pores. P granules cover most nuclear pores in *C. elegans* germ cells except during cell division and in transcriptionally quiescent oocytes or early embryos. We are interested in how retroelements confront, or circumvent, P granules. The *C. elegans* genome contains an unusually small number of retroviral and retrotransposon sequences compared to other animal genomes, suggesting that nematodes have efficient methods of combating these elements. No natural virus infections or viruslike particles (VLPs) have been reported previously in nematodes. We used electron microscopy to search for VLPs in the adult gonads of 17 wild strains of *C. elegans* and in 4 other *Caenorhabditis* species. In a typical experiment, a total of from 800 to 1200 germ cells were scored from about 40 gonads, and each gonad was analyzed from a single plane of section to provide a minimum estimate. We found that about 4% of germ cells in N2 gonads contain VLPs; these were seen in multiple laboratory strains of N2 as well as in a fresh sample of the "ancestral" N2 strain from the CGC. Most of the wild *C. elegans* strains lacked VLPs, and none had more than N2; VLPs were abundant in *C. japonica* gonads, but absent in the other *Caenorhabiditis* species tested. The VLPs appear to form on P granules in a narrow zone of late pachytene germ cells, then exit the germ cells in tight association with long microtubules that extend from the germ nuclei into the gonad core. This indicates a possible role of P granules in the formation of VLPs, analogous to recent results for yeast Ty3 assembly in P bodies. The VLPs are also present in early oogonia, presumably through cytoplasmic flow within the gonad, but generally are not detected in the most proximal oocytes or in early embryos. We did not observe an increase in VLP frequency in a strain lacking the cell death and RNAi pathways [ced-1(e1735);ced-3(n717); rde-1(ne300)], however the number of VLPs was affected markedly (both increasing and decreasing) in mutants lacking various Argonaute family members. We are testing by RNAi which retroelements contribute to the VLPs. Thus far, RNAi for either CER1 or CER2 (Gypsy/Ty3 clade) elements decrease VLP number, while RNAi against CER13 (Bel clade) does not. We are currently making GFP reporters and immunological reagents to examine CER1 and CER2, and hope to test whether strains with increased numbers of VLPs are active for retrotransposition.

747C

A dominant suppressor of the *fog-1(q253*ts*)* allele maps to *C. elegans LGII*. **Kristin R. Douglas**, Samantha M. Laskowski, Allyse J. Stombres. Dept Biol, Augustana Col, Rock Island, IL.

Germ cell fate in *C. elegans* is determined through a complex genetic pathway ending in *fog-1* and *fog-3*. *fog-1* encodes a member of the **C**ytoplasmic **P**olyadenylation **E**lement **B**inding (CPEB) protein family. In other model systems, CPEB proteins have been shown to regulate translation of target messages. FOG-1 is required for germ cell proliferation and spermatogenesis in *C. elegans*; however, its target messages and exact mechanism of activity in the worm germline remain to be elucidated.

To identify proteins that either regulate or interact with FOG-1, we performed a genetic suppressor screen. We mutagenized approximately 85,000 haploid genomes and identified two dominant suppressors of the *fog-1(q253*ts*)* allele. We have mapped one of the dominant suppressors to the cluster region of *LGII* using genetic and snip-SNP mapping. We report further characterization of the suppressor mutation including gonad morphology and broodcount data.

748A

Sex Chromosomes, Double Strand Break Repair, and Checkpoint Activation in the C. elegans Germ Line. A. Jaramillo-Lambert, **J. Engebrecht**. Molec & Cellular Biol, Univ California, Davis, Davis, CA.

Germ cells are specialized cells that undergo mitotic proliferation followed by meiosis and cellular differentiation to generate haploid gametes for sexual reproduction. In C. elegans germ cells are arranged in a spatial/temporal gradient within the syncytial gonad. C. elegans sex is determined by the X chromosome to autosome ratio (XX=hermaphrodite; X0=male). Using an S phase labeling assay we found that hermaphrodite meiotic prophase for oogenesis takes 54-60 hours whereas prophase for spermatogenesis in males is completed by 20-24 hours. Examination of meiotic progression in a number of sex determination mutants revealed that meiotic prophase timing is dictated by the sex of the germ line (oogenesis vs. spermatogenesis). Another sex-specific difference is the extent to which checkpoints monitor and safeguard the genome. In the proliferative zone, both hermaphrodite and male germ cells arrest in response to perturbation of DNA replication or DNA damage. Surprisingly, the genetic requirement for checkpoint-activated arrest appears to be distinct in males compared to hermaphrodites. In the hermaphrodite germ line some nuclei undergo physiological apoptosis, but perturbations in chromosome synapsis or DNA damage causes a checkpoint-activated increase in apoptosis. However, the male germ line has neither physiological nor checkpoint activated apoptosis. Analysis of sex determination mutants revealed that both physiological and checkpoint activated apoptosis are also dictated by germline sex. The male germ line carries a chromosome that lacks a homologous partner to pair and recombine with (the X chromosome); a condition which causes segregation defects, checkpoint activation, and apoptosis in the hermaphrodite germ line. Surprisingly, we found that a single X chromosome fails to activate a checkpoint response in a background that senses asynapsis of a single pair of autosomes. We also found that a single X chromosome incurs double strand breaks (DSBs), repair of these breaks appears to be delayed, and that this is sensed by the germ line. Synaptonemal complex axial components are assembled onto the single X and in some nuclei, the X undergoes self-synapsis. Taken together, these results suggest that there are specific mechanisms in place to prevent the single X chromosome from activating checkpoints.

749B

Sex-specific differences in heterochromatin formation on unpaired/unsynapsed chromosomes during *C. elegans* meiosis. **Alexander V. Fedotov**[1,2], William G. Kelly[1]. 1) Department of Biology, Emory University, Atlanta, GA; 2) Graduate Program in Genetics and Molecular Biology.

Meiotic Silencing (MS) is a conserved phenomenon in which chromatin that is poorly paired or unsynapsed during meiosis is targeted by repressive mechanisms. MS was initially described in *Neurospora*, where it recognizes invasive elements and directs transcriptional repression by RNAi-mediated responses. In higher eukaryotes, MS is achieved by formation of heterochromatin on unsynapsed chromosomal segments, resulting in their silencing. However, the molecular mechanism by which aberrant chromatin is recognized and targeted for repression during meiosis is unclear. We are using *C. elegans* to study the signals that trigger and control MS.

Initial studies of MS in *C. elegans* have shown that X chromosome is an endogenous substrate for silencing in XO male meiosis due to its lack of a homolog. As a result, the X chromatin becomes specifically enriched in the specific for heterochromatin mark, histone H3 lysine 9 dimethylation (H3K9me2). We characterized the pattern of distribution of H3K9me2 in both adult male and hermaphrodite gonads of *C. elegans* mutants defective for chromosome pairing, recombination, and synapsis using immunohistochemistry. Here we report that accumulation of H3K9me2 on aberrant chromatin is controlled at two different levels and this regulation of heterochromatin formation is sex-specific. We found that in males the unpaired status of homologous chromosomes in general triggers MS, but this mark can also accumulate when pairing is normal but stable synapsis is defective. In contrast, hermaphrodites also activate MS in response to defective pairing, but seem to exhibit little response when synapsis alone is abnormal. This finding suggests the presence of sex-specific regulators/checkpoints that act differently in male vs. hermaphrodite germline of *C. elegans*. Since sexual dimorphism in stringency of meiotic events has also been reported for mammalian meiosis, our findings support the conserved nature of this phenomenon. We are currently investigating whether heterologous chromatin associations may still trigger MS to determine if this process has a role in homology recognition and/or suppression of heterologous recombination. NIH-5R01GM063102.

750C

rsr-2, a gene with homology to the human splicing factor SRm300, is a novel component of the sex determination pathway in the *Caenorhabditis elegans* germ line. **Laura Fontrodona**[1], Mónica Ferrer[2], Simo Schwartz Jr[2], Julián Cerón[1]. 1) Cancer and Human Molecular Genetics, Bellvitge Institute for Biomedical Research, l'Hospitalet de Llobregat, Barcelona, Spain; 2) CIBBIM-Nanomedicine,Vall d'Hebron University Hospital, Barcelona, Spain.

Protein components of the splicing machinery are highly conserved in eukaryotes and may have additional functions apart from splicing. In a previous study, *rsr-2*, a gene encoding a SR protein that is homolog of the human splicing factor SRm300, was identified as one of the genes that interact genetically with *lin-35* Rb (Ceron et al, 2007). Through RNAi assays, we found that *rsr-2* inactivation causes a pleiotropic phenotype. One of the most striking phenotypes observed was excess of sperm and lack of oocytes that cause sterility. Such defect is produced by the fail to switch the germ cell fate from sperm to oocyte during germ line development. The genetic network that rules the germ line sex determination process has been previously studied and characterized. Performing genetic epistasis experiments, we have identified *rsr-2* as a novel component of a sex determination pathway since is acting downstream of *gld-3* and upstream of *fem-3*. In the intestine, we have found that *rsr-2* functions as repressor of *fem-3* expression through its 3'UTR. This observation classifies *rsr-2* as a putative mog (masculinisation of the germ line) gene. Upon our request, the National Bio-resource Project (Japan) has isolated two mutations in *rsr-2*, (*tm2507* and *tm2625*), which are deletions that produce viable animals and larval lethality respectively. We have already made a backcrossed and balanced strain that is suitable for rescue *tm2625* in the soma generating a tool to study the consequence of *rsr-2* absence in the germ line. To further investigate the functional mechanisms of *rsr-2*, we have observed by in situ hybridization that *rsr-2* mRNA is present in the sperm and enriched in the proximal part of the germ line. Moreover, we are cloning whole ORFs and fragments of *rsr-2* and putative functional partners to identify protein-protein interactions by Yeast Two Hybrid assays.

751A

Dynamic instability of P granules during zygote polarization. **Christopher M. Gallo**, Geraldine Seydoux. Dept Molecular Biol & Genetics, Johns Hopkins University, Baltimore, MD.

P granules are protein-RNA complexes essential for germline development. P granules are inherited maternally and segregate asymmetrically with the germline in early embryos. The distribution of P granules changes most dramatically in zygotes: in just 10 minutes, P granules go from uniform to exclusively posterior. Several mechanisms have been suggested to contribute to this change (Hird et al., 1996 and Cheeks et al., 2004). One theory is that flows that form deep in the cytoplasm during polarization sweep the P granules towards the posterior. Because P granules move in three dimensions, their dynamics are difficult to follow directly by conventional microscopy. We have used live confocal microscopy to follow P granules directly in zygotes. Our observations suggest that P granule segregation depends, not on directed movement, but on the asymmetric assembly and disassembly of P granules.

We constructed a GFP:PGL-1 fusion to track P granules. By collecting confocal slices over a 9 micron-deep section of the zygote, we were able to follow several individual P granules over the entire polarization period. We observed two behaviors: 1) P granule shrinkage and disappearance (primarily in the anterior) and 2) P granule *de novo* appearance, growth, and fusion (*exclusively* in the posterior). By following P granule tracks over time in the anterior of the embryo, we find that the majority of granules move randomly, with only a minority moving towards the posterior.

To begin to understand how these dynamics are regulated, we examined P granule behavior in zygotes depleted of MEX-5/6 (two redundant proteins that localize to the anterior cytoplasm [Schubert et al., 2000]) or of PAR-1 (a kinase enriched on the posterior cortex required for MEX-5's anterior localization [Guo and Kemphues, 1995; Schubert et al., 2000]). We found that P granules behave dramatically differently under these two conditions: in *mex-5/6(RNAi)*, P granules shrink but do not disappear, and new small P granules appear throughout the embryo. In contrast, in *par-1(RNAi)*, P granules shrink and disappear uniformly throughout the embryo. Preliminary results combining *mex-5/6(RNAi)* and *par-1(RNAi)* suggest that *mex-5/6* are mostly epistatic to *par-1*. These results suggest that P granule asymmetry depends, at least in part, on a MEX-5/6-dependent activity that promotes P disassembly (and/or blocks assembly) in the anterior cytoplasm.

752B

Cytosolic Aminopeptidase P (APP-1): a possible role in meiotic progression in Caenorhabditis elegans. **Richard Elwyn Isaac**[1], Hannah Craig[1], Enrique Martinez-Perez[2], Darren Brooks[3]. 1) Faculty of Biological Sciences, Miall Building, University of Leeds, Leeds LS2 9JT, UK; 2) Department of Molecular Biology and Biotechnology, University of Sheffield, Firth Court, Western Bank, Sheffield S10 2TN, UK; 3) Biomedical Sciences Research Institute, School of Environment and Life Sciences, University of Salford, Salford M5 4WT.

Aminopeptidase P (APP) is a metallopeptidase that specifically removes amino acids from the N-terminus of peptides with a penultimate N-terminal proline residue. The cyclic structure of the proline side chain places a conformational restraint on peptide bonds in its vicinity, which therefore tend to be resistant to hydrolysis by non-specialist peptidases. APP is one of the few peptidases capable of cleaving peptide bonds involving a proline residue and therefore has an important role in protein/ peptide processing and catabolism. In humans, at least two APP genes exist, coding for a soluble cytosolic enzyme (APP-1) and a secreted membrane-bound form (APP-2). APP-2 has been the subject of much research due to its role in the regulation of blood pressure and potential as a drug target in the treatment of hypertension, but there is little information available on the biological role of APP-1. We have previously shown that a C. elegans APP-1::GFP fusion protein is strongly expressed as a cytosolic protein in the intestinal cells of late embryos, larvae and adult worms. Immunocytochemistry using antibodies specific for C. elegans APP-1 has confirmed the initial promoter-GFP expression pattern and has revealed additional strong expression of APP-1 in the female germline. We have obtained a C. elegans app-1 mutant (tm1715), generously provided by the Japanese National Bioresource Project, that has a 452 bp deletion spanning most of exon 2 and part of exon 3. We have shown that tm1715 is a null mutant by using a specific assay for APP aminopeptidase activity and by western blot analysis. Phenotypic analysis for tm1715 revealed a higher (18-fold) than normal number of males (XO) generated on self-fertilisation of the adult hermaphrodite (XX) and a 50 percent reduction in brood size. These phenotypes are similar to those seen in a sub-set of him mutants (high incidence of males) that are defective in segregation of autosomes as well as the X chromosome during meiosis. The APP-1 antibody gives diffuse staining inside meiotic nuclei of the female gonad and staining with anti-RAD-51 antibodies of app-1 mutants suggests an altered pattern in the repair of DNA double-strand breaks (DSBs) generated during meiotic prophase. These preliminary results suggest an important biological role for APP-1 in meiotic progression in C. elegans.

753C

Maternal-effect epigenetic germ line silencing of *fem-1*. **Cheryl Lynn Johnson**, Andrew Spence. Dept Molecular Genetics, Univ Toronto, Toronto, ON, Canada.

Germ-line gene expression is restricted by many mechanisms which maintain the integrity of the germ line in *C. elegans*. We have discovered a novel form of silencing that can be prevented by maternally inherited transcripts, a rare example of a positive role for RNA in regulating gene expression. This phenomenon was discovered through its effect on the inheritance of *fem-1*, a gene required for spermatogenesis in both sexes and for male somatic development. When females carrying any of three deficiency alleles, *fem-1(Df)*, are crossed to wild type males, their heterozygous progeny often exhibit germ line feminization (the Fog phenotype). In contrast, cross-progeny of females carrying other *fem-1* null alleles, such as the nonsense mutation *e2268*, are phenotypically wild type. We found that injecting in vitro-transcribed *fem-1* RNA into the germ line of the *fem-1(Df)* females rescued spermatogenesis in their progeny, suggesting that the Fog phenotype was caused by a failure of the deficiency chromosomes to provide *fem-1* RNA to the offspring. Non-overlapping fragments of *fem-1* RNA rescued, indicating that neither a specific region of *fem-1* RNA nor functional FEM-1 protein is required. We hypothesized that maternal *fem-1* RNA may be needed in order to promote the activity of the *fem-1* locus in the zygotic germ line. This idea is supported by the observations that the cross-progeny of *fem-1(Df)* females have reduced *fem-1* transcript accumulation and genetic activity in the germ line. The reduction in *fem-1* activity is also heritable since the proportion of Fog progeny increases upon backcrossing to *fem-1(Df)* females. Together, these data suggest that *fem-1* is heritably silenced in the germ line in the absence of maternal RNA, but provision of maternal *fem-1* RNA can promote expression of the gene. This mechanism could be a means of protecting the germ line by only licensing activity of those genes that were transcribed in the germ line of the previous generation. To investigate the mechanisms involved in silencing and licensing *fem-1*, we performed an RNAi screen of candidate genes. Identification of the *C. elegans* piwi homologs *prg-1* and *prg-2* as strong suppressors of *fem-1* germ-line silencing implicates their products, perhaps in association with small RNAs whose accumulation depends on their activity, in the mechanism of germ-line silencing that is opposed by maternal *fem-1* RNA. Among the strongest enhancers were certain members of the *C.elegans*-specific branch of the Argonaute family. Thus the licensing of germ-line gene expression by maternal RNA adds to the expanding repertoire of regulatory roles served by Argonaute proteins.

754A

Germ cell development and a Deleted in Azoospermia homolog. **T. Karashima**, M. Otori, E. Hasegwa, M. Yamamoto. Dept Biophys, Biochem, Tokyo Univ, Tokyo, Japan.

The DAZ (Deleted in Azoospermia) family of RNA-binding proteins, which are highly enriched in the germ line, bind to the 3' UTR of specific mRNAs and up-regulate their translation. Deletions of the *DAZ* gene cluster on the human Y chromosome are associated with azoospermia, oligozoospermia, and testicular germ cell tumors.

DAZ-1, the single DAZ homolog in the nematode *C. elegans*, is expressed in the distal part of germ line (Maruyama et al. 2005). Depletion of DAZ-1 blocks the transition from pachytene to diakinesis during female meiosis and abolishes oocyte production (Karashima et al. 2000). We previously reported that DAZ-1 protein can bind to the 3' UTR of *gld-1* mRNA and promotes the accumulation of GLD-1 protein, a translational repressor essential for the progression of female pachytene (Francis et al. 1995; Jones et al. 1996). Interestingly, oogenesis can be restored in the *daz-1* mutant by attenuating the activity of the translational regulator GLD-3 (Otori et al. 2006). GLD-3 promotes spermatogenesis and/or inhibits oogenesis, is translationally repressed by FBF, and shows relatively high accumulation in the proximal germ line in the wild-type hermaphrodite (Eckmann et al. 2002 and 2004). Although DAZ-1 protein can bind to the 3' UTR of *fbf* mRNA (Otori et al. 2006), FBF amount was not reduced in the *daz-1* adult. Instead, we found that GLD-3 accumulation is repressed by GLD-1 in the wild-type female pachytene germ cells, and is derepressed in the *daz-1* mutant.

Biochemical and genetic analyses suggested that DAZ-1 promotes GLD-1 accumulation independently of FBF/PUF, which both negatively and positively regulates the translation of *gld-1* mRNA by polyadenylation and deadenylation (Crittenden et al. 2002; Suh et al. 2009), and that DAZ-1 directly recruits PAB-1/poly(A)-binding protein to the *gld-1* 3' UTR. On the other hand, CPB-3/cytoplasmic polyadenylation element-binding protein, an *in vitro* interactor of DAZ-1, is not likely involved in the poly(A) elongation and thus the translational activation of *gld-1* mRNA.

755B

Translational control of sperm-specific proteins by IFE-1, a germline-specific isoform of eIF4E, in *C. elegans*. **Ichiro Kawasaki**, Myung-Hwan Jeong, Yhong-Hee Shim. Dept. of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul, Korea.

The PGL proteins are constitutive protein components of P granules and function redundantly in *C. elegans* germline development. Among them, PGL-1 is the most critical component. PGL-1 has been shown to specifically interact with IFE-1 *in vitro* and *in vivo*. IFE-1 is one of the five *C. elegans* isoforms of eIF4E, the mRNA 5' cap-binding component of the translation initiation complex eIF4F. We further identified that IFE-1 is specifically required for spermatogenesis using RNAi. These findings suggest that IFE-1 and possibly PGL-1 are involved in translational control of a subset of mRNAs that are required for spermatogenesis.

To further analyze the molecular mechanism of action of IFE-1 and PGL-1 on spermatogenesis, we performed a proteomic analysis to search for proteins that are down-regulated in *ife-1(ok1978)* mutant worms, which show the same temperature-sensitive fertilization defective phenotype as *ife-1(RNAi)* worms. Worm populations of *ife-1(ok1978)* mutant (*ife-1* single or *ife-1; fem-3(q20gf)* double mutant) and wild type (N2 or *fem-3(q20gf)*) were synchronized at L1 larval stage, up-shifted and grown to young adult stage at non-permissive 25°C, and harvested. Worm protein lysates prepared from *ife-1(ok1978)* and wild type were labeled with Cy3 and Cy5 fluorescent dyes, and applied to 2D-DIGE analysis. Protein spots significantly down-regulated in *ife-1(ok1978)* mutant worms compared to wild-type control worms were further analyzed by MALDI-TOF mass spectrometry to identify the proteins.

Through this analysis, we found that some sperm-specific proteins were indeed significantly down-regulated in *ife-1(ok1978)* mutants at 25°C. Among the genes encoding the down-regulated sperm proteins, RNAi of *gsp-3* caused an *ife-1*-like phenotype. That is, *gsp-3(RNAi)* hermaphrodites produced unfertilized oocytes or early-arrested embryos, and this defect was rescued by mating with wild-type males, as observed in *ife-1* mutants. Although the *ife-1* phenotype is temperature-sensitive, the *gsp-3(RNAi)* phenotype is not. These results suggest that IFE-1 is essential for translation of some sperm-specific proteins, including the predicted phosphatase GSP-3. We are investigating whether the temperature-sensitive fertilization defect of *ife-1* mutant is caused mainly by a reduced level of GSP-3.

756C

Genetic and FRET Analyses of the Requirement for GSA-1 and ACY-4 in the Gonadal Sheath Cells for MSP Signaling of Oocyte Meiotic Maturation. **Seongseop Kim**, J. Amaranath Govindan, David Greenstein. Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN 55455 USA.

In *C. elegans*, a sperm-sensing mechanism regulates oocyte meiotic maturation and ovulation, tightly coordinating sperm availability and fertilization. Sperm release the major sperm protein (MSP) hormone to trigger meiotic resumption (meiotic maturation) and to promote contraction of the follicle-like gonadal sheath cells that surround oocytes. In the absence of *gsa-1* (encodes the $G\alpha_s$ stimulatory G protein) or *acy-4* (encodes adenylate cyclase) in the gonadal sheath cells, oocytes fail to undergo meiotic maturation, ovulation, and fertilization, resulting in sterility. GSA-1-ACY-4 signaling in the gonadal sheath cells is required for all described MSP-dependent meiotic maturation responses in the germline, including the activation of MPK-1 mitogen-activated protein kinase in oocytes and the promotion of actomyosin-dependent cytoplasmic streaming that drives oocyte growth. In the standard model for stimulatory G-protein signaling, ligand binding to a receptor results in activation of $G\alpha_s$, which then binds to and activates adenylate cyclase. We propose that unidentified MSP receptors on the gonadal sheath cells are G-protein-coupled receptors. If this model for MSP signaling is correct, then we predict that interaction between GSA-1 and ACY-4 in vivo only occurs in the presence of MSP. We are using FRET to test this model. FRET is measured using rescuing GSA-1::YFP and ACY-4::CFP fusions in female, mated female, and hermaphrodite backgrounds. Preliminary results indicate that there is robust FRET in mated, but not unmated females. Hermaphrodites also exhibit a FRET signal, however, it is lower than in mated females. Perhaps the chronic presence of sperm results in signaling downregulation. We are continuing to collect data and plan to determine the effect of MSP on FRET. To address the mechanism by which GSA-1-ACY-4 signaling in gonadal sheath cells promotes meiotic maturation, we conducted a genetic screen for mutations that suppress the requirement for *acy-4* for fertility. We mutagenized *acy-4(ok1806); tnEx37[acy-4(+) + sur-5::gfp]* hermaphrodites and screened in the F2 generation for fertile animals that lost the extrachromosomal array. We verified that the original *ok1806* deletion that removes sequences required for catalytic activity was intact and that no wild-type *acy-4* sequences are present. From a screen of ~20,000 haploid genomes, we recovered 63 suppressed strains. The suppressed strains vary in brood sizes from ~90 (strong suppression) to ~10 (weak suppression). Progress on genetic and molecular analysis of the suppressor loci will be presented.

757A

Insulin Signaling is Required for Robust Larval Germline Proliferation in C. elegans. **Dorota Korta**, David Michaelson, Josef Capua, E. Jane Albert Hubbard. Developmental Genetics Program, Skirball Institute; Kimmel Center for Stem Cell Biology; Department of Pathology, New York University School of Medicine, New York, NY.

The proper control of cell proliferation is critical for development, and understanding this control at the molecular level in the whole-organism context has broad implications for cancer and stem cell biology. We are using the *C. elegans* germ line as a model to investigate proliferation control. Notch receptor-mediated activity determines the region within the germ line where mitosis can occur and/or differentiation is inhibited. Our previous studies indicated that the distal-most pair of gonadal sheath cells is required for robust larval germline proliferation but not for the proliferation/differentiation decision *per se*, suggesting that these controls are separable. Our more recent results indicate that soma-to-germ line signaling through the insulin/IGF-like receptor (IIR) pathway influences the number of undifferentiated germ cells in the distal germ line. Establishment of proper germ cell numbers requires somatic activity of genes encoding putative insulin-like ligands INS-3 and INS-33. The insulin/IGF-like receptor DAF-2 and components of the PI3K branch of this conserved signaling pathway, including DAF-18/PTEN and DAF-16/FOXO are required in the germ line. IIR signaling in *C. elegans* is well-characterized for the dauer decision: reduction of IIR signaling causes constitutive dauer formation, during which germline proliferation is impaired. However, we find that the requirement for IIR for larval germ cells is anatomically and temporally separable from its role in the dauer decision. We further investigated the mechanism by which germ cell numbers were affected. In mutants defective in IIR signaling, cell death is not elevated, cell size is not reduced, and the distance to the proliferation/differentiation border is only slightly reduced. Rather, the primary effect of reduced IIR signaling is a reduction in the mitotic index. By contrast, reduced Notch signaling does not reduce mitotic index, but greatly reduces the distance to the proliferation/differentiation border. Reducing the activity of both IIR and Notch pathways results in both defects (reduced mitotic index and reduced distance to differentiation) without additional synergistic effects. Taken together, our results suggest that (i) the IIR pathway responds to post-dauer-decision cues, ensuring adequate larval proliferation for adult gametogenesis and (ii) two conserved pathways regulate larval germline development largely independently: Notch signaling sets up the border of differentiation, and insulin signaling stimulates robust mitosis.

758B

Divergent Evolution of Puf family Proteins: PUF-2/12 Promote Spermatogenesis and Promote Commitment to Meiosis in C. briggsae. **Qinwen Liu**, Eric Haag. Department of Biology, University of Maryland, College Park, MD.

mRNA-binding proteins play crucial roles in regulating germline sex determination in *C. elegans*. To study the function of RNA-binding proteins in the *C. briggsae* germ line and their evolutionary significance in sex determination, we have started to investigate *Cb-puf* genes, which encode members of the Pumilio/FBF (Puf) RNA-binding protein family. We developed paralog-specific RNAi assays to knock down the three *Cb-puf* genes most closely related to the *C. elegans fbf-1/2* genes, both alone and in various combinations. Single gene RNAi of *Cb-puf-1*, *Cb-puf-2* or *Cb-puf-12* didn't give any obvious phenotype. However, the synthetic phenotype of *Cb-puf-2/12(RNAi)* is feminization of the hermaphrodite germline (Fog), which contrasts with the masculinization (Mog) knockdown phenotype of its homologs, the *fbf*s, in *C. elegans*. *Cb-puf-2/12(RNAi)* worms also showed low penetrance of proximal and whole germline tumors, which is also the opposite of the reduced germline proliferation seen in *fbf* mutants. *Cb-puf-2/12(RNAi)* also transformed the germ line of *C. briggsae* males and *Cb-tra-1* and *Cb-tra-2* pseudo-males to a tumor-like state. A deletion allele of *Cb-puf-2*, *nm66*, was isolated. Unexpectedly, homozygous *Cb-puf-2(nm66)* mutant worms arrest at L3 stage, and can survive at least 15 days at 20ºC, indicating the gene has an essential somatic function required for larval progression. RNA *in situ* showed *Cb-puf-2* transcript is present in the adult germline. RT-PCR showed *Cb-puf-2* transcript increases during larval development and has a peak at L4 stage. These results reinforce the idea of convergent evolution of germline sex determination in nematode species, and at the same time, uncover the rapid evolving functions of PUF homologs. *Cb-puf-2* transgenic worms will be generated for rescue experiment to confirm mutant larval arrest phenotype. Downstream targets of *Cb-puf-2* will be sought to investigate the molecular basis of its functional transition.

759C

Regulators of meiotic silencing. **Eleanor Maine**[1], Xingyu She[1], Xia Xu[1], Alex Fedotov[2]. 1) Dept Biol, Syracuse Univ, Syracuse, NY; 2) Dept Biol, Emory Univ, Atlanta, GA.

Non-coding (nc) RNAs have been implicated in transcriptional gene silencing in a variety of contexts. During first meiotic prophase in many organisms, including C. elegans, unpaired chromosomes (e.g., the male X) become silenced. Meiotic silencing is hypothesized to function in maintaining genome integrity. In *C. elegans*, the silencing of unpaired chromatin involves enrichment of the histone modification, H3K9me2 [Kelly et al. (2002)]. We find that mutations in several proteins implicated in the biogenesis and/or function of ncRNAs disrupt the normal pattern of H3K9me2 accumulation, suggesting that it may be regulated (at least in part) via an RNA-mediated mechanism.

Meiotic H3K9me2 distribution depends on activity of EGO-1 (RdRP), CSR-1 (Argonaute), EKL-1 (a Tudor domain protein), and DRH-3 (Dicer-related helicase), all components of a functional pathway defined by Rocheleau et al. (2008). H3K9me2 accumulation on the male X chromosome is severely reduced in animals lacking EGO-1 [Maine et al. (2005)]. In contrast, H3K9me2 inappropriately spreads to the autosomes in males lacking CSR-1, EKL-1, or DRH-3 function [She et al.]. H3K9me2 distribution appears normal in XX mutants but becomes abnormal if a chromosomal duplication or unpaired chromosomes (that should be targeted for H3K9me2 enrichment) is introduced. In addition to the spreading effect, the H3K9me2 level is variably reduced on unpaired chromatin in *csr-1*, *ekl-1*, and *drh-3* mutants. Mutations in these four genes cause other meiotic defects, which we are characterizing in order to determine if/how they are related to the altered H3K9me2 pattern. We have identified a mutation in a fifth gene of this phenotypic class, *ego(om57)*, that abolishes H3K9me2 enrichment on unpaired chromatin. We are now working to clone this gene. We also find that SIN-3 activity promotes H3K9me2 accumulation on unpaired chromatin in some contexts. SIN-3 is the *C. elegans* ortholog of Sin3, known in other systems to assemble histone deacetylase complexes and to interact with other chromatin proteins. While SIN-3 has no known functions in ncRNA-mediated processes, a human histone deacetylase called HDAC-1 has been implicated in transcriptional gene silencing [Hawkins & Morris (2008)].

We are working to distinguish between alternative models for how these factors regulate H3K9me2 accumulation. Do they participate directly in the regulation of the histone-modifying machinery or, instead, regulate the expression of genes responsible for distinguishing between paired and unpaired chromatin?

760A

Activating interactions between Dicer, microRNAs and GLH-1 in the *C. elegans* germline. **Tamara Jean McEwen**, Erica Beshore, Shalin Shah, Karen L Bennett. MMI, University of Missouri, Columbia, MO.

In *Caenorhabditis elegans*, maintenance of a productive germline is dependent on the germline RNA helicase, GLH-1, an integral component of the germline-specific P granules that surround the nuclei of germline progenitor cells, germline stem cells, and developing oocytes and sperm. Our laboratory has discovered that GLH-1 and the ribo-endonuclease Dicer-1 (DCR-1) are interdependent both by genetic and biochemical analyses. These two proteins bind each other and are colocalized to P granules at the pachytene stage of oogenesis when many maternal RNAs are processed and stored. When either GLH-1 or DCR-1 is missing from the germline, levels of the other are substantially decreased. MicroRNAs (miRNAs), non-coding RNAs found in plants and animals, are processed by Dicer and are involved in development, apoptosis, and disease progression. Most reports have found miRNAs repress the translation of their target genes; however, evidence indicates miRNAs can also activate their targets. Two miRNAs, *miR-67* and *miR-83*, are predicted to bind overlapping sites in the small, 140-nt, 3'-UTR of *glh-1* mRNA. This 3'-UTR also contains multiple sites, specifically nanos response element (NRE) and adenylation control element (ACE) motifs, predicted to bind regulatory proteins. Microarray analysis by the Bass laboratory (U Utah) indicated that *glh-1* and many other germline-enriched mRNAs are down-regulated in *C. elegans dcr-1* null mutants. We have verified, through quantitative real time PCR, that *glh-1* mRNA levels are reduced an average of 2-fold when Dicer is missing. Therefore, we hypothesize that *miR-83* and *miR-67* may function to up-regulate the translation of *glh-1*; thus, GLH-1 levels would decrease when these miRNAs are absent. We obtained *miR-67* and *miR-83* deletion strains and generated the *miR-67; miR-83* double mutant. Our preliminary results indicate that GLH-1 protein levels are decreased in *miR-67* and *miR-83* mutants and in the double, when compared to wildtype animals. In addition, these miR mutants exhibit abnormally-shaped gonads, with some animals displaying the rarely-reported phenotype of a bifurcated distal gonad similar to that occasionally seen with loss of *glh-1* or *glh-4*. Based on our findings to date, we propose these two miRNAs, perhaps in conjunction with yet-to-be-defined regulatory proteins, bind the *glh-1* 3'UTR and activate *glh-1* mRNA translation, resulting in increased GLH-1 protein levels. To determine whether this effect is direct or indirect, we intend to verify that these miRNAs bind *glh-1* and hope to create transgenic worms with mutated *glh-1* 3'-UTRs. We propose the possibility that many mRNAs in the *C. elegans* germline may be regulated by similar, novel miRNA-mediated activation.

761B

NUD-1 Functions in *C. elegans* Sperm Development. Michael Meyer, Scott Gratz, Michael Large, **Jennifer Anne Miskowski**. Dept Biol, Univ Wisconsin, La Crosse, La Crosse, WI.

The *C. elegans* NUD-1 protein is the structural and functional ortholog of the fungal NudC protein. NUD-1/NudC proteins are evolutionarily conserved and have been implicated in diverse processes such as asymmetrical cell division in yeast, nuclear migration in fungi, and neuronal migration in humans; however, their exact function is unknown. When NUD-1 levels are depleted in N2 hermaphrodites by RNAi, a variety of phenotypes are found, including sterility, which supports a role for NUD-1 in *C. elegans* gonad development. Analysis of NUD-1(RNAi) animals revealed abnormalities in both the somatic gonad and the germ line. To ascertain whether the observed germ line defects were the result of a direct role for NUD-1 in the germ line, rather then an indirect consequence of somatic gonad defects, NUD-1 RNAi was performed in *rrf-1* mutants. Treated hermaphrodites were rendered sterile, although they sometimes made sperm and/or oocytes. *rrf-1* males with depleted NUD-1 levels also made sperm. To test whether these sperm were viable, the RNAi-treated males were mated to Fog females, and a significant percentage of these matings yielded no progeny compared to control matings. The expression pattern of NUD-1 further supports its function in sperm development. Observation of transgenic worms expressing a full-length NUD-1::GFP construct revealed faint staining in sperm, in addition to other tissues. Furthermore, a number of immunofluorescence experiments have been performed using two different antibodies that were generated against a phosphorylated version of mammalian NUDC and cross-react specifically in *C. elegans*. Consistently, NUD-1 is localized in perinuclear puncta in primary spermatocytes with more diffuse staining in secondary spermatocytes and spermatids. NUD-1 colocalizes with fibrous-body membrane organelles (FBMOs), which are sperm-specific organelles. Like FBMOs, NUD-1 is asymmetrically segregated to spermatids and away from residual bodies and this localization pattern requires functional SPE-15/Myosin VI protein. To further explore the relationship between NUD-1 and FBMOs, the localization of FBMOs in NUD-1(RNAi) mutants is being explored.

762C

A Genetic Screen to Identify Partners of Puf-8, A C. elegans Member of The PUF Family Of RNA-Binding Proteins. **Ariz Mohd**, Kuppuswamy Subramaniam. Biological Sciences & Bioeng., IIT Kanpur, Kanpur, Uttar Pradesh, India.

In animal gonads a balance is maintained between proliferating germ cells and differentiation. A shift in this balance leads to catastrophic consequences, either way making the animal sterile. Members of the PUF family of RNA-binding proteins are key germ cell regulators in many organisms. Some of the PUF proteins, such as Pumilio of Drosophila, FBF-1, FBF-2 and PUF-8 of Caenorhabditis elegans and DjPum of planarians, are essential for the proliferation of GSCs. FBF-1 and FBF-2 maintain GSCs, at least in part, by suppressing the translation of GLD-1, a promoter of meiotic entry during oogenesis. In contrast, PUF-8 seems to take part in both GSC proliferation and entry into meiosis. PUF-8 also functions in sperm to oocyte switch redundantly with FBF-1. Even null alleles of puf-8 exhibit temperature-dependent phenotype, raising the possibility that it may function redundantly with other proteins. In order to identify these potential partners of PUF-8, we performed a genetic screen for mutants that are synthetic sterile with puf-8(-). This screen has yielded three classes of mutants: class I mutants continue to proliferate mitotically and do not enter meiosis, class II mutants produce very few or no germ cells and class III mutant hermaphrodites make only sperm. The opposing phenotypes of class I and class II suggest that the PUF-8 may promote both mitosis and meiosis depending on the partner. These results strongly suggest a critical role for PUF-8 in controlling the balance between mitosis and meiosis. Using standard mapping techniques, we have identified one of the class II mutants, pds-37(kp37) (puf-8 dependent sterility), as an allele of toca-2. Sequencing confirmed a 316-bp deletion in this allele. Phenotypic characterization of this mutation is in progress.

763A

RNAi Spreading Mutants *rsd-2* and *rsd-6* are Deficient for Germ Cell Immortality. **Aisa Nakashima**[1], Lauren Garwood[2], Stacy Alvares[2,3], Jan LaRoque[2], Theresa Zucchero[2,3], Julie Hall[2], Shawn Ahmed[1,2]. 1) Genetics, University of North Carolina, Chapel Hill, NC; 2) Biology, University of North Carolina, Chapel Hill, NC; 3) SPIRE Postdoctoral Fellowship Program.

Germ cells represent canonical stem cells that possess the remarkable quality of being able to proliferate from one generation to the next, indefinitely, free of replicative damage. *mortal germline (mrt)* mutants initially display normal levels of fertility, but become progressively sterile when grown for multiple generations. Of 16 *mrt* mutants identified in a pilot EMS screen (Ahmed and Hodgkin, 2000), most were temperature-sensitive and only became sterile when grown at 25°C. Of the ts mutants, two were resistant to RNAi feeding constructs targeting embryonic lethal genes: *1c* and *10i*. Genetic mapping experiments, complementation tests for RNAi resistance, and DNA sequencing revealed that *1c* and *10i* contain mutations in *rsd-6* and *rsd-2*, respectively. Independently isolated alleles of *rsd-6* and *rsd-2* confer progressive sterility when propagated at 25°C, and complementation tests confirmed that these genes are required for germ cell immortality. *rsd-6* and *rsd-2* have been reported to be deficient for spreading of dsRNA-mediated RNAi from somatic cells to germ cells (Tijsterman *et al*., 2004). However, our results indicate that RSD-6 may be required in germ cells to promote germ cell immortality, whereas it functions in somatic but not germ cells to promote RNAi spreading. In addition, other RNAi-spreading defective mutants do not exhibit progressive sterility. These results indicate that RSD-6 and RSD-2 are likely to promote germ cell immortality via a mechanism that is distinct from their role in RNAi spreading.

764B

Characterizing the roles of enhanced RNAi genes *eri-1* and *eri-3* in sperm development and function. **Bernadette Nera**, Thais Cintra, Dmitry Ratner, Diana Chu. San Francisco State University, San Francisco, CA.

Generation of functional sperm depends on timely control of the gene expression at specific stages of spermatogenesis. RNA interference (RNAi) pathways are involved in regulating gene expression and are required for male fertility in other organsims. In *C. elegans*, enhanced RNAi mutants, *eri-1* and *eri-3*, are male sterile. Our lab is interested in investigating the role of these genes in proper sperm formation.

Previous studies have found *eri* mutants are sterile. However, it is unknown if the sterility is caused by a lack of germ cell production or if germ cells are produced but not functional. We have found that *eri* mutant hermaphrodites produce less progeny than wild type at both permissive and restrictive temperatures. At 25°C *eri* mutants did not produce any viable progeny but were still able to produce oocytes and sperm. This indicates that though *eri* mutant sperm cannot fertilize, they are capable of generating a signal for oocyte maturation.

In order to analyze how and where sperm formation is defective, temperature shifted *eri* mutant and wild type males were fixed and stained with the DNA dye DAPI. Overall the progression of germ cell nuclei appears to be normal; however, we observed chromosomes during late pachytene appear more diffusely compacted. In addition we observed defects in sperm meiotic chromosome segregation, with the presence of abnormally sized and shaped sperm nuclei, suggesting defects in chromosome segregation or integrity. We are currently analyzing the localization of components of the core chromosome segregation machinery to investigate how *eri-1* and *eri-3* mutants are defect in chromosome segregation.

RNAi is important for genomic integrity in other organisms such as zebrafish, mice, and flies where small RNAs are important for transposon silencing. To investigate genomic integrity in *C. elegans*, we analyzed double strand break formation and germ cell apoptosis in *eri* mutants. Our preliminary results show that at 25C eri mutants do not generate more double stranded breaks than wild-type.Thus, unlike RNAi components in other organisms, *eri* mutants may not play a role in transposon silencing in *C. elegans*.

The link between the enhanced RNAi phenotype of the *eri* mutants and chromosome defects resulting in abnormal sperm suggests that sperm-specific microRNAs may play an important role in sperm development.

765C

VBH-1 localizes to cytoplasmic granules in response to stress conditions. **Daniel Paz-Gomez**, Rosa Navarro. Departamento de Biologia Celular, IFC-UNAM, Mexico, D.F., Mexico.

Germ cells from several organisms possess specialized cytoplasmic granules made of mRNA and proteins, which are essential for its development and function commonly known in *C. elegans* as P granules. VBH-1 is a P granule associated protein that is a homolog of *Drosophila* DEAD-box RNA helicases Vasa and Belle. DEAD-box RNA helicases unwind mRNA in an ATP manner and facilitate RNA-protein interactions. VBH-1 is important for embryogenesis and gametogenesis, and plays an important role in the sperm/oocyte switch in the hermaphrodite gonad. VBH-1 associates to P granules, and is also found in the cytoplasm of all blastomeres during embryogenesis, and in the male and hermaphrodite gonad. To test the function of different VBH-1 domains on its localization, we divided this protein in three different regions. Its amino domain (first 151 aa), the helicase domain (336 aa) and carboxy-terminus domain (157 aa downstream of the helicase domain). We fused each *vbh-1* region to a gfp reporter under the control of a *pie-1* promoter, and introduced each construct into animals by biolistic transformation. We found that the helicase and carboxy-terminus domains are important for VBH-1 association to P granules. VBH-1 amino domain is less important for its localization to P granules under normal growing conditions, however we observed than under heat shock, this domain was also able to associate to P granules. We also observed that under heat shock, diffused somatic AMINO:VBH-1 protein aggregates into granules. Indeed, we observed that all three VBH-1 domains, and full-length VBH-1 protein, associate to somatic and germline granules under several stress conditions (starvation, sperm depletion and osmotic and oxidative stresses). By Western blot analysis, we found that no VBH-1 overexpression occurs under stress conditions indicating that granules are the result of VBH-1 aggregation or association with other proteins. CGH-1 is a protein usually found in small cytoplasmic foci of all blastomeres during early embryogenesis and in the adult gonad that might resemble processing bodies. Under stress conditions CGH-1 aggregates into bigger granules. We found that under stress conditions VBH-1 foci co-localizes with CGH-1. These results suggest that VBH-1 might play a role during stress conditions.

766A

SHC-1 regulates a non-cell -autonomous function of DAF-16 in germline. **Wenjing Qi**[1,2,3,4], Ralf Baumeister[1,2,3,4]. 1) 1Bioinformatics and Molecular Genetics (Faculty of Biology) Albert-Ludwigs-University Freiburg, Schänzlestr. 1, D79104 Freiburg i. Brsg., Germany; 2) Center for Biochemistry and Molecular Cell Research (Faculty of Medicine); 3) Center for Systems Biology (ZBSA); 4) FRIAS Freiburg Institute of Advanced Studies, School of Life Sciences (LIFENET).

The FOXO transcription factor DAF-16 integrates multiple signalling events involved in aging, stress response, and development. Our previous study shows that the adapter protein SHC-1, the *Caenorhabditis elegans* homolog of human p52Shc, controls the nuclear translocation and activation of DAF-16 through inhibition of insulin/IGF-1 (IIS) signalling and activation of JNK pathway (Neumann-Haefelin et al, 2008). Here we demonstrate that transgenic expression of wild type DAF-16a (isoform a) in *shc-1* mutant background causes sterility and early death of adult worms. These worms display a novel germline phenotype causing the disruption of the basement membrane of the gonad at the L3 larval stage. During leakage, germ cells spread out, accumulate in the pseudocoelom and undergo endomitosis. Feeding the worms with OP-50 mixed with 5-fluorodexoyuridine (FUDR) or knocking down the Notch receptor *glp-1* prevents the early death of the worm and extends lifespan up to 200%. Notably, FUDR treatment of wild type animals does not affect lifespan, indicating that the early death described above is caused by growth of germ cells. Transgenic expression of a DAF-16a mutant, which is constitutively localized in the nuclei, is able to reproduce the same germline phenotype in *shc-1* mutant but not in wild type worms, suggesting that nuclear DAF-16 is capable of inducing the germline defect in the absence of SHC-1. Thus, our data indicate that cell-non-autonomous signalling of DAF-16 contributes to the observed phenotype. Since transgenic DAF 16a is neither expressed in the germline nor in the somatic gonad, there must be signals from tissues outside the gonad. In summary, this work hints a novel function of DAF-16 in germline development that is controlled by SHC-1 mediated signalling pathways.

767B

puf-8's role in the proliferation versus differentiation decision facing *C. elegans* germline stem cells. **Hilary E. Racher**, Dave D. Hansen. Biological Sciences, University of Calgary, Calgary, AB, Canada.

In the *C. elegans* germ line, GLP-1/Notch signaling regulates the germline stem cell proliferation versus meiotic entry decision. Two redundant pathways, *gld-1/nos-3* and *gld-2/gld-3*, function downstream of GLP-1/Notch signaling to either inhibit proliferation and/or promote meiotic development. Enhancer screens have been performed to identify additional genes that function in regulating the proliferation versus differentiation decision. One screen was designed to find mutations that enhance the tumourous phenotype of a weak *glp-1* gain-of-function allele (gf). The genes corresponding to these mutants are likely to be either general negative regulars of GLP-1/Notch signaling, or positive regulators of meiotic entry (functioning downstream of Notch signaling). One of the genes identified in this screen was given the name *teg-2* (*t*umourous *e*nhancer of *glp-1* weak gf). Using a variety of mapping and cloning methods, we have found *teg-2* to be allelic to *puf-8* (*pu*milio and *f*bf). *puf-8* encodes an RNA-binding protein that belongs to the family of Puf proteins (homologous to Drosophila Pumilio). Previous studies have suggested roles for *puf-8* in the *C. elegans* germ line [1,2,4] and during vulva development [3]. We have identified an additional function for *puf-8* in the *C. elegans* germ line; to inhibit proliferation of germline stem cells. We have observed that loss of *puf-8* enhances the overproliferative phenotype of three *glp-1(gf)* alleles (*ar202, oz264* and *oz112oz120*) and is also able to suppress the Glp phenotype of a partial loss of function *glp-1* mutant (*bn18*). *puf-8* is not synthetically tumourous with the known genes functioning downstream of GLP-1/Notch signaling, *gld-1, nos-3, gld-2* and *gld-3*, suggesting that *puf-8* does not function in this portion of the pathway to promote meiotic development. *puf-8* mutants have previously been observed to have excess proximal proliferation due to dedifferentiation of spermatocytes [1]. To determine if the overproliferation we observe in *puf-8; glp-1(gf)* mutants is due to a disruption of the proliferation/differentiation decision or dedifferentiation, we made *puf-8; glp-1(gf); spe-6* and *puf-8; glp-1(gf); fem-3* mutants. Both *spe-6* and *fem-3* were previously shown to suppress the dedifferentiation tumour in *puf-8* single mutants [1]; however, in a *glp-1(gf)* background, we did not see suppression of the tumourous phenotype. This suggests that the *puf-8; glp-1(gf)* tumour is due to a disruption in the proliferation/differentiation decision.(1. Subramaniam, K. and Seydoux, G. (2003). 13, 134-9) (2. Bachorik, J. L. and Kimble, J. (2005). 102, 10893-7) (3. Walser, C. B., et al. (2006). 133, 3461-71) (4. Ariz, M., et al. (2009). 326, 295-304).

768C

Under stress conditions TIA-1 is required for CGH-1 granules formation and to induce germ cell apoptosis in *C. elegans*. **Carlos Silva**[1], Jorge Ramírez[2], Valerie Reinke[3], Rosa Navarro[1]. 1) Departamento de Biología Celular, IFC–UNAM, México, D. F., México; 2) Unidad de Microarreglos, IFC-UNAM, México D.F., México; 3) Department of Genetics, Yale University of Medicine, USA.

Germ cell apoptosis is an evolutionary conserved process important to maintain oocytes quality. In *C. elegans*, fifty percent of germ cells are eliminated during oogenesis by an unknown mechanism. Higher levels of apoptosis can be triggered by DNA damage and pathogen infections. The BH3-only domain protein EGL-1 participates in both types of apoptosis, but DNA damaged-induced apoptosis is also dependent of the transcriptional factor p53 (CEP-1). Heat shock, oxidative, and osmotic stresses, also induce germ cell apoptosis through the MAPKK pathway in a mechanism independent of EGL-1 and p53. Another stress condition that induces germ cell apoptosis is starvation however, its regulation is independent of EGL-1, CEP-1, or MAPKKs. In an RNAi screening to search for genes important for starvation-induced apoptosis we found C18A3.5; one of the three TIA-1/TIAR homologs in *C. elegans*. TIA-1 and TIAR are RNA binding proteins that promote the assembly of stress granules, and induce apoptosis in mammals. Stress granules are discrete cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited in cells subjected to environmental stress. C18A3.5 mRNA is enriched in the hermaphrodite gonad and decreased during starvation. C18A3.5(tm361) animals are temperature sensitive sterile, and have defects during oogenesis and embryogenesis. C18A3.5 is required to induce germ cell apoptosis in response to different stress conditions. To test if C18A3.5 is important for processing bodies and stress granules formation, we exposed wild type and C18A3.5(tm361) animals to starvation and heat shock. When nematodes are exposed to starvation or heat shock, large CGH-1 positive granules become visible in the core distal part of the gonad. In the absence of C18A3.5, CGH-1 is found in small granules and P granules, however no large CGH-1 granules were observed in the core of the gonad under stress conditions suggesting that, as in mammals, this protein is important for granules assembly. The other TIA-1/TIAR related *C. elegans* genes are not required to induce germ cell apoptosis or to participate during assembly of granules under stress conditions.

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Analysis of *mortal germline* Mutants. **Alicia N Simmons**, Aisa Nakashima, Ashley Hedges, Masa Godwin, Yan Liu, Shawn Ahmed. Department of Genetics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

A number of C. elegans mutants that posses a mortal germline phenotype have been isolated. These mutants suffer from heritable forms of damage. To gain a better understanding of the nature of the processes that promote germ cell immortality, genetic and phenotypic analysis of mortal germline mutants is being performed. Of the mutants examined thus far, all occur as a consequence of single recessive mutations. Further characterization of the genetic underprimings of these mutants, and their effects on germ cell development will be presented.

770B

Characterization of TEG-1 in C. elegans germ line. **Christopher L C Wang**[1], Lina Zhao[1], Laura Wilson-Berry[2], Tim Schedl[2], Dave Hansen[1]. 1) Department of Biological Sciences, University of Calgary, Calgary, AB, Canada; 2) Department of Genetics Washington University School of Medicine St. Louis, MO 63110, USA.

The C. elegans germline is a polar tissue with distal cells mitotically dividing, while more proximal cells enter meiosis and differentiate into gametes. Results from biochemical and genetic studies indicate that the GLP-1/Notch signaling pathway promotes distal germ cell proliferation by antagonizing two redundant pathways, one containing GLD-1 and the other GLD-2, which each promote meiotic entry (1-3). Two genetic screens were performed to identify novel genes involved in regulating the proliferation vs. differentiation decision. In one screen, mutations that enhanced the over-proliferation phenotype of a weak *glp-1(oz112oz120 gf)* allele were identified. In the other screen, mutations that were synthetic tumorous with a *gld-2* null allele were isolated. As a result, *teg-1* (tumours enhancer of *glp-1(gf)*) was identified in both screens. The open reading frame of *teg-1* encodes a protein of 370 amino acid residues. TEG-1-related proteins are found from yeast to human and contain GYF domain that is thought to play a role in protein-protein interactions (4). We have raised antibodies against TEG-1 peptides, which detect a band at about 65 kDa in N2 lysate, but not in a *teg-1* null mutant lysate, indicating the antibodies are specific to TEG-1. Subcellular localization studies demonstrate that TEG-1 is found in all germline and somatic nuclei of the gonad, with stronger staining being detected in distal tip cell, proximal germ cells, and oocytes. Moreover, we found that TEG-1 co-immunoprecipitates with UAF-1, the large subunit of U2 small nuclear ribonucleoparitcle (snRNP) auxiliary factor (5), suggesting TEG-1 forms a complex with a subset of UAF-1. This finding is reminiscent the human TEG-1 homolog CD2BP2, which interacts with a tri-snRNP bridging protein that is a component of the spliceosome complex (6), and suggests C. elegans TEG-1 may also involve in pre-mRNA splicing. We are currently conducting experiments to identify potentially misspliced mRNAs in *teg-1* mutants to better understand the mechanism of TEG-1in regulating the proliferation vs. meiotic entry decision in the germ line. 1. Hubbard, E.J. 2007. Dev. Dyn. 236: 3343-3357. 2. Kimble, J. and S.L. Crittenden. 2007. Annu. Rev. Cell Dev. Biol. 23:405-33. 3. Hansen, D. and T. Schedl. 2006. Curr. Top. Dev. Biol. 76:185-215. 4. Kofler, M.M. and C. Freund. 2006. FEBS J. 273:245-256. 5. Zorio, D.A. and T. Blumenthal. 1999. Nature 402:835-838. 6. Laggerbauer. B. et. al. 2005. RNA 11:598-608.

771C

Regulation of P granule integrity by a phosphatase. **Jennifer T. Wang**, Christopher Gallo, Ekaterina Voronina, Geraldine Seydoux. Molecular Biology and Genetics, Johns Hopkins University School of Medicine / HHMI, Baltimore, MD.

P granules are conserved ribonucleoprotein complexes that are important for germ cell development. In a screen for regulators of germ granule integrity and localization, we identified let-92, the catalytic subunit of protein phosphatase 2A (PP2A). PP2A is a heterotrimer that is composed of a catalytic subunit (let-92), a structural subunit (paa-1), and a variable regulatory subunit. Knockdown of let-92, paa-1, or a specific regulatory subunit by RNAi resulted in diffuse localization of P granule components in all cells of the embryo. PP2A knockdown did not affect localization of PATR-1, a P body component, or PAR-2, an anterior-posterior polarity determinant. These results indicate that a specific PP2A heterotrimer regulates P granule integrity in the C. elegans embryo.

772A

Identification of genes expressed in the hermaphrodite germ line of *C. elegans* using SAGE. **Xin Wang**[1], Yongjun Zhao[2], Kim Wong[2], Peter Ehlers[3], Yuji Kohara[4], Steven Jones[2], Marco Marra[2], Robert Holt[2], Donald Moerman[5], Dave Hansen[1]. 1) Dept. Biological Sciences, University of Calgary, Calgary, AB, Canada; 2) Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada; 3) Dept. Mathematics and Statistics, University of Calgary, Calgary, AB, Canada; 4) National Institute of Genetics, Yata, Japan; 5) Dept. Zoology, University of British Columbia, Vancouver, BC, Canada.

Germ cells must undergo elaborate developmental stages to progress from an undifferentiated germ cell to a fully differentiated gamete. Some of these stages include exiting mitosis and entering meiosis, progressing through the various stages of meiotic prophase, adopting either a male (sperm) or female (oocyte) fate, and completing meiosis. To increase our understanding of the genes necessary for the formation and the maintenance of functions of the germ line, we have constructed a SAGE library from hand dissected *C. elegans* hermaphrodite gonads. We found that roughly 21% of all known *C. elegans* genes are expressed in the adult hermaphrodite germ line. Genes with functions in protein turnover, energy production and translation are highly expressed in the germ line with the most highly expressed genes involved in translation, ribosomal structure and biogenesis. Expression levels for ribosomal genes are roughly 4 fold above their expression levels in the soma. We further found that 1063 of the germline-expressed genes have enriched expression in the germ line as compared to the soma. A comparison of these 1063 germline-enriched genes with a similar list of genes prepared using microarrays revealed an overlap of 460 genes, mutually reinforcing the two lists[1]. We identified 603 germline-enriched genes, supported by *in situ* expression data, which were not previously identified. We also found >4 fold enrichment for RNA binding proteins in the germ line as compared to the soma, suggesting a stronger reliance on RNA metabolism for control of the expression of genes in the germ line. Additionally, the number and expression level of germ line expressed genes on the X chromosome is lower than expected based on a random distribution. The comparison between SAGE and microarray highlighted some of the strengths and limitations of each technological platform. It also emphasized the importance of using multiple technological platforms to obtain a more complete picture of the genes that are expressed in a given tissue.

1. Reinke V, Gil IS, Ward S, Kazmer K: Genome-wide germline-enriched and sex-biased expression profiles in Caenorhabditis elegans. *Development* 2004, 131(2):311-323.

773B

Examining Pathways that Contribute to Dietary Omega-6 Fatty Acid-induced Sterility. **Christopher M Webster**, Jennifer L Watts. Washington State University, Pullman , WA.

Dietary fatty acids are vitally important to the health and fitness of many animals. Biologically, polyunsaturated fatty acids serve as precursors for a number of different signaling molecules and factors such as thromboxanes, prostacyclins, leukotrienes as well as the pro-inflammatory prostaglandins. While both omega-3 and omega-6 unsaturated fatty acids are classified as essential for mammals, it is becoming widely accepted that omega-6 fatty acids in fact may play a role in promoting certain cancers and heart disease. The nematode *C. elegans* serves as an important model for studying the roles of polyunsaturated fatty acids. When fed to *C. elegans*, the omega-6 fatty acid, dihomo-gamma linolenic acid (DGLA), leads to loss of germ cells and reduced fertility. Our preliminary studies indicate that JNK and p38 MAP kinase pathways may play a role in this DGLA-induced sterility. Inhibition of the JNK pathway, through mutation of the MAP kinase kinase mek-1, along with the MAP kinase phosphatase, vhp-1, lead to suppression of the DGLA-induced sterile phenotype. VHP-1 also serves to dephosphorylate members of the p38 pathway, raising the possibility that this pathway may remain in an activated state. Our data indicate that JNK and p38 pathways may play a role in mediating the response to the dietary omega-6 fatty acid, DGLA.

774C

Three *cib*-genes, three functions, one phenotype News in germ line development. Sophie von Elsner[2], **Anne Wiekenberg**[1], Henning Schmidt[1], Ralf Schnabel[1]. 1) Institut fuer Genetik, TU Braunschweig, Braunschweig, Germany; 2) current address: Department of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY, USA.

In *C. elegans*, the embryonic germ line is defined by the first four rounds of division. In contrast to the soma, the germ line cells (P-cells) divide asymmetrically in a stem-cell-like manner. To understand the mechanisms underlying this asymmetry, we analysed mutants in three genes with major defects in the embryonic germ line. In *cib-1* (Schnabel and Schnabel, 1990), *cib-2* and *cib-3* (changed identity of blastomeres) the germ line cell P1 to P3 divide, after a delay up to one cell cycle, symmetrically. The initial defect in *cib-2* is that the mitotic chromosomes do not condense. As a consequence, in *cib-2*, DNA is shredded during mitosis. Therefore the delay occurs (as a secondary defect) in the activation of the cell cycle checkpoint. We confirmed, that the activation of the cell cycle control depends on the damaged DNA by laser irradiation of one pronucleus in the wildtype embryo. This phenocopies the *cib-2* mutant. Since *cib-1* codes for the only thymidylate synthase (Winska *et al.*, 2005) in the worm, the P-cell problem caused by mutations in both genes are probably due to DNA integrity. The germ line phenotype suggests maybe the existence of a germ line specific DNA integrity checkpoint. CIB-3 is essential for the organisation of the cytoplasmic distribution of germ line specific factors. Nevertheless, CIB-2 and CIB-3 have identical interaction partners. This indicates a linkage between DNA integrity and the organisation of the cytoplasmic polarity. Thus, the system may serve to survey integrity of the worm population, by eliminating embryos with defect germ line DNA.

775A

Features of sperm cell differentiation that alter the meiotic program *C. elegans*. D.C. Shakes[2], **J Wu[1]**, P.L. Sadler[2], K. LaPrade[1], L.L. Moore[2], A. Noritake[2], D.S. Chu[1]. 1) Biology, San Francisco State Univ, San Francisco, CA; 2) College of William and Mary, Williamsburg VA.

The fundamental process of meiosis underlies two differentiation programs that occur at different rates and generate vastly different cell types, sperm and oocytes. There is a limited understanding in any organism, including *C. elegans*, regarding how sperm or oocyte specification either coordinates with or modifies meiosis to give rise to these disparate cell types. We have conducted an in-depth analysis of sperm cell formation to understand how gamete-specific features influence meiotic events and progression.

Our work has produced a detailed timeline of late meiotic prophase during spermatogenesis in *C. elegans*. This study is unique in that it defines a broad set of cytological and molecular landmarks that inter-relates changes in chromosome morphology and dynamics with germ cell cellularization, subcellular organelle disassembly, spindle formation, and meiotic cell cycle transitions to accurately stage nuclear progression. This analysis has uncovered differences in sperm meiotic chromatin composition and morphology compared to oocyte meiosis. Nuclei progressing past pachynema undergo distinct morphological changes after the incorporation of sperm nuclear basic proteins into sperm chromatin. Most strikingly, *C. elegans* spermatogenesis includes a previously undescribed extended stage when chromosomes form a constricted mass within an intact nuclear envelope. This karyosome stage, which is a common feature of meiosis in many organisms, follows desynapsis and is largely transcriptionally inactive. However it is highly dynamic, as multiple cell signaling pathways are sequentially activated during this stage and in an order that is distinct from that of diakinetic oocytes. Also in contrast to developing oocytes, spermatocytes exhibit centrosome-directed microtubule dynamics that are distinct in both their timing and morphology. Correspondingly, we observe that kinetochore structures that mediate chromosome segregation also exhibit sperm-specific features. Overall, several of these gamete-specific features effectively increase the efficiency and pace of meiotic progression during sperm formation. These studies identify specific features of the meiotic program that differ in sperm and oocyte meiosis, revealing that the underlying molecular machinery required for meiosis is differentially regulated in each sex.

776B

Structure-function analysis of PGL-3 that play a crucial role in P granule formation. **Masafumi Yonetani[1]**, Momoyo Hanazawa[2], Asako Sugimoto[1,2]. 1) Osaka university, Osaka, Japan; 2) RIKEN CDB, Kobe, Japan.

P granules are ribonucleoprotein (RNP) complexes and thought to play important roles in germ cell development. Although many components of P granules have been identified, how they interact to form P granules is unknown. To elucidate the mechanism of P granule formation, we recently established a granule assembly assay using cultured mammalian cells (CHO cells). We found that, among the 14 P granule components tested, only PGL-1 and PGL-3 autonomously formed granules, whereas other P granule components scattered throughout the cytoplasm in CHO cells. The PGL granules formed in CHO cells recruited other P granule components when co-expressed, as well as endogenous RNA and poly(A) binding protein. Thus, PGL proteins appeared to play crucial roles in RNP granule formation. To further investigate the roles of PGL proteins, we conducted a structure-function analysis of PGL-3 in CHO cells and *C. elegans* embryos. PGL-1 and PGL-3 contain an RNA-binding motif, RGG box, in their C termini. We found that RGG box of PGL-3 was dispensable for granule formation, but essential for recruiting endogenous RNA and other P granule components into the PGL granules in mammalian cells. We further found that 161-479aa of PGL-3 is sufficient for granule formation, and that 159-318aa is required for self-interaction. These functional domains of PGL-3 identified in mammalian cells were next examined in *C. elegans* embryo, by expressing GFP-tagged PGL-3 variants. We confirmed that 159-318aa was required for the targeting of PGL-3 to P granules, whereas RGG box was dispensable. Our results suggest that PGL-3 contains distinct domains for self-interaction/granule formation and RNA binding, both of which cooperatively contribute to P granule formation.

777C

MosSCI and Gateway compatible toolkit for germline expression of transgenes. **Eva A. Zeiser[1]**, Christian Frøkjær-Jensen[2,3], Erik Jorgensen[3], Julie Ahringer[1]. 1) The Gurdon Institute, University of Cambridge, Cambridge, United Kingdom; 2) Dep. Biomedical Sciences, U Copenhagen, Denmark; 3) HHMI, Dep. Biology, U Utah, Salt Lake City, UT.

Transgene silencing in the germline has hampered research in the germline and early embryo. The creation of “complex” extrachromosomal arrays using genomic DNA and then the development of technology for low copy number insertions using microparticle bombardment finally allowed germline expression of transgenes (Mello et al., 1991; Praitis et al., 2001). However, bombardment is very labor intensive and complex extrachromosomal arrays often are silenced. Recently, the Mos1 mediated Single Copy Insertion (MosSCI) method was developed to insert single copies of transgenes into defined sites in the genome of *Caenorhabditis elegans* (Frøkjær-Jensen et al., 2008). The technique is based on the MosTIC technique (Robert & Bessereau, 2007). Directed integration of a single copy avoids problems like overdosage, passage related loss of transgenes and locus mediated phenotypes. It also avoids extrachromosomal array related gene silencing in the germline which makes it especially attractive for studies on the gonad, and early embryos.

We are using the MultiSite Gateway system to generate transgenes for germline gene expression. This method allows easy fusion of three different sequences in a defined order (5’, middle, and 3’). We have generated a set of 5’ constructs containing the germline active promoter pmex-5 (Merritt et al., 2008) on its own or fused to the sequences of GFP, EGFP, Citrine and Tag RFP-T. Similarly, we have generated a set of 3’ constructs by fusing GFP, EGFP or Citrine to the tbb-2 3’UTR (Merritt et al., 2008). Using an appropriate combination of 5’ and 3’ constructs with a middle ORF of one’s choice allows easy generation of N- or C-terminal fluorescently tagged transgenes for expression in the germline or early embryo. We also show that the hsp-16.2 and hsp-16.41 heat shock promoters can be used for inducible germline and early embryo gene expression as single copy MosSCI generated insertions.

778A

TEG-4 is a splicing factor involved in regulating C. elegans germ line proliferation. P. Mantina, L. MacDonald, A. Kulaga, **X. Zhao**, D. Hansen. Dept Biological Sci, Univ Calgary, Calgary, AB, Canada.

Stem cells are unique cells that are capable of both self-renewing and differentiating into other cell types. It is crucial for stem cells to maintain a balance between proliferation and differentiation. Premature entry of stem cells into differentiation will deplete the pool of stem cells, resulting in an irreversible loss of the differentiated tissue. Conversely, cells that continue to proliferate, without entering into differentiation, will eventually lead to tumor formation. The C. elegans germ line serves as a good model to study how the balance between proliferation and differentiation is maintained. The adult hermaphrodite germ line contains two gonad arms, and within each gonad arm, the germ line displays a distal to proximal polarity. Cells residing at the distal end of the gonad are germline stem cells, which divide mitotically to proliferate, and as those cells move proximally, they enter into meiosis, differentiating into sperm and oocytes. The main regulatory network controlling the balance between mitosis and meiosis in the C. elegans germ line consist GLP-1/Notch signaling pathway, functioning at the distal end to promote proliferation, followed by two redundant mRNA regulatory pathways, the gld-1/nos-3 and the gld-2/gld-3 pathways that act to either inhibit proliferation or promote meiotic entry. During an enhancer screen searching for a tumourous germline phenotype using a sensitized genetic background, a gene named teg-4 was identified. We have mapped and cloned teg-4 and found that it is homologous to the SAP130 human splicing factor. We found that the teg-4 (oz210) allele partially reduces teg-4 function and shows some temperature sensitivity. 15%, 49% and 67% of homozygous teg-4(oz210) animals are sterile when grown at 15°, 20° or 25°, respectively, and most of the infertile animals make excess sperm at the expense of oogenesis. The rest of the sterile animals manifest a range of other phenotypes, such as defective oocytes, smaller than normal germ lines, or abnormal germ cells. We found that teg-4(oz210) enhances the over-proliferation phenotype of glp-1(ar202gf). It is also able to form a synthetic tumor with gld-1(q485), gld-2(q497) and gld-3(q730). These results suggest that a reduction in teg-4 activity can tip the balance towards proliferation, probably via a mechanism by which a reduction in teg-4 activity partially reduces the proper splicing of targets involved in controlling the balance between proliferation and differentiation.

779B

The discovery and Characterization of Novel Suppressors of the lin-35; slr-2 Larval Arrest Phenotype. **Stanley R. Polley**, David S. Fay. Molecular Biology, University of Wyoming, Laramie, WY.

Previous research in our laboratory has identified a novel role for LIN-35/Rb, the lone C. elegans retinoblastoma ortholog, in the regulation of intestinal gene expression. Furthermore, we have shown that LIN-35 acts in concert with SLR-2, a Zn-finger protein, to co-regulate the expression of many genes required for nutrient utilization and intestinal functions. In lin-35; slr-2 double mutants, L1 larva undergo uniform arrest and display many hallmarks of starvation. In order to shed additional light on the role of LIN-35 in intestinal functions, we are conducting a genome-wide RNAi feeding screen to identify suppressors of the L1 larval arrest. This approach has resulted in the identification of 14 candidate suppressors, which make-up at least three functionally distinct suppressor classes: (1) ribosome biogenesis genes, (2) mitochondrial prohibitins, and (3) a subset of SynMuv suppressors. Notably, the first two classes do not suppress the SynMuv phenotype, consistent with distinct roles for LIN-35 in the intestine and hypodermis. The majority of suppressors identified thus far likely control transcription or translation of a wide range of genes, consistent with the proposed mechanism of lin-35; slr-2 larval arrest, which results from the synergistic misregulation of intestinally expressed genes. Continued RNAi screening and a concomitant genetic screen for lin-35; slr-2 suppressors may identify integral components acting upstream or in parallel to LIN-35 and SLR-2. Identification and characterization of these genes could significantly expand on the developing role of LIN-35 in non-cell cycle-related developmental functions.

780C

Sexually dimorphic control of organ shape in the early *C. elegans* gonad. **Ngan Lam**[1], 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.

Somatic gonadal precursor (SGP) cells divide asymmetrically but do so in a sexually dimorphic fashion with respect to both daughter size and daughter fate. Importantly, "leader" function, which governs organ morphogenesis, is allocated to distal SGP daughters in hermaphrodites but to proximal daughters in males. To understand the sexually dimorphic control of SGP divisions and its effect on organ shape, we screened for male-specific leader mutants. From this screen, we identified a mutation in *hlh-3*, which encodes a bHLH transcription factor homologous to Drosophila Achaete-scute.

Our analysis demonstrates that HLH-3 controls specification of the male leader cell, which is called the "linker cell". No linker cell is made in *hlh-3* mutant males. Interestingly, overexpressing HLH-3 results in generation of cells with linker cell morphology in hermaphrodites. To ask if *hlh-3* expression is sex-specific, we used a HLH-3-GFP translational reporter transgene controlled by the *hlh-3* promoter (M. Krause et al, Development, 124:2179-89, 1997). The reporter gene was expressed in male but not hermaphrodite gonads. Moreover HLH-3-GFP was first seen in proximal SGP daughters and remained visible in the male linker cell. Therefore, the expression of *hlh-3* is controlled in a sex-specific and daughter-specific manner. We conclude that HLH-3 is a key regulator of male-specific organ shape via its role in linker cell specification. A parallel study found that HLH-2/E/Daughterless controls leader function in both sexes (M. Chesney, N.Lam et al, manuscript submitted). Because HLH-2 acts by binding different HLH partners, we suggest that the control of sexually dimorphic organ shapes relies, at least in part, on the sex-specific control of distinct HLH partners in distinct SGP daughters.

781A

MSP AND GLP-1/NOTCH SIGNALING COORDINATELY REGULATE OOCYTE GROWTH. **Priah Nadarajan**[1], J. Amaranath Govindan[1], Marie McGovern[2], E. Jane Albert Hubbard[2], David Greenstein[1]. 1) Dept Genetics, Cell Biol & Dev, Univ Minnesota, Minneapolis, MN 55455 USA; 2) Developmental Genetics Program, Skirball Institute NYU School of Medicine, New York, NY 10016 USA.

Fertility depends on germline stem cell proliferation, meiosis, and gametogenesis, yet how these key transitions are coordinated is unclear. We present a model in which the two major signaling centers in the adult hermaphrodite gonad, GLP-1/Notch distally and major sperm protein (MSP) proximally, while individually functioning in a separate process-germline proliferation and meiotic maturation, respectively-work in concert to regulate the differentiation of female germ cells into functional oocytes. In *C. elegans*, a sperm-sensing mechanism coordinates oocyte meiotic maturation and fertilization. Sperm release MSP to trigger meiotic resumption (meiotic maturation) and to promote the contraction of the follicle-like gonadal sheath cells. All known MSP-dependent responses in the germline require GSA-1 ($G\alpha_s$)-ACY-4 (adenylate cyclase) signaling in the gonadal sheath cells, including the promotion of actomyosin-dependent cytoplasmic streaming that drives oocyte growth. Thus, MSP signaling via the gonadal sheath cells links oogenesis and embryo production. These findings prompted us to conduct a genetic analysis of the MSP-dependent oocyte growth process. Surprisingly, we found that *glp-1* signaling functions in the germline to ensure proper oocyte growth when the MSP hormone is present. Reduction-of-function mutations in *glp-1* cause oocytes to grow abnormally large in a manner that is dependent on the presence of MSP and $G\alpha_s$-adenylate cyclase signaling in the gonadal sheath cells. By contrast, gain-of-function *glp-1* mutations result in the production of small oocytes. Further, MSP-dependent oocyte growth depends on DTC signaling involving the redundant function of GLP-1 ligands LAG-2 and APX-1. GLP-1 signaling affects two cellular oocyte growth processes, actomyosin-dependent cytoplasmic streaming and oocyte cellularization. *glp-1*(rf) mutants exhibit elevated rates of cytoplasmic streaming and delayed cellularization. MSP is sufficient to promote cytoplasmic streaming, and we show that MSP, but not GLP-1, promotes the phosphorylation of the regulatory myosin light chain (rMLC-4) of NMY-2 smooth muscle myosin throughout the germline. GLP-1 signaling in oocyte growth depends in part on the downstream function of the FBF-1/2 PUF RNA-binding proteins. Interestingly, abnormal oocyte growth in *glp-1* mutants, but not premature meiotic entry of germline stem cells, requires the function of the cell death pathway. These data illustrate how germline processes are intertwined when MSP is present and the adult gonad is switched into a reproductive mode.

782B

Global analysis of the genes necessary for gonadal sheath development using RNAi. Helaina Skop, Lindsay Eisemann, Tara Spencer, Tiffany Pica, Kostas Ballas, Andrumedia Persaud, Rina Amin, **Laura G. Vallier**. Department of Biology, Hofstra University, Hempstead, NY.

The gonadal sheath is essential for correct development of gametes and for fertility. Five pairs of cells encircling the proximal two thirds of each gonadal arm comprise the gonadal sheath. The gonadal sheath and the distal tip cell (DTC) both contribute to the establishment of a continuously dividing stem cell pool for the germline; if the DTC is missing, no germ cells are produced and if the distally located sheath pair 1 is missing, only the initially produced set of stem cells go into meiosis but they are not replenished. Additionally the sheath contributes toward three other processes that are crucial for fertility: progression past pachytene, ovulation into the spermatheca through rhythmic contractions of the muscular proximal pairs 4 and 5, and finally determination of the sperm cell fate. Deletion of the gonadal sheath via laser ablation results in almost complete sterility due to disruption of these processes. A number of genes have been identified that are important for sheath specification or function and include *emb-30, lin-9, lin-26* and *unc-52*. Other genes are expressed in one or more sheath cells and include lim-7*, ceh-18, ceh-32, myo-3* and *unc-54*, among others. We would like to identify all the genes necessary for gonadal sheath development. We used the Ahringer library to screen for genes that were necessary for sheath development by looking for loss of fluorescence and sterility in a *lim-7::GFP* (DG1575) background, which is expressed in part in the gonadal sheath. Currently, we are examining the candidates we recovered and have previously reported on for aberrant shape, rearrangement or absence of one or more of the gonadal sheath cells via gonad dissection and staining of the cells. An update on this aspect of the project will be presented at the meeting.

783C

Distal tip cell migration requires the transcriptional cofactor, CBP-1. **Ming-Ching Wong**, R. Daniel Sloan, Jean E. Schwarzbauer. Department of Molecular Biology, Princeton University, Princeton, NJ.

The *C. elegans* hermaphroditic gonad consists of two U shaped arms. The morphology of this organ relies on the migration of the distal tip cells (DTC). DTCs must first migrate longitudinally away from the mid-body along the ventral basement membrane. The cells then turn to migrate dorsally and turn again to migrate longitudinally back toward the mid-body along the dorsal basement membrane. To identify genes required for DTC migration, an RNAi screen was conducted. This screen revealed that the gene encoding the transcriptional cofactor, *cbp-1*, is necessary for DTCs to properly turn during migration (Cram et al., 2006, J. Cell Sci, 119:4811). 76% of worms treated with *cbp-1* RNAi exhibited gonads that either failed to achieve the first ventral-to-dorsal turn or turned away from the mid body during the second turn, which resulted in gonad arms that extended to the pharynx or the tail. Knock down of *cbp-1* expression specifically in DTCs during larval stages revealed that CBP-1 is required in the DTCs and acts cell autonomously. Therefore, we propose that CBP-1 is crucial for the transcriptional control of genes essential for the DTCs to direct gonad arm turns. Using a candidate gene approach, we are in the process of uncovering CBP-1 target genes necessary for proper DTC migration.

784A

Fate Determined by Committee: How the X:A Ratio Controls Sexual Identity with Precision. Benhom Farboud, John Gladden, Margaret Jow, **Todd Slaby**, Paola Nix, Barbara Meyer. Dept Molecular & Cell Biol, HHMI/UC-Berkeley, Berkeley, CA.

In C. elegans, sexual fate is determined by the ratio of X chromosomes to sets of autosomes (X:A). 2X:2A embryos develop as hermaphrodites, while 1X:2A embryos develop as males. Several genes on the X chromosome termed X Signal Elements (XSEs) act cumulatively in a dose-dependent manner to repress the master switch gene xol-1, the level of which determines the sex of the animal. Genes on autosomes termed Autosomal Signal Elements (ASEs) signal the animal's ploidy and antagonize XSE function by activating xol-1. In a 2X:2A animal, two doses of XSEs overcome ASEs to repress xol-1, thereby promoting the hermaphrodite fate. The hermaphrodite fate includes the activation of dosage compensation, the process that halves expression from both hermaphrodite X chromosomes to equal expression from the single male X. In a 1X:2A animal, the single dose of XSEs permits xol-1 to be active, thereby promoting the male fate and preventing the activation of dosage compensation. Three XSEs have been characterized molecularly. The nuclear hormone receptor SEX-1 and the ONECUT homeodomain protein CEH-39 repress xol-1 transcription by binding to multiple sites in the xol-1 promoter, while the RNA-binding protein FOX-1 prevents the proper splicing of xol-1 pre-mRNA by binding to multiple sites in a xol-1 intron. To date, two ASEs have been characterized: the T-box transcription factor called SEA-1 and the Zinc-finger protein called SEA-2. Both antagonize XSEs by binding to multiple sites in the xol-1 promoter. Thus far, variations of canonical transcription factor binding sites appear required for XSE function, while a unique T-box recognition sequence is needed for SEA-1 action. In sum, antagonistic molecular interactions carried out through multiple binding sites on a single promoter explain how only a two-fold difference in the concentration of XSEs can be translated into the high or low activity state of xol-1. The fidelity of X counting is then enhanced through xol-1 splicing control. We have characterized further the contributions of individual components of the X:A ratio, identified a candidate third ASE, and addressed the molecular nature of the cumulative dose-dependent antagonism between X and A signals.

785B

Isolation of *Caenorhabditis elegans* mutants defective in sperm function. **Gunasekaran Singaravelu**[1], Diane Shakes[2], Andrew Singson[1]. 1) Waksman Institute of Microbiology, Piscataway, NJ; 2) College of William and Mary, Williamsburg, VA.

The functional components required during fertilization can be conveniently studied in *Caenorhabditis elegans*. Here we describe a screening strategy for isolation of *C. elegans* mutants defective in sperm function (Spe) or egg function (Egg). We mutagenized the hermaphrodite population of the genotype *sem-2(n1343) Is[Pelt-7::gfp + rol-6 (su 1006)] I* using Ethyl methyl sulfonate (EMS) and screened for the self sterile mutants that could be rescued by allowing them to mate with wild type males. The *sem-2(n1343)* genetic background prevents the worm from laying eggs and the progeny are hatched inside the body. The transgene, *Pelt-7::gfp*, drives the expression of *gfp* under *elt-7* promoter, which is active in intestine. Thus, amid the mutagenized population, sterile mutants can be readily identified by the absence of fluorescing eggs and/or larvae in their uterus. In our most recent screen, we isolated twenty-four non-conditional sterile Spe mutants and eleven temperature sensitive Spe mutants. Our preliminary characterization suggests that various aspects of *C. elegans* reproductive biology such as sperm development, differentiation and/or functions are compromised in these mutants. A slightly modified strategy can be used to identify mutants that cause defective oogenesis or oocyte function. We are currently mapping and further characterizing these mutants with the aim of unraveling the genetic determinants behind the normal, reproductively competent sperm.

786C

A Role for *C. elegans* Eph RTK Signalling in PTEN Regulation. **Sarah Brisbin**, Jun Liu, Jeffrey Boudreau, Ian Chin-Sang. Dept Biol, Queen's Univ, Kingston, ON, Canada.

The *C. elegans* Eph Receptor Tyrosine Kinase, VAB-1, has known roles in neuronal and epidermal morphogenesis as well as oocyte maturation through interaction with its Ephrin ligands. In humans, Eph receptors are involved in nervous and vascular system development and have been implicated in cancer formation and progression. DAF-18, the *C. elegans* ortholog of the human tumour suppressor gene, PTEN, is a negative regulator of the DAF-2/Insulin receptor-like signalling pathway which has roles in dauer formation and adult longevity. Mutations in human PTEN have been associated with numerous cancers. We show that VAB-1 binds DAF-18 in a kinase independent manner, VAB-1 is a negative regulator of DAF-18 at the protein level and that VAB-1 is able to phosphorylate DAF-18 *in vitro*. Western blots and immunostaining revealed that DAF-18 expression is low in wild-type animals and DAF-18 protein levels increase in both the *vab-1(dx31)* null allele and the weak kinase inactive *vab-1(e2)* allele . VAB-1 and DAF-18 are both expressed in head neurons, oocytes and the germline precursor cells, Z2/Z3. *vab-1* mutants show increased longevity and sensitivity to dauer conditions which is consistent with increased DAF-18 activity. Lastly, a *daf-18(ok480)* null allele suppresses both oocyte maturation and the increased MAPK expression displayed in *vab-1* mutant worms. By indentifying the tissues these proteins are co-expressed in and confirming with multiple analyses, we propose that the VAB-1 Eph Receptor Tyrosine Kinase functions in neurons to regulate DAF-18 during ageing and dauer formation. In addition, DAF-18 functions in the oocytes during oocyte maturation downstream of VAB-1 signalling.

787A

Genetic mapping and characterization of a *C. elegans* Sma mutant. **Ryan Gleason**, Rich Byrne, Richard Padgett. Waksman Inst, Rutgers Univ, Piscataway, NJ.

The transforming growth factor-β (TGFβ) superfamily controls various processes of development and homeostasis of most tissues in metazoan organisms by exploiting the multifunctional TGFβ signal transduction pathway. The TGFβ superfamily consists of 42 structurally related members including members of the BMP family, TGFβ family, activin family, glial derived neurotrophic factor (GDNF) and Mullerian Inhibitory Factor (MIF). The members of this superfamily act most commonly as local mediators to regulate a wide range of biological processes in all organisms. They have been functionally identified as essential regulators of developmental processes including; pattern formation, proliferation, cell division (positive and negative regulators), differentiation, extracellular matrix production, cell migration, cell death, tissue repair, and immune regulation. Aberrant expression of members of the TGFβ superfamily has been detected in many diseases and cancer. The TGFβ signaling pathways are conserved, and genetic screens in both *C. elegans* and *D. melanogaster* have been essential in delineating the transduction pathway. To identify novel members of this signal transduction pathway including receptors, intracellular components, extracellular regulators, and transcription factors we have characterized Sma mutants identified in genetic screens within the lab. *wk94*, a new allele identified as a novel Sma/Mab mutant has been mapped to chromosome I. *wk94*, regulates a gene reporter wkEx52 (spp-9::GFP), a reporter for Dbl-1 TGFβ signaling, further characterizing its role in TGFβ signaling. Efforts are underway to map and clone *wk94*. Current status will be presented at the meeting.

788B

Global genome survey of genes required for germ line stem cell quiescence during dauer. **Emily C. Wendland**, Richard Roy. Department of Biology, McGill University, Montreal, Quebec, Canada.

The germ line stem cells divide throughout reproductive development in *C. elegans* to eventually generate all the gametes of the adult hermaphrodite. However, these divisions can be halted during nutrient stress and/or during dauer development. This cellular quiescence of germ line stem cells in dauer is tightly regulated by at least two intracellular signalling cascades that require the function of tumour suppressor genes such as PTEN/*daf*-18 and LKB1/*par*-4. Mutations in genes involved in these pathways lead to the inappropriate proliferation, or hyperplasia, of the germ line stem cell population during dauer. Mutations in both PTEN and LKB1 are known to affect aspects of cell growth and polarity and give rise to syndromes that result in an increased frequency of hamartomatous tumours. Whether these pathways cause tumours by affecting TOR-mediated cell growth or aspects of cell polarity, or both, remain to be determined. We have conducted a global genome survey using an RNAi feeding approach in order to identify additional genes that act in this pathway to regulate germline stem cell quiescence to better understand how these genes might affect tumourigenesis. This systematic knockdown of genes has identified a number of genes with functions in the control of cellular proliferation and cell polarity pathways. Further molecular characterization of each of these genes will help us to better define how these pathways converge to affect germ line stem cell quiescence and to better understand their roles in Peutz-Jeghers and other related hamartoma-associated pathologies.

789C

Analysis of breakage and meiotic instability in attached-X-chromosome strains. **Jonathan Hodgkin**, Nirmal Jethwa, Delia O'Rourke. Dept Biochem, Univ Oxford, Oxford, United Kingdom.

The isolation of an attached X chromosome (*X^X*), consisting of two apparently intact X chromosomes joined at their left telomeres, was previously reported (Hodgkin and Albertson 1995, Genetics 141: 527). In contrast to attached X chromosome constructs in other organisms, and also to X-autosome fusions in *C. elegans*, this *X^X* configuration is unstable, frequently breaking down to give an apparently normal X chromosome, or else an X chromosome with a deletion of the left end, or an X chromosome carrying a duplication of the left end. The extent of these deletions and duplications is variable, and they are generated at high frequency (in at least 5% of all *X^X* oogenic meioses). *X^X* strains therefore provide a useful source of aberrations in this region of the genome, which contains both a meiotic pairing site and numerator sites for sex determination.

It seemed likely that *X^X* breakage was occurring at meiosis, and might be dependent on the meiotic recombination machinery. To test this hypothesis, *X^X* was crossed onto a series of meiosis-defective backgrounds (*him-1, him-3, him-5, him-6, him-8, him-14, xnd-1*, etc.). *X^X* breakage, as measured by the production of self-progeny males, was significantly or greatly reduced in most of these backgrounds, indicating that the breakage events are partly or wholly dependent on meiotic recombination.

Previous FISH studies using YAC probes indicated that the end junction between the two X chromosomes was symmetrical and involved no deletion. We attempted to clone the junction fragment by single primer PCR, in order to determine its exact structure. However, PCR amplification was not successful, perhaps because the junction may include a substantial stretch of telomere repeats or local rearrangements. Further investigation of the structure and properties of the end junction is in progress.

790A

Polo-like kinase 2 (PLK-2) is required for crossover formation during meiosis. **Sara Labella**, Monique Zetka. Biology, McGill University, Montreal, PQ, Canada.

The accurate segregation of homologous chromosomes at the end of meiosis I relies on the formation of crossovers during homologous recombination. Meiotic recombination initiates with the Spo-11 catalyzed double-strand breaks (DSBs) and the repair of these breaks through homologous recombination can have two different outcomes, yielding either crossover (CO) or non-crossover (NCO) products. Little is known about the molecular details that regulate the formation of the double Holliday junction (dHJ) recombination intermediate and its resolution into CO or NCO products. EMS screening for meiotic mutants in our laboratory isolated a recessive mutation, *vv44*, which results in 77% embryonic lethality and 31% males. Mapping and subsequent complementation analysis indicated that *vv44* is an allele of *plk-2*, a serine/threonine kinase member of the conserved PLK family, already known to have important roles as mitotic and meiotic cell cycle regulators. *plk-2(vv44)* results in a P197L substitution in the activation loop of the kinase domain, raising the possibility that the protein encoded by this allele is kinase dead. PLK-2 is expressed at wild-type levels in *plk-2(vv44)* mutants and localizes to the synaptonemal complex in both wild type (WT) and *plk-2(vv44)* mutants, indicating that the protein is expressed and localized normally in mutant germlines. While homologous chromosomes show extensive alignment and synapsis at pachytene in *plk-2(vv44)* germlines, the 6 bivalents characteristic of wild-type diakinesis nuclei are never observed (0/55), and instead 75% show 11 or 12 univalents. Consistent with a requirement for PLK-2 in crossover formation, the frequency of crossing over in autosomal and X chromosome intervals in *plk-2(vv44)* mutants was reduced from 43-62% of the wild-type frequency. This deficit in crossing over is not a consequence of reduced recombination initiation since *plk-2(vv44)* mutant germlines exhibit elevated levels of RAD-51-marked early recombination intermediates throughout pachytene compared to WT. Furthermore, the disappearance of RAD-51 foci from late pachytene nuclei and the presence of intact univalents at diakinesis in *plk-2(vv44)* germ lines is consistent with the interpretation that these recombination intermediates are repaired by a non crossover pathway. Taken together these data indicate that *plk-2(vv44)* mutants are able to initiate recombination and are competent to form crossovers, but fail to do so at appropriate levels. Our results raise the possibility that PLK-2 may function in the conversion of dHJs into crossovers, similar to the function attributed to *S. cerevisiae* Cdc5, the sole PLK family member in budding yeast (Sourirajan and Lichten 2008). (Supported by CIHR).

791B

Suppressor analysis of mutants defective in meiotic chromosome alignment and synapsis. **Ka-Lun Law**, Monique Zetka. Dept Biol, McGill Univ, Montreal, PQ, Canada.

Proper chromosome segregation at meiosis I depends on the alignment of homologous chromosomes, the establishment of the synaptonemal complex (SC), and the formation of chiasmata between homologs. Previous studies have demonstrated that HIM-3, a component of the meiotic chromosome core, is required for these processes (Zetka *et al*. 1999). The *him-3(vv6)* mutation results in the substitution of a highly conserved residue of the HORMA domain (Couteau *et al*. 2004), believed to mediate protein-protein interactions (Aravind *et al*. 1998). HIM-3 levels in *him-3(vv6)* mutant germ lines appear to be normal and the protein is loaded to the chromosome core. However, *him-3(vv6)* mutant germ lines exhibit defects in homologue alignment, extensive non-homologous synapsis, and defects in recombination progression, resulting in defects in chiasma formation that lead to a high embryonic lethality (*emb*) and a high incidence of male (*him*) phenotype as consequence of chromosome mis-segregation. To identify proteins that interact with HIM-3 or function in the HIM-3 pathway, we performed an EMS-based suppressor screen using the *him-3(vv6)* allele to isolate candidates that suppress the embryonic lethality characteristic of *him-3(vv6)*. We isolated a strong extragenic suppressor, *vv39*, which increases chiasma formation on the autosomes. In *him-3(vv6); vv39* mutants, successful homologue alignment is stabilized by synapsis and the non-homologous synapsis defect of *him-3(vv6)* is suppressed. Antibody staining against the SC components SYP-1 and HIM-3 revealed a reduction of synapsed chromosome cores at pachytene. Furthermore, recombination initiation and progression are restored to a wild-type kinetic in the suppressed background. Interestingly, *vv39* is also able to suppress the homologue alignment defect associated with the loss of the HIM-3 paralogue HTP-1, which interacts with HIM-3 and is required for the coordination of homologue alignment and synapsis (Couteau *et al*. 2005; Martinez-Perez *et al*. 2005). Successful homologue alignment is also stabilized by synapsis in the *htp-1(gk174); vv39* double mutants. I hypothesize *vv39* is able to rescue the non-homologous synapsis defects in *him-3(vv6)* and *htp-1(gk174)* mutants, therefore homologous chromosomes that are aligned undergo homologous synapsis, and early recombination intermediates can be converted into crossovers. I am currently further characterized the meiotic functions of *vv39* through RNAi and other biochemical methods. This work is supported by NSERC.

792C

Cloning and Characterization of a New Meiotic Mutant *vv33*. **Yvonne Quan**, Monique Zetka. Biology, McGill University, Montreal, PQ, Canada.

We initially isolated a novel recessive meiotic mutant, *vv33*, in an EMS-based genetic screen in search of meiotic mutants defective in chromosome segregation. Mutants were screened for meiotic defects, distinguished by embryonic lethality (Emb) and high incidence of males (Him) due to chromosome missegregation. *vv33* mutants exhibit high embryonic lethality (80%) and an increase in male progeny (8%) among the surviving offspring. DAPI staining of *vv33* mutants revealed the presence of abnormally large nuclei and micronuclei throughout the mitotic and meiotic regions of the germline. Immunofluorescence, FISH analysis, and live imaging studies suggest that *vv33* causes chromosome missegregation in premeiotic nuclei, resulting in the formation of gametes with abnormal numbers of chromosomes, and aneuploidy in the progeny of mutant homozygotes. Further characterization of the precise role of *vv33* in the mitotic germline divisions will be presented. (Supported by the Canadian Institutes of Health Research).

793A

Identification of novel components of the *C. elegans* meiotic machinery. **Simona Rosu**, Angela Tam, Anne Villeneuve. Dept Genetics, Stanford Univ, Stanford, CA.

Faithful inheritance of chromosomes during sexual reproduction requires the coordinated action of multiple meiosis-specific chromosome structural components, meiotic recombination proteins, and regulatory factors. Mutagenesis-based genetic screening approaches in *C. elegans* have proven highly fruitful for identifying key components of this meiotic machinery. Here we report two novel meiosis components identified using such strategies.

The *me96* mutant was identified in a cytological screen for meiotic abnormalities visible in oocytes at diakinesis, the last stage of meiotic prophase. Overall, *me96* worms produce 60% dead embryos and 11% male progeny and exhibit a mixture of univalents and bivalents at diakinesis. Notably, the phenotype becomes progressively more severe as the worms get older. Mapping, RNAi and sequencing revealed that the *me96* allele contains an early stop mutation in the *F26H11.4* gene, thus defining a novel component of the meiotic machinery. F26H11.4 has three paralogs in *C. elegans*; interestingly, whereas F26H11.4 exhibits oogenesis-enriched expression, its three paralogs are expressed in spermatogenesis, suggesting sex-specific functional diversification. Immunofluorescence experiments show that loading of the synaptonemal complex appears normal in the *me96* mutant, but RAD-51 foci, which are markers of DNA double strand breaks (DSBs), are reduced. This suggests that F26H11.4 is involved in regulating DSB formation or processing, steps that are critical for meiotic recombination. Further characterization of this mutant will provide new insights into the complex regulation of meiotic recombination.

The *tn309* mutant was isolated in a screen for temperature-sensitive embryonic lethal mutants in the Greenstein lab. At 25 degrees, the *tn309* mutant exhibits achiasmate chromosomes in diakinesis-stage oocytes and produces both dead embryos (70-80%) and a high incidence of males (10%), phenotypes indicative of a meiotic defect. The *tn309* mutation has been mapped to a 0.68 cM region on chromosome I containing 34 genes, none of which are previously known meiosis genes. Further genetic mapping, RNAi experiments and sequencing are under way to identify the molecular change responsible for the *tn309* phenotype.

794B

him-5 regulates meiotic break formation. **Judith Yanowitz**[1], Frazer Heinis[1], Nathan Favani[2], Sara DiRienzi[2], Cynthia Wagner[1], Philip Meneely[2]. 1) Dept Embryology, Carnegie Institution, Baltimore, MD; 2) Dept of Biology, Haverford College, Haverford, PA.

Chromosome segregation in meiosis requires the proper number and position of crossovers. Yet chromosomes show characteristic distributions and numbers of crossover events suggesting that chromosome-specific factors are shaping the crossover landscape. A growing body of evidence implicates chromatin structure as a major determinant of crossover distribution. Nevertheless, the genes involved in this process have remained largely unknown. Many meiotic mutants, known as precondition mutants, alter the crossover landscape. It is likely that analysis of these mutants will reveal the underlying state of chromatin along the chromosomes.

We have been analyzing the *C. elegans* precondition mutant *him-5*. *him-5* was identified in Hodgkin's original HIM screen and was shown to have strong specificity for the X chromosome. We have analyzed his alleles *e1467* and *e1490*, as well as a null allele, *ok1896*. All three mutants cause an increase in male self-progeny ranging from 20-40% caused by a failure of recombination on the X chromosomes. In addition, all three alleles show a slight increase in autosomal nondisjunction, with age-related changes in the spectrum of severity.

We show that *him-5* is defective in making the double strand breaks that initiate recombination. We can completely rescue the X and autosomal nondisjunction phenotypes by creating breaks with ionizing radiation. In the mutant, chromosomes are completely paired and synapsed, but they prematurely disassemble the synaptonemal complex on the X chromosome, a phenotype that is also rescued by irradiation. These results are striking because it was previously thought that chromosome-specific meiotic functions were confined to homologue recognition and pairing. Together with our work on *xnd-1* (which has a similar phenotype), we have defined a novel step at which chromosome-specific identities are essential for proper disjunction.

him-5 corresponds to D1086.4 and encodes a protein with no known homologues outside of C. elegans. Two-hybrid analysis suggests that HIM-5 acts in a protein complex but the precise function of this complex is unknown. HIM-5 and XND-1 act independently of one another suggesting that further analysis of HIM-5 will elucidate novel aspects of chromosome-specific crossover control.

795C

Cardiolipin, a mitochondria specific phospholipid is essential for *C. elegans* gonad development. **Taro Sakamoto**[1], Yukae Ohtomo[1], Takao Inoue[2,3], Hiroyuki Arai[2,3], Yasuhito Nakagawa[1]. 1) Sch. of Pharm. Sci., Kitasato Univ; 2) Grad. Sch. Pharm. Sci., Univ. of Tokyo; 3) CREST, JST.

Cardiolipin (CL), also termed diphosphatidylglycerol, is a unique phospholipid that contains four fatty acyl chains. CL localizes to the mitochondrial inner membrane. During recent years CL has been attracting increasing attention as a functional phospholipid. CL plays a key role in the mitochondrial energy production, mitochondrial membrane dynamics, and regulation of mitochondrial apoptotic pathway. Although the biochemical features of CL are well investigated, its *in vivo* physiological functions are poorly understood. In CL *de novo* biosynthetic pathway, the rate-limiting and final steps are catalyzed by phosphatidylglycerophosphate (PGP) synthase and CL synthase, respectively. We identified *pgs-1* and *crls-1* genes in *C. elegans* genome sequences as the putative homologue of PGP synthase and CL synthase.

To investigate about the physiological functions of CL, we examined the phenotypes of *pgs-1* and *crls-1* gene deletion mutants. In this meeting, we present the phenotypic data of these mutants focused on gonad developmental defects. Both mutants, *pgs-1(tm2211)* and *crls-1(tm2542)*, showed sterile phenotype in hermaphrodites with impaired oogenesis and germ cell proliferation. The length and diameter of distal gonad of *pgs-1(tm2211)* were shortened to about the half of wild type, and this mutant could not generate normal oocytes. *crls-1(tm2542)* were able to generate several oocytes and lay fertilized eggs. However, the brood size of *crls-1(tm2542)* was extremely low compared to wild type, and all of these eggs failed to hatch.

To further characterize this phenotype, we examined the germ cell proliferation defects of *pgs-1(tm2211)* and *crls-1(tm2542)* by the inhibition of *gld-1* pathway. In wild type, *gld-1* RNAi caused the reentry of the germ cells in the early stages of oogenesis into the mitotic cell cycle and over-proliferation. On the other hand, both mutants suppressed these germ cell phenotypes by *gld-1* RNAi.

Growth and development rely on the mitochondria as the major source of ATP, and gonad has abundant mitochondria compared to other somatic tissues. One of the characteristic features of the germ cell is the continuous proliferation throughout the life. Therefore, we speculate that CL has an important role for *C. elegans* gonad development through the maintenance of proper germ cell proliferation.

796A

The Ras/MPK-1 pathway controls mRNPs and their regulators during oogenesis. **Arnaud Hubstenberger**, Scott Noble, Thomas C. Evans. Department of Cell and Developmental Biology, University of Colorado, Denver Health Science Center, Aurora, CO 80045, USA.

Oogenesis requires the translational repression/activation of specific mRNAs at different steps of meiotic progression. The pathways that connect mRNA translation patterns to germ cell development remain largely unknown. The MPK-1 pathway controls many biological events during meiosis progression, including pachytene exit, oocyte organization, and oocyte differentiation. During these events, different mRNA regulators arise and function at specific stages. For example, the translation repressor GLD-1 functions in early meiosis and disappears upon pachytene exit, whereas the RNA binding protein PUF-5 is made beginning at pachytene exit and controls late steps of oogenesis. Does the MPK-1 pathway influence these RNA control factors? We found that Ras and MPK-1 functionally cooperate with PUF-5 during oogenesis. A *mpk-1(ts-lf)* mutant synergizes with *puf-5(RNAi)* to cause severe oocyte misorganization. Furthermore, puf-5(RNAi) partially suppresses oocyte growth defects of a Ras hyperactive *(let-60(gf))* mutant. Finally, MPK-1 promotes PUF-5's ability to repress GLP-1 translation during oogenesis. These results suggest that PUF-5 may act downstream of MPK-1 to mediate part of Ras/MPK's biological effects. Two observations are consistent with this idea. First, MPK-1 activity controls the ratio of two forms of PUF-5 protein. Second MPK-1 activity controls the timing of GLD-1 loss and PUF-5 onset during meiosis progression. Previous work showed that repressed mRNA, PUF-5 and GLD-1 localize to P body-like granules. Interestingly, Ras/MPK activity influences P body-like granule structure, and PUF-5 and MPK-1 together control localization of the P body protein CGH-1. These studies suggest that Ras/MPK controls localizatjon pattern, composition, and function of mRNP complexes in the cytoplasm of the C. elegans germline.

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797B

Role of the MAP Kinase Cascade in the MSP Signaling Response. **Y. Yang**, M.A. Miller. University of Alabama at Birmingham, Birmingham, AL.

The MSP domain is an evolutionarily conserved immunoglobulin-like structure of about 120 amino acids. A P56S mutation in the MSP domain of the human vapB gene causes a dominantly inherited form of Amyotropic Lateral Sclerosis (ALS) or sometimes Spinal Muscular Atrophy (SMA) (Nishimura et al., 2004). In C. elegans, MSP is the most abundant protein in sperm and is the founding member of MSP domain family. MSP functions as an intracellular cytoskeleton protein and a secreted hormone. Secreted MSP binds to the VAB-1 Eph receptor and other receptors on oocyte and ovarian sheath cell surfaces to induce oocyte maturation and sheath contraction. We have recently shown that worm, fly, and human VAPB MSP domains are secreted ligands for Eph receptors and other receptors. The human VAPB MSP domain is found in blood serum, consistent with a hormonal function. In fly cells, the P56S mutation prevents secretion of the VAPB MSP domain, suggesting that the signaling function is important for ALS and SMA pathogenesis (Tsuda et al., 2008). To better understand how MSP domains transduce signals, I have been studying the PTP-2 tyrosine phosphatase, an SHP homolog that is thought to couple receptor tyrosine kinase (RTK) activation to the MAPK cascade. Previous studies have shown that MSP promotes MPK-1 MAPK phosphorylation during oocyte maturation (Miller et al., 2001) and that PTP-2 is required for normal fertility (Gutch et al., 1998). I have shown that PTP-2 is expressed in the gonad, where it is required for MSP-induced oocyte maturation and MAPK activation, but not MSP-induced sheath contraction. Expressing GFP::PTP-2 specifically in the germ line can partially rescue the oocyte maturation defects, embryonic lethality, and larval lethality of ptp-2 null mutants. Furthermore, these ptp-2 null defects can be rescued by inactivating multiple RAS GAPs, as well as by a RAS gain of function mutation. MSP binding studies show that MSP receptors are present on the oocyte and sheath plasma membranes in ptp-2 null mutants. These results are consistent with a model in which MSP regulates the activity of a canonical RTK/MAPK pathway to induce oocyte maturation. The identity of the RTK is not known and it is not clear whether MSP regulates RTK activation through a direct interaction (i.e. the RTK is an MSP receptor) or indirectly. VAPB homologs may also regulate the MAPK pathway because PTP-2 is expressed in muscle and loss of PTP-2 causes muscle mitochondrial defects that are similar to loss of worm VAPB (called VPR-1).

798C

Protein degradation in the *C. elegans* germ line can regulate entry into meiosis. **Lindsay D. MacDonald**, Aaron Knox, Dave Hansen. Dept Biological Sciences, University of Calgary, Calgary, AB, Canada.

In any population of stem cells a delicate balance between proliferation and differentiation must be maintained to ensure proper stem cell function and tissue homeostasis. In the *C. elegans* germ line, this balance is controlled by the GLP-1/Notch signaling pathway. GLP-1/Notch signaling promotes proliferation, at least in part, by negatively regulating two redundant downstream pathways (GLD-1/NOS-3, GLD-2/GLD-3), which inhibit proliferation or promote meiotic entry. We have recently identified a weak loss of function allele of one of the proteasome alpha subunits (*pas-5(oz237)*), that enhances an overproliferation phenotype in the *C. elegans* germ line. Through genetic epistasis we have found that the proteasome has two separate functions in regulating the decision between proliferation and meiotic entry. First, the proteasome acts as a general negative regulator of Notch signaling. *sel-10*, an E3 ubiquitin ligase, has been implicated in GLP-1 INTRA degradation by targeting INTRA to the proteasome. However, when proteasome function is reduced and SEL-10 is no longer present in the cell, an enhancement of Notch signaling is still seen, suggesting a second E3 ubiquitin ligase is required for proper ubiquitination and subsequent degradation of INTRA. Second, the proteasome is involved in negatively regulating proliferation downstream of GLP-1/Notch signaling, preferentially in the GLD-1/NOS-3 pathway. GLD-1 is a KH domain containing protein that has been shown to bind the 3'UTRs of specific transcripts to prevent their translation to promote meiotic entry. These findings lead us to a model in which the proteasome is responsible for degrading proteins that are also regulated by GLD-1 to more efficiently remove them from the cell and promote entry into meiosis as the cells move out of the mitotic zone of the gonad. In order to identify the proteins whose degradation is necessary for proper entry into meiosis we are performing an RNAi screen of all substrate recognition subunits (SRS). These SRS's are responsible for binding to the specific target protein to allow that protein to be ubiquitinated and subsequently degraded by the proteasome. If we knockdown the function of the SRS, its target protein will not be degraded by the proteasome, resulting in an overproliferation phenotype similar to that seen in the *pas-5(oz237)* mutant. If we can identify the SRS we can then use biochemical methods to identify the protein that is required for proliferation and whose degradation is required for entry into meiosis.

799A

Exo-1, a multi-tasking nuclease guarding genome stability. **Bennie Lemmens**, Marcel Tijsterman. Genome dynamics and stability, Hubrecht Institute, Utrecht, NL.

DNA double strand break (DSB) repair is essential, not only for the maintenance of genome stability and therefore animal survival, but also for completion of meiosis. Exonucleases, such as Exo1, play key roles in DNA repair. Exo1 was first isolated in fission yeast extracts that possessed 5' to 3' exonuclease activity. Since then Exo1 has been implicated in a multitude of eukaryotic DNA metabolic pathways, such as telomere maintenance and DSB repair[1]. Moreover, Exo1 is implicated in mismatch repair (MMR); a process crucial for removing misincorporated nucleotides during DNA replication. MMR deficiency results in instability of simple repeat sequences, a phenomenon referred to as microsatellite instability (MSI). Several MMR factors also affect homologous recombination (HR) and DNA damage signaling[2]. Here, we introduce the C. elegans homologue of Exo1 and investigate how it contributes to genome stability.

The C. elegans F45G2.3 gene (hereafter referred to as *exo-1*) encodes a protein that is highly similar to yeast and mammalian Exo1. We show that *exo-1* deletion mutants have elevated MSI, suggesting a conserved role in MMR. In addition, we found that *exo-1* mutants are hypersensitive to γ-irradiation, in a manner that is epistatic with the well-conserved HR factor, *brc-1*. This suggests a role for *exo-1* in HR, which is the principle DSB repair pathway active in the worm germline. In contrast, deletion mutants of *mlh-1*, which is a non-redundant factor in MMR, are not sensitive to γ-irradiation, which implies that *exo-1* has an MMR-independent role in DSB repair in the germline.

During meiosis, programmed DSBs are introduced by the topoisomerase *spo-11* and repair of these breaks is essential for completion of meiosis. Under normal conditions, *exo-1* mutants are able to complete meiosis, indicating *exo-1* is either not involved in the repair of *spo-11* induced DSBs or that it acts redundantly with other factors. In order to identify factors that may be working redundantly with *exo-1*, we performed a genome-wide synthetic lethal RNAi screen. This screen identified several genes required for development of *exo-1* deficient animals and their contribution to DSB repair is being investigated. Additionally, we are searching for redundant factors via a candidate gene approach, focusing on the worm's RecQ helicases. This was prompted by recent studies showing that Sgs1, the sole RecQ helicase in yeast, acts in parallel with Exo1 in processing DSBs in mitotic cells[3].

1) Tran PT et al. EXO1: a multi-tasking eukaryotic nuclease, DNA Repair (2004)
2) Jiricny J. The multifaceted mismatch-repair system, Nat. Rev. Mol. Cell Biol. (2006)
3) Klein HL. DNA endgames, Nature (2008).

800B

Recombination pathway and partner choice during meiotic double strand break repair in *C. elegans*. **Diana E. Libuda**, Anne M. Villeneuve. Dept of Developmental Biology, Stanford University, Stanford, CA.

Recombination is critical for generating genetic variation, for maintaining genome integrity through repair of DNA breaks, and for ensuring chromosome segregation during meiosis, the specialized cell division program by which diploid organisms generate haploid gametes. Meiotic recombination is initiated by double strand DNA breaks (DSBs), which are repaired using meiosis-specific mechanisms that favor utilization of the homologous chromosome (instead of the sister chromatid) as the recombination partner and that promote a crossover (rather than noncrossover) outcome of the DSB repair (DSBR) process. Crossover recombination between homologous chromosomes is required to create temporary physical connections that promote proper meiotic chromosome segregation. Our lab has built on a recent analysis of DSBR following heat-shock induced excision of the Mos1 transposon (Robert *et al.* 2008) to develop assays to monitor meiotic recombination events between homologous chromosomes initiated at a defined DSB site (see abstract by Rosu and Villeneuve). Since the *C. elegans* germline is organized in a spatial-temporal gradient, DSBs can be induced simultaneously at all meiotic stages, and the repair outcomes for these DSBs induced at different stages can be subsequently assessed. Currently, we are using a novel Mos1-based assay system to investigate the nature of gene conversion tracts associated with both crossover and noncrossover homologous recombination events generated by DSBs induced at various stages of meiotic prophase. In addition, we are constructing a second-generation version of the assay system of Rosu and Villeneuve; this modified assay system incorporates features that also allow for the detection of recombination events between sister chromatids. With this new meiotic DSBR assay, we will directly test the hypothesis that germ cells undergo a switch in DSBR mode during prophase progression to enable use of the sister chromatids as repair partners during late prophase. Moreover, we will investigate the roles of meiotic recombination machinery components and meiotic chromosome structures in promoting specific outcomes of DSBR by assessing repair events in mutants defective in the genes encoding these proteins. Furthermore, we are developing genetic and RNAi-based screens incorporating this second-generation assay to identify components that play roles in inhibiting the formation of either crossover or intersister recombination events. Overall, these studies will elucidate how meiotic chromosomes are able to utilize specific recombination pathway and partner preferences to yield desired recombination outcomes, thereby enabling proper chromosome segregation and maintaining genome integrity.

801C

C. elegans HIM-18/SLX-4 interacts with SLX-1 and XPF-1 and maintains genomic integrity in the germline by processing recombination intermediates. **Takamune T. Saito[1]**, Jillian L. Youds[2], Simon J. Boulton[2], Monica P. Colaiácovo[1]. 1) Dept Genetics, Harvard Medical Sch, Boston, MA; 2) DNA damage Response Laboratory, Cancer Research UK, Clare Hall, EN6 3LD South Mimms, UK.

DNA double-strand breaks (DSBs) can arise in various ways, including as a result of the collapse of stalled replication forks, exposure to DNA damaging agents and the formation of programmed meiotic DSBs. The importance of DSB repair is therefore highlighted by its critical roles in replication fork restart, the maintenance of genomic integrity and promoting faithful meiotic chromosome segregation. Homologous recombination (HR) provides an efficient and accurate repair of DSBs, in part through the use of an intact donor template for repair. Through the process of HR, two four-way DNA junction intermediates referred to as double Holliday junctions (dHJ) are formed. dHJ resolution is essential for the repair of blocked or collapsed replication forks and for the production of crossovers between homologs that promote accurate meiotic chromosome segregation. In *D. melanogaster*, MUS312 is thought to be required for dHJ resolution along with XPF-ERCC1 known as a structure specific endonuclease complex. In *S. cerevisiae*, the Slx4-Slx1 complex cleaves branched DNA substrates in vitro.

Through a functional genomics approach, we identified HIM-18, an ortholog of MUS312/Slx4, as a critical player required in vivo for dHJ resolution in *C. elegans*. HIM-18 is localized to mitotic nuclei at the premeiotic tip and to meiotic nuclei from late pachytene through diakinesis in wild type germlines. DNA damage sensitivity and an accumulation of recombination intermediates (RAD-51 foci) during premeiotic entry in *him-18* mutants suggest that HIM-18 is required for DNA duplex resolution at stalled replication forks. A reduction in crossover recombination frequencies, accompanied by an increase in recombination intermediates during meiosis, germ cell apoptosis, unstable bivalent attachments and subsequent chromosome nondisjunction, support a role for HIM-18 in meiotic HJ resolution. The evidence of a physical interaction of HIM-18 with SLX-1 and XPF-1, accompanied by the synthetic lethality of *him-18* with *him-6*, the *C. elegans* BLM homolog, suggest that the proposed function of MUS312/Slx4 in DNA repair is conserved in *C. elegans*. We propose that HIM-18 facilitates resolution of recombination intermediates resulting from replication fork collapse and programmed meiotic DSBs in the *C. elegans* germline.

802A

Studies on localization mechanisms of the maternal pos-1 mRNA in C. elegans embryos. **Kouki Noguchi**, Yuji Kohara. Genome Biol Lab, Natl Inst Genetics, Mishima, Japan.

mRNA localization has been observed in diverse cells and organisms. In C. elegans, there are also several maternal mRNAs, which is known to be localized during early development. Distribution patterns of these mRNAs suggest involvement of mRNA degradation in the localization. However, there is little information about cis- and trans-elements for the mRNA localization. To understand how cells regulate the localization, identification of these elements are desired. In this study, we are focusing on the maternal pos-1 mRNA. The pos-1 mRNA is localized to the posterior half of embryos during the 1st cleavage and is localized to germ lineage in early development. To investigate cis-elements of the localization, we first developed a vector, which maternally expresses fusion mRNA of VENUS CDS and arbitrary sequences from the pos-1 promoter. By using the vector, we generated transgenic worms that express the VENUS::pos-1 3'UTR fusion mRNA and tested localization of the mRNA. In situ hybridization revealed that the mRNA is localized as intrinsic pos-1 mRNA. Thus, we analyzed the pos-1 sequences by using the vector. Deletion analysis of the pos-1 3'UTR revealed that 59 nt of the pos-1 3'UTR (bases 178-236 downstream of the pos-1 stop codon.) is required for the localization. Interestingly, there are evolutionary conserved CYCACA tandem repeats (191-196 and 199-204) and 30nt (207-236). Hence, we next tested whether these sequences are involved in the localization by making series of constructs, which contain substitutions in these sequences. This analysis revealed that these conserved sequences are actually required for the localization. We also found that, the VENUS protein is also localized in the transgenic embryos of VENUS::pos-1 3'UTR mRNA. In the embryos, the VENUS protein was first detected just after fertilization and was uniformly expressed at the time. After that, the protein was preferentially accumulated in posterior half by the four-cell stage. Such localization is not observed in the VENUS::mel-11 3'UTR worms; in these worms, the VENUS protein was expressed in adult gonad and was uniformly distributed in early embryos. Thus, the VENUS protein localization is pos-1 3'UTR dependent and should be accomplished by local translation rather than post-translational protein localization. Strikingly, the pos-1 3'UTR dependent protein localization is abolished in mex-5 mutant, in which the pos-1 mRNA localization is also abolished. It suggests a close correlation between pos-1 3'UTR dependent local translation and mRNA localization. We are now trying to identify trans-elements of the pos-1 mRNA localization by using yeast three-hybrid assay. At the meeting, we will also presents the result.

803B

Several RNA granule components are required to induce apoptosis under stress conditions. **Laura Láscarez**, Carlos Silva, Rosa Navarro. Departamento de Biología Celular. IFC–UNAM, México, D. F., México.

Germ cell apoptosis is an integral part of oogenesis in several organisms. Conditions, like heat shock, oxidative and osmotic stresses, increase germ cell apoptosis in *C. elegans* by the MAPKK pathway, and through an EGL-1 and CEP-1 independent mechanism. Starvation also induces apoptosis, but through a different mechanism. To find out genes that regulate stress-induced apoptosis, we first identified in a microarray analysis genes that alter their expression levels when animals are starved for 6 h. Using RNAi we are testing if any of these genes are required for germ cell apoptosis. Interestingly, we observed that several genes that change their expression levels during starvation encode for proteins that associate with RNA granules. RNA granules are non-membranous cytoplasmic ribonucleoprotein particles that have been implicated in post-transcriptional regulation of RNAs. Some examples of RNA granules are: P granules, processing bodies (PBs) and stress granules (SGs). P granules store RNA and proteins important for germ cell function and embryogenesis. PBs contain components of the mRNA decay machinery, and stored and degraded RNA. SGs are cytoplasmic aggregates of stalled translational preinitiation complexes that accumulate during stress. RNA granules share some components and may use similar mechanisms to regulate mRNA translation or decay. Among genes that alter their expression level under starvation conditions are *car-1*, *cgh-1*, *cey-2*, *pgl-1* and *mex-5*. *cgh-1* and *car-1* encode for RNA binding proteins that are PBs and SGs components, and in their absence physiological germ cell apoptosis is increased. *cey-2* encodes an RNA binding protein with a cold-shock/Y-box domain that associates with CGH-1 and CAR-1. PGL-1 is also an RNA binding protein that is a key P granule component, and MEX-5 is a zinc finger protein that localize to CAR-1 and CGH-1 granules in late stage oocytes. We found that RNAi in *cey-2*, *mex-5* and *pgl-1* increase significantly the physiological germ cell apoptosis. As we previously described, stress conditions increase germ cell apoptosis. However, when *car-1*, *cgh-1*, *cey-2*, *mex-5* and *pgl-1* are silenced by RNAi no increase in stress-induced apoptosis is observed. Our results suggest that these genes play an important role on germ cell apoptosis. We are currently testing if other RNA granule components that were found in our microarray analysis are also required for germ cell apoptosis, and if any of these genes play a role in RNA granule aggregation under stress conditions. We are also studying if there is a direct link between stress conditions, RNA granule aggregation and apoptosis.

804C

Identification of direct targets of the global sexual regulator TRA-1 by chromatin immunoprecipitation. **Matthew Berkseth**, David Zarkower. Department of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN.

Caenorhabditis elegans naturally occurs as two sexes, the XX hermaphrodite and the X0 male, with sexual specialization evident in roughly one third of somatic cells, making it an ideal organism in which to study the control of sexual differentiation. The extensive sexual dimorphism observed in the worm results from the differential activity of the global sex determination pathway, which culminates in the transcription factor TRA-1, the worm homologue of the vertebrate GLI proteins. While the global sex determination pathway upstream of TRA-1 has been well understood for many years, little is known of the genes that function downstream of TRA-1 to direct sexual differentiation throughout the worm. Only a few direct targets of TRA-1 have been identified, all of which are genes whose expression is required in specific cells for male development and whose transcription is repressed by TRA-1 in the hermaphrodite. The combined actions of these few known TRA-1 targets cannot fully account for the observed sexual dimorphism in *C. elegans*, so additional biologically important targets remain to be identified. It is unknown whether TRA-1, like its vertebrate homologues, can function as both a transcriptional repressor and an activator, or what chromatin modifications may be recruited by TRA-1 to target genes. Likewise, it is unknown whether TRA-1 binding to its targets occurs only at the specific developmental times and in the specific tissues necessary to direct proper sexual differentiation, or occurs even when its regulation is not required. To address these questions and to identify additional TRA-1 binding sites throughout the genome, we are performing chromatin immunoprecipitations using an affinity purified TRA-1 antibody. We have successfully identified known TRA-1 binding sites with this approach, including those in the promoter regions of *mab-3* (Yi *et. al.*, 2000) and *fog-3* (Chen and Ellis, 2000), and we are employing whole genome tiled microarrays to identify other TRA-1 targets throughout the genome.

805A

Defining the pathway that controls hermaphrodite development in *C. briggsae* . **Xiangmei Chen**[1], Yiqing Guo[2], Ronald Ellis[2]. 1) Graduate School of Biomedical Sciences, UMDNJ, Stratford, NJ 08084; 2) Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ 08084.

Phylogenetic studies suggest that hermaphroditism might have evolved independently in *C. elegans* and *C. briggsae*. In *C. briggsae*, *she-1* is a novel gene that promotes spermatogenesis in XX animals. Surprisingly, null alleles of *she-1* are temperature sensitive, which implies that other genes help control hermaphroditic development. To identify these genes, we isolated 12 *she-1* suppressors.

The dominant suppressors *v93* and *v98* are *tra-2* alleles. Homozygous *XX* animals are males, just like null allele of *tra-2*. Furthermore, a null allele of *tra-2* also suppresses *she-1*. Thus, *she-1* acts upstream of *tra-2* and is very sensitive to changes in *tra-2* dosage.

The mutations *v94, v99, v100, v102* and *v103* are dominant. Even after extensive backcrossing, two of them have additional phenotypes. Homozygous *she-1; v100* animals are sterile and have very small germlines, and homozygous *v103* mutants are dead. We mapped *v99* to a region between 7 Mbp and 11 Mbp on LG *III*, and *v100* to a region between 2 Mbp and 7 Mbp on LG *I*.

Five suppressors *v92, v95, v96, v97* and *v101*, are recessive; they restore *she-1(lf) XX* self-fertility, but do not appear to affect the soma. We mapped *v95* and *v101* to LG *I*, since they complement to each other, which indicates that they define two different genes. We also mapped *v97* to LG *V*, *v92* to a region between 2 Mbp and 8 Mbp on LG *II*, and *v95* to a region between 0.4 Mbp and 7 Mbp on LG *I*.

Mutations that suppress *she-1* might promote spermatogenesis in all situations, or might specifically correct the defect caused by loss of *she-1* activity. To distinguish between these possibilities, we built strains that lacked the *she-1* mutation. The brood size of *cby-7 mip-10; v92* is 30% lower than that of *cby-7 mip-10*, and the brood size of *cby-7 mip-10; v99* is 55% lower than that of *cby-7 mip-10*, suggesting that neither suppressors increase the spermatogenesis. Finally *cby-7 mip-10; v100 XX* animals are sterile with a very small germline, just like *cby-7 she-1(v35) mip-10; v100* animals, so *v100* is likely to define an essential gene. We are now using RNAi to see if any suppressors also affect other mutations in the sex-determination pathway.

806B

FOG-3 phosphorylation by MAPK controls the *C. elegans* sperm fate. **Myon-Hee Lee**[1], Keith Nykamp[2], Judith Kimble[1,2]. 1) Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI; 2) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI.

FOG-3 TOB/BTG family protein is essential for sperm fate specification in *C. elegans* (Chen et al. 2000) and the primary *C. elegans* ERK/MAPK, known as MPK-1, has also been implicated in sperm fate specification (Lee et al. 2007). We have obtained several lines of genetic evidence that suggest a key role for MPK-1 in controlling the sperm fate. To learn how MPK-1 might function in this important cell fate decision, we examined the FOG-3 amino acid sequence and found a potential MAPK-docking site and four predicted MAPK phosphorylation sites (i.e S/TP). We then test the idea that MPK-1 might control the sperm fate by FOG-3 phosphorylation. *In vitro*, we have found that FOG-3 can be directly phosphorylated by murine ERK/MAP kinase; *in vivo*, we have generated a battery of FOG-3 transgenes and found that FOG-3 phosphorylation is critical for sperm fate specification. Importantly mammalian Tob is also a substrate of MAPK (Maekawa et al. 2002; Suzuki et al. 2002). We suggest therefore that the *C. elegans* germline may use an ancient regulatory cassette for control of the sperm/oocyte decision.

References:

Lee et al. (2007) Multiple functions and dynamic activation of MPK-1 extracellular signal-regulated kinase signaling in *Caenorhabditis elegans* germline development. Genetics 177, 2039-62.

Maekawa et al. (2002) Identification of the anti-proliferative protein Tob as a MAPK substrate. J Biol Chem 277, 37783-7.

Suzuki et al. (2002) Phosphorylation of three regulatory serines of Tob by Erk1 and Erk2 is required for Ras-mediated cell proliferation and transformation. Genes Dev 16, 1356-70.

Chen et al. (2000) A novel member of the Tob family of proteins controls sexual fate in *Caenorhabditis elegans* germ cells. Dev Biol 217, 77-90.

807C

A new member of the spermiogenesis inhibition pathway: allele *hc198*. **Misa U. Austin**, Craig W. LaMunyon. Dept Biological Sci, 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 against *spe-27* uncovered a pathway that inhibits spermiogenesis. Members of the spermiogenesis inhibition pathway include *spe-4* and *spe-6*. Here, I report on a mutation in another member of the inhibitory pathway, allele *hc198*. While *hc198* was isolated as a suppressor of *spe-27*, it also suppresses other members of the *spe-8* group, bypassing the spermiogenesis signal transduction pathway in a manner similar to mutations in *spe-4* and *spe-6*. This, and premature activation defects indicate the gene affected by *hc198* is also an inhibitor of spermiogenesis. Although *hc198* is a recessive suppressor of *spe-27*, it does not restore full fertility. In a wild-type *spe-27* background, *hc198* mutants have compromised fertility that is restored by mating, characteristic of a spermatogenesis defective phenotype. Genetic mapping places the *hc198* mutation at -2.30 on Chromosome I. We are currently performing complementation tests with sperm genes in the mapped region. Identification of this gene will add another member to the spermiogenesis inhibition pathway, which, combined with the *spe-8* group signal transduction pathway, provides for exquisite control over the timing of sperm activation. Furthermore, spermiogenesis in *C. elegans* involves regulated fusion of vesicles called membranous organelles and the controlled polymerization of the Major Sperm Protein. One gene, *spe-4*, whose role in the inhibitory pathway was discovered in our lab, is homologous to human Presenilin-1, an Alzheimer's Disease gene. Therefore, the spermiogenesis inhibition pathway involves mechanisms shared by many cellular systems.

808A

Diverse roles for conserved mRNP granule factors during C. elegans oogenesis. **Scott L. Noble**, Arnaud Hubstenberger, Tom Evans. Dept Cell & Developmental Biol, Univ Colorado Denver, Aurora, CO.

The regulation of mRNA/protein complexes (mRNPs) is required for development of complex organisms. In multiple organisms, mRNPs localize to large cytoplasmic granules including processing bodies (P bodies) and stress granules (SGs). These mRNP granules are all related and are likely sites of mRNA control. Recent reports showed that *C. elegans* contains diverse RNP granules with unique functions. Germlines with arrested oogenesis form large granules we call “grP bodies”, which contain the P body proteins CGH-1 and CAR-1, and smaller “dcP bodies” that lack CGH-1 and CAR-1 but carry the P body protein DCAP-2. We are conducting in situ and RNAi-based assays to understand the factors that control these conserved RNP granules and how they influence mRNA fate.

Recent work in mammals showed that some stress granule (SG) proteins are modified by O-linked N-acetylglucosamine(OGN) and that OGN modification promotes SG and P body formation. OGN-modified proteins reside in SGs but not P bodies. Interestingly, we find in the worm germline that OGN-modified proteins localize to dcP bodies, but not to grP bodies. However, loss of the OGN transferase (ogt-1) does not appreciably alter either grP or dcP bodies, and does not dramatically impair oogenesis or embryogenesis. Together with our previous studies, this suggests that neither grP bodies nor dcP bodies are strictly analogous to SGs or P bodies, and that OGN modification of RNP granules is conserved but may serve a specialized function.

Previous work showed distinct functions for CGH-1 and CAR-1. CGH-1 loss induces elongated crystal-like granules, whereas loss of CAR-1 causes reduction of grP body size. We now find that *car-1(RNAi)* prevents elongated crystal-like granule formation in a *cgh-1(ts-lf)* mutant. Similarly, we find that loss of another Sm domain protein, ATX-2, also prevents crystal formation in *cgh-1(ts-lf)*. ATX-2 like CAR-1 associates with CGH-1 complexes. These results argue that CAR-1 and ATX-2 may have some similarity in function distinct from CGH-1. However, the different phenotypes of *car-1* and *atx-2* mutants indicate these proteins also have unique functions. In human cells, the related Ataxin-2 controls SGs and P bodies but in distinct ways. We found that ATX-2 is required for grP body formation in C. elegans. This suggests possible conservation of ATX-2’s control of mRNP granules. Ongoing studies aim to dissect the molecular functions of these conserved RNP granule proteins in an organismal context.

809B

Translational Initiation Factor IFE-2 is Required for Formation of Meiotic Crossovers. **A. SONG**[1], S. Labella[2], N. Korneeva[1], E. Aamodt[1], M. Zetka[2], R. Rhoads[1]. 1) Biochemistry & Molecular Biology, LSUHSC-S, Shreveport, LA; 2) Biology, McGill University, Montreal, QC.

eIF4E is an mRNA cap-binding protein known for its role in promoting translational initiation but more recently also shown to participate in mRNA-specific translational repression and mRNA decay. *C. elegans* expresses five eIF4E-family members, IFE-1 through IFE-5, whose individual roles remain largely unknown. We found that *ife-2* deletion mutants exhibit low brood size, low embryonic viability, and a *Him* phenotype at 25 ºC but not 20 ºC. Consistent with a general germline defect, both spermatogenesis and oogenesis are defective at 25 ºC. While DAPI-stained *ife-2* mutant germlines at 20 ºC contain the normal 6 bivalents at diakinesis, at 25 ºC they are consistently marked by 12 univalents, indicating a failure to form chiasmata. Measurement of the pairing kinetics of chromosome V by FISH and immunolocalization of the synaptonemal complex marker SYP-1 indicated that the defect in crossing over in *ife-2* mutants is not a consequence of defects in meiotic chromosome alignment or synapsis. Time-course analysis of RAD-51-marked early recombination intermediates revealed that recombination initiates at wild-type levels, indicating that failure to form chiasmata is not due to a defect in meiotic DSB formation. The number of RAD-51 foci declined as pachytene progressed, and intact and well-condensed univalents were observed at diakinesis, indicating that *ife-2* mutants are competent to repair meiotic DSBs by homologous recombination (HR) but fail to convert HR intermediates into crossovers. Induction of exogenous breaks by irradiation revealed 12 intact univalents at diakinesis, consistent with a requirement for IFE-2 in crossover formation but not HR-mediated repair. Three conserved *C. elegans* proteins are required for crossover formation but not meiotic recombination, initiation, or HR-mediated repair: MSH-5 and MSH-4/HIM-14, thought to form a heterodimer that stabilizes the crossover-specific HR intermediate, and the central region protein ZHP-3. Similar to the previous analysis of *msh-4/him-14* and *msh-5* mutants, *ife-2* mutants also show persistence of RAD-51 foci into late stages of pachytene. Real-time RT-PCR analysis of *ife-2* mutants revealed that at 25 °C, the mRNAs for *msh-4/him-14* and *msh-5* were shifted from large to small polysomes and untranslated mRNPs, but not those of *gpd-3, spo-11*, or *zhp-3*, indicating an mRNA- and eIF4E-specific defect in translational initiation. We speculate that IFE-2 may upregulate translation of *msh-4/him-14* and *msh-5* mRNAs at higher temperatures to stabilize Holliday junctions. (Supported by NIH funding to R.E.R. and CIHR funding to M.Z.).

810C

An alternative way to construct a nematode–Embryogenesis of *Romanomermis culicivorax* differs considerably from *C. elegans*. **Jens Schulze**, Einhard Schierenberg. Zoological Inst, Univ Cologne, Germany.

Nematodes constitute excellent candidates for comparative studies of early embryogenesis. However, our picture of their embryonic development is mainly shaped by *C. elegans* and *Ascaris megalocephala*. Their pattern of development is therefore considered typical for the whole taxon. For the first time a comprehensive description of embryogenesis in the basal nematode *Romanomermis culicivorax* is presented and found to differ considerably from the standard *C. elegans*. The segregation of colored cytoplasm into specific somatic founder cell and its descendants, a so far unique phenomenon in nematodes, resembles the distribution of pigmented myoplasm in certain ascidians. Polar cortical interphase microtubules in early blastomeres suggest a new MTOC involved in the asymmetric distribution of the colored cytoplasm. The cell lineage is less complex in that many branches generate predominantly cells with monoclonal fates. New is also that hypodermal and neuronal cells form duplicating rings along the a-p axis resembling segmentation and may give embryonic support for the Ecdysozoa hypothesis. Furthermore, we detected a global shift in fate assignment of the somatic founder cells in *R. culicivorax* compared to *C. elegans*. In summary, our data give evidence that in the course of nematode evolution massive modifications of the developmental program took place and embryogenesis of *C. elegans* is just one option within the taxon of nematodes.

811A

Evolution of embryonic development in nematodes. **Jens Schulze**, Einhard Schierenberg. Zoological Inst, Univ Cologne, Germany.

Nematodes are well suited for a comparison study of early embryogenesis. Analyzing development of a model system like *C. elegans* alone allows no conclusions about evolutionary modification within the taxon nematoda. For better understanding evolution of development among nematodes and identification of plesiomorphic and apomorphic characters, data from suitable representatives have to be related to their phylogenetic position. The approach of using developmental markers constitute a unique chance to compare our findings with outgroups like tardigrades and rotifers, in search of embryonic support for the Ecdysozoa or Articulata hypothesis. Our data show that embryogenesis in nematodes is unexpectedly variable with floating transitions which can be interpreted as frozen images of evolutionary change. However, some basic similarities with respect to embryogenesis are found among them, e.g. the general existence of cell lineages and early separation of soma from germline along the a-p axis, whereby a future ventral midline is established. The comparison between the basal nematode *Tobrilus stefanskii* and published data on the tardigrade *Hypsibius dujardini* concerning early pattern formation revealed obvious similarities in contrast to rotifers. This may be considered as an embryological support for the Ecdysozoa hypothesis.

812B

Wide diversity in structure and expression profiles among members of the *Caenorhabditis elegans* globin protein family. **S De Henau**, D Hoogewijs, B P Braeckman, J R Vanfleteren. Department of Biology and Center for Molecular Phylogeny and Evolution, Ghent University, B-9000 Ghent, Belgium.

Globins are small globular proteins, with a characteristic 3-over-3 α-helical sandwich structure that encloses an iron-containing heme group. Organisms can express multiple globin molecules with variant properties and functions (Vinogradov and Moens, 2008). In silico analysis of the genome of *Caenorhabditis elegans* revealed an unexpectedly high number of globin genes featuring a remarkable diversity in gene structure, amino acid sequence and expression profiles (Hoogewijs *et al.*, 2004). The availability of full genomic sequences and EST data from several other nematode species presents a unique opportunity to explore the evolutionary globin dynamics of these organisms. *Caenorhabditis* species contain a very large number of globin genes, and even distantly related nematodes harbor orthologs to many of them. Bayesian phylogenetic analysis resolves all nematode globins into two distinct globin classes. Analysis of the globin intron-exon structures suggests extensive loss of ancestral introns and gain of new positions in deep nematode ancestors, and mainly loss in the *Caenorhabditis* lineage. Also, our analysis provides some evidence for a number of gene duplication events giving rise to a class of globin genes that is likely unique to the nematode phylum (Hoogewijs *et al.*, 2008). Secondly, to reveal tissue-specific expression profiles of this protein family, we constructed gene fusions of the promoter regions for all 33 globin genes to the coding region of GFP. The majority of these fusion products is expressed in neuronal cells in the head and tail portions of the body. The other globin genes seem to be expressed in non-neuronal tissues, including body wall muscle, vulval muscle and the pharynx. Based on these results, most globin genes are likely to have a cell-specific function (Hoogewijs *et al.*, 2008). At this moment, we are extending this expression analysis by including the complete coding region of each globin gene. By doing this we will be able to determine whether the globin gene introns and 3'UTR contain additional *cis*-regulatory information. Lastly, we used real-time PCR (qPCR) to analyze expression patterns of this globin family under hypoxia stress condition. We found that oxygen shortage (0,5% O_2) modulates the expression of several globin-like genes. This seems to indicate that these globins are involved in the protection of tissues from hypoxia. Strikingly, there is little overlap in genes responsive to hypoxia and genes responsive to anoxia (12h <0,001% O_2) (Hoogewijs *et al.*, 2007), which might illustrate functional diversity in this protein family.

813C

Quantifying the robustness and evolvability of a developmental system. **Christian Braendle**[1], Charles F Baer[2], Marie-Anne Felix[3]. 1) Institute of Developmental Biology & Cancer, CNRS, Universite of Nice, Nice, France; 2) Dept. of Biology, University of Florida, Gainesville, FL, USA; 3) Institut Jacques Monod, CNRS-Universities of Paris 6/7, France.

Many developmental processes generate invariant phenotypes despite environmental or mutational perturbations. Such robustness is a fundamental biological property, yet its extent, limits and adaptive significance have rarely been assessed empirically. Here we tested how environmental variation and accumulation of spontaneous random mutation impact the developmental system underlying vulval formation in *Caenorhabditis* nematodes. In different environments, a correct vulval pattern develops with high precision but rare deviant patterns reveal the system's limits and how its mechanisms respond to environmental challenges. Key features of the apparent robustness are functional redundancy among vulval precursor cells and tolerance to quantitative variation in Ras, Notch and Wnt pathway activities. These environmental responses and the precision of the vulval patterning process further vary within and between Caenorhabditis species. To quantify how developmental precision responds to mutational perturbations, we used a set of mutation accumulation (MA) lines derived from two *C. briggsae* and two *C. elegans* genotypes. Developmental defects and variants increased after MA treatment for all tested genotypes, yet the type and proportion of the mutationally induced variation varied among genotypes. Thus, the mutability of this developmental system evolves, so that the mutationally induced phenotypic space is biased depending on the genetic background. Comparison of the standing genetic variance (V_G) for deviant vulva phenotypes with the mutational variance (V_M) leads to the conclusion that strong natural selection acts to maintain the robustness of this developmental process.

814A

Toward characterization of bacteria-to-nematodes HGT. **Amir Sapir**[1,2], Alon Zaslaver[1,2], John DeModena[1], Paul Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA; 2) Equal contribution.

Horizontal gene transfer (HTG) is a fundamental process among unicellular organisms for acquiring new traits. Although initially thought to be extremely rare in metazoans, recent whole-genome sequencing projects reveal extensive gene transfer from prokaryotes to metazoans. This type of gene transfer is particularly relevant for symbiotic organisms that occupy new niches, where survival requires acquisition of new genes not previously present in the organism's gene pool. For example, hemi-cellulose hydrolysis, induced by plant parasitic nematodes, is thought to have been acquired by the transfer of bacterial genes to the plant parasites' bacteriovorous ancestors. In contrast to HGT between bacteria, the sequence of events leading to bacteria-to-nematodes HGT, as well as the molecular details of this process, remain elusive. So far, mechanistic studies of HGT in metazoans have been hindered by its rare occurrence, and the fact that symbiotic organisms are usually not suitable for long in-lab evolutionary studies. Our aim is to study HGT by combining the powerful genetics of E. coli (the donor) and C. elegans (the recipient). Specifically, we use the transfer of the unc-119 rescuing gene from E. coli to unc-119 mutant worms as an indicator for successful HGT. Rescued worms are examined to validate that gene transfer indeed happened, and these worms will be further analyzed to decipher the exact mechanism by which HGT occurred. We predict that the problem of identifying rare HGT events can be overcome by our experimental settings that involve growing multiple generations of worms in large numbers under specific selection. In a pilot experiment, we grew 4x106 worms per generation over 7 generations on E. coli carrying the unc-119 rescuing gene and validated that this approach is suitable for HGT studies. If HGT will not be identified, we will employ different conditions and genetic backgrounds that might increase HGT probabilities: i) Use of C. elegans mutants promoting bacterial propagation in the worm's gut. ii) Exposing the cultured worms to various stress conditions. iii) Inducing HGT by unc-119 gene transposition in the E. coli donor. Ultimately, this system will serve as an empirical framework to elucidate the enigmatic process of bacteria-to-nematodes HGT.

815B

Geographic and genetic variation in fecundity under temperature stress in *C. briggsae*. Melanie Croydon-Sugarman, Anisha Prasad, **Asher D. Cutter**. Dept Ecology/Evolutionary Biol, Univ Toronto, Toronto, ON, Canada.

In *C. briggsae*, patterns of genetic diversity among strains from across the globe correlate perfectly with the geographic origin of the natural isolates, corresponding to clades of worms from temperate regions, the tropical circles of latitude, and near the equator (Cutter et al. 2006; Dolgin et al. 2007). Ecologically, these geographic regions differ dramatically in temperature regime, begging the question of whether heritable phenotypic differences might also conform to the geographic partitioning of variation in a potentially adaptive manner. An association between the temperature at which a particular isolate is optimally fecund and the temperature of the isolate's clade of origin could indicate local adaptation and provide insight into *C. briggsae* ecology and evolution. To address this issue, we tested the thermal tolerance, as quantified by self-fecundity, of 10 wild-isolate strains originating from the three latitudinal regions when the strains were subjected to extreme high and low temperatures. Our results demonstrate a decline to zero progeny production at 32°C that was exhibited by worms from all three regions, indicating an upper fertile limit between 30°C and 32°C for *C. briggsae* as a species. However, at 30°C we observed a significant 4-fold difference in lifetime fecundity for strains from the Tropic circles of latitude clade compared to those of both the temperate and equatorial clades, suggesting a tolerance of the tropical isolates to higher temperatures. Ongoing work explores fecundity at low temperatures (12°C–16°C) to test for heritable differences among strains at cooler temperatures.

Cutter, A.D., M.A. Felix, A. Barriere & D. Charlesworth. 2006. Patterns of nucleotide polymorphism distinguish temperate and tropical wild isolates of *Caenorhabditis briggsae*. *Genetics*. 173: 2021-2031.

Dolgin, E.S., M.A. Felix & A.D. Cutter. 2008. Hakuna nematoda: genetic and phenotypic diversity in African isolates of *Caenorhabditis elegans* and *C. briggsae*. *Heredity*. 100: 304-315.

816C

Natural variation and selection in locomotory phenotypes. **Adler R Dillman**[1], Christopher J Cronin[1], Charles F Baer[2], Paul W Sternberg[1]. 1) Biology, California Institute of Technology, Pasadena, CA; 2) Zoology, Florida State University, Gainesville, FL.

A major motivation behind establishing *C. elegans* as a model system was to understand the influence of genes on behavior. Some behaviors however, have proven to be difficult to quantitatively evaluate. In this study we used a well established automated tracking system, which analyzes the body posture of nematodes over time and extracts quantitative information concerning their movement. Quantitatively assessing phenotypic differences in locomotory data provides a unique opportunity to better understand the phylogenetic diversity of Caenorhabditis nematode locomotion. Using this system, we evaluated the level of detectable variation in locomotory phenotypes in 9 different Caenorhabditis species and 8 natural isolates of *C. elegans*. Aside from phenotypic variation among and between species, we are interested in understanding the relative roles of mutation and natural selection involved in driving phenotypic variation in locomotion. We report direct measurements of trait variation in natural isolates and trait variation resulting from mutation alone by utilizing 7 different mutation accumulation (MA) lines; allowing us to quantify the extent to which purifying selection promotes stability in locomotory behaviors. Our data suggest that strong stabilizing selection acts to shape locomotory behavior in *C. elegans*.

817A

Characterization of the cryptic genetic variation in the vulva system of *C. elegans*. **Fabien Duveau**, Josselin Milloz, Marie-Anne Félix. Institut Jacques Monod–CNRS & University of Paris 7, Paris, France.

Many biological systems appear insensitive to a certain degree of perturbation. For instance, the output of vulval cell fate patterning is quasi-invariant among *C. elegans* individuals grown in different environmental conditions. In theory, a system robust to some environmental perturbations should also be robust to certain genetic mutations. Consequently, the signalling network underlying the formation of such robust system could accumulate **cryptic genetic variations** that do not affect the final product. Prior to this work, the presence of cryptic genetic variation in the vulval signaling network among *C. elegans* wild isolates was uncovered through different approaches. One of them was to introduce several mutations of EGF/Ras, Wnt or Delta/Notch pathways affecting vulval cell fate patterning into different *C. elegans* wild isolates. For a specific mutation, the severity of the effect on vulva development depends significantly on the wild genetic background, revealing the presence of cryptic genetic variation among the tested wild isolates. In the work presented here, we attempt to characterize the **genetic architecture** of this cryptic variation and to **identify the underlying molecular variation** using a quantitative genetic approach. This should inform us about the kind of selective pressures driving the evolution of cryptic variation and about its possible evolutionary consequences. We decided to study the genetic variation between JU605 and JU606, two strains that carry the *let-23(sy1)* mutant allele of EGFR in different wild genetic backgrounds: N2 for JU605 and AB1 for JU606. The effect of *let-23(sy1)* on vulval induction is stronger in JU605 compared to JU606 in animals grown at 25°C, likely due to cryptic genetic variation existing among N2 and AB1 wild isolates. To identify the factors involved, we first constructed a set of 60 Recombinant Inbred Lines from a cross between JU605 and JU606. The 60 RILs were all scored for vulval induction and genotyped for 49 N2/AB1 SNP markers. A QTL (Quantitative Trait Loci) analysis detected a region in the middle of chromosome I that would explain about 30% of the phenotypic variance. Another region, located on the left arm of chromosome II, was less significantly associated with the phenotypic variation and presented a smaller effect (about 8%). We then established several independent Near Isogenic Lines that helped us to restrain the position of the major effect QTL on chromosome I to a 579 kb interval containing 125 genes. Finding the molecular polymorphism explaining the QTL effect will allow us to know whether the cryptic variation directly affects the vulval signalling network and whether its activity is changed in a tissue-specific manner.

818B

Loss of the insulator protein CTCF during nematode evolution. **P. Heger**[1], B. Marin[2], E. Schierenberg[1]. 1) Zoological Institute, University of Cologne, Kerpener Strasse 15, 50937 Koeln, NRW, Germany; 2) Botanical Institute, University of Cologne, Gyrhofstrasse 15, 50931 Koeln, NRW, Germany.

The zinc finger (ZF) protein CTCF (CCCTC-binding factor) is highly conserved in *Drosophila* and vertebrates where it has been shown to mediate chromatin insulation at a genomewide level. A mode of genetic regulation that involves insulators and insulator binding proteins to establish independent transcriptional units is currently not known in nematodes including *Caenorhabditis elegans*. We therefore searched in nematodes for orthologs of proteins that are involved in chromatin insulation.

While orthologs for other insulator proteins were absent in all 35 analysed nematode species, we find orthologs of CTCF in a subset of nematodes. As an example for these we cloned the *Trichinella spiralis* CTCF-like gene and revealed a genomic structure very similar to the *Drosophila* counterpart. To investigate the pattern of CTCF occurrence in nematodes, we performed phylogenetic analysis with the ZF protein sets of completely sequenced nematodes. We show that three ZF proteins from three basal nematodes cluster together with known CTCF proteins whereas no zinc finger protein of *C. elegans* and other derived nematodes does so.

Our findings suggest that CTCF and possibly chromatin insulation are more ancient than previously assumed and that they have been secondarily lost during nematode evolution. We propose a switch in regulating gene expression during nematode evolution, from the common vertebrate and insect type involving distantly acting regulatory elements (e. g. silencers or insulators) and chromatin insulation to a so far poorly characterised mode present in more derived nematodes lacking all or some of these components and involving operons instead.

819C

Evo-Dev-Omics–embryonic gene expression analysis across six nematode species. **Michal Levin**, Tamar Hashimshony, Itai Yanai. Faculty of Biology, Technion Israel Institute of Technology, Haifa, Israel.

Metazoan development is a highly conserved process governed by different gene regulatory circuits comprised of different genes across species. Precise temporal and spatial gene regulation plays a major role in developmental systems. Comparing developmental regulatory programs of both phenotypically similar and divergent organisms is a powerful test of the hypothesis that morphological evolution is predominantly based upon modulation of gene expression across time and space. Further it can provide insights into the plasticity of the genomic program for encoding phenotypically similar organisms with different underlying programs. We are exploring embryonic gene expression programs of a stable set of orthologs and quickly evolving paralogs across a phylogeny of nematodes. In a preliminary experiment–performed in Craig Hunter's lab–custom-designed species-specific microarrays were used to measure transcript abundance in precisely staged *C.elegans* and *C.briggsae* embryos. The examined stages spanned the first quarter of embryonic development (4-cell to 190-cell stage). Despite sharing an almost identical developmental program these species show a highly divergent set of gene expression profiles. To further explore the relationship between distant species and their underlying developmental gene expression networks we are expanding our analysis to a set of six completely sequenced nematode species (*C.elegans, C.briggsae, C.remanei, C.brenneri, C.japonica* and *P.pacificus*) and to embryological stages spanning the whole embryonic development (4-cell stage to hatching). A comparison of the timing of four embryonic milestone stages (4-cell, gastrulation, morphogenesis and hatching) has been completed for all of the six nematode species using live imaging and DAPI staining techniques. Based on the sequenced genomes of all the six species, custom-designed microarrays bearing the whole gene set of each of the species will be used to obtain expression levels of all genes along embryonic development for each species. The obtained data set will enable us to screen for gene expression conservation and divergence across gene families. Further we will map regulatory sequences for specific expression profiles both within and across species. An essential aim is to model the degree of expression divergence as a function of both gene functionality and sequence alterations in regulatory sequences. This approach will provide a two-dimensional picture of gene expression patterns along both time and species allowing an in-depth analysis of the underlying principles of morphological evolution.

820A

De-canalizing effect of new mutations evidenced in transcriptome of *C. elegans*? **Dejerianne Ostrow**, Dustin Blanton, Chikako Matsuba, Matthew Salomon, Charles Baer. Department of Biology, University of Florida, Gainesville, FL.

Why some phenotypic traits are highly plastic or otherwise variable and others are highly invariant (=canalized) in the face of environmental variation remains a puzzle. Data from whole-genome microarray analysis of cDNA collected from large worm cultures suggests that spontaneous mutations reduce the environmental variability of transcript number. In an attempt to better understand the role of transcriptional modulation as a means of maintaining canalized phenotypes, we are investigating the variance between isogenic *C. elegans* lines as evidenced by the variability in gene expression in much smaller samples. The motivation for using very small samples is to minimize variance propagated by small differences in timing of development. For each of 6 biological replicates, RNA was extracted and pooled from 5 L3 worms, converted to cDNA, amplified, labeled, and hybridized to an Affymetrix GeneChip. Measures of variability between biologically replicated transcriptomes allow us to estimate transcriptional variance when genetic variation is minimized. These data are the foundation for future research comparing ancestral control lines to mutation accumulation lines for which mutational parameters have been well characterized.

821B

Persistence time of mutations affecting fitness and body size in Caenorhabditis briggsae and C. elegans. **Matthew Salomon**[1], Dejerianne Ostrow[1], Naomi Phillips[2], Dustin Blanton[1], Whitney Bour[1], Thomas Keller[1], Laura Levy[1], Thamar Sylvestre[1], Ambuj Upadhyay[1], Charles Baer[1]. 1) Department of Biology, University of Florida, Gainesville, FL; 2) Department of Biology, Arcadia University, Glenside, PA.

The level of genetic variation present in a population is a composite function of mutation, population size, and natural selection. Historically, efforts to understand differences (or similarities) between groups in levels of genetic variation have focused on the interplay between population size and natural selection. However, much less attention has been paid to the alternative possibility that differences among groups are due to systematic differences in the underlying rate of mutation. Much of the difficulty in interpreting the role of mutation stems from the fact that most of what is known about genomic mutational properties, for quantitative traits in multicellular eukaryotes, comes from a handful of phylogenetically distant and biologically dissimilar model organisms, making meaningful comparisons difficult. Over the past several years our lab has been investigating the properties of new mutations in a model nematode system within a comparative phylogenetic framework. Mutations have been allowed to accumulate in the (relative) absence of natural selection, thus allowing us to estimate the genetic variance introduced by new mutation (VM) for two species of rhabditid nematodes, Caenorhabditis elegans and C. briggsae. Previous work in this system suggests that the mutation rate in C. briggsae is on the order of twice that of C. elegans for quantitative traits and dinucleotide repeats. Here we report the standing genetic variance (VG) for two quantitative traits, lifetime reproduction and body size, in worldwide collections of C. briggsae and C. elegans natural isolates. Comparisons of VG to VM between the natural isolates and our mutation accumulation lines allow us to infer the magnitude and pattern of constraint on phenotypic evolution in these two species. Taking the results from the two species together, the persistence time (VG/VM) of new mutations affecting fitness is on the order of tens to perhaps hundreds of generations, with an average selection coefficient against homozygotes of a few per-cent. Furthermore, the pattern of persistence time for mutations affecting adult body size is onsistent with that of fitness in both species. These results suggest that idiosyncratic selection, perhaps due to random hitchhiking–"genetic draft"–is paramount in shaping the standing genetic variance of these traits in these species.

822C

Characterization and localization of C.elegans PDE3, a homolog of the mammalian PDE3 family. **A Samidurai**[1], T Cai[2], A Faiyaz[1], T Fukushige[3], V Manganiello[1]. 1) TMB,NHLBI, NIH, Bethesda, MD; 2) NIDCR, NIH, Bethesda, MD; 3) NIDDK,NIH,Bethesda,MD.

Cyclic nucleotides Phosphodiesterases (PDEs) regulate the intracellular concentrations of the second messengers, cAMP and cGMP, by controlling their rate of hydrolysis.The mammalian PDE3 family is known to play an important role in insulin signaling pathways, and in cardiovascular tissues, oocytes and adipose tissues. Caenorhabditis elegans represents a unique model for easy genetic manipulation, as well as identification and functional analysis of genes involved in regulation of signal transduction. Insulin signaling pathway is well characterized in C.elegans, and is thought to be functionally similar to the mammalian cascade. We report here the expression and characterization of the C.elegans Phosphodiesterase 3 gene family, a homolog of the mammalian PDE3 family. In contrast to the mammalian PDE3 gene family, which consists of two closely related subfamilies, PDE3A and PDE3B, that are generated from separate genes located on human chromosome 11 and 12, respectively, the single nematode PDE3 gene is present on chromosome II, spaning about 20.2kb on the genome, and encodes two different PDE3 isoforms. The CEPDE3 long form consists of 11 exons and codes for a 63.5 kda protein, and the short form has 8 exons and codes for a 51.9 kda protein. Both isoforms have the characteristic Pfam and phospho diester domain and also contains the HD metal binding motif, which is unique for the PDE family of genes. The CEPDE3 long form and short form show an overall 97 % homology between each other and 100 % homology among their catalytic domains, similar to the 80 % similarity found in the catalytic regions of mammalian PDE3A and 3B isoforms. We also compared the unique signature insert present in the mammalian PDE3 catalytic domain and found 11 out of 44 amino acids matched with CEPDE3. We have cloned both long and short CEPDE3 isoforms, and expressed them in Sf21 insect cells. The PDE activity assay showed that both PDE3 long and short forms are inhibited by cilostamide (a specific inhibitor of mammalian PDE3 isoforms) and not by rolipram (a specific inhibitor of PDE4 isoforms). Furhter the IC50 value of cilostatmide and rolipram for PDE3 long and short form are comparatively closer to that of the mammalian PDE3. The MS/MS protein sequence analysis showed evidence for the presence of predicted phosphorylation sites. The molecular mechanisms underlying catalysis by PDE3 and its inhibitor selectivity still remains unsolved. Further characterization of the CEPDE3 gene may enhance our understanding of these properties of PDE3 enzymes, as well as their role in cellular physiology.

823A

Comparative proteome analysis between *Caenorhabditis elegans* and *Caenorhabditis briggsae*. **Dai Sasahara**, Shizuka Hino, Ayako Terasawa, Masahiro Ito. Bioinformatics, Ritsumeikan University, Kusatsu, Shiga, Japan.

Caenorhabditis elegans and *Caenorhabditis briggsae* diverged from a common ancestor roughly 100 million years ago. These 2 *Caenorhabditis* species differ in some aspects like thermal sensitivity, gene (intergenic region), vulval-uterine, excretory duct and male tail, and at the same time, also share characteristics such as genes and proteins, hybridization system, and morphological body structure. Therefore, we investigated whether or not the expression patterns of gene products were conserved in these species. Comparative proteome analysis between *C. elegans* and *C. briggsae* was conducted using two-dimensional difference gel electrophoresis (2D-DIGE).

L1 stage larvae of *C. elegans* and *C. briggsae* were synthesized using the alkali-bleach method and were collected thrice. Two-dimensional electrophoresis was performed using the Ettan DIGE system and the spots (proteins) were detected using an image analysis software. The spots that showed different expression levels between the 2 species were analyzed using Student's *t*-test (p=0.05).

In this study, 2D electrophoresis of the 3 obtained samples, showed that 599 spots differed between the 2 species. A total of 301 and 298 spots showed high expression levels in *C. elegans* and *C. briggsae*, respectively. In addition, 209 spots—119 spots in *C. elegans* and 90 spots in *C. briggsae*—showed a significant difference in Student's *t*-test (p=0.05). Although ~95% of the genes were similar between *C. elegans* and *C. briggsae*, the protein expression patterns were very different because of the following reasons: (1) difference in amino acid sequences (composition), (2) post-translational modification, and (3) difference in the protein expression pattern. Consequently, to further elucidate these reasons, the proteins were subjected to matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and liquid chromatography mass spectrometry (LC-MS).

824B

Examining Changes in Gene Function in Caenorhabditis nematodes using RNAi libraries. **Adrian Verster**[1], Arun Ramani[1], Marie-Anne Felix[2], Sheldon Mckay[3]. 1) Molecular Genetics, University of Toronto, Toronto, Canada; 2) Institut Jacques Monod, CNRS–University Denis Diderot, Paris, France; 3) Cold Spring Harbor Laboratory, Cold Spring Harbor, USA.

Gene functions change throughout evolutionary history and understanding how this occurs is a key goal of molecular evolutionary biology. To date researchers have systematically looked at changes in gene function by comparing loss of function phenotypes in orthologs from different model organisms, such as *S. cerevisiae* and *C. elegans*. However, since there has been such a large evolutionary divergence between these organisms their body plans are so different and many phenotypes are impossible to compare. It is much more practical to compare phenotypes from organisms with similar body plans and ecological niches. Here we use *Caenorhabditis* nematodes to systematically look at changes in gene function through evolution because loss of function phenotypes map easily between species.

In order to address this problem we are building an RNAi library for *C. briggsae* to look for changes in gene phenotype from those that were observed in *C. elegans*. Genome scale RNAi has been successfully used in *C. elegans* and thus we are going to use it in *C. briggsae*. In order to do this we take advantage of the *sid-2* transgenic *C. briggsae* line (gratefully contributed by Marie-Anne Felix's group) which has been shown to uptake RNAi by feeding. We will present preliminary screening data from the *C. briggsae* RNAi library and showcase the changes in gene function we have found so far.

825C

Characterization of recombinant cysteine synthase in *Caenorhabditis elegans*. **Roman Vozdek**[1], Ales Hnizda[1], Jakub Krijt[1], Milan Kodicek[2], Viktor Kozich[1]. 1) Institute of Inherited Metabolic Diseases, Charles University, First Faculty of Medicine, Prague 2, Czech Republic; 2) Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Institute of Chemical Technology, Prague.

Nematode *Caenorhabditis elegans* (*C. elegans*) could be a suitable model to study metabolic and cellular consequences of homocystinuria due to cystathionine β-synthase (CBS) deficiency. However, metabolism of sulfur amino acids in *C. elegans* is as yet unknown, namely the steps in cysteine biosynthetic pathways. Cysteine can be synthesized either via the transsulfuration pathway which utilizes homocysteine by CBS or via the assimilation pathway which uses sulfide by cysteine synthase (CS).

In silico analysis of *C. elegans* database identified four homologs of human CBS, namely *ZC373.1*, *C17G1.7*, *K10H10.2* and *R08E5.2*. The aim of this study was to express the gene *C17G1.7* (predicted CS) in prokaryotic system, to purify and further characterize this recombinant protein. Molecular weight of polypeptide chain was determined to be 37,2 kDa by MALDI-TOF MS. Blue Native electrophoresis revealed a molecular weight of 70 kDa suggesting that recombinant CS is a dimer. Purified protein contains pyridoxal 5'-phosphate (PLP) as determined by UV/VIS absorption spectrometry; circular dichroism showed characteristic PLP maximum confirming its localization in a centre of organized globular protein. We determined that purified enzyme has very specific enzymic activity for CS reaction; other possible activities were not detected. Recombinant CS exhibited K_M values for *O*-acetyl-L-serine and sulfide of 5.54 and 4.23 mM, respectively, and a turnover number of 139 and 134 s^{-1}, respectively.

These data show that *C17G1.7* could play an important role in cysteine biosynthesis since *C. elegans* genome contains also a CBS gene, we hypothesize that nematode utilizes both cysteine biosythesis pathways–sulfur assimilation and transsulfuration pathway.

This work was supported by Wellcome Trust International Senior Research Fellowship in Biomedical Science in Central Europe.

826A

Variations in sensitivity to external RNA interference in the *Caenorhabditis* genus. **Isabelle Nuez**, Marie-Anne Félix. CNRS, Inst J Monod, Paris, France.

The introduction of dsRNA into *C. elegans* triggers sequence-specific gene silencing (RNAi) that can spread between cells and into the progeny. RNAi can be triggered externally by soaking of the worms in dsRNAs or by feeding them with *E. coli* bacteria that express dsRNAs. Winston et al. (Hunter lab) isolated a mutant defective in RNAi through externally administered dsRNAs. The corresponding *sid-2* gene encodes a transmembrane protein, which localizes at the luminal membrane of intestinal cells. SID-2 is required for the uptake of dsRNA from the environment. In contrast to *C. elegans* wild isolates, *C. briggsae* AF16 is naturally insensitive to external RNAi, yet can be complemented by transgenesis with the *Ce-sid-2* gene (Winston et al, 2007).

In the lab, we keep a large collection of wild and transgenic nematodes, which allows us to study vulva development in many species, including microevolution among wild isolates of a given species. We recently isolated seven new *Caenorhabditis* species in rotting fruits and flowers.

We tested the natural sensitivity to RNAi administered by feeding of different *Caenorhabditis* species. Several new species showed natural sensitivity to RNAi by feeding. We mapped the distribution of the natural sensitivity to external RNAi onto the phylogenetic tree of the *Caenorhabditis* genus (a collaboration with K. Kiontke and D. Fitch at New York University). Our results suggest that evolution of this feature in the *Caenorhabditis* genus may have been complex. Sensitivity to RNAi by feeding is a feature that may have disappeared during evolution in some species or have appeared in others.

"Resistant" species that were found to be naturally insensitive to external RNAi are currently tested for their sensitivity to external dsRNAs after transgenesis with *Ce-sid-2*. We already succeeded in rendering *C. remanei* sensitive to external RNAi.

Winston, W., et al. (2007). *Caenorhabditis elegans* SID-2 is required for environmental RNA interference. Proc. Natl. Acad. Sci. USA 104, 10565-10570.

827B

Independent Recruitment of F-box Genes to Specify Hermaphrodite Development. **Yiqing Guo**, Ronald E Ellis. Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ.

Although sexual reproduction is found throughout the animal kingdom, the mechanisms that control it change rapidly. To elucidate how sex-determination pathways evolve, we are comparing two related nematodes, *C. elegans* and *C. briggsae*. Both species make *XX* hermaphrodites, which are female in appearance, but produce sperm during larval development that can be used for self-fertilization. Phylogenies suggest that each species evolved hermaphroditism independently.

In *C. elegans*, *fog-2* is essential for *XX* animals to become hermaphrodites. However, the Schedl group showed that FOG-2 is a novel F-box protein with no homolog in *C. briggsae*. This result implied that *C. briggsae* must use another mechanism to specify hermaphrodite development. Thus, we screened for *C. briggsae* mutants that became female rather than hermaphrodite. Four mutations created *XX* females but did not affect males, and all failed to complement each other, so they define the new gene *she-1*, for spermless hermaphrodites. Two alleles isolated in non-complementation screens have the same phenotype. Genetic tests place *she-1* upstream of *tra-2* in the sex-determination pathway, at the same position that *fog-2* occupies in *C. elegans*. Furthermore, both *she-1* and *fog-2* are sensitive to changes in *tra-2* dosage. These results imply that *tra-2* acts at a key point in the sex-determination pathway, and that two species have evolved hermaphroditism by modulating its activity.

Positional cloning revealed that SHE-1 is an F-box protein that is unrelated in structure to FOG-2. Since SHE-1 can bind SKR-1, a component of the E3 ubiquitin ligase complex, and a missense mutation in the F-box domain inactivates SHE-1, it is likely to promote hermaphrodite development by regulating the stability of a target protein. We are currently using the yeast two-hybrid system to find the target.

Thus, two different species have recruited novel members of the F-box family of genes into the sex-determination pathway to create hermaphrodites. Since FOG-2 regulates the translation of *tra-2* messages by interacting with GLD-1, and SHE-1 does not bind GLD-1, these proteins act by different molecular mechanisms.

828C

Comparative genomic analysis of the nematodes in Antarctica. **Hiroshi Kagoshima**, Junko Kajiwara, Tadasu Shin-i, Yuji Kohara. Genome Biol Laboratory, National Inst Genetics, Mishima, Shizuoka, Japan.

Antarctica is an extreme environment for life. There, low temperature and lack of liquid water severely restrict biological activities. Nematodes are the exceptional multicellular organisms which can inhabit the Antarctic environment. The aim of this project is to elucidate the molecular and genetic mechanisms of nematodes to adapt to the Antarctic environment.

We took two approaches for this project: 1) the establishment of molecular taxonomy of Antarctic nematodes, 2) comparative expression analysis between the Antarctic nematode, *Panagrolaimus davidi*, and the model nematode, *C. elegans*.1) To work on Antarctic nematodes, we first tried to identify wild isolates of nematode, because very limited molecular information is available for Antarctic nematodes. We successfully established a method to obtain 18S and 26S rRNA gene sequences from single wild nematodes for molecular phylogenic analysis, in collaboration with Drs. Convey, Maslen (British Antarctic Survey, UK) and Dr. Kito (Sapporo Medical University, Japan). We are currently re-evaluating the taxonomy of Antarctic nematodes based on morphological classification by the taxonomy based on the nucleotide sequences. 2) For the second approach, we have analyzed 25,000 cDNA sequences of *P. davidi* in collaboration with Drs. Wharton and Marshall (University of Otago, New Zealand). The comparison of the expression profiles in *P. davidi* and *C. elegans* illuminated the possibility that the difference between the two reflects their life styles. *P. davidi* expressed higher level of stress response genes, such as cold shock response gene (*cey-2*), heat resistant gene (*lea-1*). We are particularly interested in *lea-1* gene and examining biochemical properties of the gene product.

829A

Function and phylogenetics of the NR2E nuclear receptors. **Christopher Alvaro**[1], Tiffany Zehner[1], Katherine Weber[1,2], Bruce Wightman[1]. 1) Biology Department, Muhlenberg College, Allentown, PA; 2) MRC Laboratory of Molecular Biology, Cambridge, UK.

The NR2E subclass of nuclear receptors is conserved from cnidarians to vertebrates. Family members such as *tailless* and *fax-1* have been shown to function in nervous system development and body patterning. Phylogenetic analysis identifies at least three major clades of NR2E-related nuclear receptors: the NR2E1/2 clade, which includes *nhr-67* and *tailless*; the NR2E3/5 clade, which includes *fax-1* and PNR; and a new group that includes *nhr-239* of *Caenorhabditis* and related genes in insects and the echinoderm *Stongylocentrus*. The existence of a possible *nhr-239* ortholog in both protostomes and deuterostomes suggests that this class could have an ancient origin. A fourth clade, NR2E6, appears to be represented only in some insects. We are recovering NR2E orthologs from mollusks and annelids to study the relationship of Lophotrochozoan nuclear receptors to the known vertebrate and Ecdysozoan genes.

While ligand-binding domains (LBD's) of nuclear receptors in insects and vertebrates share modest sequence similarity, the *Caenorhabditis* LBD's are considerably more diverged. The *nhr-111* gene, which is present in *C. elegans* but not *C. briggsae*, includes a FAX-1-related LBD. We have used a gene fusion approach to show that the LBD of *C. briggsae* FAX-1, *C. elegans* NHR-67, and *C. elegans* NHR-111 can substitute for *C. elegans* FAX-1 LBD function. These data demonstrate that LBD function may be equivalent (or at least similar) among some nematode nuclear receptors, despite relatively low sequence conservation. Gene-threading models support structural conservation of *Caenorhabditis* LBD's with vertebrate nuclear receptors.

Deletions of *nhr-111* or *nhr-239* cause no obvious phenotype, suggesting that both perform subtle or redundant functions. *nhr-111* is expressed in a subset of neurons and the somatic gonad precursor cells. We are exploring possible roles for *nhr-111* and *nhr-239* in *C. elegans* behavior and development.

This work is supported by a grant from the NSF.

830B

Regulation of Gene Expression: Where Did That Noise Come From!? **Alexander R. Mendenhall**[1], Alexander K. Seewald[2], James R. Cypser[1], Patricia M. Tedesco[1], Thomas E. Johnson[1]. 1) Integrative Physiology, University of Colorado, Boulder, CO; 2) Seewald Solutions, Vienna, Austria.

Isogenic populations of worms show surprising variation in lifespan (Rea et al., 2005). In isogenic worm populations, the level of gene expression of an integrated *Phsp-16.2::gfp* on the second day of adulthood functions as a very good predictor of subsequent longevity. In an effort to understand how this stochastic variation (also called "noise" and measured as the coefficient of variation [SD/Mean]) in gene expression can correlate with lifespan, we are beginning a systematic study of sources of noise. We have created multiple strains of *C. elegans* in which we have independently integrated the same *Phsp-16.2::gfp* reporter gene construct. After heat shock we see considerable variation in the level of GFP expression among both strains and individual worms. In each animal, we observe variation across tissues and among cells within the same tissue (the intestine), indicating that there are factors responsible for gene expression, even within a tissue, which are variable between cells in the same animal. Furthermore, we have constructed strains expressing red and green fluorescent reporter genes both under *Phsp-16.2* control in two distinct loci. These strains allow us to understand how different loci containing the same sequence information are, nonetheless, regulated differently, both among individual worms and within an individual. When analyzing whole worms, the brightest red animals are also the brightest green animals, indicating a significant co-specification of overall expression levels. This data suggests that there are common elements (transcription factors, etc.) within individual worms that have an intrinsic effect on level of expression. However, when animals are analyzed in detail under the microscope, some individuals exhibit striking differences in which reporter is activated both within and between tissues, which demonstrates that there is cellular autonomy in determining which locus to express. We are currently working to understand how promoter sequence, transgene copy number, flanking DNA sequence, genetic background, chromatin structure, and other variables contribute to this noise. We are also determining the predictive capabilities of each of the *Phsp-16.2* reporter gene strains with regard to frailty and longevity.

See Rea et al., 2005. A stress-sensitive reporter predicts longevity in isogenic populations of *Caenorhabditis elegans*. Nat. Genet. 37: 894-898.

831C

Regulation of protein homeostasis genes is required for long-term survival at cool temperatures. **Syuichi Takano**, Angela Sanchez, Pamela Larsen. Dept Cellular and Structural Biol, UTHSCSA, San Antonio, TX.

Animals have adapted to survive ambient temperature fluctuations in their environment. Animals living in cool or warm conditions display physiological responses to the temperature. Two examples are the increase metabolic rate with increased temperature and the increase life span with decreased temperature. Generally, it is believed that these physiological changes are due to thermodynamic effects. We have found that the temperature-dependent changes in metabolic rate and life span are mutable. Certain mutant *daf-2* alleles are abnormal for an increased of metabolic rate with increased temperature or for a decreased of life span with increased temperature. We are interested in understanding the biological mechanisms triggered by a cool or warm temperature shift and whether the biological responses contribute to the enhanced or reduced survival for the individual living in the new climate.

In the laboratory, cultivation temperatures from 15°C to 25°C are used for *C. elegans*. We found significant differential expression of 338 genes in a microarray analysis of worms acclimatized to 15°C or 25°C (unpublished S. Takano, C. Curtis, S. Tavaré and P. L. Larsen). Among these genes the categories of protein synthesis and proteolysis were over-represented, suggesting protein homeostasis is critical to temperature acclimatization. Quantitative RT-PCR was used to establish that differential expression does occur for specific genes related to protein synthesis and proteases. The steady-state expression level is higher at 15°C than 25°C. This result is counterintuitive as the expectation had been that cellular processes are generally higher at 25°C, yet this is not the case for the more than 30 genes related to protein synthesis or proteolysis tested.

There are hundreds of genes in the genome in these two categories and the majority of these genes are not differentially expressed. The apparent specificity of the temperature-dependent regulation raised the possibility that an increase of protein synthesis and particular proteolysis contributes to the enhanced long-term survival at the cool temperature. To test this, adult worms cultivated at 15°C were fed RNAi *E. coli* of the protein-homeostasis-related genes. The knockdown of some of these genes caused severe herniation while others caused paralysis. This suggests that while thermodynamics may play a role in enhanced long-term survival in cool conditions, it is not sufficient. The regulation of specific protein synthesis-related and proteolysis genes is necessary for healthy long-term survival in cool conditions.

832A

Natural variation of the dynamics of global gene transcription regulation in aging worms. **A. Viñuela**, L.B. Snoek, J.A.G. Riksen, J.E. Kammenga. Laboratory of Nematology, Wageningen University, Wageningen, Netherlands.

Over the past decades the study of induced mutants has revealed major genes affecting lifespan and aging in the fly Drosophila melanogaster and the worm Caenorhabditis elegans. But so far it is unclear if and how gene regulation changes with age and how this is affected by natural variation. High-throughput gene transcription profiling in mutant and wildtype worms and flies showed that gene transcription variation did not decrease with age, suggesting that gene regulation remains constant throughout life. But currently detailed insight into genome-wide regulatory patterns in aging organisms is lacking. Here we present the first study of the temporal dynamics of global gene transcription regulation in a recombinant inbred line population of aging worms. These SNP genotyped recombinant inbred lines (RILs) were derived from a cross between two of the most genetically divergent wildtypes N2 and CB4856. We extracted mRNA from juvenile, reproductive and old worms and measured genome-wide gene expression in all RILs using 60 mer microarrays. In addition we measured average lifespan for all RILs. We show that gene transcription decreased monotonically for ~19% of all genes and increased for ~16 % of the genes across the RIL population. Variation in gene transcription decreased with age in ~2% of all genes, whereas for ~9% the variation was increased. Genetic mapping of gene transcription (eQTL) revealed age specific as well as age independent eQTLs. The number of eQTL and their effect decreased with age. But the effect of a few eQTL increased with age indicating "old-age" specific transcription regulation. Combined mapping of the three time points yielded 470 age independent eQTLs and 74 age dependent eQTLs. These age dependent transcripts have a different temporal expression depending on which parental allele harboring the age eQTL is present. Research is ongoing to see if these time-dependent eQTLs can explain the genetic variation in lifespan across the CB4856 x N2 RILs.

833B

Aging Transcription Factors *lin-11* and *let-711* in *C. elegans*. **Tseten Yeshi**, Jim Lund. Dept Biol, Univ Kentucky, Lexington, KY.

Aging is a complex physiological process regulated by genetic mechanisms and environmental factors. Microarray experiments have identified thousands of genes that have altered expression as a function of age and it is reasonable to assume that the activity of a smaller group of transcription factors underlie the expression changes of these aging genes. Transcription factors that were progressively up-regulated or down-regulated as a function of age were identified from aging time course microarray data. We hypothesize that these transcription factors regulate the expression of subsets of aging genes.

let-711 and *lin-11* are transcription factors that display expression changes during aging. *let-711* is the *C. elegans* ortholog of NOT1, the key component of the CCR4/NOT complex which is involved in negative regulation of gene expression in yeast, flies, and mammals. It shows down-regulated expression in worm populations older than nine days. *lin-11* is a LIM homeodomain transcription factor that is involved in embryogenesis and vulval development. This gene is strongly up-regulated in worm populations older than 16 days.

RNAi knockdown of *lin-11* and *let-711* initiated in adult worms produced significant reduction of lifespan compared to control worms. We then performed microarray experiments with RNAi knockdown populations to study their effect on genome expression profiles. Seventeen genes are differentially expressed when *lin-11* was knocked down and 103 genes upon *let-711* knockdown. Four genes were up-regulated and three were down-regulated in both experiments. The targets of these aging transcription factors include genes involved in the aging related Insulin/IGF-1 pathway, the TGF-beta pathway, and germline proliferation. A significant number of these targets have also been shown to be differentially regulated by the Insulin/IGF-1 signaling pathway as well as during aging by previous microarray experiments.

834C

Dissecting the spatiotemporal expression patterns of Wnt and Frizzled genes to obtain insight into Wnt-dependent processes in *C. elegans*. **Teije C Middelkoop**[1], Dong Hyun Kim[2], Alexander van Oudenaarden[2], Hendrik C Korswagen[1]. 1) Hubrecht Institute, KNAW, University Medical Center Utrecht, Utrecht, Netherlands; 2) Department of Physics, Massachusetts Institute of Technology, Cambridge, USA.

During *C. elegans* development the Wnt signaling pathway is used repeatedly to dictate distinct developmental decisions. The *C. elegans* genome encodes five Wnt ligands and four Frizzled receptors that can function partially redundantly in a variety of processes. Although many events were shown to depend on Wnt signaling, little is known about the endogenous expression patterns of Wnts and Frizzleds throughout worm development. Therefore we sought to analyze the expression of Wnt ligands and their receptors using a novel single molecule fluorescence in situ hybridization method (FISH). Using this technique we are now analyzing endogenous expression of all the *C. elegans* Wnt and Fz homologs at the mRNA level. Ultimately, we will generate a detailed spatiotemporal map of Wnt and Fz expression throughout development. Furthermore, we are generating Wnt::protA fusion constructs to visualize the distribution of Wnt ligands at the protein level, as we have previously reported for EGL-20. Together, these data will give molecular insights into the complex signaling network of Wnt molecules and Frizzled receptors and may provide clues about the role of individual Wnt and Fz homologs in *C. elegans* development. In addition, we will extrapolate these data to Q neuroblast migration, a process known to depend on both canonical and non-canonical Wnt signaling. We will focus on the initial migration of Q neuroblasts since little is known about this process. By combining single molecule FISH, mutational analysis and cell biology we will provide molecular insights into the developmental control of Q neuroblast migration.

835A

Identification of molecules in specific neurons required for pheromone perception. **Yuan ZHOU**, Ching K. LI, King L. CHOW. Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Hong Kong.

Chemosensation is important function for animals to perceive ongoing changes in the environment and coordinate appropriate responses. The chemosensory system detects different chemical cues, including sex pheromone, using one of the hundreds of chemosensory receptors (CRs). We have previously shown that *C. remanei* females can produce a sex pheromone with long range attractiveness towards both *C. remanei* and *C. elegans* males. The attractive response of males towards the chemical involves the neural circuit composed of the two ciliated neurons, CEM, AWA, and the interneuron, AIZ. Our data also implicate the presence of G protein-coupled receptors involved in this perception process acting in AWA. In order to identify these receptors and related molecules acting in AWA, we have initiated the experiments to identify AWA-enriched mRNA through cell-specific pull down experiment. At the same time, we are characterizing the AWA-specific *odr-7* promoter and *odr-10* promoter by a serial deletion study to define the AWA-specific *cis*-element. We hope that the consensus definition can help identify the GPCRs that are uniquely expressed in AWA neurons and allow functional testing to be conducted.

Similarly, very little information about CEM neuronal function has been reported. Since they are also required in pheromone perception, we take a similar approach described above for AWA characterization and will identify key components acting uniquely in CEM. The details about systematic deletion of CEM-specific promoters and preliminary results of identifying key regulator acting in CEM will be reported. Collectively, we hope to define the molecular identities of these two cell types and understand how these two cell types function to coordinate the pheromone perception process. (This study is supported by Research Grants Council, Hong Kong.).

836B

Suppressor of presenilin genes uncover a position effect on transcription at the *hop-1* locus. Alisson Gontijo[1,2], Julien Cottineau[1], Philippe Yakanowsky[1], **Bernard Lakowski[1]**. 1) Dept Neuroscience, Pasteur Inst, Paris, France; 2) Instituto de Neurociencias, CSIC-UMH, Unidad de Neurobiología del Desarrollo, Alicante, Spain.

Mutations in the *sel-12* presenilin gene cause an Egg-laying (Egl) defect due to reduction of signaling through the *lin-12*/Notch receptor. The phenotype of *sel-12* mutants can be strongly suppressed by mutations in the suppressor of presenilin (*spr*) genes. The SPR-1, SPR-3, SPR-4 and SPR-5 proteins resemble components of the REST-CoREST mammalian transcriptional repressor complex. SPR-5 in an ortholog of the mammalian histone demethylase LSD1. LSD1 is able to demethylate mono or di-methyl lysine 4, or lysine 9, on Histone H3. SPR-1 is an ortholog of the scaffold and nucleosome binding protein CoREST while SPR-3 and SPR-4 are C2H2 zinc finger proteins that weakly resemble the transcription factor REST. The similarity of the SPR proteins to components of the REST-CoREST complex suggests that the *spr* genes should play a broad role in regulating histone modifications and chromatin in *C. elegans*. Our results suggest that we recovered mutations in these genes in *sel-12* suppressor screens because one of the genomic regions they affect is the *hop-1* locus another, largely functionally equivalent, *C. elegans* presenilin gene. Mutations in *spr-3, spr-4* and *spr-5* suppress *sel-12* by de-repressing the transcription of *hop-1*. However, why the *hop-1* gene is normally transcriptionally repressed by the SPR proteins has been unclear. We have found that the transcript levels of *hop-1* in *C. briggsae* are much higher in the early larval stages than in *C. elegans* and that the transcript levels of *hop-1* in *C. briggsae* are comparable to the *hop-1* transcript levels in the strongest *C. elegans spr* mutant. This suggests that the *spr* genes, which are conserved in *C. briggsae*, do not repress the transcription of *Cbri-hop-1*. This suggests that some difference in the genomic structure around the *hop-1* locus between *C. elegans* and *C. briggsae* leads to *hop-1* being repressed by the SPR proteins in *C. elegans* but not in *C. briggsae*. Other evidence is also consistent with a position effect on *hop-1* transcription in *C. elegans*. Independently, we have found that two polymorphisms in the CB4856 mapping strain from Hawaii can partially suppress the phenotype of *sel-12* mutations. We have mapped these polymorphisms to the clusters of LG I and LGIV. Our evidence suggests that the polymorphism on LG I may be in the *hop-1* gene itself. We will present a hypothesis to explain the position effect at the *hop-1* locus and why the SPR proteins may be recruited to the *hop-1* locus in *C. elegans*.

837C

Identification of HPL-2/HP1 target genes in post-embryonic development. **S. Schott[1]**, S. Rohner[2], P. Meister[2], E. Oakley[2], S. Gasser[2], F. Palladino[1]. 1) LBMC–CNRS UMR5239, Ecole Normale Supérieure Lyon, LYON, France; 2) Institut Friedrich Miescher, Bâle, Switzerland.

HP1 proteins play an important context dependent function in the epigenetic regulation of gene expression. We have shown that C. elegans HPL-2, one of the two nematode HP1 homologues, acts in distinct differentiation pathways, presumably through the regulation of specific genes. In order to identify downstream targets of HPL-2, we performed microarray analysis comparing the expression profile of wild type and hpl-2 null mutants. We will present data showing that hpl-2 target genes include developmentally regulated transcription factors, cell cycle regulators, and extracellular matrix proteins. Chromatin immunoprecipitation analysis is being used to identify direct targets and characterize the epigenetic profile associated with HPL-2 recruitment to chromatin. This data should contribute to a better understanding of how epigenetic regulation contributes to the establishment and maintenance of developmental programs.

838A

Information and structural properties of *C.elegans* gene regulatory networks. **Tetsuya Maeshiro[1]**, Shin-ichi Nakayama[1], Masahiro Ito[2]. 1) SLIS, University of Tsukuba, Tsukuba, Japan; 2) Ritsumeikan University, Shiga, Japan.

We have analyzed the structure of experimentally elucidated and predicted gene regulatory networks, obtained by various experimental methods including microarray experiments, using quantitative methods. These networks consist of nodes that represent genes, and nodes are connected when represented genes are related by some transcription factor, whether clarified or not. Actually known networks were compared with fictitious networks based on quantitative properties that measure network structure, and values to discriminate real and fictitious networks were extrapolated. Analyzed properties include local density, path length, centrality, and the flux. The last property, flux, is a new structural property which is analogous to information flow over the network connections. The flux value is defined for each connecion among nodes, and the flux density distribution function is computed. Then the classification of network is based on the discrepancy of distribution from the distribution calculated from actual biological networks. The properties measure globally the networks, and masks details of individual links. Given a predicted gene regulatory network, although the reliability of individual transcription relationships are difficult to be estimated, global evaluation is statistically possible. The proposed method uses solely the structural parameters of network, and no explicit biological knowledge is included. Explicit biological information denotes function of proteins, gene sequence data, species name, for instance. Methods that depends heavily on such information may score higher discrimination rate of plausible networks from nonbiological networks. However, biological information is still incomplete, and may result in partial use of information, which seems to be difficult. The proposed network evaluation criteria is useful to reject unreal or nonbiological networks, and can be used to judge the plausibility of networks generated by gene regulatory network prediction systems.

839B

Analysis of gene regulation and cell fate from single-cell gene expression profiles in C. elegans. **Xiao Liu**[1], Fuhui Long[2], Hanchuan Peng[2], Sarah Aerni[3], Min Jiang[1], Adolfo Sánchez-Blanco[1], John Murray[4], Elicia Preston[4], Barbara Mericle[4], Serafim Batzoglou[3], Eugene Myers[2], Stuart Kim[1]. 1) Dept Developmental Biol, Stanford Univ, Stanford, CA; 2) Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA; 3) Department of Computer Science, Stanford University Medical Center, Stanford, CA; 4) Department of Genome Sciences, University of Washington, Seattle, WA.

To understand gene regulatory networks in development, we developed an experimental pipeline to create a gene expression dataset using images of wCherry-expressing worms as a proof-of-principle that demonstrates important new biological insights can be extracted from single cell gene expression data. Each of the steps in the pipeline can be scaled up, enabling one to generate data much faster in the future. The expression database currently contains images from L1 stage larvae of 93 reporter genes, including 61 transcription factors. We generated their expression profiles in 363 specific cells, and were able to quantitatively analyze expression of each gene as well as the molecular expression signature for each cell. We analyzed the pattern of expression of every gene to determine the relative effect of cell fate and cell lineage. Expression of most genes was strongly correlated with cell fate. However, some cells with identical fate expressed different sets of genes based on their lineage. The most striking examples are nuclei of syncytium hypodermal 7. Twelve hyp7 nuclei are derived from the C lineage and eleven are derived from the AB lineage. The molecular signature for nuclei derived from the C lineage shows a significant difference from that of nuclei derived from the AB lineage. Thus, nuclei in the same syncytial cell can show large differences in gene expression pattern, indicating that there is different transcriptional control in different nuclei. The level of expression of the 93 reporter genes in each cell is a molecular signature for that cell, and can be used as a quantitative measure to determine whether cells have different or related cell fates. We created a developmental activity map based solely on molecular signatures, in which we identify regions of the cell lineage where developmental fates begin to diverge. For each gene, we used the cell lineage and the observed expression levels at the L1 stage to predict when that gene became committed to be expressed in the embryonic lineage. We then searched for embryonic cell divisions in which daughter cells become committed to express a different battery of genes, thereby identifying cell divisions that are asymmetric and revealing when developmental potentials begin to diverge in the embryonic lineage.

840C

Larval arrest upon cholesterol starvation is mediated by DAF-16 nuclear localization and DAF-12 activity. **Myung-Hwan Jeong**, Ichiro Kawasaki, Yhong-Hee Shim. Dept. of Bioscience and Biotechnology, and Bio/Molecular Informatics Center, Konkuk University, Seoul, Korea.

Nematodes including free-living *C. elegans* require sterol for their growth as a nutritional source because *C. elegans* is unable to biosynthesize sterol *de novo*. Cholesterol starvation (CS) lowered brood size and induced larval arrest. We found that *daf-12* expression was increased in CS so were *daf-9* and *daf-16*. DAF-9, a cytochrome P450, produces a ligand for DAF-12, a hormone receptor. DAF-16 is a key factor in the *daf-2*/insulin-like signaling. When *daf-2*/insulin-signaling is inactivated, DAF-16 moves to nucleus and regulates gene expression of genes that are responsible for dauer formation. Ligand-activated DAF-12 inactivates DAF-16 nuclear localization and dauer formation. The larval arrest induced upon cholesterol starvation appears to be mediated by DAF-16 translocalization in the presence of DAF-12 activity. And activated DAF-16 in turn up-regulates *daf-12* expression and maintains the high level of DAF-12. Despite of DAF-16 translocalization, worms grown in CS did not lead to *sod-3* expression and life span extension. Life span of worms grown in CS was shortened, even in *age-1* mutants. In conclusion, cholesterol starvation activates DAF-16 translocalization through DAF-12 hormone signaling and induces downstream gene expression for the larval arrest.

841A

Autoregulation of *mab-22* expression takes place during *Caenorhabditis elegans* male sensory ray. **David C K Leung**, King L. Chow. Dept Biol, HKUST, Hong Kong.

We have reported that *mab-22* encoding a T-box containing transcription factor, TBX-2, is indispensable for ray cells assembly. *mab-22(bx59)* mutant exhibits extensive ray-missing phenotype in a temperature-sensitive fashion, which also infers its action in all rays. However, the *mab-22::gfp* reporter containing key regulatory elements to rescue the mutant ray phenotype displays activity only in the structural cells of rays 1, 5 and 7 in wild-type animals. We hypothesize that *mab-22* expression is under its own negative regulation in other rays. To test this notion, *mab-22::gfp* reporter line was crossed into *mab-22* mutant. The incidence of expression and intensity of GFP signals in the structural cells of rays 1, 5 and 7 surged. In addition, substantial level of reporter expression was detected in all rays.

We probe into this hypothesis of feedback regulation and test if it is mediated by direct binding of the *mab-22* product onto the *mab-22* locus. By adjusting the *mab-22* dosage with the *bx59* allele cultured at different temperatures or by changing the copy of wild-type and *bx59* alleles, the *mab-22* reporter expression level was examined. The results consistently indicated that reduction of *mab-22* activity boosted the *mab-22* reporter expression level in the structural cells. We have identified two putative MAB-22 binding sites on its own intron 1. We show that when these sites were deleted altogether, the *mab-22* reporter expression in wild-type animals could be detected in all structural cells with a higher level in rays 1, 5 and 7. Besides, site-directed mutagenesis of the two binding sites individually was performed to confirm the *bona fide* MAB-22 effective sequence. Both the mutant reporters with only one of the two binding sites mutated lead to full expression of the reporter in all the structural cells. The observation substantiates that *mab-22* exerts a repressive effect on its own expression *via* this intron 1 with two MAB-22 recognition sequences. To the best of our knowledge, it is the first report that a T-box gene expression is modulated by a negative autoregulatory mechanism. Biochemical analyses are in progress to decipher the binding specificity of MAB-22 to yield additional insights into the mechanism of the sequence-specific DNA binding of T-box protein. (This study is supported by the Research Grants Council, Hong Kong).

842B

Infrared laser-mediated gene induction in targeted single cells in *C. elegans*. **Motoshi Suzuki**[1], Yasuhiro Kamei[2], Yoichi Oda[1], Syunsuke Yuba[3], Shin Takagi[1]. 1) Nagoya University, Japan; 2) Osaka University, Japan; 3) Research Institute for Cell Engineering, National Institute of Advanced and Industrial Science and Technology, Japan.

Methods for manipulating gene expression at the experimentalist's discretion would provide a useful means to analyze diverse biological processes. Heat shock promoters drive the expression of downstream genes in response to heat stimuli. Previous studies showed that irradiation with the visible laser light (440 nm) can induced heat shock response-mediated expression of transgene in targeted cells. However, irradiation with a 440 nm laser, which was originally used for cell ablation, may have detrimental effects on cells. We have developed a novel microscope system called infrared laser-evoked gene operator (IR-LEGO)(Kamei et al., Nat Methods 2009 Jan;6(1):79-81.). Because the absorption coefficient of water is higher in the infrared (IR) region, the IR light would heat cells more efficiently than the visible light. Application of IR-LEGO to *C. elegans* revealed that irradiation for 1s was sufficient for gene induction in a targeted single seam cell. Under appropriate conditions, 40% of irradiated seam cells expressed GFP under the control of a heat shock promoter, and all irradiated cells developed normally, indicating that the IR irradiation did not cause cell damage. We also found that the expression of MAB-5 and MIG-24 protein induced with IR-LEGO each rescued the phenotypes of the corresponding mutants. We also report on current attempts to induce gene expression in targeted cells of early embryos and in single neurons of the nerve ring.

843C

SMA-9 Mediates Regulation of DBL-1/BMP Target Genes. **Jianghua Yin**[1,2], Edlira Yzeiraj[1], Ling Yu[1], Cathy Savage-Dunn[1]. 1) Dept Biol, Queens Col, CUNY, Flushing, NY; 2) Biochemistry Program, the Graduate Center at CUNY, New York, NY.

The transforming growth factor β (TGF-β) family comprises a large number of structurally related polypeptide growth factors, each capable of regulating a critical array of cellular processes including cell proliferation, differentiation, adhesion, and death. Mutations of the component in TGF- β pathway are found to be responsible for many human genetic disorders. Our lab is focused on BMP/TGFβ signaling in *C. elegans*, composed of the ligand DBL-1, transmembrane receptors SMA-6 and DAF-4, and intracellular transducers SMA-4, SMA-3 and SMA-2, regulating body size and male tail development. Mutants of DBL-1 pathway components lead to small body size and abnormal male tail ray patterning. SMA-9, the homolog of *Drosophila schnurri*, functions as a transcriptional cofactor in DBL-1 pathway. How SMA-9 fulfills its regulatory functions becomes the key question we want to answer. *sma-9* undergoes extensive alternative splicing. Whether these splicing variants play particular roles in development is still largely unknown. However, we have the evidence that at least one mRNA variant has important regulatory function in male tail patterning. It can partially rescue the male tail defects in all three *sma-9* mutant backgrounds when we overexpress it using hs promoter. To determine how SMA-9 contributes to the selection and regulation of DBL-1 target genes, we have studied potential SMA-9 target genes. T27F2.4 gene, a bZip transcription factor, is a potential target gene candidate. Microarray analysis, RT-PCR and GFP reporters show that its expression is upregulated in *sma-9* mutants. Knockdown of this gene results in long body size phenotype. *ptr-22* and *wrt-4* are also potential SMA-9 target genes. *ptr-22p::2xNLSmCherry* transgenes show the intestinal expression and those expressions are upregulated in different *sma-9* mutant backgrounds. *col-41* is another potential SMA-9 target gene from our microarray data. Knockdown of this gene through RNAi results in small body size phenotype. GFP reporter shows low but detectable expression in hypodermis in wild-type background, which is the essential organ in body size regulation. Significantly, the expression of *col 41p::2xNLSmCherry* transgene is eliminated or reduced after RNAi of *sma-3* and *sma-4*. In addition, we align the minimal upstream regulatory sequences with upstream sequences of *col-41* homologs from other *Caenorhabditis* species, *C. briggsae*, *C. remanei*, and *C. brenneri*. The strong conservation in the -500bp to 0 bp upstream of the start codon gives us a clear clue for future deletion series analysis of potential regulatory sequences. In summary, we now have the tools in place to study the mechanisms of DBL-1 pathway transcriptional regulation in *C. elegans*.

844A

Regulation of GLD-1 turnover in the *C. elegans* germ line. **Sarah E. DeGenova**[1], Tim Jarevela[2], Sudhir Nayak[1]. 1) Department of Biology The College of New Jersey 2000 Pennington Rd. Ewing, NJ 08628; 2) Department of Biological Sciences Carnage Mellon University 4400 Fifth Avenue Pittsburgh, PA 15213.

The KH domain containing RNA binding protein GLD-1 (defective in germ line development) has multiple functions in germ line development. The tightly regulated accumulation of GLD-1 at the distal region and loss of GLD-1 at the loop are critical for its functions and is essential for maintaining germ line polarity and oocyte development. Unlike the requirements for GLD-1 accumulation in the distal region, the mechanisms by which GLD-1 levels fall abruptly and are sustained at low concentration in oocytes are unknown. To identify components involved in regulation of GLD-1 levels, we performed a Po RNAi screen. Since reduction of function in GLD-1 by RNAi results in Po sterility and F1 embryonic lethality, we focused on genes that resulted in the same phenotypes in high throughput screens or were expressed in the germ line based on microarray data contained in *ORFeome-RNAi* v1.1 (OpenBiosystems, WS180, 1498 genes). To increase the sensitivity of the screen, we utilized a transgenic line carrying a functional GLD-1::GFP that offered a real-time readout of germ line polarity and health. Animals with altered GLD-1::GFP expression were DAPI (4',6-diamidino-2-phenylindole) stained to asses both GLD-1 accumulation and nuclear morphology. Our screen identified genes involved in two major cellular processes: targeted degradation (*pbs, cul, skr*) and translation (*ife, eft*). Importantly, in both cases, we observed dramatic changes in GLD-1 accumulation prior to changes in germ line morphology. The focus of this presentation will center on the rapid turnover of GLD-1 in the germ line. Since the blocking of translation via RNAi (*ife* and *eft* genes) resulted in the rapid down regulation of GLD-1, we suspected a rapid turnover rate. To confirm these findings, we tested chemical translational inhibitors for their ability to phenocopy the RNAi results. Our preliminary data indicates that when strains were exposed to chemical translational inhibitors, GLD-1 accumulation decreased rapidly within 2 hours and was essentially absent after 6 hours with minimal effect on germ line morphology. In total, our data suggests that down regulation of GLD-1 prior to oocyte development is mediated by inherent instability and the action of the 26S proteasome. We are confirming these results on a subset of genes isolated from the screen with complementary approaches.

845B

Toward the understanding the rules of GLD-1 binding specificity. **Jung Hoon Doh**[1], Yuchae Jung[1], Valerie Reinke[2], Min-Ho Lee[1]. 1) Department of biology, University at Albany, Albany, NY; 2) Yale University School of Medicine, Genetics, New Haven, CT.

Considering the general suppression of transcription during late oogenesis and early embryo development, post-transcriptional control of maternal mRNAs by RNA binding proteins emerges as an important mechanism in controlling late oogenesis and early embryo development. GLD-1 (defective in GermLine Development) is a maxi-KH motif containing RNA binding protein, which controls many different stages during germline development, suggesting that GLD-1 likely controls many mRNA targets. Previously, we identified multiple mRNA targets of GLD-1 that are coimmunoprecipitated with GLD-1 from the cytosol extract. 129 potential mRNA targets of GLD-1 are enriched more than two fold in a GLD-1::FLAG IP / microarray analysis. To validate functional relationship of these putative GLD-1 mRNA targets during development, we categorized the putative targets into distinct functional groups based on public databases. Statistically enriched functional gene groups with significance levels ($P < 0.01$) compared to the total C. elegans proteome distributions are “embryonic development ending in birth or egg hatching (GO:0009792)”, “cytokinesis (GO:0000910)”, “protein binding (GO:0005515)”, “embryonic cleavage (GO:0040016)”, and “DNA replication (GO:0006260)”, consistent as GLD-1 mRNA targets. However, the exact mechanism of how GLD-1 specifically recognizes these mRNA targets from non-targets in vivo remains unclear. Thus we selected 42 of these GLD-1 mRNA targets and identified GLD-1 binding regions in each target. We were able to detect GLD-1 binding to either 5’, 3’untranslated region (UTR) or both ends in 40 out of 42 mRNA targets examined, suggesting most mRNA targets identified in our GLD-1::FLAG IP / microarray analysis are likely true targets. Then we searched for over-represented and evolutionarily conserved sequence motifs in these binding regions and in the corresponding other nematode orthologous regions. We identified two over-represented sequence motifs, a novel AG rich motif and the other essentially similar to the previously identified hexanucleotide motif. Currently, we are examining the importance of these over-represented sequence motifs in several GLD-1 binding regions in vitro and in vivo.

846C

mRNA targets of GLD-3-containing ribonucleoprotein complexes (RNPs). **Britta Jedamzik**, Ryuji Minasaki, Christian R. Eckmann. MPI-CBG, Dresden, Germany.

Germline and early embryonic development of higher eukaryotes rely extensively on post-transcriptional mRNA regulation. The control mechanisms of translational mRNA activation or repression are crucial for cell fate specification and seem to be largely conserved. For example, *gld-3* (*g*erm*l*ine development *d*efective-3) of *C. elegans*, a member of the conserved Bicaudal-C family of RNA-binding proteins, is an important regulator of germline and embryonic development (1). GLD-3 is part of larger mRNP complexes, which are expected to regulate specific sets of target mRNAs. Interestingly, two distinct cytoplasmic poly(A) polymerases (PAPs), GLD-2 and GLD-4, are proposed to rely on GLD-3 activity (2). In our model, GLD-3 provides an RNA-binding activity to both non-canonical PAPs and may even serve as a platform for other regulatory proteins to bind and influence the target specificity of different GLD-3-containing complexes. However, it is unclear what the individual complexes are composed of and what the respective target mRNAs are. To test directly the RNA-binding ability of GLD-3 we employed RNA homopolymer binding assays. GLD-3 specifically bound to poly(G) RNA and not to poly(U), (C) or (A), a feature shared with *Drosophila* Bicaudal-C (3). To identify in vivo mRNA targets of the GLD-2/3 complex we used a candidate gene approach and performed RNA co-immunoprecipitation (RNA co-IP) experiments. We identified and characterized *gld-1* as the first mRNA target of the GLD-2/GLD-3 PAP in collaboration with the Kimble lab (4). However, we learned from this study that *gld-1* mRNA translation depends only partially on GLD-2 PAP activity and that *gld-1* mRNA regulation is fully compensated for in *gld-3* mutants. Recently, we found that *gld-1* mRNA is a target of a second cytoplasmic PAP, the GLD-4/GLS-1 complex (2). We now report that GLD-3 is also a member of the GLD-4/GLS-1 PAP in vivo, suggesting that *gld-1* mRNA activation shares a common component, GLD-3, and two in parallel acting but distinct PAP enzymes, GLD-2 and GLD-4. Finally, to find more GLD-3-RNP targets in an unbiased way we performed RIP-CHIP (RNA co-IP and microarray analysis) experiments. We have now a preliminary list of ~70 potential GLD-3 targets, which are currently validated with various biochemical techniques. The confirmation of their biological significance to be a target of GLD-3 and its associated PAPs in vivo is still pending.

(1) Eckmann CR et al. (2004) Genetics 168(1), pp147-60. (2) Schmid M et al. (2009). Genes and Dev. (in press). (3) Saffman EE et al. (1998) Mol Cell Biol. 18(8), pp4855-62. (4) Suh N et al. (2006) Proc Natl Acad Sci USA 103 (41), pp15108.

847A

Roles for SMG-1 in NMD and DNA damage signaling. **Luciana Leopold**[1], Matthew Eckler[1,2], Mia Lowden[1,2], Yan Liu[1], Shawn Ahmed[1,2]. 1) Department of Genetics, University of North Carolina, Chapel Hill, NC, USA; 2) Department of Biology, University of North Carolina, Chapel Hill, NC, USA.

SMG-1 is a phosphatidylinositol kinase-related protein kinase that functions as a key component of the nonsense-mediated RNA decay (NMD) pathway. An allele of *smg-1, yp3*, was isolated from a *mortal germline* mutant strain that becomes progressively sterile and displays germline tumors at the non-permissive temperature of 25°C. However, the progressive sterility phenotype of this strain is a synthetic effect that occurs as a consequence of an additional mutation. Although outcrossed strains homozygous for *smg-1(yp3)*, or for the null *smg-1* alleles *r904* and *e1228*, do not become progressively sterile, these strains do display a low level of germ cell tumors at 25°C. Mammalian SMG proteins function in both NMD as well as the S-phase DNA replication checkpoint. Treatment of *C. elegans smg-1* mutants with hydroxyurea, which perturbs the S-phase checkpoint, can elicit germ cell tumors at the permissive temperature of 20°C, mimicking the effect of growth at 25°C. We present evidence that *smg-1(yp3)* is a separation-of-function mutation that disrupts the S-phase checkpoint, but not NMD. Thus, the DNA damage signaling function of *smg-1* can suppress germ cell tumors, and the role of *smg-1* in NMD is mechanistically separable from its role in DNA damage signaling.

848B

RNA sequence requirement for GLD-1 mediated translational repression in vivo. **Jane Wright**, Mathias Senften, Rafal Ciosk. FMI, Maulbeerstrasse 66, CH 4058, Basel, Switzerland.

Many of the key decisions in germline development such as the mitosis to meiosis transition and the switch between spermatogenesis and oogenesis involve translational regulation mediated by the GLD-1 (defective in Germ Line Development) protein. GLD-1 is a KH domain RNA-binding protein of the STAR family. GLD-1 is known to regulate several mRNAs in the gonad and in vitro analysis has identified putative GLD-1 binding RNA elements. However it is not known if these elements are important for GLD-1 mediated translational repression in vivo. We have studied GLD-1 binding and repression on a subset of GLD-1 target mRNAs, including cye-1 (encoding cyclin E) and rme-2 (encoding a yolk receptor). We find that point mutations of putative GLD-1 binding elements within the 3'UTRs alleviate GLD-1 binding in vitro and translational repression in vivo. Remarkably, introduction of a single GLD-1 binding element into an unrelated 3'UTR is sufficient to confer GLD-1 mediated regulation. Our findings demonstrate that RNA sequences preferentially recognized by GLD-1 in vitro are also critical for GLD-1 binding and regulation in vivo. Interestingly, such RNA elements are very frequent in 3' UTRs and are found in some mRNAs that are not regulated by GLD-1. This suggests that additional constraints determine which mRNAs are associated with and regulated by GLD-1.

849C

SUMO and the T-box factor TBX-2. **Tanya L. Crum**, Paul B. Huber, Peter G. Okkema. Department of Biological Sciences and the Laboratory for Molecular Biology, University of Illinois at Chicago.

T-box transcription factors are crucial developmental regulators in all multicellular organisms, but little is known regarding the mechanisms they use to regulate target gene expression. In *C. elegans*, the phylogenetically conserved, Tbx2 subfamily member TBX-2 is specifically required for ABa-derived pharyngeal muscles, and TBX-2 function depends on post-translational SUMOylation. Recently, SUMOylation has also been implicated in T-box factor function in humans, and this modification may serve as a conserved regulatory mechanism for these proteins in all species. We are investigating the role of SUMOylation in T-box factor function using *C. elegans* TBX-2. TBX-2 contains multiple consensus SUMOylation sites, and these sites are conserved in TBX-2 orthologs from related nematodes. TBX-2 is conjugated to multiple SUMO-1 peptides in *in vitro* reactions using human SUMOylation enzymes and when co-transfected with human SUMO-1 in COS-1 cells, suggesting multiple TBX-2 consensus SUMOylation sites are functional. Using yeast 2-hybrid assays, we have found two consensus SUMOylation sites that mediate specific interactions with the E2 SUMO conjugating enzyme UBC-9 and the E3 SUMO ligase GEI-17. Mutation of both of these sites eliminates TBX-2 SUMOylation in COS-1 cell assays, and mutation of one of these sites interferes with TBX-2 SUMOylation in *in vitro* assays. We are currently testing mutants affecting these sites for function in *C. elegans*. We have begun asking if other T-box factors are SUMOylated, and have found *C. elegans* MAB-9 is also SUMOylated *in vitro*. Consensus SUMOylation sites are found in many T-box transcription factors in *C. elegans* and other organisms, and we suggest function of these T-box factors similarly involves SUMOylation.

850A

Identifying factors required for specification of muscle sub-types in the *C. elegans* pharynx. **Brittany C. Logan**, Shoubin Wen, Jeb Gaudet. Biochemistry & Molecular Biol, University of Calgary, Calgary, Alberta, Canada.

The bulk of the *C. elegans* pharynx consists of eight muscle groups (pm1-8), each with unique morphology that give the pharynx its distinct bilobed structure. Muscle group pm6 is unique in that it produces the pharyngeal grinder, a chitinous structure involved in the mechanical breakdown of food. How the different pharyngeal muscle sub-types are specified is largely unclear and little is known about the specification and development of the pm6 cells. To begin to examine pm6 development, we first identified a set of pm6-specific genes by searching available gene expression databases (in particular, the Kohara group's NEXTDB). From a list of candidate pm6-specific genes, we selected three genes (*C04G6.10, F41G3.3, F45B8.3*) for further study, and constructed GFP reporters for all three genes to verify that each was expressed specifically in the pm6 cells. We next performed a detailed analysis of the *C04G6.10* promoter and identified two *cis*-regulatory elements (PM6-2 and PM6-3) that are critical for pm6-specific expression. The first element, PM6-2, contains a predicted PHA-4 binding site. Consistent with regulation by PHA-4, extensive mutation of the PM6-2 sequence abolishes *C04G6.10* expression. However, more discrete point mutations indicate that PM6-2 contains an element in addition to the PHA-4 site. Interestingly, when placed in multiple copies upstream of a "promoterless" GFP, PM6-3 is sufficient for pm6-specific expression. Thus, two mechanisms appear to regulate pm6-specific expression of *C04G6.10*. The first, acting through PM6-2, appears to be a factor that represses expression in non-pm6 cells, possibly by interfering with PHA-4 binding and/or function. The second, acting through PM6-3, appears to respond to a pm6-specific activator. We are currently examining the other two pm6-specific reporters (*F41B3.3* and *F45B8.3*) to determine whether their expression is regulated by the same *cis*-elements present in the *C04G6.10* promoter. Preliminary data suggest that expression of both is dependent on a PM6-3-like sequence, arguing that expression of many pm6-specific genes is regulated by a common upstream activator. To identify the putative pm6-specific transcription factor acting through PM6-3, we will perform a yeast one-hybrid screen, test candidate factors by RNAi and perform a forward genetic screen for mutants that lack expression of a pm6-specific reporter. This work will ultimately lead to the identification of factors required for specification of the pm6 muscle group and formation of the pharyngeal grinder.

851B

RNA recognition by the cell fate determinant MEX-3. **John M Pagano**, Sean P Ryder. Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, MA.

Post-transcriptional regulation of gene expression governs the development of the *Caenorhabditis elegans* germline and early embryo. The putative RNA binding protein MEX-3 is essential for maintaining totipotency of germline stem cells and for specification of the anterior founder cell (AB) during embryogenesis. *mex-3* mutants are maternal effect lethal with a terminal phenotype that includes posterior muscle proliferating into the anterior (1). In addition, worms that are deficient in MEX-3 and a second RNA binding protein, GLD-1, are sterile and form transdifferentiated germline tumors (2). Genetic studies reveal that two genes involved in early development, *pal-1* and *nos-2*, are dependent upon *mex-3* for their protein expression pattern (1, 3). MEX-3 contains two conserved KH domains and is thought to control mRNA stability, translation efficiency, and/or mRNA localization through specific interactions with target maternal mRNAs. However, the RNA binding specificity and network of regulatory targets have not been determined. Here, we used *in vitro* selection (SELEX) and quantitative biochemical methods to define the consensus MEX-3 recognition element (MRE). We demonstrate that MEX-3 binds specifically to a bipartite element consisting of two four-nucleotide elements with variable spacing between each half-site. The putative mRNA targets *pal-1* and *nos-2* each contain two MREs in their 3'UTR. Furthermore, the transcript *nos-2* has an MRE within a critical *cis*-regulatory element required to repress NOS-2 expression in early embryos (4). Our results show that MEX-3 binds specifically to this element. The MRE is present in several other genes that are required for germline development and embryogenesis. Candidate regulatory targets include a number of RNA binding proteins (*mex-1, gld-1, spn-4, puf-6, puf-7*) and membrane proteins (*glp-1, ooc-3*). Target prediction based on the MRE will be used to further elucidate the role of MEX-3 in early development.

1. Draper BW, et al. (1996) Cell 87, 205-216
2. Ciosk R, et al. (2006) Science 311, 851-853
3. Jadhav S, et al. (2008) Development 135, 1803-1812
4. D'Agostino I, et al. (2006) Dev. Biol. 292, 244-252.

852C

Hunting for targets of TBX-2 in the developing pharynx. **Lynn M. Scrogham**[1], Tom J. Ronan[2], Peter G. Okkema[1]. 1) Dept Biological Sci, Univ Illinois, Chicago, Chicago, IL; 2) Dept Bioengineering, Univ Illinois, Chicago, Chicago, IL.

T-box transcription factors are crucial developmental regulators, and they have been more recently implicated in a variety of human diseases and cancers. Despite their importance, relatively few direct targets of T-box transcription factors have been identified. *C. elegans* TBX-2 is required for the development of ABa-derived pharyngeal muscles and is a member of the conserved Tbx2 sub-family of T-box factors, which includes both transcriptional activators and repressors. We are interested in identifying TBX-2 targets that function in pharyngeal muscle development, and in determining if TBX-2 is a transcriptional activator or repressor. To identify targets of TBX-2, we have compared mRNA expression levels in wild-type and *tbx-2(bx59)* mutant embryos using Affymetrix microarrays. Of 22,500 probe sets examined, we found 991 mRNAs that were significantly up-regulated in *tbx-2(bx59)* relative to wild-type and 195 mRNAs that were significantly down-regulated. Using clustering analysis, comparisons to existing data sets on pharyngeal gene expression, and phylogenetic foot-printing to identify promoters with consensus T-box factor binding sites, we are initially focusing on a subset of these genes that may be direct targets of TBX-2. Preliminary results of semi-quantitative PCR analysis indicate these genes are differentially expressed in *tbx-2(bx59)*, and we are currently examining the spatial and temporal expression patterns of these genes in wild-type and *tbx-2(bx59)* mutants. These analyses should identify the first direct targets of TBX-2 in the *C. elegans* pharynx.

853A

Identification and Characterization of *hlh-6* Independent Gland Genes. **Ryan Bart Smit**, Jeb Gaudet. Dept Biochem & Mol Biol, Univ Calgary, Calgary, AB, Canada.

The pharynx (or foregut) of the nematode *Caenorhabditis elegans* is involved in initiation of digestion and transportation of its food source. We previously showed that pharyngeal gland cells secrete mucin-like proteins (PHAT proteins) that aid in transportation of the bacteria along the lumen. We identified *hlh-6* as a critical regulator of *phat* gene expression and subsequent gland function. Mutations in *hlh-6* result in a starvation phenotype presumably due to the lack of PHAT protein coating and lubricating the pharyngeal lumen. However, not all gland expressed genes are dependent on *hlh-6* for expression and three out of five glands are still present in *hlh-6* mutants, suggesting the presence of other gland gene regulators and potentially other gland functions. By identifying other gland transcription factors and examining their mutant phenotype we hope to further our understanding of the development and function(s) of these cells.

Searching for *cis*-acting elements in *hlh-6* independent gland genes will lead to the identification of an *hlh-6* independent transcription factor. A list of all known gland expressed genes was compiled by searches of the literature and available expression databases (NEXTDB, the BC Gene Expression Database, the Hope Lab Expression Database). The promoters of these genes were then examined for the presence of a consensus *hlh-6* binding motif. Thirty genes had promoters lacking a probable HLH-6 binding site and were classified as possible *hlh-6*-independent genes. Reporters were made for ten of these genes and we are currently testing which of these reporters are indeed *hlh-6* independent by examining their expression in *hlh-6* mutants. Once we have a list of confirmed *hlh-6*-independent gland genes, the promoters of these genes will be examined computationally for the presence of shared motifs. The gland expression of one gene (*B0507.1*) has been shown to be only partially dependent on *hlh-6*. Further analysis of this promoter has identified a critical region of 60 bp in the *B0507.1* promoter that is responsible for the *hlh-6* independent gland expression. We are currently defining the specific *cis*-acting element(s) within this region, which may suggest probable *trans*-acting factors. From the list of all known gland expressed genes, ten are transcription factors (zinc finger and nuclear hormone receptor families) and eight do not contain probable HLH-6 binding sites. As a complementary approach we are examining the expression of known HLH-6 independent genes in mutants for these transcription factors.

854B

Coordinate regulation of pharyngeal morphogenesis in *C. elegans* by LIN-35, UBC-18, ARI-1 and PHA-1. **Kumaran Mani**, David Fay. Dept Molecular Biol, Univ Wyoming, Laramie, WY.

We have previously described a role for the *lin-35*/Rb tumor suppressor in the organogenesis of the *C. elegans* pharynx. Specifically, LIN-35/Rb, a tumor suppressor ortholog, in conjunction with UBC-18-ARI-1, a conserved E2/E3 complex, and PHA-1, a novel protein, coordinately regulate an early step of pharyngeal morphogenesis involving cellular re-orientation. Functional redundancy among these proteins is indicted by the observation that *lin-35; ubc-18* double mutants, as well as certain allelic combinations of *pha-1* with either *lin-35* or *ubc-18*, display similar defects in pharyngeal development, whereas single mutants do not. Earlier work by Schnabel and colleagues had identified three loci, *sup-35*, *sup-36*, and *sup-37*, which act as strong recessive genetic suppressors of *pha-1* single-mutant lethality. By genetic analyses, we show that *sup-35*, *sup-36* and *sup-37* also reverts the synthetic lethality of *lin-35; ubc-18*, *lin-35; pha-1*, *ari-1; pha-1* and *ubc-18 pha-1* double mutants. To gain a better understanding of the networks regulating pharyngeal development, we have cloned *sup-35*, *sup-36* and *sup-37*. SUP-35 contains C2H2-type Zn-finger domains as well as a conserved RMD-like motif and shows a dynamic pattern of subcellular localization during embryogenesis. Mutations in *sup-35* specifically suppress hypomorphic alleles of *pha-1* and other synthetic phenotypes as well. Genetic and molecular data suggest that SUP-35 negatively regulates *pha-1* transcription by acting upstream of or in complex with SUP-36 and SUP-37. We further demonstrate that LIN-35, a transcriptional repressor, and UBC-18-ARI-1, a complex involved in ubiquitin-mediated proteolysis, in turn negatively regulates SUP-35 abundance through distinct mechanisms. While SUP-37, another C2H2-type Zn-finger protein, is also a likely transcription factor, SUP-36 is predicted to play a role in microtubule polymerization. Our studies have also identified a phylogenetically conserved protein as a novel suppressor that may suppress *pha-1* and other synthetic lethalities through regulation of the LIN-35 binding partner, EFL-1/E2F. Our cumulative findings piece together the components of a novel regulatory network that includes LIN-35/Rb, UBC-18-ARI-1 and PHA-1 which functions to control pharyngeal morphogenesis. Our results also shed light on general mechanisms that may underlie developmental genetic redundancies as well as principles that may govern complex disease traits.

855C

Transcription factors binding the gonadal sheath enhancer in *lim-7* intron 1. **Michael Kiedrowski**, Laura G. Vallier. Biology Department, Hofstra University, Hempstead, NY.

Initiating and maintaining fertility in an organism is a complex process, relying on the proper timing and expression of many components derived from both somatic and germline tissue sources. In *C. elegans*, the gonad is composed of two U-shaped armed joined centrally at the uterus. Its reproduction system includes the germline and the somatic gonad, the latter being composed of the distal tip cell, the gonadal sheath, and the spermatheca, the spermatheca-uterine valve and the uterus. The ten cells comprising the gonadal sheath mostly enclose each arm of the gonad and are responsible for four crucial functions to maintain fertility. A common fluorescent reporter, *tnIs6* (*lim-7::GFP*), expresses GFP within the gonadal sheath. The reporter construct contains 2.3 kb upstream of the *lim-7* start site and the first 2 exons and intron 1 that lies between them; the presence of intron 1 is necessary for GFP expression in the sheath. Recently, through intron bashing, the first 45 bp in intron 1 was found to be necessary and sufficient for expression of GFP in the gonadal sheath. Therefore, we wanted to discover what was binding to that enhancer element within intron 1, which allowed expression of GFP in the gonadal sheath. To do this we have begun a one-hybrid analysis to look for the transcription factors that bind to this enhancer element. In this way, we will begin to understand the regulation of expression of the LIM-7 LIM-homeodomain transcription factor in non-neuronally derived tissues. Preliminary results will be presented at the meeting.

856A

Global analysis of suppressors of the lethality of *lim-7*(*tm674*) by RNAi. **Robert Labiento**, Gina Destefano, Laura G. Vallier. Biology Department, Hofstra University, Hempstead, NY.

The LIM-7 LIM-homeodomain (LIM-HD) transcription factor belongs to the evolutionarily conserved LIM-HD family, whose members have in common two N-terminally located LIM domains and a homeodomain located C-terminally to them; each LIM domain is composed of two modified zinc fingers and the two LIM domains are the site of interaction with other transcription factors, whereas the homeodomain binds DNA. There are six subclasses of LIM-HD transcription factors and LIM-7 falls within the Islet subclass. The LIM-7 protein is expressed in motor neurons, the gonadal sheath, and muscles cells, based on different reporter constructs. A sole allele exists for *lim-7*, *tm674*, which is an in frame deletion of the majority of both LIM domains; the resulting phenotype is L1 lethality. Although in other systems, the role of Islet class transcription factors in the organogenesis of pancreas, heart and pituitary, and the differentiation and function of motor neurons has been established, much less is known about how Islet class transcription factors are regulated and what they regulate. One way to identify the players in the pathway is to look for suppressors of the *tm674* lethal phenotype. We chose to use a reverse genetics approach via RNAi. Starting with *tm674*/ *hT2[GFP let]* heterozygous hermaphrodites, we are screening chromosomes 1 and 2 of the Ahringer library for those genes that allow growth to adulthood of non-GFP worms: those not carrying the *hT2[GFP let]* balancer chromosome are homozygous for *tm674*, yet have the lethality suppressed such that they can grow to adulthood. In a small pilot screen, we found a transcription factor, a serine protein kinase and two novel genes, which each act (when depleted via RNAi) as suppressors of *tm674* lethality. Additional progress on this research will be presented.

857B

Inhibition of TPA-1 protein kinase C by Enzastaurin: Implications for lung cancer and mesothelioma. **Shahid S. Siddiqui**, Sivakumar Lognathan, Ravi Salgia. Dept Med, Univ Chicago, Chicago, IL.

G-alpha 12/13 types of G-proteins play an important role in development, and are known to function as potent oncogenes when expressed in cultured cells. We have previously identified a homologue of Rho-guanine nucleotide exchange factor (GEF) in C. elegans (CeRhoGEF), which functions downstream of gpa-12, the C. elegans homologue of G alpha 12/13. CeRhoGEF contains a PSD-95/Dlg/ZO-1 domain and a regulator of G protein signaling (RGS) domain upstream of the Dbl homology-pleckstrin homology region similar to mammalian RhoGEFs with RGS domains, PSD-95/Dlg/ZO-1-RhoGEF and leukemia-associated RhoGEF (Yau et al., 2003). Ronald Plasterk and colleagues used suppressor screen for gpa-12 and identified suppressors in the tpa-1 gene that encodes two protein kinase C isoforms (TPA-1A and TPA-1B) that are homologous to PKC theta/delta (Tabuse et al. 1995; van der Linden et al., 2003). TPA-1 mediates the action of tumor promoter PMA and results in feeding defects and developmental arrest. In human cancers including lung cancer and mesothelioma, PKC function is closely associated with vascular endothelial growth factor receptor (VEGFR). The VEGF (vascular endothelial growth factor) and its receptor VEGFR are up-regulated in a variety of malignant tissues, including lung cancer and mesothelioma. Our results on malignant cell lines (H513, H28, H2461, H2373, H2596, and MSTO) show that PKC and VEGFR are overexpressed in lung and mesothelioma and also in tumor tissues. Inhibition of PKC by Enzastaurin inhibits the phosphorylation of PKC-beta 1 and 2 induced by rhVEGF, and Enzastaurin is under clinical trials for lung cancer. Expression of a reporter GFP under the tpa-1 promoter in C. elegans transgenic lines show expression of the reporter in nervous system sensory neurons, muscle cells, and somatic vulva, and this expression is reduced in animals exposed to Enzastaurin. This system provide high throughput screening platform for evaluating potential cancer drugs that mediate conserved signal transduction pathways across C. elegans and humans, and reveal novel genes that interact with PKC, VEGF and G-protein signaling. We thank J. Miwa and Y. Tabuse for discussion and strains.

858C

DNA binding activity of LIN-54 is essential for proper function of the DRM complex in transcriptional repression and development. **Tomoko M. Tabuchi[2,4]**, Bart Deplancke[1,4], M. Inmaculada Barrasa[1], Melissa M. Harrison[3], H. Robert Horvitz[3], Kirsten A. Hagstrom[2], Albertha J.M. Walhout[1]. 1) Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA 01605; 2) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA 01605; 3) Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; 4) authors contributed equally.

Some transcription factors are global regulators of many genes. A candidate of such a factor is LIN-54, which emerged as a “hub” in C. elegans gut and neuron transcriptional networks. LIN-54 is a member of the conserved DRM protein complex, which includes the tumor suppressor Rb (LIN-35) and the heterodimeric DNA-binding proteins E2F/DP (EFL-1/DPL-1). In fly and human, DRM regulates transcription of genes essential for cell cycle, DNA replication, and cell fate specification. In C.elegans, members of the DRM complex have multiple functions, including regulation of vulva development as part of the “synMuvB” set of chromatin factors. LIN-54 associates with chromatin; however, little is known about the molecular function of LIN-54 within the DRM complex, nor about the genome-wide distribution of LIN-54 and its effect on transcription of candidate target genes. Here, using chromatin immunoprecipitation (ChIP-chip), we show that LIN-54 is enriched at promoters of approximately 10% of protein-coding genes. This provides the first genome-wide distribution profile of a synMuv protein, and reinforces the idea that synMuv proteins associate with a broad range of targets outside vulva development. Gene ontology analysis indicates that LIN-54 associated genes function primarily in the cell cycle and development. We show that LIN-54 binds DNA in vitro and in vivo via its tesmin domain, a conserved cysteine-rich motif. Strains carrying a lin-54 tesmin domain mutation have an intact DRM complex, but DRM shows reduced association with target promoters, many genes involved in cell cycle and development are inappropriately upregulated, and developmental defects are observed. These results suggest that the DNA binding activity of LIN-54 is essential for the DRM complex to regulate gene expression, and imply that conserved DRM complexes may use both E2F and LIN-54 to recognize and bind targets. These studies illustrate that the global regulator LIN-54 anchors the DRM complex to DNA via its tesmin domain and is a critical coordinator of developmental gene expression programs in C. elegans.

859A

A *C. elegans* model of CFTR. **Mario F Neto[1,2]**, Susana M Garcia[3], M Catarina Silva[1,2], Richard I Morimoto[2], Margarida D Amaral[1]. 1) Dept Chemistry and Biochemistry, Faculty of Sciences, University of Lisboa, Portugal; 2) Dept Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL; 3) Dept Molecular Biology, Massachusetts General Hospital and Dept Genetics, Harvard Medical School, Boston, MA.

Cystic Fibrosis (CF) is a monogenic disease caused by mutations in the gene coding for the CF transmembrane conductance regulator (CFTR). This protein is a chloride channel expressed mainly at the apical membrane of epithelial cells. F508del, the most common mutation in CFTR, challenges the cellular proteome by alterations in its folding, leading to retention of the mutant protein in the endoplasmic reticulum (ER), translocation to the cytoplasm, and subsequent degradation by the proteasome. Expression of CFTR in *C. elegans* does not yield a phenotype which led us to develop a strategy using chimeric CFTR fusion proteins with a closely related member of the ABC transporter family, hMDR1 (Human Multidrug Resistance Protein 1 or P-gp) that confers resistance to drugs and heavy metals. This phenotype, for this CFTR-like protein, provides an assay to monitor folding and clearance in *C. elegans*. Replacement of the region in CFTR surrounding the F508 residue by a 9 amino acid region of the Nucleotide Binding Domain 1 (NBD1) of hMDR1 mimics the folding properties of wt- and F508del-CFTR in HEK 293 cells (Loo *et al*., 2002). Expression of this CFTR/MDR1 chimera in *C. elegans* defective for multidrug resistance [*pgp-1*(*pk17*) and *pgp-3*(*pk18*)] resulted in different arsenite resistance phenotypes. We have also initiated studies with chimeras expressed under the *vha-6* intestinal gene promoter, to direct the expression of the transgenes to an epithelial tissue. This will allow us to examine expression and cell biological properties in relevant tissue types using live cell imaging techniques. These lines will be used for genome-wide RNAi screens to identify modifiers of folding and stability of these CFTR/hMDR1 chimeras. The candidate genes will likely be involved in the folding and degradation of CFTR, revealing critical components of the systems maintaining organellar proteostasis, namely from the ER quality control.

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860B

Investigating the Role of the Dosage Compensation Protein DPY-21 as a Scaffold for the Recruitment of Chromatin Remodeling Complexes. **William S. Kruesi**, Barbara J. Meyer. HHMI / University of California, Berkeley.

The *Caenorhabditis elegans* dosage compensation complex (DCC) binds discrete sites along the X chromosome and reduces X-linked gene expression in XX hermaphrodites to equalize transcript levels with those of XO males. Studies are ongoing to uncover the mechanism by which the DCC acts. Recent immunoprecipitation and mass spectrometry experiments have suggested a physical interaction between the DCC and a number of chromatin remodeling complexes. One of the complexes identified is the Nucleosome Remodeling and Deacetylase (NuRD) complex. NuRD has both nucleosome remodeling and histone deacetylase functions that are associated with transcriptional repression. Studies from mammals have identified a conserved motif within several transcription factors that is necessary for NuRD recruitment to specific genes. This NuRD interaction motif is homologous to a stretch of amino acids found within the proline-rich N-terminal tail of the DCC protein DPY-21. It is known that DPY-21 is required for proper dosage compensation, but not for localization of the DCC to the X chromosomes. Using a combination of genetic, biochemical, and cell biological techniques I am investigating whether DPY-21 acts as a scaffold for recruiting NuRD and other chromatin remodeling complexes to *C.elegans* X chromosomes to facilitate dosage compensation.

861C

HTZ-1 Function is Important for Limiting the Dosage Compensation Complex to the X chromosome. **Emily L. Petty**, Alysse Cohen, Györgyi Csankovszki. Dept of MCDB, University of Michigan, Ann Arbor, MI.

Dosage compensation is a specialized form of gene regulation resulting in balance of sex-chromosome linked gene expression between sexes in species that utilize a chromosome-based sex determination strategy. In C. elegans, dosage compensation is achieved by activity of the dosage compensation complex (DCC). The DCC binds along both X chromosomes in the hermaphrodite to down-regulate gene expression by half, thus limiting X-linked gene products to levels produced in XO males. Sequence motifs enriched on the X chromosome play an important role in targeting the DCC resulting in a chromosome-specific enrichment of the complex, but other unknown factors are predicted to aid in targeting the DCC to the X chromosomes. One possible factor involved in the targeting step may be the chromatin environment of the X chromosome.

To investigate this possibility we have tested candidate chromatin-related genes for function in dosage compensation and found that reduction of H2A histone variant, *htz-1* impairs dosage compensation. By immunofluorescence, we observe reduced HTZ-1 levels on the dosage compensated X chromosomes, but do not observe such a reduction in the absence of dosage compensation. In *htz-1* knock-down animals we do not observe a reduction in expression of known dosage compensation components, arguing against the possibility that *htz-1* indirectly affects dosage compensation by regulation of dosage compensation genes. We do, however, observe a change in DCC localization upon knock-down of htz-1. At the resolution of single cells we observe an expansion of DCC localization beyond the territory of the X chromosomes as marked by fluorescent *in situ* hybridization (FISH). We propose that HTZ-1 may be an important regulator of dosage compensation by aiding in X-chromosome targeting of the DCC to the X chromosomes. The autosomal enrichment of HTZ-1 may serve as a DCC repellant and combined with the relative low HTZ-1 levels on the X chromosome, may help limit DCC binding to primarily the X chromosomes.

862A

Histone H4 lysine 16 acetylation and dosage compensation in *Caenorhabditis elegans*. **Michael Braxton Wells**, Gyorgyi Csankovszki. MCDB, University of Michigan, Ann Arbor, MI.

Dosage compensation is the process by which X chromosome gene expression is balanced between males and females or hermaphrodites. Mechanisms of dosage compensation vary between organisms. Dosage compensation has been well studied in mammals, flies, and worms; however, many mechanistic details of worm dosage compensation remain to be elucidated. Dosage compensation is achieved in *Caenorhabditis elegans* via two-fold downregulation of gene expression from both hermaphrodite X chromosomes by the Dosage Compensation Complex (DCC). The roles of chromatin modifications in mammalian and fly dosage compensation are well documented, but relatively little is known concerning the role of chromatin modifications in worm dosage compensation. Acetylation of histone H4 lysine 16 (H4K16-Ac) is an activating histone modification known to be responsible for the two-fold upregulation of gene expression required for dosage compensation in *Drosophila*. Our research examines potential roles for H4K16-Ac in worm dosage compensation using a combination of fluorescence microscopy and RNAi analysis. In wild type hermaphrodites, H4K16-Ac is underrepresented specifically on the X chromosomes in mixed stage embryos, gut nuclei, and other cell types. H4K12-Ac, which has been linked to transcriptionally silent β-heterochromatin, is present only at low levels on all chromosomes. Levels of two additional activating marks, H4K5-Ac and H4K8-Ac, appear similar on X chromosomes and autosomes. These data indicate that the depletion of marks associated with active transcription of the X chromosomes is specific to lysine 16. We are currently determining which histone acetyltransferase(s) and deacetylase(s) act at H4K16. We are also continuing to investigate the nature of the relationship between H4K16-Ac depletion and dosage compensation. Our data could suggest a method of dosage compensation in worms mechanistically opposite of that which acts in *Drosophila*.

863B

The T-box gene *tbx-2* is negatively regulated by the NF-Y complex and through autoregulation. **A.C. Milton**, P.G. Okkema. Department of Biological Sciences, Laboratory of Molecular Biology, University of Illinois at Chicago, Chicago, IL.

The *C. elegans tbx-2* gene encodes a conserved member of the T-box transcription factor family that is essential for development of ABa-derived pharyngeal muscles. The *tbx-2* promoter is active in pharyngeal precursors prior to formation of the pharyngeal primordium, as well as in a subset of neurons and in bodywall muscle. We are examining regulation of the *tbx-2* promoter to understand how pharyngeal muscle is specified in the ABa lineage. Based on results from a feeding RNAi screen of an expanded transcription factor library, we have found that *tbx-2* promoter activity is repressed by the NF-Y heterotrimeric CCAAT-binding complex. RNAi knockdown of the NFYA-1, NFYB-1 and NFYC-1 subunits results in strong ectopic expression of *tbx-2::gfp* in gut and seam cells. In comparison, RNAi knockdown of NFYA-2 results in relatively weak ectopic expression of *tbx-2::gfp*, suggesting an NF-Y complex containing this subunit plays a smaller role in repressing *tbx-2*. NF-Y is widely expressed, and we hypothesize it is a direct repressor of *tbx-2* expression. We also found that TBX-2 itself negatively regulates *tbx-2::gfp* expression. In both *tbx-2*(RNAi) and hypomorphic *tbx-2* mutants, *tbx-2::gfp* is strongly expressed in the gut and seam cells. TBX-2 is not normally expressed in the gut and seam cells, suggesting it indirectly represses its own expression by a cell non-autonomous mechanism. To identify additional mechanisms regulating *tbx-2* expression we are also characterizing the *tbx-2* promoter region. We have identified a *tbx-2* transcriptional enhancer that is necessary and sufficient for expression in the embryonic pharynx. This enhancer contains a number of sequences and consensus transcription factor binding sites that are conserved in other *Caenorhabditis* species, and we are currently examining the function of these sequences.

864C

Mutational and environmental variance and the transcriptional (?) control of phenotypic canalization in C. elegans. **Charles F. Baer**[1], Dee R. Denver[2]. 1) Department of Biology, Univ Florida, Gainesville, FL; 2) Department of Zoology Center for Genome Research and Biocomputing Oregon State University 2000 Cordley Hall Corvallis, OR 97331 USA.

The development of many phenotypic traits is canalized (robust) with respect to environmental variation, i.e., the same phenotype develops irrespective of the environmental circumstances in which the developing individual finds itself. Canalization is adaptive when natural selection favors the same phenotype in different environmental contexts. We have previously documented that spontaneous mutations de-canalize several phenotypic traits–fecundity, body volume, and vulval development–in a predictable way. Canalization of a phenotypic trait necessarily requires variability in some underlying mechanism, but the nature of the controls is rarely known. Here we present evidence that the accumulation of spontaneous mutations actually REDUCES environmental variance in gene expression. We compared transcript abundance of > 7000 genes in four lines of C. elegans that had accumulated mutations for ~280 generations ("MA lines") to that of the common (presumably unmutated) ancestor of those lines, using standard dye-swap microarray methodology. Contrary to our a priori expectation, MA lines exhibited significantly LESS environmental variance for transcript abundance than did their common ancestor. This unexpected result is consistent with what would be predicted if variability in gene expression provides the controlling mechanism underlying phenotypic canalization. These results must be considered highly preliminary for several reasons, which we discuss, but a plausible mechanism underlying phenotypic canalization is obvious.

865A

Laser-Capture Microdissection and Microarray Profiling of *C. elegans* Male Tail Tip Morphogenesis. **R. Antonio Herrera**, Sindhoora S, David H. A. Fitch. Center for Developmental Genetics, Dept Biology, New York University, New York, NY.

Regulation of *C. elegans* male tail tip morphogenesis involves the input of several distinct molecular pathways. The heterochronic [1], Wnt signaling [2], Hox patterning [4] and sex determination [3] pathways each contribute to the proper development of the male tail tip syncytium. Mutations in certain genes result in unretracted adult male tail tips. Our lab and others have confirmed the intersection between these molecular pathways by examining the expression of transgenic reporters in various mutant backgrounds [3]. Our current understanding of the genetic network is limited, as many of these interactions are indirect.

To identify candidate genes that are involved in tail tip retraction, we are performing a microarray analysis of gene expression in tail tips isolated from synchronized males and hermaphrodites prior to male L4 tail tip morphogenesis. We are using laser-capture microdissection to obtain tail tips, from which total RNA is isolated for hybridization onto Affymetrix *C. elegans* GeneChips. The tail tip lends itself particularly well to analysis of post-embryonic gene expression in specific somatic cells because the four tail tip cells can be removed by a single slice perpendicular to the anterior-posterior axis. Work is in progress to identify genes that are differentially expressed and/or change in expression profile from late L3 to middle L4. To confirm their role in morphogenesis, we will examine tail tip phenotypes in RNAi knockdowns or mutants of these genes.

[1] Del Rio-Albrechtsen et al. 2006, Dev. Biol. 297:74.
[2] Zhao et al. 2002, Development 129:1497.
[3] Mason et al. 2008, Development 135:2373.
[4] see MD Nelson Abstract.

866B

Valproic acid affects expression of specific metabolic genes in C. elegans. **Marketa Kostrouchova**[1], Marta Kostrouchova[2], Zdenek Kostrouch[1]. 1) Laboratory of Molecular Pathology, Inst Inherited Metab Disorders, Charles Univ, Prague, Czech Republic; 2) Laboratory of Molecular Biology and Genetics, Inst Inherited Metab Disorders, Charles Univ, Prague, Czech Republic.

Valproic acid (VPA, 2-propylpentanoic acid), a classical drug widely used in the treatment of bipolar disorder and epilepsy was recently shown to have anti-proliferative and differentiation inducing effect on cancer cells both in vitro and in vivo. This effect is connected with the inhibitory effect of VPA on histone deacetylases of class I and class II. We examined the effect of 1 and 5 mM VPA on larval development and transcription profile in genome wide DNA microarrays. Although developmental defects including body morphology changes and dpy phenotypes were observed at high concentration of VPA, most larvae were able to develop without a morphological phenotype. The most pronounced effect of VPA was the delay in developmental timing of larval stages. Analysis of the most dramatically upregulated genes indicated pronounced changes in the transcription of metabolically active genes including enzymes, p450 genes, transporters and an uncharacterized putative metabolism-related kinase. We are currently exploring the possible link between the developmental timing and the effect of VPA on the transcription of metabolically active genes. Acknowledgement: We thank Dr. M. W. Krause for support and advice. We thank the NIDDK Microarrays facility for performing the microarrays analysis. The work was supported by the grant 304/08/0970 and 304/07/0529 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

867C

C. elegans supplementary nuclear receptors characterized by the P box sequence CNGCKT regulate development of C. elegans. **Marta Kostrouchova**[1], Katerina Simeckova[1], Michal Pohludka[2], Zdenek Kostrouch[2]. 1) Laboratory of Molecular Biology and Genetics, Inst Inherited Metabolic Dis, Charles Univ, Prague 2, Czech Republic; 2) Laboratory of Molecular Pathology, Inst Inherited Metabolic Dis, Charles Univ, Prague 2, Czech Republic.

Multiplied nuclear hormone receptors (NHRs) have similarities in functional domains in the DNA binding as well as in the putative ligand binding domains. The sequence analyses indicate that the mutational changes are propagated to further multiplied and diversified genes. From the total number of 289 sequences that are recognized by sequence analysis as potential NHRs, 18 sequences are characterized by the P-box (the DNA binding domain protein sequence CNGCKT). In this project, we further characterized 12 nuclear receptors that belong to this group of NHRs: nhr-1, nhr-10, nhr-17, nhr-47, nhr-68, nhr-101, nhr-116, nhr-120, nhr-129, nhr-137, nhr-141, and nhr-168. We show that all analyzed genes are expressed and have a highly diversified pattern of expression. Surprisingly, half of the studied genes show developmental phenotypes in RNAi experiments at standard laboratory conditions. This indicates that multiplied NHRs are indispensable for normal C. elegans development and supports the concept that diversified NHRs were introduced into the nematode genomes for regulation of specific developmental functions. Acknowledgement: We thank Drs. A. Fire for vectors and the host used in RNAi and M.W. Krause for support and advice. The work was supported by grants 304/08/0970 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

868A

GEI-8: Possible nuclear hormone receptor corepressor in C. elegans. **Pavol Mikolas**[1], zdenek Kostrouch[2], Marta Kostrouchova[1]. 1) Laboratory of Molecular Biology and Genetics, Inst Inherited Metab Disorders, Charles Univ, Prague, Czech Republic; 2) Laboratory of Molecular Pathology, Inst Inherited Metab Disorders, Charles Univ, Prague, Czech Republic.

N-CoR and SMRT co repressors are vertebrate proteins that mediate transcriptional repression by nuclear hormone receptors. This occurs through assembly of the repressor complex composed of nuclear receptors, HDACs and other components. Interaction of N-CoR and SMRT with HDACs determines the chromatin remodeling function of the repression complex. gei-8 contains a region homologous to SANT domain which is the HDAC interacting domain in N-CoR and SMRT. We were able to clone a large part of predicted isoform A containing the SANT domain. Isoforms B and C were not confirmed due to the lack of expression at the predicted start. GFP expression from predicted promoters is found predominantly in neurons. Analysis of gei-8 mutant line VC1213 revealed a severe developmental phenotype marked by progressive locomotion defect, low pharynx pumping, structural defects of intestine and germline and most significantly L3/L4 arrest resulting in infertility. Increased sensitivity to acetylcholinesterase inhibitor Aldicarb suggests that impaired synaptic transmission causes impaired locomotion and feeding defect. Our data suggest that gei-8 has an indispensable role in development and may possibly act as a co repressor. Further experiments will be targeted at its effects on transcriptional regulation. Acknowledgement: We thank Drs. A. Fire for vectors and host used in RNAi and M.W. Krause for support and advice. The work was supported by grants 304/08/0970 and 304/07/0529 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic. We thank the C. elegans Gene Knockout Consortium for providing us with the VC1213 mutant line.

869B

Ultra-high throughput sequencing of amplified transcripts from individually dissected cells. **Erich M. Schwarz**[1], Miriam B. Goodman[2], Ali Mortazavi[1], Brian A. Williams[1], Lorian Schaeffer[1], Mihoko Kato[1], Martin Chalfie[3], Barbara J. Wold[1], Paul W. Sternberg[1,4]. 1) Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125; 2) Department of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305; 3) Department of Biological Sciences, Columbia University, New York, NY 10027; 4) Howard Hughes Medical Institute, Pasadena, CA 91125.

Gene expression in individual cell types of *C. elegans* has been studied through microarray analysis of populations, either by sorting of GFP-labelled embryonic culture cells or by precipitation of tagged poly(A)-binding proteins. Ultra-high throughput sequencing of cDNA (RNA-seq) allows gene expression data to be quantitated and mapped across the genome with unprecedented precision, and onto unannotated transcripts or splicing isoforms; amplification of transcripts from individually dissected cells allows these data to be generated from cells in their native context (rather than on a culture dish) and from single cells at precise moments of their development (e.g., from a migrating postembryonic cell). We are therefore developing RNA-seq on postembryonic neurons. Our preliminary data from the AFD, ASER, and PLML neurons indicate that this technique is usable for transcript discovery: ~11,000 protein-coding genes show expression in at least one isolate, and transcripts known to be representative of these neuron types, such as *gcy-8*, *gcy-19*, and *mec-7* are strongly expressed. Globally, though, our individual data sets are quite noisy: for multiple isolates of a given cell type, activity for only ~500+ genes is repeatedly observed, and overall variability of observed expression is high (r-squared ≤ 0.4). To overcome this, we are testing improved amplification techniques and small pools of cells.

870C

Pigment Dispersing Factor in *C. elegans*: a combined full genome and proteome study. **Liesbet Temmerman**, Annelies Bogaerts, Lies Franssens, Dries Cardoen, Ellen Meelkop, Tom Janssen, Liliane Schoofs. K.U.Leuven, Leuven, Belgium.

Innumerate organisms rely on internal biological clocks in order to prepare themselves for periodic environmental changes. The best studied and perhaps most appealing molecular timekeeper is the circadian clock, which keeps track of the day-night rhythm. In invertebrates, Pigment Dispersing Factor (PDF) is the key molecule that links the circadian clock to a fit output. Deletion of the *pdf* gene results in animals with aberrant rhythmic behaviour.

Despite the discovery of *C. elegans* daily rhythmic behaviour, its molecular basis remains elusive, and might even be fundamentally different from the currently known timekeeping mechanisms. Our lab has been able to identify two *pdf* genes in *C. elegans*, which could be coupled to locomotor activity. It has however never been shown that *C. elegans pdf* influences the circadian activity rhythm. It is therefore likely that unravelling the PDF-system in *C. elegans* will provide insight into a more primitive or basic function of this neuropeptide.

To assess the molecular effects of the *pdf-1* gene, we opted for a combined full genome and proteome study in which a *pdf-1* null mutant was compared to wild type worms. After RNA isolation, transcript levels were investigated by means of microarray analysis and validated by real-time PCR. Protein isolates were labelled and separated by 2D-DIGE. Differentially expressed proteins were excised from the gels, trypsin digested and identified by Peptide Mass Fingerprint after MALDI-TOF.

Using the discriminating power of these techniques, we were able to identify a limited set of differentially transcribed and/or expressed genes; thereby providing a more complete insight into the *C. elegans* PDF-system.

871A

A High Resolution Map of C. elegans Gap Junction Proteins. Zeynep F. Altun[1], Bojun Chen[2], Zhao-Wen Wang[2], **David H. Hall**[1]. 1) Ctr C Elegans Anatomy, Albert Einstein Col Med, Bronx, NY; 2) Dept of Neuroscience, U. Conn. Health Center, Farmington, CT.

The innexin family of gap junction proteins has 25 members in C elegans. We analyzed in vivo expression patterns of the entire family at various developmental stages by expressing GFP under the control of innexin-specific promoters. Innexins are differentially expressed in embryos, larvae and adults, and are found in virtually all cell types and tissues, including early blast cells, during tissue morphogenesis, and in most larval and mature tissues. Complex tissues such as the pharynx express many different innexins in overlapping or complementary patterns, which may be important in their organization and function. Several innexins are co-expressed in the same cell in many instances, suggesting that they may form heteromeric and/or heterotypic channels. Some innexins are expressed in cells that are not known to have morphologically defined gap junctions (motile distal tip cell, anchor cell, sperm), suggesting that they may be involved in signaling by forming hemichannels. The highly complex patterns of expression, in combination with the known broad distribution of gap junctions from anatomical studies, suggest that innexins likely play roles in almost all body functions, including embryonic development, cell fate determination, oogenesis, egg laying, pharyngeal pumping, excretion and locomotion. Supported by NIH RR12596 (to DHH) and NSF 0619427 and NIH GM083049 (to ZWW).

872B

RNA expression analysis of the LIM-homeodomain gene, *lim-7*, in the life cycle of *Caenorhabditis elegans* using FISH. **Tomasz J. Jodlowski**, Laura G. Vallier. Biology Department, Hofstra University, Hempstead, NY.

LIM-homeodomain (LIM-HD) proteins comprise an evolutionarily conserved family of transcription factors, whose members are characterized by two N-terminal LIM domains and a homeodomain located C-terminally to them. One the six subclasses of LIM-HD proteins is defined by transcription factor, Islet1, which was identified in a screen for proteins that bound to the insulin gene enhancer in the β cells of the rat pancreas. Among other functions, Islet1 is necessary for motor neuron differentiation and function and for heart and pancreas organogenesis, in diverse organisms. In *C. elegans*, LIM-7 is the sole LIM-HD Islet subclass member; it is expressed in the gonadal sheath, muscle cells, and motor neurons, among other tissues. An in-frame deletion of the majority of both LIM domains in *lim-7* (*tm674*) results in lethality during the L1 stage. Rescue of *tm674* restores viability but the resulting adult hermaphrodites are sterile. To date it is not known whether the transcript is maternally provided or zygotically transcribed. In this study, the RNA profile of *lim-7* is being determined. In addition, since mouse Islet isoforms have been identified, the presence of any *C. elegans lim-7* isoforms is being sought from available cDNA libraries. Using the amplified PCR product of the cDNA(s) found, a fluorescent probe will be created to allow the in-situ visualization of the mRNA expression patterns and of any isoforms if found. FISH expression patterns of *lim-7* mRNA in early embryos will determine maternal or zygotic contribution. Ongoing results will be presented at the meeting.

873C

Functional Analysis of Sp Transcription Factors in *Caenorhabditis elegans* . **Eva Krpelanova**, Suzanne Rademakers, Sjaak Philipsen, Gert Jansen. Dept of Cell Biol & Genetics, Erasmus MC, Rotterdam, Netherlands.

Sp (specificity protein) transcription factors are important regulators of many cellular processes, such as cell cycle, metabolism and morphogenesis. The Sp family is united by a specific combination of three conserved Cys2His2 zinc fingers that form the DNA-binding domain and a Buttonhead (BTD) box CXCPXC, just N-terminal to the zinc fingers. There are nine Sp genes both in humans and in mice. C. elegans has three Sp-related transcription factors: *sptf-1, sptf-2* and *sptf-3*.

Our aim is to characterize the functions of these three transcription factors and to find some of their interacting partners. Therefore we have made GFP fusion constructs to determine their expression patterns and we set out to analyze the phenotypes of knockout, knockdown and transgenic animals carrying additional copies of a particular sptf-gene.

The gene structure of *spft-1* has been confirmed previously. *sptf-1::GFP* expression is first observed in late embryos; from L1 to adult GFP is expressed in the intestine, rectum, 3 neurons in the head and from L3 onwards in the vulva. *sptf-1(tm784)* knockout animals are homozygous viable, but with a low rate of survival until adulthood. We rescued the phenotype in knockout animals by injecting a low dose of *sptf-1*. RNAi experiments show no obvious phenotype. High level of overexpression of *sptf-1*, *sptf-1XS*, caused effects on movement and body morphology. *sptf-2* gene structure has been confirmed. *sptf-2* is expressed post embryonically in the intestine, body muscles, and two neurons in the head. *sptf-2(tm1130)* knockouts are viable, no obvious phenotype was observed. RNAi experiments show no obvious phenotype. High levels of *sptf-2* overexpression is probably lethal since F1 *sptf-2XS* animals did not produce viable transgenic progeny. We have identified two splice forms of *sptf-3*, one confirms the prediction, the second splice variant has three additional upstream exons. *sptf-3::GFP* is expressed ubiquitously in early embryos and at the comma stage; from late embryos to adults GFP is restricted to the intestine, neurons of the ventral and dorsal nerve cords as well as two neurons in the head close to the posterior pharyngeal bulb. *sptf-3(tm607)* knockout animals are lethal, 20% embryonically, 77% as L1, 3% as L2. RNAi screens of *sptf-3* show a spectrum of mutant phenotypes, comprising embryonic lethality, maternal sterility, abnormal both body morphology and locomotory behaviour. We have observed growth defects in *sptf-3XS* transgenic animals.

Currently we are setting up a screen to identify suppressors of sptf-induced lethality.

874A

Lipid Synthesis Targets of *C. elegans* SREBP. **Monika Tzoneva**, Kyle Ann Brooks, Jennifer Watts. School of Molecular Biosciences, Washington State University. Pullman, WA 99164.

We have used expression microarrays to examine on a genome-wide scale the range of targets regulated by SBP-1, the *C. elegans* homolog of SREBP1 and SREBP2 (the Sterol Response Element Binding Proteins). In mammals, SREBP1 and SREBP2 have been well established as central to the regulation of cholesterol and fatty acid homeostasis.

Lipid analysis of *sbp-1*(RNAi) and *sbp-1* mutants has revealed low levels of triacylglycerides, and a range of fatty acid composition defects. In order to find the genes that act downstream of *sbp-1*, we used the Affymetrix genomic microarray to perform comparisons between gene expression levels of the following strains: wild-type; *sbp-1(ep79)*, a strong loss-of-function allele; and *epEx307*, a strain carrying a stable extrachromosomal array of SBP-1:GFP. As expected, the most significant differences were found between *sbp-1(ep79)* and *epEx307*: 530 genes were downregulated in *sbp-1(ep79)*, while 342 were upregulated. Among the downregulated targets are a number of genes involved in fatty acid synthesis, desaturation, elongation and beta oxidation, indicating that the *C. elegans* SBP-1 acts at least partially on targets similar to those of its mammalian homologs.

We further analyzed a subset of 40 of the downregulated genes using RNAi, followed by a number of phenotypic assays, and chose to concentrate on F22E10.5, a choline/ethanolamine phosphatidyltransferase (CEPT) and C31E10.7, a cytochrome b5.

Depletion of C31E10.7(cytochrome b5) by RNAi causes very high levels of stearic acid, and low levels of polyunsaturated fatty acids, a phenotype similar to that of the *fat-6; fat-7* double mutant, which is deficient in stearoyl-CoA desaturase (SCD) activity. Because cytochrome-b5 mediated electron transfer is necessary for the desaturation reaction mediated by *fat-6* and *fat-7*, we propose that C31E10.7 encodes the cytochrome b5 that acts in concert with one or both of these genes.

Inhibition of F22E10.5 (CEPT) by RNAi leads to low levels of phosphatidylcholine (PC), and marginally lower levels of phosphatidylethanolamine (PE). The fatty acid profile is similar to that of the three other genes predicted to be involved in the PC synthesis pathway: F08C6.2 (CTP-phosphocholine cytidylyltransferase), *cka-1*, and *cka-2* (choline kinases). Interestingly, RNA inhibition of F08C6.2 leads to very strong nuclear localization of SBP-1:GFP, a step required for activation. Taken together, these results identify a novel role for SBP-1 in PC biosynthesis.

875B

Genome-wide RNAi screen for regulators of lipid homeostasis in *C. elegans*. **Neal A. Dach**[1], Veerle Rottiers[1,2], Anne C. Hart[1,3], Anders M. Näär[1,2]. 1) Cancer Center, Massachusetts General Hospital, Boston, MA; 2) Dept. of Cell Biology; 3) Dept. of Pathology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA.

Metabolic syndrome, which occurs in about 47 million Americans, is characterized by a group of risk factors including high cholesterol, hypertension, abdominal obesity, and insulin resistance. Metabolic syndrome is thought to largely result from lifestyle factors, such as excess energy intake and decreased exercise, however the underlying molecular mechanisms are still poorly understood. Defects in the regulation of fatty acid and cholesterol homeostasis may be involved in the development of metabolic syndrome. The SREBP family of transcription factors regulates genes responsible for fatty acid and cholesterol synthesis and uptake. SREBP activates gene expression by recruiting the chromatin-targeting CBP/p300 acetyltransferases and the ARC/Mediator complex which in turn recruits RNA Polymerase II. This mechanism of activation is conserved in the nematode *C. elegans* and depletion of the nematode SREBP ortholog *sbp-1* or *mdt-15* (ortholog of the ARC105 ARC/Mediator subunit) results in decreased polyunsaturated fatty acid and triacylglyceride levels as well as a buildup of stearic acid. The expression of *fat-6* and *fat-7*, two stearoyl-CoA desaturases (SCDs) which convert stearic acid to oleic acid, is decreased in *sbp-1* and *mdt-15* mutants. Animals lacking *fat-7, fat-6, sbp-1* and *mdt-15* show similar phenotypes. Dietary supplementation with oleic acid significantly ameliorates their defects, indicating that the SCDs are physiologically important targets of SBP-1 function. To identify novel regulators of SCDs and lipid homeostasis in general, we have performed a genome-wide RNAi screen for modifiers of *fat-7* expression. Employing the COPAS Biosorter, we have identified genes whose RNAi knockdown increases or reduces the expression of a *fat-7* promotor::*gfp* fusion reporter construct. We find that RNAi of *sbp-1* and *mdt-15* reduces p*fat-7*::*gfp* expression, serving as positive controls for GFP expression reduction. RNAi of *fat-6* and *fat-7* results in up-regulation of the p*fat-7*::*gfp* reporter due to a compensatory mechanism, providing positive controls for GFP expression increase. Finally, we used *unc-22* RNAi and L4440 empty vector as negative controls. We are now retesting and assessing the specificity of about 1,200 putative hits from the genome-wide screen. Our unconfirmed list of modifiers includes expected hits: *sbp-1, nhr-49, cbp-1, fat-7*, and C31E10.7, a putative cofactor for FAT-7, suggesting that functionally important genes will be identified. We expect many of the genes that emerge from this screen to expand our understanding of how animals maintain lipid homeostasis.

876C

Genomic survey for zinc transport proteins and transcriptional regulation of zinc homeostasis in *C.elegans*. **Krupa Deshmukh**, Kerry Kornfeld. Developmental Biology, Washington University in Saint Louis, Saint Louis, MO.

Zinc is a vital trace element required by all organisms. Extreme levels of dietary zinc lead to disorders including *acrodermatitis enteropathica*, a severe case of skin inflammation in humans. Organisms have evolved mechanisms to alleviate adverse effects of high and low zinc levels. We use *C.elegans* as model system to investigate mechanisms of zinc homeostasis by identifying the proteins involved and also by examining transcriptional changes of genes associated with dietary zinc variations. Zinc transport proteins in *C. elegans* were identified by surveying its genome, for putative members of two key zinc transport protein families, namely Cation Diffusion Facilitator (CDF) and Zrt/Irt-like Protein (ZIP). We identified 14 CDF and 14 ZIP family proteins in *C.elegans*, which includes novel CDF and ZIP proteins in addition to those reported by previous studies. Putative CDF and ZIP proteins were classified based on protein sequence similarity with orthologs from other eukaryotes. This classification suggests presence of *C.elegans* ortholog for every human CDF and ZIP family protein. Transcriptional changes associated with dietary zinc variations have not been systematically studied in *C.elegans*. We examined changes in mRNA level of candidate genes in response to varying levels of dietary zinc. The genes analysed include *ttm-1*, a member of CDF family and two metallothionein genes (*mtl-1* and *mtl-2)*. Metallothioneins in most eukaryotes chelate excess zinc, cadmium and copper. By manipulating dietary zinc levels in fully defined chemical media (*C.elegans* Maintenance Media [CeMM]), and by using Quantitative Real Time PCR (qPCR) we analysed relative abundance of the gene transcripts . We find that abundance of *ttm-1* transcript increases up to 5-fold with 1000-fold increase in dietary zinc. Increased levels of *ttm-1* transcripts at high zinc level is consistent with role of CDF genes in reducing excess levels of cytosolic zinc. mRNA levels of *mtl-1* and *mtl-2* increased up to 100- and 1000-fold respectively with 1000-fold increase in dietary zinc. Metallothionein genes in *C.elegans* therefore respond to zinc stress similar to that observed in higher eukaryotes. We generated transgenic worms carrying 5'-upstream regions of *mtl-1* and *mtl-2* fused to GFP. Green fluorescence signal was induced in worms upon zinc supplementation in their diet. Increased levels of *mtl-1* and *mtl-2* mRNA is therefore due to upregulation of their transcription under high zinc conditions. We are analysing potenitial regulatory elements responsible for zinc mediated induction of metallothionein genes. By microarray analysis we are further examining whole genomic transcriptional profiles of *C.elegans* under varying conditions of dietary zinc.

877A

NHR-49 cooperates with multiple partners to selectively modulate distinct aspects of lipid metabolism. **Pranali P. Pathare**[1,2], Tessie Ng[1,2], Marc Van Gilst[1]. 1) Dept Basic Sci, Fred Hutchinson Research Ctr, Seattle, WA; 2) Molecular and Cellular Biology, University of Washington-Seattle.

HNF4α, a human nuclear receptor transcription factor that is involved in diabetes, governs the expression of a wide range of metabolic genes in different tissues. The C.elegans homolog of HNF4α is NHR-49 which has been demonstrated to coordinate fatty acid beta-oxidation and desaturation. In a gene expression analysis, we found that NHR-49 is also essential for repressing the expression of genes predicted to participate in sphingolipid processing and lipid remodeling. Thus, NHR-49 coordinately influences several genes in multiple fat metabolism pathways, through both activation and repression. This differential regulation of NHR-49 modules led us to propose a model whereby NHR-49 controls its different sectors of lipid metabolism by interacting with specific transcriptional cofactors. We evaluated potential NHR-49 interacting proteins (ref: Brock, T and Watts, J et al. and Taubert, S et al.) for their impact on global NHR-49 gene activation or repression. We found 4 potential co-factors that controlled distinct NHR-49 target genes: NHR-66, NHR-80, NHR-13 and MDT-15. Notably, we found that knockout of nhr-66 specifically abrogated NHR-49 repression of sphingolipid and lipid remodeling targets. In contrast, deletion of nhr-66 did not impact NHR-49's activation of beta-oxidation and desaturase genes. Our findings thus support a model whereby NHR-49 collaborates with NHR-66 to regulate a system of genes involved in modulating the glycosphingolipid and phospholipid membrane composition. In addition, we found that knockout of nhr-80 and nhr-13 affected the expression of NHR-49-regulated fatty acid desaturase genes, but had no impact on any of its other gene targets. Thus, NHR-49 partners with NHR-80 and NHR-13 to regulate the ratio of saturated and unsaturated fat in lipid membranes. Therefore, in characterizing NHR-49's transcriptional network, we found that the control of distinct NHR-49 regulatory modules is based on NHR-49's association with distinct interacting partner proteins.

878B

IDENTIFICATION AND CHARACTERIZATION OF A HEME RESPONSIVE ELEMENT IN THE hrg-1 PROMOTER. **J Sinclair**, I Hamza. Department of Animal and Avian Sciences, University of Maryland, College Park, MD.

Despite its biological significance, little is known about how animals sense and respond to heme to maintain homeostasis. C. elegans is a heme auxotroph, which makes it an excellent model to identify and dissect heme homeostasis pathways. Using C. elegans we have identified HRG-1, a vesicular heme transporter that is transcriptionally upregulated when environmental heme is low. The current study seeks to address how hrg-1 is regulated by heme. Here, we show that a putative 23 base pair (bp) heme-responsive element (HRE) and GATA-binding motifs are necessary for heme-dependent regulation of hrg-1. The HRE comprises both enhancer and repressor elements and works in conjunction with ELT-2 to regulate hrg-1 expression. We propose that the HRE could be used as a molecular tool in C. elegans to tightly regulate internal gene expression by modulating environmental heme. Our ultimate goal is to identify the transacting factor to eventually create a whole animal sensor for monitoring organismal heme homeostasis.

879C

Conserved SREBP Gene Regulatory Mechanisms Controlling Lipid Homeostasis During Feeding and Fasting. **Amy K. Walker**[1], Fajun Yang[1,2], Karen Jiang[1], Jun-yuan Ji[1,3], Jennifer Watts[4], Joseph Rodgers[5], Pere Puigserver[5], Nicholas Dyson[1,3], Anne Hart[1,3], Anders Näär[1,2]. 1) MGH Cancer Center, Charlestown, MA; 2) Dept. of Cell Biology, Harvard Medical School, Boston, MA; 3) Dept. of Pathology, Harvard Medical School, Boston, MA; 4) School of Molecular Biosciences, Washington State University, Pullman, WA; 5) Dana-Farber Cancer Institute, Boston, MA.

Human metabolic disorders such as obesity and metabolic syndrome are linked to changes in fatty acid or cholesterol biosynthesis; processes controlled by the sterol regulatory element binding protein (SREBP) transcription factors. To study SREBP function *in vivo*, and to delineate SREBP transcriptional functions, we have examined SREBP (SBP-1) gene regulatory mechanisms in *C. elegans*, combined with complementary mechanistic studies in mammalian cell lines.

SREBP activates genes important in cholesterol, fatty acid (FA) and phospholipid biosynthetic (PL) pathways and in production of co-factors such as Ac-CoA and NADPH. We have identified a novel set of SREBP-responsive genes in *C. elegans* and mammals, the methyl-group producing enzymes of the 1-carbon cycle (1-CC). We hypothesize SREBP regulates these genes because PL biogenesis requires methylation. Interference with the 1-CC in *C. elegans* disrupts lipid homeostasis, resulting in intestinal lipid accumulation. Importantly, this phenotype resembles lipid accumulation in human fatty liver disease. Co-regulation of the 1CC and lipid homeostasis may be a common impact point in metabolic disease.

We have also investigated mechanisms of SREBP inhibition during fasting and found that SIRT1(*sir-2.1*) limits fat production through direct deacetylation of SREBP, attenuating SREBP-dependent expression of FA biosynthetic genes. Furthermore, we found that *C. elegans* lacking functional *sir-2.1* (SIRT1), or treated with sirtuin inhibitors, retain fats during fasting. SBP-1 targets such as FA desaturase *fat-7* are inappropriately expressed during fasting in *sir-2.1(lof)* animals or after treatment with sirtuin inhibitors. These mechanism are conserved; fasted Drosophila larvae lacking dSir2 (*sir-2.1*) fail to downregulate dSREBP-dependent genes and retain fat body lipids. SREBP target gene expression increases after RNAi depletion or inhibition of SIRT1 in human cells and after SIRT1 depletion in murine liver during fasting. Thus, SIRT1/SIR-2.1/Sir2 plays an important conserved role in lipid homeostasis by shutting down SREBPs during fasting. These studies highlight the complex relationships between metabolic pathways in humans; delineating common control points will be key for designing treatments.

880A

Conserved transcription factor networks link fatty acid and one carbon metabolism. Karen Jiang[1], Veerle Rottiers[1], Jennifer Watts[2], Anne Hart[1,3], Anders Näär[1,4], **Amy Walker**[1]. 1) MGH Cancer Center, Charlestown, MA; 2) School of Molecular Biosciences, Washington State University, Pullman, WA; 3) Department of Pathology, Harvard Medical School, Boston, MA; 4) Department of Cell Biology, Harvard Medical School, Boston, MA.

Human metabolic disorders such as cardiovascular disease, obesity and metabolic syndrome are linked to changes in fatty acid or cholesterol biosynthesis; processes controlled by the sterol regulatory element binding protein (SREBP) family of transcription factors. To examine SREBP function *in vivo*, and to delineate SREBP transcriptional functions, we have examined SREBP (SBP-1) gene regulatory mechanisms in *C. elegans*, combined with complementary studies in mammalian cell lines.

SREBP activates genes important for cholesterol, fatty acid and phospholipid biosynthetic pathways, in addition to ensuring production of co-factors such as Acetyl-CoA and NADPH. We have identified a novel set of SREBP-responsive genes in *C. elegans*, the methyl-group producing enzymes of the 1-carbon cycle (1CC). Multiple genes in this pathway, including *sams-1*(MAT1A) are responsive to SBP-1/SREBP in both *C. elegans* and mammalian cells. *sams-1*/MAT1A encodes a s-adenosyl methionine transferase, which provides the methyl donor (SAMe) for most methylation reactions. We hypothesize these genes are important components of SREBP function because methylation is necessary for phospholipid biogenesis, particularly the synthesis of phosphatidylcholine. Interference with the 1CC in *C. elegans* through RNAi or in *sams-1(ok3033)* disrupts lipid homeostasis, resulting in the accumulation of fat droplets in the intestine and body cavity. Importantly, this phenotype is highly reminiscent of lipid accumulation in fatty liver disease in humans and similar to the MAT1A KO in mice (Lu et al PNAS, 2001). Furthermore, we have found that phosphatidylcholine levels are low in *sams-1(RNAi)* animals suggesting that defects in phospholipid metabolism could contribute to the phenotypes of these animals. SInce *sbp-1* is necessary for fat storage, it is surprising that a SBP-1 regulated gene causes increases in lipids. We have found that several SBP-1 target genes that also important for lipid metabolism (fatty acid desaturases *fat-5*, *fat-6* and *fat-7*) are strongly upregulated in *sams-1(RNAi)* animals. In addition, *sbp-1* and the FA desaturases are necessary for lipid accumulation in *sams-1(RNAi)* animals, suggesting that changes in 1C metabolism could impact key aspects of lipid biosynthesis through SBP-1. Alterations in 1C metabolism and SREBP function are associated with similar diseases, suggesting that co-regulation with lipid homeostasis may be a common impact point in metabolic disorders.

881B

A novel antibiotic selection system for nematode transgenesis. **R. Giordano**[1], S. Milstein[2], N. Svrzikapa[2], M. Vidal[2], D. Dupuy[1]. 1) Genome Regulation and Evolution, Institut Europeen de Chimie et Biologie, Pessac, France; 2) Center for Cancer Systems Biology (CCSB) and Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Genetics, Harvard Medical School, Boston, Massachusetts 02115, USA.

The generation of transgenic animals has been instrumental to study many biological aspects of C. elegans biology. Transgenic animals can be obtained by either microinjection or ballistic bombardment. Both techniques rely on the use of genetic markers to facilitate the recovery of transformed animals. Most commonly used markers include dominant phenotype markers such as *rol-6*, and rescue of non-lethal mutations (*dpy-5*, *unc-119*). These systems have inherent limitations: *rol-6* dominant mutation markers can be used on wild-type animals but the obtained transgenic strains have phenotypic defects that need to be manually selected for strain maintenance. *dpy-5* rescued strains are phenotypically wild-type, which can be advantageous for some studies, but also needs manual maintenance. *unc-119* rescued animal finally have the added advantage of providing the transgenic animals with a selectable advantage in starvation conditions.

We developed a nematode expression vector carrying the neomycin resistance (neoR) gene as a selection marker. This gene confers resistance to geneticin, an antibiotic that normally inhibit protein synthesis in eukaryotes and is lethal for wild-type animals. Here we show that the neoR marker is a potent tool that allows hands-off maintenance of populations of transgenic worms on geneticin plates. This system does not imply any prerequisite on the original genotype of the recipient strain and can therefore be used on mutants lines as well as transgenic strains obtained with common markers.

Moreover, we placed the neoR gene under the control of the *rps-27* promoter, a highly conserved ribosomal protein throughout the nematode phylogeny. Therefore, we expect this vector to permit transformation of a wide variety of nematode species, thus allowing for gene expression conservation and evolution studies.

882C

Developing a reversible, cell-specific system for inhibiting protein synthesis. Young Eun Choi[1], **Maxwell G. Heiman**[2], Valeri J. Thomson[3], Shai Shaham[2]. 1) Bard College, Annandale-on-Hudson, NY; 2) Rockefeller University, New York, NY; 3) Bard High School Early College II, Elmhurst, NY.

The ability to block protein synthesis in a tissue- and time-specific manner would be a powerful tool for studying the role of a given cell type in a particular biological process. To begin establishing such a system, we asked whether protein synthesis in *C. elegans* could be reversibly inhibited by cycloheximide, an antibiotic that binds to eukaryotic ribosomes and blocks amino-acid chain elongation. We found that first larval stage (L1) animals synchronized by starvation-induced arrest and exposed to 800 mg/L CHX remained at the L1 stage for more than a week despite the presence of food. Presumably, this reflects a requirement for new protein synthesis for exit from the L1 arrest. When CHX was removed, animals returned to normal development; thus, the effects of CHX are reversible.

We reasoned that we might be able to render these effects tissue-specific by combining CHX-resistance mutations with cell-specific promoters to generate animals with a mix of CHX-sensitive and -resistant tissues. Such CHX-resistant mosaics may allow one to ask, for example, whether new protein synthesis is required in specific neurons or glia in learning-related paradigms. To isolate CHX-resistance mutants, we performed random mutagenesis and selected for animals that escaped L1 arrest in the presence of CHX. We recovered two mutants that developed normally in the presence of CHX. This was not due to non-specific drug resistance because they remained sensitive to geneticin, another translation-blocking antibiotic. As CHX resistance is dominant in both of the mutants we isolated, these mutations may also prove useful as selectable markers for transformation.

A surprising result of this study is that, although protein synthesis is considered part of basal metabolism, L1-arrested animals continued to move and appeared healthy after one week on CHX. This suggests that many basic cell functions, including neuronal excitation and muscle contraction, may not require protein synthesis. Surviving a protein synthesis block presumably demands compensatory inhibition of protein degradation. Indeed, the L1-arrested state is special in this sense, as animals at other developmental stages (L2 to adult) died when incubated on CHX. Thus, although protein synthesis is surely required for growth and differentiation, it may not be required for life, and may be dispensable in cells that are not growing or differentiating.

883A

Investigation of Low-cost GFP Microscopy. **Andy Papp**, Srikanth Bangalore, Chris Aldrich, David Perry. Tritech Research, Los Angeles, CA.

The Green Fluorescent Protein (GFP) gene from the fluorescent jellyfish *Aequorea victoria*, and its variants (such as EGFP), are used extensively to study the location and timing of gene expression in transgenic animals and plants (Chalfie et al, 1994). Resultant GFP fusion proteins are especially well-suited to studying *in vivo* gene expression patterns in the transparent nematode, *C. elegans* and the transparent larva of the fly *D. melanogaster*. Perhaps one of the most significant limitations to its use in large-scale genetic screens is the high cost of equipment needed to observe GFP microscopically — namely the Fluorescence Dissecting Stereomicroscope for screening and picking mutants and upright and inverted epi-fluorescent compound microscopes for more detailed studies.

Commercially available Fluorescence Dissecting Stereomicroscopes typically sell in the midrange between US$10,000 and US$20,000. They are offered by only the “high-end” microscope companies, based upon their most expensive dissecting scopes, and incorporate their premium-priced mercury arc-lamp illuminators, power supplies and epi-fluorescence modules. This leads us to wonder whether it is possible to cut corners without sacrificing utility, and which corners can be cut.

Since the inception of GFP-based expression screens, a variety of researchers have produced custom-made and home-made GFP dissecting scopes. These include, for example, Welcome Bender (Harvard Medical School, pers. comm.) and Ian Chin-Sang (Chin-Sang, 2004). There are several possibilities to consider toward lowering the cost of a Fluorescence Dissecting Stereomicroscope. It may be possible to get sufficient light from relatively inexpensive, long-lived, lower-power-consuming Light Emitting Diodes (LEDs) vs. mercury arc lamps. Depending upon the spectral specificity of the LEDs, filters or dichroic mirrors might be omitted. Getting enough light intensity of the precise wavelengths that excite GFP fluorescence focused onto the sample is important. The exciting beam can be provided directly or focused backward through the microscope using “epi-illumination”. We will explore these possibilities and report (and possibly demonstrate) the results.

Chin-Sang, Ian. (2004) GFP Stereoscope Using LED light source. Queen’s University, Kingston, ON, Canada. http://130.15.90.245/gfp_stereoscope.htm.

884B

A simple drug selection system for C. elegans. **Jennifer Isabel Semple**, Rosa Garcia-Verdugo, Ben Lehner. Systems Biol Unit, Ctr Genomic Regulation, Barcelona, Spain.

The commonly used transgenesis selection markers in worms involve the introduction, or rescue, of mutant phenotypes. Such mutations make the growth of the worms more labor intensive and can possibly interfere with the analysis of the transgene of interest. In addition, rescue of a mutant phenotype, such as the widely used unc-119 system, is limited to species that contain an appropriate mutant worm strain. Here we report the development of a drug selection system in order to address these issues. Our experiments were carried out with mosaic animals expressing the gene from an extra-chromosomal array, suggesting that this system would be extremely useful for large scale genomic or proteomic studies where one could combine the ease of generating worms carrying extra-chromosomal transgenes with an easy protocol for enriching large populations of transgenic worms. We also anticipate that this selection system will be useful for other applications, such as stable worm transformation by bombardment, which requires selection of rare transformation events from among a large number of worms.

885C

IR-LEGO: a tool for manipulating gene expression in targeted single cells *in vivo* . **Shin Takagi**[1], Motoshi Suzuki[1], Yasuhiro Kamei[3], Shunsuke Yuba[2]. 1) Dept Biol, Nagoya Univ Sch Sci, Nagoya, Japan; 2) NEDO, Kawasaki, Japan; 3) Dept Rad Biol, Osaka Univ Fac Med, Osaka, Japan.

We have developed a novel microscope system, Infrared Laser Evoked Gene Operator (IR-LEGO), for inducing gene expression *in vivo*. By depositing heat under the microscope with irradiation of infrared laser, IR-LEGO induces the heat shock response in targeted single cells, thereby eliciting the expression of a gene of interest under the control of a heat shock promoter in transgenes.

Using C. elegans as an experimental material, we have been testing applicability of IR-LEGO to living organisms, and determined the optimum condition for gene induction. A comparison with a conventional visible laser (440-nm dye laser) system for cell ablation experiments showed that the IR-laser (1480 nm) is much more suitable for inducing gene expression; With the IR-laser, gene induction can be induced in larval seam cells more efficiently under a wider range of conditions, with an irradiation period of less than one second. While the visible laser often has detrimental effects on irradiated cells, the IR-laser is far less harmful.

Thus, IR-LEGO would be a useful tool for C. elegans research, particularly in analyzing development as well as manipulating the function of nervous system. Future prospects as well as current technical limitations of this method will be discussed.

886A

The UTRome project: A resource to study 3'UTR biology in *C.elegans*. **Marco Mangone**[1], Oliver Attie[1], Emily Mis[1], Philip MacMenamin[1], Charles Zegar[1], Kourosh Salehi-Ashtiani[2], Marc Vidal[2], Kris Gunsalus[1], Fabio Piano[1]. 1) Center for Genomics and Systems Biology, Department of Biology, New York University, 1009 Silver Center, New York, USA; 2) Center for Cancer Systems Biology (CCSB), Department of Cancer Biology, Dana-Farber Cancer Institute, and Department of Genetics, Harvard Medical School, Boston, MA.

Three-prime untranslated regions (3'UTRs) are widely recognized as important post-transcriptional regulatory portions of mRNAs. RNA-binding proteins and small non-coding RNAs such as microRNAs (miRNAs) bind to functional elements within 3'UTRs to influence mRNA stability, translation and localization. To characterize and clone *C.elegans* 3'UTRs, we have developed a high throughput 3'RACE strategy and have characterized an initial target set of 7,014 genes. For each gene, we use a transcript-specific forward primer and a universal polydT(23) anchored reverse primer. The primers are designed to generate products compatible with the Promoterome and ORFeome resource. We cloned the 3'UTRs into an entry vector ready to be used for the third position in the multi-site Gateway system suitable for downstream functional analysis. In our first cycle, we cloned 3'UTRs of ~ 6,000 genes and sequence verified these as mixed bacterial transformants (or "minipools"). For half of these genes, the analysis of their 3'ends identified new 3'UTRs compared to data present in Wormbase WS190 (http://wormbase.org). We subsequently separated these minipools into ~56,000 isolated colonies and re-sequenced our library by deep sequencing (Illumina/Solexa and 454/Roche). We obtained unique cloned 3'UTR isoforms for over 90% of our target set, and observed multiple 3'UTR isoforms for over half. Most of the alternative isoforms are produced by differential PAS site usage. Our data and annotations are being deposited into the modENCODE website (http://www.modencode.org), and are also viewable in our 3'UTR-centric website (http://www.utrome.org). We will present the status of this project and its applications to study 3'UTR biology in *C.elegans*.

887B

Endrov and Virtual-Worm Base–A new standard platform and workflow for storage, viewing and analysis of microscopy images and related data. **Johan Henriksson**[1,2], Jurgen Hench[1,2], Thomas R Burglin[1,2]. 1) Dept Biosciences & Nutrition, Karolinska Institutet, Stockholm, Huddinge, Sweden; 2) Dept of life sciences, Södertörn University, Huddinge, Sweden.

The current software for microscopy has many short-comings; the lack of standardization makes communicating data hard. Information from experiments gets lost affecting repeatability. Technical limitations make some experiments impossible. There is no common way of handling data extracted from the images. By extracting data from images in a standard manner, comparability can be increased and more people get use of the data. We have developed a new framework (www.endrov.net) that covers the entire microscopy chain: * A new open file format (OST) which handles arbitrary metadata and annotation, 6D recordings (3D+channel+time+well), mixed resolutions and compression (lossy and lossless). It is based on existing standards and is simple to implement. Access time is fast even for very large datasets. * Methods to make existing microscopy software support complex recording schemes and output into our format (or other formats). * An extensible framework to view, annotate, process and analyze recordings. It is a plug-in framework written in Java and hence works on all operating systems. Endrov can be used as a library, making heavy data analysis simple. * Most common proprietary formats are also supported through Bio-formats. The OME framework is also supported. * Scripting in Java and a simple graphical language * Our framework is open and free. Runs on Mac OS X, Linux, and Windows. * Currently we are adding support for direct control of the microscope, a bioinformatics platform and efficient storage of data on central servers As an application, we have used our framework to follow the lineage in *C. elegans* embryos, and to quantify gene expression levels. See the two abstracts by Jurgen Hench et al and Lois Tang et al. We propose that our framework will be used for exchange of expression data in the worm community. Recorded expressions are extracted and uploaded to a central database. They can then be downloaded for viewing and analysis by everyone else.

888C

Coordinate transcription controls of body wall, pharynx and intestine muscle-gene expressions in *Caenorhabditis elegans*. **Hiroaki Kagawa**[1], Tetsuya Bando[1,2], Frederick Anokye-Danso[1,3]. 1) Biology, Okayama University, Okayama, Japan; 2) JST, Tokusima University, Tokushima, Japan; 3) MCDB, University of Pennsylvania, PA.

We present over all views how muscle genes express during muscle development in the worm. Especially results are described on the transcription factors controlling the tropomyosin gene, *tmy-1/lev-11* in pharynx and intestine and CeMyoD gene, *hlh-1* in body wall. The single tropomyosin gene of *C. elegans, tmy-1/lev-11* produces four isoforms of protein: two body wall types from the external promoter and two pharynx types from the internal promoter. The internal promoter of tropomyosin regulates expression of *tmy-1* in the pharynx and intestine. By promoter deletion of *tmy-1* reporters, a 100 bp fragment was identified that contained binding sequences for a GATA factor, for a chicken CdxA homolog and for a forkhead factor. Both the forkhead and CdxA binding sequences contributed to pharyngeal and intestinal expression. In addition, the GATA site also influenced intestinal expression of *tmy-1* reporter. We showed that ELT-2 and PHA-4 proteins interact directly with the GATA and forkhead binding sequences, respectively, in gel mobility shift assays. RNAi knockdown of *elt-2* diminished *tmy-1::gfp* expression in the intestine. In contrast to RNAi knockdown of *pha-4*, expression of *tmy-1::gfp* in *pha-4;smg-1* mutants was slightly weaker to that of the wild type. Ectopic expression of PHA-4 and ELT-2 by heat shock were sufficient to elicit widespread expression of *tmy1::lacZ* reporter in embryos. We present models by which ELT-2, PHA-4 and CdxA control expression from the internal promoter of*tmy-1* in intestine and pharynx. Combination of two of three factors controlled tissue-specific expression of the *tmy-1* gene. We found that the essential promoter sequence of the body wall troponin C gene, *pat-10/tnc-1* was similar to one of three enhancers of CeMyoD gene, *hlh-1*. We have isolated three *hlh-1* enhancer binding proteins including CeMyoD. Other two are CeTIS11, one of Zn finger protein and a NHR family protein. A NHR family protein also has a binding site of the second intron of *pat-10/tnc-1*. This is the reason that NHR stimulates the expression of muscle genes. Interestingly ZYX-1 bound enhancer-binding proteins CeMyoD and CeTIS-11, and also affected muscle gene expressions. ZYX-1 consists N-terminal proline rich and C-terminal three LIM domains. We will present a model on how these enhancer-binding proteins control body wall muscle gene expressions and orchestrate appropriate number and correct stage of muscle proteins.These regulation mechanisms will help to understand how muscle genes are expressed under the control of transcription factors and chemicals.

889A

Transcriptional control of dorsal-ventral polarity in *C. elegans*. **Rossio K. Kersey**, Thomas Brodigan, Tetsu Fukushige, Mike Krause. NIDDK/NIH, Bethesda, MD.

Spatial polarity cues in animals are used repeatedly during development for many processes, including cell fate determination, cell migration, and axon guidance. Once formed, the body wall muscle cells of *C. elegans* are a great source of polarity cues because they extend the length of the animal in four quadrants, occupying left and right positions on both the dorsal and ventral side of the animal. One cue known to originate from body wall muscle is UNC-129/TGF-beta that is produced at higher levels in dorsal versus ventral muscle resulting in a dorsal-ventral gradient. This pattern of *unc-129* expression is, in turn, generated by the activity of the transcriptional repressor UNC-130 that is preferentially produced in ventral body wall muscle. To understand how these dorsal ventral differences in expression are established, we have analyzed the 10.5 Kb promoter region of *unc-130* with a series of deletions. We found two regions in the promoter that drive ventral body wall muscle expression when isolated. These two promoter regions are also sufficient to impose ventral polarity on the *unc-54* promoter that normally is expressed uniformly in all body wall muscle. Results from the present and future studies will contribute to elucidating the mechanisms of transcriptional repression by *unc-130*. By identifying the trans-acting factors regulating *unc-130* expression we hope to understand the logic behind the generation of polarity cues.

890B

Identifying Components and Connections of the Muscle Differentiation Transcription Factor Network in *C. elegans*. **Steven G. Kuntz**[1,2], Lorian Schaeffer[1], John DeModena[1,2], Brian Williams[1], Paul W. Sternberg[1,2], Barbara Wold[1]. 1) Div Biol, California Inst Technology, Pasadena, CA; 2) HHMI, California Inst Technology, Pasadena, CA.

Several evolutionarily ancient gene regulatory networks (GRNs) control tissue differentiation in *C. elegans*. We want to learn which components and connections of tissue differentiation GRNs are invariant over evolution, which are variable, and what functional consequences come from the variations. Embryonic body-wall muscle differentiation in C. elegans is very accessible and several key transcriptional regulators are known, including *hlh-1* (myogenic regulatory factor), *hnd-1* (TWIST/hand protein), and *unc-120* (SRF/MEF protein). Nevertheless, we do not know all the components, nor how they are connected with each other and their target genes. Also, the network appears to be resistant to mutations, continuing to drive differentiation even when core transcriptional regulators are mutated. To expand our knowledge of myogenic factors, we first performed a synthetic lethal screen to identify additional core network transcription factors. By feeding RNAi knock down in an *hlh-1* mutant background, we identified multiple transcription factors required cooperatively for muscle differentiation. We have found a number of potentially interesting new components–including *ceh-51* and *hmg-1.2*–and are asking how these factors interact with each other to drive muscle differentiation, as well as how they affect downstream muscle gene expression. To further understand factor-factor interactions, we are knocking down each core factor (by dsRNA microinjection) in all available core factor mutant backgrounds, thus revealing cross-interactions. For understanding of downstream expression, we are looking fluctuations in transcript level through RNA-seq in the mutant backgrounds. Finally, to understand how these genetic cross-interactions between transcription factors appear to 'buffer' the downstream transcript changes, we are using ChIP-seq to look at differential binding of RNA Polymerase II in mutants for several myogenic transcription factors.

891C

Defining the embryonic muscle gene regulatory network using chromatin immunoprecipitation and an analysis of potential cis-acting elements. **Haiyan Lei**, Tetsunari Fukushige, Michael Krause. LMB, NIH/NIDDK, Bethesda, MD.

Previous work in C. elegans has shown that posterior embryonic body wall muscle lineages are regulated through a genetically defined transcriptional cascade that includes PAL-1/Caudal activation of muscle-specific transcription factors, including HLH-1/MRF and UNC-120/SRF, which together orchestrate specification and differentiation. Using chromatin immunoprecipitation (ChIP) in embryos, we recently demonstrated direct binding of PAL-1 in vivo to an hlh-1 enhancer element (Lei et al., 2009). Through mutational analysis of the evolutionarily conserved sequences within this enhancer, we identify two cis-acting elements and their associated trans-acting factors (PAL-1 and HLH-1) that are critical for the temporal-spatial expression of hlh-1 and proper myogenesis. Our data demonstrates that hlh-1 is indeed a direct target of PAL-1 in the posterior embryonic C. elegans muscle lineages, defining a novel in vivo binding site for this critical developmental regulator. We find that the same enhancer element is also a target of HLH-1 positive auto regulation, underlying (at least in part) the sustained high levels of CeMyoD in bodywall muscle throughout development. In order to further identify the embryonic muscle gene regulatory network, we combined HLH-1 ChIP with whole-genome tiling arrays to identify binding sites throughout the genome. We are currently validating our ChIP-chip data with transgenic reporters. These types of approaches will provide a molecular framework for the gene regulatory network activating the muscle module during embryogenesis.

892A

Body wall muscle in *C. elegans*–from expression profiling to profiling expression. **Barbara Meissner**[1], Laure Granger[2], Teresa Rogalski[1], Ryan Viveiros[1], Adam Warner[1], Adam Lorch[1], Laurent Segalat[2], Don Moerman[1]. 1) Dept Zoology, Univ British Columbia, Vancouver, BC, Canada; 2) CNRS-CGMC, Villeurbanne Cedex, France.

The large size of body wall muscle cells and the beautifully organized muscle sarcomeres within *C. elegans* muscle offer an opportunity to identify the precise position of proteins within cell substructures and the possibility of determining how such structures are assembled. The close relationship between subcellular localization and function is such that determining the preferential localization of a protein is often an essential step towards determining its function. GFP tagging has been shown to be a powerful tool in large-scale protein localization studies[1-3]. Our goal is to generate a comprehensive 'localizome' for the *C. elegans* body wall muscle by systematic GFP-tagging and localization of proteins expressed in muscle.

We have used Serial Analysis of Gene Expression (SAGE) to generate a comprehensive profile of late embryonic muscle gene expression. To date, four SAGE libraries are available generated from FACS sorted embryonic muscle cells (http://elegans.bcgsc.bc.ca). For this subcellular localization project, we are focusing on genes which a) exhibit expressed tags in the muscle SAGE libraries b) display a muscle phenotype in the RNAi screen performed previously (#981A, 16th IWM 2007) and c) are orthologs or at least homologs of human genes. We are using Gateway cloning technology to express protein::GFP fusions of candidate genes exclusively in muscle cells. To date we have analyzed the expression of about 300 genes, 240 of which display localized expression in the *C. elegans* body wall muscle (e.g. dense bodies, M-lines, golgi, mitochondria, cell membrane, nucleus or nucleolus). For most proteins localized in this study no prior data on localization was available. In addition to discrete subcellular localization we observe overlapping patterns of localization including the presence of protein in the dense body and the nucleus, or the dense body and the M-lines. In total we discern more than 15 subcellular localization patterns within nematode body wall muscle.

The localization of all proteins within a muscle cell will be an invaluable resource in our attempt to understand how proteins interact within muscle to form properly organized and regulated sarcomeres.

[1]Ding et al, *Genes Cells* 2000 [2] Huh et al, *Nature* 2003 [3]Heazlewood et al, *Nucleic Acids Res* 2007.

893B

Functions of biopterin synthesis and recycling genes in *C. elegans*. **Curtis Loer**[1], Ana Calvo[2], Ernst Werner[3], Gabriele Werner-Felmayer[3], Aurora Martinez[2]. 1) Dept of Biology, Univ San Diego, San Diego, CA; 2) Dept of Biomedicine, Univ of Bergen, Bergen, Norway; 3) Institute of Medical Chemistry & Biochemistry, Univ of Innsbruck, Innsbruck, Austria.

In mammals, the pterin cofactor tetrahydrobiopterin (BH4) is required for the function of aromatic amino acid hydroxylases (AAHs), nitric oxide synthases (NOSs), and glycerol ether monooxygenase (GEM). BH4 is synthesized *de novo* from GTP via 4 steps catalyzed by 3 enzymes: GTP cyclohydrolase I (GCH), pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR). The last enzyme, SR, carries out two final reducing steps. When used as a cofactor in the AAH reaction, BH4 is oxidized in the catalytic cycle. Reduced BH4 can be regenerated by 2 enzymes: pterin-4-a-carbinolamine dehydratase (PCBD) and quinonoid dihydropteridine reductase (QDPR). The AAHs requiring BH4 include the rate-limiting enzymes in the synthesis of serotonin and catecholamines (TPH and TH, respectively), and in phenylalanine catabolism (PAH, phenylalanine hydroxylase). *C. elegans* has clear orthologs of most biopterin synthesis and recycling genes. Some BH4-using enzymes are clearly present: all the AAHs exist (*tph-1, cat-2* and *pah-1* genes), but no clear NOS homologs are found in *C. elegans.* Mutants in the *cat-4*/GCH gene are serotonin- and dopamine-deficient, and are hypersensitive, perhaps due to a 'leaky' cuticle; mutants in the *ptps-1* gene appear to have an identical phenotype. The hypersensitivity phenotype may be caused by dysfunction of an unrecognized *C. elegans* enzyme that requires BH4. *C. elegans* lacks a clear SR ortholog; its function may be mediated by other aldo-keto-type reductases/short chain dehydrogenases. There are many candidate genes for this function in the *C. elegans* genome; none we have examined to date likely function as SR. We are also examining putative *pcbd-1* and *qdpr-1* mutants. Preliminary results with *cat-4* and *ptps-1* mutants show the predicted reduction or loss of GCH and PTPS activity, respectively, and accompanying reductions in pterin content. We are also characterizing the expression pattern of *cat-4*::GFP reporters (kindly provided by A. Chisholm); the reporter is expressed in 5HT and DA neurons, hypodermis and intestine.

894C

Two sets of inverted repeat sequences are required for alternative splicing of the cholinergic locus *unc-17/cha-1*. **Ellie Mathews**, Greg Mullen, Jim Rand. Genetic Models of Disease Research Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

Acetylcholine (ACh) is a major neurotransmitter in both vertebrate and invertebrate nervous systems. A single phylogenetically conserved locus encodes both the acetylcholine biosynthetic enzyme choline acetyltransferase (ChAT; *cha-1*) and the vesicular acetylcholine transporter (VAChT; *unc-17*) proteins. The VAChT coding region is contained within the first intron of the *cha-1* gene, and alternative splicing of the pre-mRNA gives rise to separate ChAT and VAChT transcripts. In *C. elegans*, most cholinergic neurons robustly express both proteins, although a few neurons appear to preferentially express either ChAT or VAChT. We identified two distinct sets of inverted repeat sequences (designated R1 and R2) in the non-coding sequences flanking the *unc-17* coding region which could potentially form RNA stem-and-loop secondary structures. These repeats are conserved in other nematodes, and in each species, the repeats are much better matches to each other than they are between species, so they are likely to be important. We have also found structural counterparts of the R1 sequences in the mouse and rat genomes. We speculated that these sequences might play a role in regulating alternative splicing. We therefore 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. To validate the reporter, we introduced the *cn355* mutation, which disrupts splicing of the endogenous *unc-17* gene, and found that it had comparable effects on expression of the dual reporter. We have now shown 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 instead merely requires sequence complementarity (i.e. the ability to form secondary structures), while the function of the R2 repeats shows some sequence dependence in addition to requiring sequence complementarity. In an effort to identify trans-acting factors regulating this process, we screened for mutations affecting alternative splicing of *unc-17/cha-1* using the GFP/wCherry reporter, and identified a single mutation, *md3360*, that alters the ratio of GFP:wCherry expression. (Supported by a grant from NIGMS).

895A

BIR-1, the homologue of human Survivin affects chromatographic pattern of cytoskeletal proteins. **David Kostrouch**[1], Marta Kostrouchova[2], Zdenek Kostrouch[1]. 1) Laboratory of Molecular Pathology, Inst Inherited Metab Disorders, Charles Univ, Prague, Czech Republic; 2) Laboratory of Molecular Biology and Genetics, Inst Inherited Metab Disorders, Charles Univ, Prague, Czech Republic.

bir-1 (the orthologue of vertebrate Survivin) is a critical regulator of mitosis progression involved primarily in the formation of complexes mediating phosphorylation of spindle microtubules and histone H3. bir-1 is expressed from an operon together with transcription and splicing cofactor CeSKIP (skp-1) that is critical for a wide spectrum of developmental changes. BIR-1, similarly as CeSKIP, is important for proper larval development and affects transcription from thyroid hormone responsive promoters in a heterologous transcription system. Based on the differential display of complete control proteomes and the proteome of L1 larvae over expressing bir-1, we identified fractions containing different amounts of protein. Mass spectrometry identified peptides specific for cytoskeletal and motor proteins in paired fractions exhibiting marked differences in control or bir-1 over-expressing larvae. This indicates cytoskeletal and motor proteins as possible targets of BIR-1 in non-dividing cells. Acknowledgement: We thank Dr. M.W. Krause for support and advice. The work was supported by grants 304/08/0970 and 304/07/0529 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

896B

Functional analysis of the F56D2.6 gene, the putative homologue of the yeast PRP43. **Jonathan E. Karpel**, Sarah Primrose. Joint Sci Dept, Claremont Colleges, Claremont, CA.

F56D2.6 is a putative helicase of the "DEAH box" family that is required for the release of the lariat intron from the spliceosome. Recent studies involving the yeast homologue of this gene, PRP43, have shown that the protein also plays a significant role in ribosome biogenesis. Proteins in this family can be grouped according to the sequences of seven conserved motifs, including the sequence D-E-A-H in motif II. Although the F56D2.6 protein only has 56% identity to the yeast PRP43 protein, these seven motifs are well conserved between the two proteins. Within the protein are three putative domains: a central region that encompasses NTPase motif I, an N-terminal segment of 118 residues upstream of motif I, and a C-terminal segment of 337 amino acids downstream of motif VI. Our research aimed to prove that F56D2.6 was the functional homologue of the yeast PRP43 protein. We used a plasmid shuffle experiment and an ATPase assay, respectively, to clearly show that F56D2.6 cDNA could substitute for Prp43p function *in vivo* and that the protein contained ATPase activity. Future research will include identification of the precise molecular substrates of F56D2.6, analysis of ribosomal protein expression in F56D2.6 mutants, and mutational analysis of the undefined C-terminal segment of the protein.

897C

Auto-regulation of *asd-1*, a Fox-1 family alternative splicing regulator. **Hidehito Kuroyanagi**[1,2,3], Yuriko Kikuchi[1], Masatoshi Hagiwara[1,2]. 1) Sch Biomed Sci, Tokyo Med Dent Univ, Tokyo, Japan; 2) Med Res Inst, Tokyo Med Dent Univ, Tokyo, Japan; 3) PRESTO, JST, Kawaguchi, Saitama, Japan.

Alternative splicing of pre-mRNAs is a major source of proteome diversity in metazoan. Recent studies on alternative splicing regulators in cultured vertebrate cells suggest a complex regulatory network of alternative splicing regulators. However, the splicing regulatory network *in vivo* still remains to be elucidated. We have recently developed a transgenic reporter system that enables visualization of alternative splicing patterns in living organisms by utilizing multiple fluorescent proteins and demonstrated that Fox-1 family proteins, Alternative Splicing Defective-1 (ASD-1) and FOX-1, and muscle-specific regulator SUP-12 coordinately regulate muscle-specific selection of *egl-15* exon 5A. The Fox-1 family proteins are evolutionarily conserved alternative splicing regulators that specifically bind to UGCAUG stretch in target pre-mRNAs. Here we report that expression of functional *asd-1* mRNA is negatively auto-regulated. We identified an alternative *asd-1* mRNA isoform, E2Δ, in which skipping of exon 2 caused a frame-shift near N-terminus. E2Δ mRNA was accumulated in *smg-2* mutant, indicating that it is a target of nonsense-mediated mRNA decay (NMD) and therefore is non-functional. The amount of E2Δ mRNA was remarkably reduced in *asd-1; fox-1* double mutant in the *smg-2* background, indicating that skipping of *asd-1* exon 2 depends on the Fox-1 family. In order to analyze the *asd-1* splicing regulation *in vivo*, we constructed a pair of *asd-1* alternative splicing reporter mini-genes that visualize inclusion and skipping of exon 2 by expression of RFP and GFP, respectively. When expressed under *asd-1* promoter, RFP expression was observed in a wide variety of tissues that express *asd-1*, while expression of GFP was restricted to muscles and some neurons but not in intestine. Consistent with the requirement of the Fox-1 family for exon 2 skipping, GFP expression was remarkably reduced in *asd-1; fox-1* double mutant, indicating that reporter expression from the mini-genes reflects regulation of endogenous exon 2. We also found that only one out of four UGCAUG stretches in the upstream and downstream introns from exon 2 is required for efficient GFP expression. These results indicate that *C. elegans* Fox-1 family RNA binding proteins ASD-1 and FOX-1 negatively regulate *asd-1* expression by repressing inclusion of exon 2 via a UGCAUG stretch in intron 2 in a tissue-specific manner.

898A

Chemotherapeutic activation of CEP-1-dependent and CEP-1-independent Cell Death in Caenorhabditis elegans. Sandy Gamss[1,3], Alicia Meléndez[2,3], **Jill Bargonetti**[1,3]. 1) Department of Biological Sciences, Hunter College, New York, NY 10065; 2) Department of Biology, Queens College , Queens, NY; 3) The Graduate Center CUNY, New York, NY.

The p53 tumor suppressor protein is a regulator of apoptosis, growth arrest and autophagy. In response to DNA damage, functional p53 activates transcription of genes involved in cell cycle arrest, autophagy and apoptosis. Not surprisingly, p53 is mutated or deleted in over 50% of all mammalian tumors. We are investigating p53-independent cell death pathways in order to identify cell death mechanisms that can be induced in human cancer cells without functional p53. The C. elegans ortholog of p53, cep-1, functions during normal meiotic chromosome segregation and germ line cell death. We would like to identify chemotherapeutic drugs that induce cell death in animals that lack cep-1 activity. γ-irradiation and UV-C have been shown to induce an increase in germ cell death in wild-type and not in cep-1 mutant worms. However, whether the same occurs for standard chemotherapeutics has not been examined. We are testing whether different DNA damaging drugs activate the CEP-1 pathway. We detected activation by quantitative PCR of the egl-1 target gene. Previously we have shown that 10-decarbomyl mitomycin C (DMC) can kill human cancer cells independently of p53 activity by a mechanism of action that includes the down-regulation of Chk1. Etoposide, mitomycin C (MC) and Nutlin-3 require an active p53 checkpoint pathway to elicit cell death. Interestingly, we have found that etoposide, mitomycin C, DMC and Nutlin-3 can elicit CEP-1 mediated activation of egl-1 transcription, similar to what has been shown with γ-irradiation and UV-C. In addition, etoposide, MC and DMC exposure decreased the brood size of wild-type worms but only DMC exposure decreased the brood size of cep-1 mutants. We are also investigating whether autophagy is a possible mechanism of p53 independent cell death. Interestingly, exposure to the mitomycins and Nutlin-3 treatment in autophagy deficient animals activated egl-1, but exposure to etoposide did not. These results suggest that autophagy participates in a specific DNA damage signaling pathway. We will report on our experiments on germ cell death induced by the compounds in wild type and autophagy deficient animals using Nomarski optics and cell death markers. This work was supported by a NIH SCORE Grant (1SC1CA137843-01) and was facilitated by a NIH Research Centers in Minority Institutions award from the Division of Research Resources (RR-03037).

899B

Characterization of three DNase II activities during the development of C. elegans. **Hsiang Yu**[1], Juey-Jen Lai[1,2], Ding Xue[2], Szecheng Lo[1]. 1) Department of Life Science, Chang Gung University, Taoyuan city, Taiwan; 2) Department of MCD, University of Colorado, Boulder, Colorado, USA.

DNase II, an acidic DNase, is known to play a prominent role in digestion of DNA from apoptotic cells across a wide spectrum of animals, including invertebrates and mammals. In contrast to two DNase II, α and β, in human, there are three homologous DNase II, NUC-1, CRN-6, and CRN-7, in C. elegans. By using the metachromatic agar-diffusion assay (MADA), we first characterized embryonic extract from N2, and three single mutants (nuc-1, crn-6, or crn-7) that exhibited the optimal pH at 4.5 for DNA digestion, indicating that NUC-1, CRN-6, and CRN-7 are likely acid DNase in worms. This observation was further supported by the result of a triple mutant (crn-7; crn-6; nuc-1) which showed no DNA digestion activity. Interestingly, the optimal temperature was at 20°C but not 37°C for the worm's DNase II activity since worms grow around 20oC in nature. MADA results of the embryonic extracts' DNase activity from three single mutants and three double mutants (crn-7; nuc-1, crn-6; nuc-1 and crn-7; crn-6) indirectly showed that NUC-1 had the highest DNase II activity while CRN-6 and CRN-7 exhibited 10 fold less activity, In the L4 stage, NUC-1 remained the highest activity but CRN-6 and CRN-7 had 4 to 8 fold less DNase II activity than NUC-1.

900C

A Forward Genetics Screen for Enhancers of ksr-1 lethality. **Phil Cheng**, Christian Rocheleau. Department of Experimental Medicine, McGill University, Montreal, PQ, Canada.

Kinase suppressor of Ras (KSR) is a scaffold protein that is required to localize components of the Ras/MAPK pathway to the plasma membrane to activate Raf kinase and to facilitate interactions between Raf and downstream kinases, MEK and ERK. C. elegans has two genes that encode for KSR proteins, ksr-1 and ksr-2, which are redundantly required for specification of the excretory duct cell. Less than 1% of ksr-1 single mutants die as a "rod-like" lethal, but is strongly enhanced by removal of ksr-2 or other positive regulators of the pathway, such as a scaffolding protein involved in Raf activation, cnk-1. A genome-wide RNAi screen was conducted to find enhancers of ksr-1 lethality (ekl genes) (Rocheleau et al. 2008). Most of the ekl genes identified in this screen encode regulators of gene expression and display pleiotropic phenotypes and were unlikely candidates to be direct regulators of Ras/MAPK signaling. Some of the ekl genes found like ubc-25, an E2 ubiquitin conjugating enzyme, and ekl-5, a novel uncharacterized gene, did not have pleiotropic phenotypes and might have a more direct role with ksr-1 to facilitate signaling in the Ras/MAPK pathway. We predict that a forward genetic ekl screen would identify a different but partially overlapping set of ekl genes, mostly those without pleiotropic phenotypes and more likely to have a direct role in regulating Ras/MAPK signaling.

I performed a forward genetic screen using EMS mutagenesis to isolate genes that enhance the "rod-like" lethal phenotype of ksr-1 mutants. From this screen, I have isolated ten candidates, seven of which are confirmed enhancers. Single Nucleotide Polymorphism (SNP) mapping was used to map these mutants to a chromosomal location on the C. elegans genome. I have mapped three mutants to chromosome I, one mutant to chromosome III, and three mutants to chromosome X. vh1 and vh18, two of the mutants mapped to chromosome I, failed to complement ubc-25. ubc-25(vh1) contains a nonsense mutation that causes a premature stop codon early in the gene and is likely a null allele. ubc-25(vh18) is a slightly less penetrant enhancer and contains a missense mutation that causes a Histidine to Arginine substitution adjacent to the UBC domain. vh2, vh22, and vh23 were mapped to chromosome X close to ekl-5 and but they all complement ekl-5, suggesting that they might be mutations in new regulators of the Ras/MAPK pathway that function closely with KSR.

901A

Identifying cadmium-response transcriptional regulators of the *C. elegans* metallothionein gene, *mtl-1*. **Julie Hall**, Jonathan Freedman. Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC.

Humans are constantly exposed to the carcinogenic metal cadmium through various environmental routes including diet and cigarette smoke. Cadmium accumulates in cells resulting in a variety of responses including oxidative stress, altered protein activity, inhibition of DNA repair, and the activation of cellular response pathways. In response to cadmium and other metals, cells increase the expression of small cysteine-rich metal-binding proteins that function in metal detoxification and homeostasis known as metallothioneins (MTs). MTs are highly conserved and *C. elegans* has two MT genes: *mtl-1* and *mtl-2*. Interestingly, *C. elegans* MT genes lack metal response elements, which are evolutionarily conserved metal regulatory regions found in the promoters of most eukaryotic MT genes. Additionally, a homolog of the cognate transcription factor has not been identified in *C. elegans*. To identify regulatory factors that control *mtl-1* transcription, integrated transgenic strains of *C. elegans* containing GFP under the control of the 5'-regulatory region of *mtl-1* were constructed, p*mtl-1::GFP*. Similar to what has been reported for other *mtl-1* transgenic strains, constitutive GFP expression was observed in the pharynx and following cadmium exposure in the intestine. To identify transcriptional regulators of *mtl-1*, genes involved in the aging/stress response and oxidative stress pathways were tested to see if they affected GFP expression in p*mtl-1::GFP*. *akt-1(mg144)*, a gain of function allele, resulted in an increase in GFP expression. AKT-1 is a serine/threonine kinase involved in the insulin signaling pathway. The *akt-1* loss of function allele, *akt-1(ok525)*, and other members of the insulin signaling pathway were tested. AKT-1 and AKT-2 act as a complex to regulate the transcription of various genes. *akt-1(ok525)* or *akt-2(ok393)* did not affect GFP expression, however, the *akt-1(ok525);akt-2(ok393)* double-mutant caused an increase in GFP levels. PDK-1 directly interacts with the AKT-1/AKT-2 complex and the *pdk-1(sa709)* mutation caused an increase in GFP levels in p*mtl-1::GFP*. Both *daf-2*, the insulin signaling receptor, and *daf-16*, the transcription factor that is inhibited by the AKT-1/AKT-2 complex, did not affect GFP expression. Recent evidence has shown that PDK-1 and the AKT-1/AKT-2 complex can regulate the transcription of various genes independent of the insulin signaling pathway. Our data suggests this may be such the case for the transcriptional regulation of *mtl-1* in response to cadmium and further pathway analysis and RT-PCR is being conducted to confirm this is the case.

902B

The transcriptional mechanisms of organophosphorus pesticide mixtures in C. elegans. **A. Viñuela**, L.B. Snoek, J.A.G. Riksen, J.E. Kammenga. Laboratory of Nematology, Wageningen University, Wageningen, Netherlands.

Organophosphorus pesticides (OP) are insecticides that are widely used to control agricultural and household pests. Initially, OP were designed to affect the nervous system by inhibiting acetylcholinsterase (AChE). However, this is not the only mode of action that leads to toxic effects, as many OPs alter immune functions in mammals by oxidative damage, metabolism modifications, and stress-related immune-suppression. In addition, the mode-of-action of single OP has been thoroughly studied, but little is known about the effects of different combined OP in a mixture. To clarify the underlying mechanisms of OP toxicity and their possible interactions in mixtures, we determined genome-wide transcription profiles of C. elegans exposed to two OP: chlorpyriphos, diazinon and a mixture of both. The influence of temperature was determined by replicate experiments at 16°C and 24°C. For all treatments, we indentified significantly enriched GO terms and protein domains associated with detoxification, general stress response, ion transport, transport and metabolism of fatty acids, and immune response. Nevertheless, less than 10% of the regulated genes were common to all the treatments. Between the regulated genes we found cadmium responsive genes, genes downstream the *daf-2/daf-16* insulin like pathway, and genes associated with innate immune response. Furthermore, the comparison between single treatments of OPs and their mixtures suggest that, on the gene transcript level, the effect of the OPs mixture is not a summed effects of the single components. This emphasizes a specificity in mixture response involving similar mechanism of response (e.g.: detoxification, stress response or lipid mobilization) by dissimilar gene transcripts compared to single compound exposures. Moreover, we are further studying the influence of the temperature in OPs toxicity by comparison of our data at 16°C and 24°C. Initial results indicated a larger influence of temperature on the number of genes differently expressed than the toxicants. In other words temperature induces a much less specific gene transcription response than the specific response to toxicants.

903C

cis-regulatory elements in promoters of the *let-7* family. **Axel Bethke**[1,2], Mary Wiese[1], Adam Antebi[1]. 1) Huffington Ctr Aging, Baylor Col Med, Houston, TX; 2) University of Osnabrück, Fachbereich Biologie/Chemie, Barbarastrasse 11, 49069 Osnabrück, Germany.

microRNAs play a critical role in development and disease, yet their transcriptional regulation is not well understood. Only a handful of transcription factors are known to directly activate their transcription. Among them, the nuclear hormone receptor DAF-12 regulates transcription of *let-7* family homologs in a ligand-dependent manner, to influence developmental progression in the heterochronic circuit. Our studies show that DAF-12 binds to specific *cis*-regulatory response elements (REs) present within the microRNA promoters. However, genetic experiments also reveal that DAF-12 is not the only factor involved. To better understand the transcriptional networks regulating microRNA expression, we have undertaken a genetic and biochemical analysis of the *cis*- and *trans*-acting factors governing their transcriptional control. In promoters of the *let-7* family members, *daf-12* activated REs appear separate or as dimers in inverted or direct repeats with various base pairs spacing between each element. To investigate the individual influence of each *daf-12* RE and the sequence directly surrounding it, we generated repeats of 100 to 150bp long fragments surrounding the REs and fused these repeats to a minimal promoter and a YFP reporter. We found isolated REs that show complete *daf-12* and ligand dose dependence, while other REs are only moderately influenced by *daf-12*. Moreover, different REs typically have non-overlapping tissue specificity, and activate transcription only in a subset of tissues compared to the full length promoter. We are now using this approach to investigate nuclear hormone receptor-mediated transcriptional regulation of separate REs isolated from the full-length promoter. We are also screening for other transcription factors that bind to REs in direct proximity to *daf-12* dependent REs to influence stage and tissue specific expression. These studies should shed light on transcription factor-microRNA networks that regulate temporal and spatial aspects of development.

904A

Predicted 3D Structures of the DNA-Binding Domain for the Ten *C. elegans* GATA Transcription Factors and Their Preferred DNA Sequence. **Max E. Boeck**[1], James Thompson[1,2], Phil Bradley[1,3], Robert Waterston[1]. 1) Dept Genome Sci, Univ Washington, Seattle, WA; 2) Dept Biochemistry, Univ Washington, Seattle, WA; 3) Fred Hutchinson Cancer Research Center, Seattle WA.

Based on the paradigm that structure dictates function we used a computational approach to determine the preferred DNA motif bound by each of the GATA transcription factors found in *C. elegans*. GATA transcription factors have been shown to be important for organogenesis and cell fate specification in every metazoan from *C. elegans* to humans. The ability of GATA factors to specify cell-fate decisions is dictated by their ability to bind a DNA motif, WGATAR, with high fidelity. GATA factors have been shown to bind the motif through the actions of a conserved DNA-binding structure, CXXC(X)17-19CXXC [1]. While the basic DNA-binding structure is conserved between members of the GATA family,numerous specificity-determining residues vary between GATA genes. Using a maximum-likelihood phylogenetic approach [2] we estimated a phylogenetic tree in order to model the evolutionary relationships between GATA factors in the sequenced Caenorhabditis species. We then used Rosetta homology modeling [3] to construct structural models of the DNA-binding structures of all the GATA factors found within *C. elegans*. By tracking the specificity-determining residues that have changed along the phylogenetic tree of these GATA factors, we have defined a prediction of which GATA factors are likely to bind very similar segments of DNA. [1] Lowry, Atchley J Mol Evol. 2000 50:103-115 [2] Guindon S, Gascuel O. Syst Bio. 2003 52(5):696-704. [3] Qian et al, Nature. 2007 Nov 8;450(7167):259-64.

905B

Identifying direct transcriptional regulators of *hlh-6* expression in the pharyngeal glands. **Vikas Ghai**, Jeb Gaudet. Dept Genes & Development, Univ Calgary, Calgary, AB, Canada.

An important problem in development is how individual cell fates are specified during organ development. We use the pharynx (or foregut) of *C. elegans* as a simple model to study this issue. Work in our lab has identified a bHLH transcription factor, HLH-6, that regulates a battery of pharyngeal gland-specific genes (Smit et al. 2008). Expression of *hlh-6* is itself gland-specific, raising the question of how this distinct pattern of expression is generated. We have identified three critical regulatory elements in the hlh-6 promoter that function combinatorially to activate gland-specific expression. These three elements include a binding site for PHA-4 (the "master regulator" of pharynx development), and three other sites: HRL1, HRL2 and HRL3 (Hlh-6 Regulatory eLements 1-3). These sites employ a simple logic to generate gland specific expression: PHA-4 activates expression broadly in the pharynx, HRL2 restricts expression to the MS-derived posterior pharyngeal cells (except the glands), HRL3 turns on expression in 'neurosecretory cells' including the pharyngeal glands and HRL1 represses expression in non-gland cells. We have previously shown that the Notch effector LAG-1 acts through HRL1 to repress expression of *hlh-6* in most cells, and that the combined activities of PHA-4, HRL2 and HRL3 are sufficient to overcome repression by LAG-1 and activate *hlh-6* expression in pharyngeal glands (Ghai and Gaudet, 2008). This mechanism for LAG-1 is independent of Notch signaling and is strictly negative, which differs from the canonical mechanism, in which CSL factors respond to Notch signaling by activating expression of target genes. Currently, we are working towards identifying the trans-acting factors that function through HRL2 and HRL3. The HRL2 sites in the promoter of *hlh-6* are required for *hlh-6* expression and are sufficient to drive expression in the posterior cells of the pharynx. The cells in which HRL2 is active are all derived from the MS blastomere, suggesting that HRL2 responds to a lineage or position-specific factor. HRL3 is also required for expression of *hlh-6* and appears to be active in the pharyngeal glands and a subset of neurons. We are presently taking several approaches to identify the trans-acting factors for HRL2 and HRL3, including RNAi of candidate factors, yeast one-hybrid, forward genetics screening and biochemistry.

906C

Modeling condition-specific gene expression using conserved cis-regulatory elements. **Nnamdi E Ihuegbu**[1,2], Gary D Stormo[1,2]. 1) Department of Genetics; 2) Center for Genome Sciences, Washington University School of Medicine, St. Louis, MO.

We recently performed conservation-based motif-finding on regulatory non-genic sequences within and around every gene in the C. elegans genome. We uncovered an array of putative binding sites that are conserved in at least two other nematode species. After removing low complexity motifs, we sought associations between resulting motifs and expression studies. Specifically, we inquired if a multivariate regression model comprising of a subset of these motifs can be constructed to explain a condition-specific transcriptome response. Preliminary analysis of differential gene expression due to heat shock has implicated several motifs in the upstream (likely transcription-factor binding sites) and 3'UTR (likely miRNA targets) portions of genes. One of these significant motifs corresponds to the previously discovered Heat-Shock Element (HSE). Additionally, we are are seeking to detect interactions between motifs that suggest putative synergistic regulation of their nearby genes. Studies are ongoing to test the ability of these motifs, and their cooperation, to produce gene expression responses to heat shock and other environmental conditions.

907A

Systematic analyses of AFD-specific enhancers in *C. elegans*. **Hiroshi Kagoshima**, Junko Kajiwara, Yuji Kohara. Genome Biol Laboratory, National Inst Genetics, Mishima, Shizuoka, Japan.

How can transcription factors control gene expression in the right place and at the right time? To investigate the mechanism of the cell-specific transcriptional regulation, we chose AFD thermosensory neuron for our target cell. We performed systematic deletion analyses of nine AFD-specific genes using promoter::GFP reporters, and narrowed down the DNA sequences required for AFD expression to relatively small regions (from 50 bp to 600 bp). Among these AFD-specific promoters, *gcy-8* and *gcy-18* promoters are particularly interesting, because these genes may have arisen from gene duplication and most probably they were controlled by the same regulatory mechanism. This notion is supported by following data: their expression were similar in spatial pattern (exclusively expressed in AFD) [Inada et al. 2006] and in temporal control (initiated at pre-comma stage), and were downregulated by either of the mutants, *ttx-1* or *ceh-14* (both of which encode homeobox transcription factors expressed in AFD) [Satterlee et al. 2001 and this work]. Moreover, the expression of *gcy-8* and *gcy-18* are completely abolished in *ttx-1; ceh-14* double mutant background. We also showed that forced expression of both transcription factors in AWB chemosensory neuron could induce ectopic expression of *gcy-8* and *gcy-18* in AWB. These results suggest that *ttx-1* and *ceh-14* genes play pivotal roles in *gcy-8* and *gcy-18* expression. We found these promoters shared common 7-8 bp DNA motifs, which are conserved in five *Caenorhabditis* species (*C. elegans, C. briggsae, C. brenneri, C. remanei* and *C. japonica*). We are currently analyzing the promoters of *gcy-8* and *gcy-18* orthologs of the *Caenorhabditis* species in *C. elegans*. Further analysis will be reported at the meeting.

908B

High-throughput transcription regulatory network mapping by yeast one-hybrid assays with high-density transcription factor arrays. **John S. Reece-Hoyes**[1,2], M. Inmaculada Barrasa[1,2], Ashley Carraher[1], Katie Brown[1], Amanda Kent[1], Amelie Dricot[2], David E. Hill[2], A.J. Marian Walhout[1,2]. 1) Program in Gene Function and Expression & Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; 2) Center for Cancer Systems Biology & Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, MA.

An organism's development and response to the environment is governed by transcription regulatory networks that control the differential regulation of the genome. This process is largely driven by the activation or repression of transcription induced by the specific binding of a regulatory transcription factor (TF) within each locus, often within gene promoters. We aim to characterize the transcription regulatory networks of the model nematode Caenorhabditis elegans. The worm genome contains about 20,000 genes, of which 941 encode TFs. In contrast to other groups, we use gene-centred ('gene-to-protein'), rather than TF-centered ('protein-to-gene') methods for the identification of promoter-TF interactions. We will use high-throughput yeast one-hybrid assays with our resource of 795 TF and 31 putative TF clones to screen the ~6000 cloned promoters of the Promoterome. We will describe improvements that utilize a Singer HDA RoToR robot that enables the interrogation of all TFs multiple times with only three plates. The data gained from this study will primarily elucidate the networks that underlie the process of gene regulation, but additionally can be used to identify individual DNA motifs bound by the TFs studied.

909C

Dissecting the structure-function relationship of NHR-49 through mutagenesis. **Alison Brooks**, Marc Van Gilst. Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Nuclear receptors are a family of transcription factors that regulate a wide variety of gene sets. HNF-4α is a mammalian nuclear receptor that regulates expression of genes involved in development and lipid and glucose metabolism. Its targets have also been implicated in multiple cancers and diabetes. Like all nuclear receptors, HNF-4α exerts its control by coordinating the effects of ligands, coregulators, and specific response elements through their binding to distinct conserved domains. Because of its importance in basic metabolic function, its role in disease, and the potential to dissect its function based on subdomain, HNF-4α is an excellent target for pharmacologically-mediated selective modulation. However, the role of each domain in transcriptional regulation must be better understood before this may be accomplished. To this end, I propose to focus my efforts on elucidating the structure-function relationship of the *C. elegans* homolog, NHR-49, via site-directed mutagenesis. Working with nematodes will allow me to easily create a large panel of domain-specific mutants and test their *in vivo* effects. Furthermore, I will use the tractability of forward genetics in worms to look for constitutively active NHR-49 mutants to test my hypothesis that selective modulation of targets is possible.

910A

Investigation of alternative transcription factor gene transcripts in *Caenorhabditis elegans* by recombineering. **Hannah L. Craig**, Julia Wirtz, Sophie Bamps, Ian A. Hope. Institute of Integrative and Comparative Biology, University of Leeds, Leeds, United Kingdom, LS2 9JT.

Of about 1000 potential transcription factor genes in the *C. elegans* genome, 126 are thought or confirmed to have alternative transcripts that would be translated to give different protein isoforms. These isoforms differ due to alternative initiation or termination of transcription, or alternative splicing. Whether these modifications in protein sequence reflect functional variation is not yet clear for the majority of transcription factor genes. To this end, recombineering has been employed to seamlessly tag individual isoforms of selected transcription factors with GFP to examine any differences in expression pattern. The forkhead transcription factor genes *fkh-7* and *fkh-9* both have 2 transcripts with alternative 5' ends, based on EST data, and may therefore be transcribed under the control of alternative promoters. Examination of expression patterns generated from N- and C-terminal gfp-tagged fusions suggests that for both these genes, the downstream promoter is responsible for driving the majority of expression. Knock out of individual transcripts for the reporter tagged genes is underway. HLH-30 is reputedly a helix loop helix transcription factor and has an orthologue in the human genome. The *hlh-30* gene encodes at least 4 alternative isoforms due to 2 or 3 alternative promoters and an optional internal exon. Previous experiments showed an *hlh-30* promoter reporter fusion to be expressed in larvae and adults in many cells types. A C-terminal *gfp* fusion generated, by fosmid recombineering, to tag all HLH-30 isoforms, gave the same expression pattern. Tagging of transcripts specifically generated from the alternative promoters gave similar expression patterns. Interestingly, the fusion proteins encoded by recombineered fosmids were cytoplasmic rather than nuclear, suggesting that the function of HLH-30 may not be regulation of transcription. Previous experiments on the homeodomain transcription factor UNC-62 focused on its functions in development and locomotion, but its expression pattern is yet to be fully characterized. The *unc-62* locus encodes at least 4 isoforms due to 2 alternative promoters and 2 alternate internal exons. The upstream promoter drives GFP expression in the developing vulva and uterus, several nerve cells in the posterior of larvae and in the VC neurons in late L3s. A recombineered fosmid reporter fusion, which should tag all UNC-62 isoforms at the C-terminus, drove visible GFP expression only in a single posterior nerve cell, identified as DVA, plus faint vulval expression. Further data from tagging of individual transcripts is being generated.

911B

Characterization of the homeobox gene *C02F12.10* and a transcriptional regulatory cascade acting in the interneuron DVC. **Huiyun Feng**, Ian A. Hope. Institute of Integrative and Comparative Biology, University of Leeds, LS2 9JT, UK.

Transcription factors are important cellular determinants of specification and development. We describe the transcript and expression pattern analyses of the *Caenorhabditis elegans* homeobox gene *C02F12.10*. The predicted exon-intron structure of *C02F12.10* would encode a small homeodomain protein of 152 amino acids but had no transcript data support. Sequence directed PCR amplification on a cDNA library, generated from mixed staged *C. elegans*, confirmed the predicted single transcript. GFP expression driven by the promoter of this transcript and a recombineered-fosmid encoded N-terminal translational GFP fusion for *C02F12.10* showed specific expression in the single interneuron DVC, in the dorso-rectal ganglion, from late embryo to adult and, as yet unidentified, uterus cells in young adults only. In contrast, a C-terminal translational GFP fusion, expressed from a recombineered fosmid, displayed a broader distribution, including DVC, uterus and other nerve cells in the head and tail region. The inconsistent expression patterns of N- and C-terminal fusions indicate that either there are alternative transcripts of *C02F12.10* present at very low abundance in the cDNA library or there are extra *cis*-acting elements, located in the intron/coding sequence of *C02F12.10*, whose action is disrupted by GFP insertion near the promoter. The possibility of an alternative *C02F12.10* isoform, initiating from an internal ATG in the second exon, is being tested. Loss-of-function *C02F12.10* deletion mutants are viable and fertile and do not show any obvious locomotion defects. We are further characterizing possible DVC identity defects in the *C02F12.10* deletion mutants. Another two homeodomain transcription factors CEH-14 and MBR-1, expressed broadly in *C. elegans* nerve cells including DVC, were found to act upstream and downstream of *C02F12.10* respectively. Expression of *mbr-1::gfp* in DVC was eliminated by the *C02F12.10* deletion and expression of *C02F12.10::gfp* in DVC was greatly attenuated or abolished in the *ceh-14 (ch3)* deletion mutant. The major synaptic outputs of DVC are to the main command interneurons AVA and AVB, which drives backward and forward locomotion respectively. Using the DVC-specific *C02F12.10* promoter we specifically targeted the light-gated cation channel channelrhodopsin-2 (ChR2) to DVC without interfering with other neurons in the circuit. While light stimulation of body wall muscles, the pharyngeal MC motor neurons and the ventral cord VB motor neurons, *via* ChR2 driven by the promoters from *myo-3*, *ceh-19b*, and *ceh-12* respectively, displayed specific and robust behavioural responses, we have not so far observed any specific response upon light stimulation of DVC.

912C

Positional analysis and functional genomics of clustered C. elegans and C. briggsae Nuclear Hormone Receptors. **Marta Kostrouchova**[1], Jaroslav Vohanka[1], Jan Majdan[2], Vlasta Poliackova[2], Eliska Machalova[1], Zdenek Kostrouch[2]. 1) Laboratory of Molecular Biology and Genetics, Inst Inherited Metabolic Dis, Charles Univ, Prague 2, Czech Republic; 2) Laboratory of Molecular Pathology, Inst Inherited Metabolic Dis, Charles Univ, Prague 2, Czech Republic.

Nuclear Hormone Receptors (NHRs) form a large family of transcription factors that are especially numerous in nematode genomes. In C. elegans, NHRs are spread over all autosomes and the chromosome X. The majority of apparently multiplied NHRs are localized on chromosome V where 179 NHRs are unevenly distributed. In this project, we analyzed the position and sequence similarity by application of mathematical statistics–R. The sequence similarity was evaluated by reciprocal comparison of similarity of each NHR compared to the set of all sequences denominated as NHRs in the Wormbase or recognized by Blast searches in the C. elegans genome. Application of Blast algorithm generated approximately 350 000 e-values. From that more than 80 000 values were used for calculation of the relative similarity of each NHR to all other NHRs. Positional analysis identified clusters in all chromosomes and indicated the orientation of genes in all clusters. This approach identified clusters of several orders based on the gene orientation and density. We have chosen a cluster containing nhr-206, nhr-208, nhr-207, nhr-209, nhr-154, nhr-153, and nhr-136, positioned at 12092023–12109115 on the chromosome V for functional analysis. The expression pattern based on the expression of gfp-fusion transgenes shows overlapping but clearly diversified expression in pharynx, intestine, intestinal sphincter, neurons, epidermal cells, rays and ray cells in males. Although the inhibition of individual genes as well as combinations of clustered genes did not induce a visible developmental phenotype, the reduction of brood size was observed in the case of genes that have orthologues in C. briggsae genome. Our data support the concept that an evolutionary pressure is keeping the multiplied genes functional in nematode genomes. Acknowledgement: We thank Drs. A. Fire for vectors and host used in RNAi, M.W. Krause for support and advice. The work was supported by grants 304/08/0970 and 304/07/0529 from the Czech Science Foundation and by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

913A

NHR-8 and the Regulation of both Basal and Xenobiotically-Induced Metabolism. **Tim H. Lindblom**, Brittany Young, Katee Castleman. Division of Science, Lyon College, Batesville, AR.

Chemicals in the environment, including pollutants, prescription drugs, and dietary ingredients have a dramatic effect on the physiology of the body by changing the biochemical makeup of many cell types. These foreign compounds, collectively called xenobiotics, alter the suite of enzymes present in cells through induced changes in the regulation of gene expression. Central players in this induction are the so-called “xenobiotic-sensing” receptors which are members of the nuclear receptor (NR) superfamily of ligand-regulated transcription factors. Members of the NR1 subfamily of NRs directly link the presence of toxins to the production of phase I and II metabolizing enzymes and drug transporters responsible for xenobiotic detoxification and efflux. The structure of these NRs, possessing both ligand and DNA binding domains, reflects their function and the almost canonical way in which they operate: xenobiotic binding, receptor translocation, and target gene transcriptional activation. Yet, increasing evidence suggests that the molecular pathway of xenosensation is hardly this simple. Crosstalk between xenosensing NRs, NRs mediating other signaling events, and even non-NR transcriptional regulators demonstrates the intersection of xenosensation with other physiologies such as lipid and carbohydrate metabolism and hormonal signaling. A *C. elegans* member of the NR1 subfamily, NHR-8, is required for wild type resistance to toxic challenges. We probed *C. elegans* Affymetrix GeneChips with RNA from *nhr-8* mutants in the presence of the xenobiotic, primaquine. Among the genes potentially regulated by NHR-8 are those that encode detoxification enzymes and those encoding basal metabolic enzymes. Thus, NHR-8 is uniquely situated to provide details of the combinatorial control of toxin induced gene expression and how this regulation impacts normal cellular biochemistry. We will report on our microarray results and our efforts to verify these findings.

914B

Regulating the transcription of a transcription factor. **Stephany G. Meyers**, Ann K. Corsi. Biology, Catholic Univ of America, Washington, DC.

An important issue in developmental biology is understanding the temporospatial regulation of genes encoding transcription factors. The *hlh-8* gene encodes a basic helix-loop-helix transcription factor called Twist that is involved in mesoderm development in *C. elegans* and other metazoa. Specifically, *hlh-8* is expressed in the undifferentiated M lineage, 2 coelomocytes, sex muscles, enteric muscles and head mesodermal cell (hmc). In humans, misregulation of Twist is implicated in cancer metastasis and mutations cause an autosomal dominant craniosynostotic disorder. Studies in multiple organisms, including *C. elegans*, reveals that Twist also provides an interesting paradigm of transcriptional control, functioning as both a homo- and heterodimer and often in a dose-dependent manner. Consequently, understanding how *hlh-8* expression is regulated is key to understanding its function in tissue-specific processes. Extensive analysis has revealed that the upstream promoter region of *hlh-8* only controls expression in a subset of tissues where it functions; namely in the coelomocytes and undifferentiated M lineage. Thus, important cis-acting elements must exist elsewhere. We are exploring the large first intron of *hlh-8* for additional regulatory elements using *gfp* reporter genes and a mutant, *hlh-8 (tm726),* with a large deletion (646 bp) in the first intron of the gene. In contrast to *hlh-8* null mutants, the intron deletion allele displays a subset of phenotypes affecting only certain tissues, suggesting intron sequences are involved in regulating expression. Through an analysis of intron 1 sequence elements, we have found two elements that appear to control *hlh-8* autoregulation, implicating Twist dosage effects that seem to be restricted to the differentiated tissues of the hmc, sex and enteric muscles. These results underscore the complexity underlying the regulation of a transcription factor with multiple modes of regulation that directly impact its function. Such complexity would be difficult to discern from high throughput, genome-wide studies.

915C

Fluorescent reporter expression analysis of *C. elegans* transcription factors with multiple isoforms. **Julia Wirtz**, Hannah L. Craig, Sophie Bamps, Ian A. Hope. Institute of Integrative and Comparative Biology, University of Leeds, LS2 9JT, UK.

C. elegans achieves tight regulation of the expression of its ~20,000 genes with ~1,000 transcription factors. Multiple isoforms of transcription factors could increase the transcriptional regulatory potential, but no concerted effort has been made to examine the scale of the significance of alternative transcripts for transcription factor gene function. As a first step, we sought to clarify which transcription factor genes in the *C. elegans* genome were likely to encode multiple proteins. In WormBase 195 transcription factor genes are annotated as encoding different isoforms. Upon scrutiny of the data 36 were excluded from our attention because their alternative transcripts appeared to arise from sequencing errors in the EST data or were predicted but not confirmed. Amongst the 159 genes that remained, 59 had alternative promoters, 26 had alternative 3' ends, 14 had alternative exons, 6 had alternative introns, 26 had minor splice site alternatives that would generate proteins differing by a few amino acids, and 28 were complex with combinations of mechanisms for producing distinct transcripts. An initial set of 7 genes (*egl-13, vab-3, ZC376.7, nhr-23, nhr-66, egl-44, jun-1*) was targeted. These genes are annotated as being driven from at least 2 promoters, except for *ZC376.7* which retains an intron in one of its 2 transcripts. A *gfp* reporter was inserted at the ends of the protein-coding regions by seamless recombineering of fosmids harboring these genes in a central position. Expression patterns had been examined previously for all of these genes, apart from *ZC376.7*. However, only conventional transcriptional or translational reporter gene fusions or in situ hybridization had been applied in these studies and not all of the predicted transcripts had been investigated. Expression of *gfp* for *egl-13, vab-3, nhr-23* and *jun-1* matched well to described patterns except that for *vab-3* no intestinal expression was observed. For *nhr-66* and *egl-44*, no expression was detected. *ZC376.7::gfp* was expressed in adult vulval cells. While expression of *gfp* inserted at the 3' end of these genes with alternative promoters should represent the sum of the expression of each transcript, *gfp* placed at the very 5' end of the coding regions would be expected to reveal the contribution of a single transcript. Expression of 5' tagged *egl-13.a* matched the 3' fusion. For *nhr-23.a* expression was seen only in head and tail hypodermal cells and neurons, and not throughout the body. For *nhr-66.b* expression was now seen, and seen in hypodermis, pharynx and intestine. Further dissection of the contribution to expression made by each transcript of these 7 genes will involve disruption of specific exons in the *gfp* fusions.

916A

Analysis of C-terminal Binding Protein in *C. elegans*. **Duygu Yucel**, Hannah Nicholas. School of Molecular and Microbial Biosciences, University of Sydney, Sydney, NSW, Australia.

C-terminal Binding Protein (CtBP) is a transcriptional co-repressor which has been widely investigated in both vertebrates and invertebrates. CtBP plays roles in a variety of biological processes including development and apoptosis. There are two highly related CtBP genes in vertebrates. Knock-out of both CtBP1 and CtBP2 genes results in lethality early in embryogenesis. Recently, a single CtBP gene has been found in *Caenorhabditis elegans*. Like its mammalian counterparts, the worm CtBP, called CTBP-1, functions as a transcriptional co-repressor and docks onto transcription factors containing an amino acid motif of the form PIDLS. *In vitro* experiments have shown that CTBP-1 can directly contact the PIDLS motifs in PAG-3 and ZAG-1. We have commenced an analysis of the expression pattern of the CTBP-1 using a translational reporter construct which has shown that CTBP-1 is localised to the nucleus of numerous cells, some of which have been identified as neurons. By defining the cells and tissues in which CTBP-1 is expressed, these studies will give further insights into the potential functions of CTBP-1.

917B

Translational repression and derepression of *pal-1* maternal mRNA. **Jacqueline M. Brooks**, Craig P. Hunter. Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Post-transcriptional regulatory events control the timing and spatial expression of proteins that act in a wide range of developmental events. In *C. elegans*, post-transcriptional control of a repertoire of maternal mRNAs is mediated via their 3' UTRs. This regulation is fundamental for proper germline and embryonic development yet the precise mechanism(s) are not well understood. Work in our lab has suggested that at least one mechanism for this regulation is post-initiation translational repression of *pal-1*, a maternally inherited mRNA that controls posterior embryonic development. In keeping with this, *pal-1* mRNA is present throughout the oocyte and early embryo yet PAL-1 protein first accumulates in the two posterior blastomeres at the four-cell stage of embryogenesis. Published work from our lab has shown that the RNA-binding proteins, GLD-1 and MEX-3, restrict PAL-1 translation via a 107 nucleotide Germline Repression Element (GRE) present in the *pal-1* 3' UTR. We have further explored the mechanisms of *pal-1* translational repression and derepression in strains expressing reporters regulated by the GRE. We have developed a new method for translation run-off by which we challenge *in vivo* repressed polysomes with translationally competent worm extracts. Our data suggests a link between *pal-1* post-initiation translation repression and incomplete peptide production thus defining a novel mechanism of reversible mRNA quiescence. We propose that spatial and temporal signals in the early embryo release this post-initiation block leading to the rapid synthesis and release of PAL-1 in the posterior blastomeres. Such mechanisms may be particularly critical in the early *C. elegans* embryo where key regulatory factors are induced in successive and brief cell cycles. We are using RNAi to target select components implicated in translational repression to identify additional molecules that regulate *pal-1* translation. Interestingly the GRE contains predicted miRNA (microRNA) binding sites that may be involved in selecting the *pal-1* mRNA for repression. We will show results that suggest that a candidate miRNA as well as components of the RNA induced silencing complex (RISC), may provide target specificity. The mechanism by which these postulated regulators attenuate the translational competence of *pal-1* mRNA is under investigation.

918C

Genetic Study of Translation Start Codon Recognition in C. elegans. **Yinhua Zhang**, Lisa Maduzia, Sebastien Charffre, Nausicaa Poullet. New England Biolabs, Ipswich, MA.

Recognition of AUG start codon during translation initiation is critical for decoding the genetic information on mRNAs. In prokaryotes, the Shine-Dalgarno sequence located 5' to the AUG on the mRNA complements to a region on the 16S rRNA and this base-pairing interaction positions the mRNA so that the initiator tRNA finds the AUG efficiently. In contrast, there is no evidence in eukaryotes that such base-paring occurs, and instead eukaryotes appear to rely mainly on the base pairing of the AUG with the anticodon of the initiator tRNA. It is postulated that recognition is achieved through ribosomal scanning, in which a pre-initiation complex, consisting of the small ribosomal subunit, multiple translation initiation factors (eIFs) and initiator tRNA, moves along the mRNA to examine each codon by base pairing with the anticodon. Perfect base pairing triggers the hydrolysis of GTP by the heterotrimeric G-protein eIF2 and leads to a cascade of molecular interactions that allow translation to start. We have developed a genetic system in C. elegans to study how the start codon AUG is recognized. Our strategy is similar to that employed in the isolation of S. cerevisiae sui (suppressor of initiation codon) mutants, whereby a selectable reporter that contained an altered initiation codon was utilized (Donahue et al., Cell 54:621, 1988). We changed the ATG translation initiation codon of an abundantly expressed GFP reporter to that of a GTG codon and found that the GFP signal is almost completely eliminated in wild type worms. With this silent reporter, we performed genetic screens and obtained mutants that showed varying degrees of GFP expression. Mutations in two mutant strains have been identified. Both are missense mutations; one in the β subunit of eIF2 and the other in eIF1. We further studied if this newly identified eIF2β mutation and the yeast SUI3 mutations when introduced into the corresponding sites of the C. elegans gene could initiation translation with other non-AUG codons. We found that all of these mutants allow GFP to be synthesized from reporters that contain altered start site codons that differ by one base either in the first or third base position. Changes in the second base position result in much less GFP expression and changes of two or three bases reduce expression to undetectable levels. Our results differ from what was previously observed in S. cerevisiae. The SUI3 mutations only allow translation from the non-AUG codon UUG in the yeast reporter assay (Huang et al., Genes Dev 11:2396, 1997). Our analyses in C. elegans suggest that eIF2β plays a role in recognizing proper codon-anticodon pairing and/or in relaying the signal of correct base pairing which leads to GTP hydrolysis.

919A

Identification of transcription factors regulating *lin-39* expression. **Wan-Ju Liu**, David Eisenmann. Biological Science, University Maryland, Baltimore County, Baltimore, MD.

The *C. elegans* Hox gene, *lin-39*, which patterns the midbody region of the worm, is required for vulval formation during development. During vulval development, *lin-39* expression is regulated by two extracellular signaling pathways, the RTK/Ras signaling pathway and the Wnt signaling pathway. To further understand how LIN-39 regulates cell fate specification and pattern formation, especially in vulva formation, we wish to identify the transcription factors that regulate *lin-39* expression. Previous work by our lab and others has identified several transcription factors and chromatin regulatory proteins that regulate *lin-39* expression. To broadly identify other transcription factors necessary for *lin-39* expression, the Yeast-1-Hybrid system was used with a *C. elegans* transcription factor library. The *lin-39* promoter region from *C. elegans*, *C. briggsae* and *C. remanei* was aligned, and 33 conserved regions were found and referred to as triple conserved regions (TCRs). 12 fragments (frf-1~frf-12) containing the TCRs were cloned into *LacZ* and *HIS3* reporter constructs that were integrated into yeast, which were transformed with the transcription factor library to identify transcription factors binding to particular *lin-39* promoter regions. We have found three transcription factors that interact with different fragments of the *lin-39* genomic region: *nhr-43*, a nuclear hormone receptor, *ztf-17*, a zinc finger protein, *alr-1*, a transcription factor containing Hox domain. We are doing further experiments to validate these interactions in *C. elegans* and determine a role for the factors. To determine if the protein regulates *lin-39* in worms, we are performing RNAi in worms carrying *lin-39::GFP* constructs. To identify the interaction between transcription factors and the *lin-39* promoter regions, we will perform gel shift assays. We will also perform RNAi on wild type worms to observe whether there is any phenotype in lin-39 dependent processes. Currently we found *lin-39* expression seems to go down in P5,p, P6,p and P7,p when treated with *ztf-17* RNAi.

920B

Site-specific insertion of transgenes into *C. elegans*. **Marcus L. Vargas**[1], Iva Greenwald[1,2]. 1) Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

In *C. elegans*, transgenes are generated by germline injection of DNA, which forms extrachromosomal arrays that are composed of many copies of the injected DNA. The nature of extrachromosomal arrays makes them problematic in many circumstances, and there is no single, simple, reliable approach that circumvents all of the problems with these arrays.

The ideal method would be homologous recombination, which so far has not been shown to be practical. The next-best would be an approach that would allow for different transgenes to be reliably and easily inserted in single copy into a defined site in the genome. This would allow for different transgenes to be compared to each other without concerns about copy number and integration site effects. The bacteriophage ϕC31 integrase system has been used successfully in *D. melanogaster* and in other heterologous systems for high-efficiency single copy insertion of transgenes into the genome (Groth et al., *Genetics* 2004; Bateman et al., *Genetics* 2006; Bischof et al., *PNAS* 2007). We have been assessing its utility for transgene insertion in *C. elegans*.

The ϕC31 integrase mediates sequence-specific recombination between two largely different sequences, called attB and attP. The basic method involves recombination between an attB site encompassing the DNA to be inserted and an attP site integrated into the genome. In pilot experiments, we found that ϕC31 mediated integration works in *C. elegans* by using an attP platform, which had been obtained by bombardment to insert a plasmid containing an attB site and *Pmyo-3::mCherry*. However, the bombarded attP platform contained multiple copies of the landing site and hence is not useful for a general method. Furthermore, this initial approach did not incorporate a selection system to facilitate the identification of integrants.

We are currently working on obtaining the attP site into the genome at single copy. We are trying two methods for single copy insertion of the attP site into the *C. elegans* genome: MosTIC recombination (Plasterk and Groenen, *EMBO J.* 1992; Robert and Bessereau, *EMBO J.* 2007) and the piggyBac transposable element, which has been shown to be mobile in *D. melanogaster* and other systems (Handler et al., *PNAS* 1998; Lobo et al., *Mol Gen Genet* 1999). If piggyBac transposition works in *C. elegans*, it may provide an alternative method for random single-copy insertion of transgenes and transposon tagging. We are also evaluating different selection systems. We will provide our progress and plans at the meeting.

921C

Investigation of the molecular function of the chromatin factor Zinc Finger Protein-1, ZFP-1, in C. elegans. **Daphne Anastasiades**, Germano Cecere, Andres Mansisidor, Alla Grishok. Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

The discovery of RNA interference (RNAi) has led to a new understanding of the regulation of gene expression. Currently, both nuclear and cytoplasmic RNAi have been observed in C. elegans. Zinc Finger Protein 1 (ZFP-1), a PHD finger containing protein that is the C. elegans homolog of the human chromatin-associated protein AF10, is one of the genes affecting nuclear RNAi. Our additional data suggest that ZFP-1 may be central for the regulation of endogenous genes by a large class of short interfering RNAs (siRNAs) antisense to their targets (endo-siRNAs). The molecular function of ZFP-1 is not known and is thus the primary focus of this investigation. We find via histone binding assays in vitro that the first PHD finger of ZFP-1 binds specifically to the dimethylated state of lysine 4 on histone H3 (H3K4me2). Using a ZFP-1::GFP translational fusion reporter, we observe nuclear localization of ZFP-1 in most cell types as well as ZFP-1 localization on the chromosomes in oocytes. Staining in oocytes with anti-H3K4me2 antibodies shows co-localization of ZFP-1 with the H3K4me2 mark, which correlates with our in vitro findings. Furthermore, because methylation of H3K4 accompanies transcription, we propose that ZFP-1 may connect RNAi factors with transcriptional machinery.

922A

Characterization of the expression pattern of ZFP-1, a chromatin factor involved in RNAi. **Germano Cecere**, Andres Mansisidor, Alla Grishok. Department of Biochemistry and Molecular Biophysic, Columbia University, New York, NY.

RNA interference (RNAi) in Caenorhabditis elegans has been shown to occur both in the cytoplasm and in the nucleus. In the past several screens have been done in order to identify the genetic components required for RNAi and several chromatin factors have been found, suggesting that a chromatin-based silencing process occurs in C.elegans. To better elucidate the mechanism of this phenomenon we started to biochemically characterize one of the putative chromatin factors required for the silencing process, zfp-1. We therefore have created transgenic lines expressing the recombinant GFP and FLAG tagged versions of ZFP-1 by using fosmid recombineering coupled with DNA bombardment in order to have all the regulatory information of the gene and to avoid silencing of the transgenes in the germline. We confirmed that the recombinant proteins are functional and the spatial-temporal expression pattern reveals that ZFP-1 is a nuclear protein expressed during all the developmental stages in all tissue including the germline. By western blotting analysis we observed at least two isoforms that are differentially expressed during the development and in different culture conditions and we started to characterize the transcriptional unit of the zfp-1 gene and using a proteomic approach to identify protein interactors of ZFP-1 variants in order to better elucidate the function of these proteins.

923B

Characterizing distinct functions of synMuv B genes in RNAi and related processes. **Xiaoyun Wu**[1,2], Gary Ruvkun[1,2]. 1) Dept Molecular Biol, Massachusetts General Hosp, Boston, MA; 2) Dept. Genetics, Harvard Medical School, Boston, MA.

Synthetic multivulva class B (synMuv B) genes prevent excess RAS signaling during vulva development. Many of them also affect RNAi related processes including the response to feeding RNAi and the silencing of transgenes. Some synMuv B genes encode transcriptional repressors, namely the components of the DRM complex, the NuRD complex and several heterochromatin factors that modify or bind to histone tails, while others were poorly characterized. We sought to understand how individual synMuv B genes function in RNAi and related processes.

Not all synMuv B genes show the same effect on RNAi related processes, suggesting that more than one mechanism may be involved. To understand these mechanisms, we surveyed all known synMuv B genes and classified their effects on RNAi and transgene silencing. Results revealed two subclasses with clear phenotypic distinctions. One subclass consists of components of the DRM complex plus *lin-15b*, where mutations led to strongly enhanced RNAi and enhanced transgene silencing. The other subclass represents the heterochromatin factors and *lin-65*, where mutations led to weakly enhanced RNAi and transgene desilencing. This classification provided insights to the functions of previously uncharacterized synMuv B genes. More importantly, it suggests that synMuv B genes may provide two separate biochemical functions that potentially act at multiple steps in RNAi related processes. We are now conducting genetic analyses to better understand the relationships between them.

synMuv B genes negatively regulate RNAi via different mechanisms than other *eri* genes. They are additive with other *eri* genes, giving rise to further enhanced feeding RNAi. Other known *eri* mutants lack several endo siRNAs, which is thought to be the basis of their enhanced RNAi phenotypes. We found that synMuv B mutants do not affect the levels of these endo siRNAs or their targets, and thus likely function at a different step. The efficiency of transgene silencing usually correlates with the efficiency of feeding RNAi, as in most known *eri* and *rde* mutants. The synMuv B heterochromatin mutants, however, show reduced transgene silencing *while* enhanced feeding RNAi, which is paradoxical and suggests that these chromatin factors act in a unique place in RNAi related processes. We are currently taking genetic, molecular and biochemical approaches towards pinpointing the steps of action in the RNAi process for synMuv B genes and understanding the unique mechanisms by which these genes negatively regulate RNAi.

924C

Examining the role of ALG-2 in *C. elegans* Dosage Compensation. **Emily Crane**[1], John Gladden[1], Amy Pasquinelli[2], Barbara Meyer[1]. 1) HHMI/UC, Berkeley, Berkeley, CA 94720; 2) Department of Biology, UC San Diego, La Jolla, CA 92093.

We have found a connection between dosage compensation and the small RNA processing machinery in *C. elegans*. Dosage compensation is achieved by targeting a dosage compensation complex (DCC) to X chromosomes of hermaphrodites. The DCC is a multi-subunit complex including 5 proteins homologous to condensin subunits. Evidence exists in many organisms that non-coding RNAs play a role in dosage compensation. For example, Drosophila requires roX RNAs to upregulate the single male X, while mammals require Xist RNA to silence a random female X. We have shown in *C. elegans* that ALG-2, one of the two argonautes that specifically processes miRNAs, plays a role in dosage compensation. Initial experiments showed that in XX embryos sensitized by a weak dosage compensation mutation [*sdc-2(y93*)] and RNAi to *alg-2*, two effects were noticed (1) hermaphrodite specific synergistic lethality that maps in the pathway at the level of dosage compensation activation, (2) disrupted localization of the DCC to X. *sdc-2* is pivotal to both sex determination and dosage compensation, is expressed only in the hermaphrodite, and is essential for targeting the DCC to the X chromosome. While neither *alg-2(RNAi)* nor *sdc-2(y93)* has a major affect on X chromosome expression, the double mutant combination has a strong effect on X chromosome expression in a manner similar to a strong *sdc-2* mutation. ChIP-Chip analysis with an antibody against a DCC component in an *alg-2(RNAi); sdc-2(y93)* mutant revealed a strong selective loss of DCC binding to sites essential to DCC recruitment to X. The *sdc-2* null mutant shows a very similar loss of DCC binding. Surprisingly, mutations in the second *C. elegans* micro RNA specific argonaute gene *alg-1* do not disrupt DCC localization or function in the *sdc-2(y93)* background.

These results suggest that *alg-2* may affect the expression or stability of one or several DCC members through an unknown regulator of dosage compensation. We are currently investigating whether *alg-2* mutations affect dosage compensation by altering a micro RNA that represses an inhibitor of dosage compensation, by causing selective chromatin silencing, or by a novel mechanism.

925A

A new synthetic lethal screen lead to the indentification of a new component of the microRNA pathway. **Gabriel D. Bossé**, Évelyne L. Rondeau, Alejandro R. Vasquez, Martin J. Simard. Laval University Cancer Research Centre, Québec, Québec, Canada.

Found in all metazoans, the newly discovered microRNA pathway is critical to regulate the expression of various genes essential for proper cell proliferation and differentiation during development. Despite its importance, so far only a few genes have been implicated in this pathway and many others have yet to be discovered. In order to uncover new components of the microRNA pathway, we performed a genetic screen to identify synthetic lethal genes with the Argonaute *alg-2*. From a pilot screen, we isolated ten candidates that lie into five complementation groups. The initial characterization of one of this complementation group suggests that we have identified a new component of the microRNA pathway. Members of this group display strong heterochronic defects as previously observed with the loss-of-function of known components of the microRNA pathway such as *dcr-1* and *alg-1/alg-2* (alae and seam cells defects, abnormal gonads and embryonic lethality). Enhanced phenotypes are observed in these mutants when the expression of the alg-2 gene is abrogated supporting a synergistic role with this Argonaute gene. We are currently performing various biochemical approaches to identify at which step(s) of the microRNA pathway this new gene is acting (i.e. microRNA production and stability, direct effect on microRNA-regulated mRNAs). At this meeting, we will present our complete characterization and identification of this new genetic interactor of *alg-2*. This work is supported by the Canadian Institutes of Health Research.

926B

A putative DEAD-box RNA helicase DDX47 plays a role in the *let-7* function in *C. elegans*. **Shih-Peng Chan**, Frank Slack. Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

RNA helicases from the DEAD-box protein family are found in almost all organisms and have been shown to participate in every aspect of RNA metabolism, including small RNA-mediated gene silencing. Here we show that a homolog of human DEAD-box RNA helicase DDX47 plays a role in *let-7* function in *C. elegans*. In *let-7(n2853)* mutant animals, reduced expression of DDX47 by feeding RNAi suppressed aberrant vulval and hypodermal development and increased the expression of the adult-specific marker *col-19::gfp*, which is indirectly activated by *let-7*. *DDX47(RNAi)* also partially restored the regulation of a *lacZ* reporter bearing the *lin-41* 3'UTR, a direct target of *let-7*, in *let-7(n2853)* animals. Furthermore, we detected DDX47 in a pull-down experiment using biotinylated 2'-*O*-methyl oligonucleotides antisense to *let-7* after *in vivo* UV-crosslinking, suggesting possible physical interactions between DDX47 and *let-7*.

927C

Small, non-coding RNAs, and the role of *mir-34* in DNA damage response. **Masaomi Kato**[1], Joanne Weidhaas[2], Frank Slack[1]. 1) Dept MCDB, Yale Univ, New Haven, CT; 2) Dept Therapeutic Radiology, Yale Univ School of Med, New haven, CT.

Small non-coding RNAs serve an important role in controlling gene expression during development. However, little detailed information exists concerning the relative expression patterns of small RNAs during development of any animals. We performed a deep analysis of small RNA expression in *C. elegans* using the Solexa sequencing technology, and found that a significant number of miRNAs showed major changes in expression during development and aging, and in different sexes of *C. elegans*. Additionally, we identified many novel miRNA candidates and hundreds of novel piRNA/21U-RNAs with dynamic expression during development, together with many longer transcripts encompassing mature 21U-RNAs that may represent intermediates in 21U-RNA biogenesis. Also, approximately half of the novel miRNA candidates showed transcripts from their "star sequence", suggesting that they are bona fide miRNAs. Our analysis reveals extensive regulation of small, non-coding RNAs during development and aging, and suggests their possible involvement in these biological processes in *C. elegans*. Identification of further transcripts and their biological roles will lead to a better understanding of the control of gene expression during development and disease.

Additionally, we have been focusing on the role of miRNAs in cancer-related events. A well-conserved miRNA, *mir-34*, is transcriptionally regulated by the tumor suppressor *p53* in mammalian cells, suggesting the possible role of *mir-34* in the cancer-related DDR (DNA damage response) network. However, these were *in vitro* studies and the role of *mir-34* in the DDR has not been defined *in vivo*. We found that the miR-34 expression level rose post-irradiation in *C. elegans*, as observed in mammalian cells. Also, *mir-34* loss-of-function mutants were radiosensitive in non-apoptotic cell death in the vulval model. Conversely, they were resistant to radiation-induced apoptotic germline cell death in *C. elegans*. These findings suggest that *mir-34* is necessary for both apoptotic and non-apoptotic cell death *in vivo* in *C. elegans*, similar to *cep-1*, a homologue of *p53* in *C. elegans*. In order to better understand the relationship if any between *mir-34* and *cep-1* in the DDR, we tested the effect of double mutations in both of these genes on cell survival in *C. elegans*. The *mir-34;cep-1* double mutants were significantly more sensitive to non-apoptotic cell death in the vulva than either single mutant, suggesting that *mir-34* and *cep-1* function in parallel in the DDR pathway to modulate the response to DNA damage in *C. elegans*.

928A

The Cold Shock Domain Protein, CEY-3, regulates miR-1 target expression and AIN-1 localization to P bodies in the body muscle. **Vishal Khivansara**[1], Jim Moresco[2], Dongping Wei[1], John Yates[2], John Kim[1]. 1) Department of Human Genetics, Life Sciences Institute, Ann Arbor, MI; 2) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

The microRNA-induced silencing complex (miRISC) guides microRNAs to the 3' UTR of target mRNAs through partial base pairing and leads to translational inhibition and/or target degradation. However, the steps of target recognition and binding, and the role of miRISC and other RNA-associated factors remain active areas of investigation. We have identified a novel protein complex that binds microRNAs and their targets in the body muscle of C.elegans. This complex includes AIN-1, a conserved member of miRISC, as well as three highly conserved RNA binding proteins with Cold Shock Domains (CSD): CEY-2, CEY-3, and CEY-4. To elucidate the function of the CSD proteins, and in particular, CEY-3, in the regulation of microRNA targets, we have focused our studies on miR-1 rcontrol of its mRNA target, mef-2, in the body muscle (1). RNAi inactivation or deletion (tm2839) of cey-3 causes a significant increase in GFP::mef-2 3'UTR reporter expression, compared to vector control RNAi, and was similar to GFP reporter expression observed after RNAi-inactivation of ain-1, which is essential for the regulation of microRNA targets (2-3). Secondly, the P-body localization of AIN-1::GFP was completely abolished in the cey-3(tm2839) deletion mutant and by cey-3 RNAi. Endogenous levels of AIN-1 were also significantly reduced when cey-3 was inactivated, suggesting that cey-3 is required for AIN-1 expression and recruitment to P-bodies. All CSD proteins possess two conserved motifs (RNP1 and RNP2) within the cold-shock domain that are essential for binding RNA substrates. Substitutions of the following residues of CEY-3 to Ala: Tyr76 and Phe78 of RNP1 and Phe90 and His92 of RNP2 disrupt the recruitment of CEY-3::GFP to P bodies, especially during later stages of development, suggesting that RNA-substrate binding is a requirement for CEY-3 localization to P bodies. Current analysis is focused on determining whether miR-1 targets are substrates of CEY-3 and whether CEY-2,- 3 and -4 form distinct AIN-1 complexes in the body muscle. We expect that these studies will provide insights into the conserved mechanisms of how microRNA targets are recognized and designated for translational inhibition and/or degradation.

1.Simon, D.J. et al., Cell 133: 903-915 (2008).
2.Ding, L. et al., Mol Cell 19:437-447 (2005).
3.Zhang, L. et al., Mol Cell 28: 598-613 (2007).

929B

The miR-51 Family Of microRNAs Is Required For The Maintenance Of Pharyngeal Attachment. **W. Robert Shaw**, Eric A. Miska. Wellcome Trust Cancer Research UK Gurdon Institute, The Henry Wellcome Building of Cancer and Developmental Biology, University of Cambridge, Cambridge, CB2 1QN, United Kingdom.

A deletion screen (1) has developed a resource of mutants carrying deletions in most C. elegans microRNAs (miRNAs), short regulatory RNAs that act to repress gene expression post-transcriptionally. The function of most miRNAs in C. elegans is unknown and the majority of mutants show no gross abnormal phenotype (1).

The miR-51 family is a redundant and conserved family of six miRNAs whose putative promoters drive GFP in overlapping but not identical spatial patterns. Mutants lacking multiple members of the miR-51 family show gradually more severe defects in growth and animals lacking all members of the family arrest as larvae with an unattached pharynx (Pun) phenotype. These mutant phenotypes can be rescued by short genomic regions encoding any of the miR-51 family miRNAs, demonstrating their redundancy.

The pharynx of C. elegans forms from a ball of cells specified in the interior of the developing embryo and extends anteriorly to attach to the hypodermis (2). This attachment requires the arcade cells, which become polarised during pharyngeal attachment and form a continuous epithelium between the pharyngeal and hypodermal cells (2). In the early development of *mir-51* family mutant larvae, this epithelium is labelled with a DLG-1-mCHERRY fusion protein indicating that the arcade cells have polarised. However, the attachment of the cells to the pharynx is not maintained and arcade cells separate from the anterior pharynx.

We have identified the first target mRNA of the miR-51 family of microRNAs as the cadherin, *cdh-3*. A reporter for cdh-3 drives GFP expression in the arcade cells and the 3'UTR of cdh-3 mRNA confers direct regulation by the miR-51 family of miRNAs. However, mutations in *cdh-3* or *cdh-3*(RNAi) fail to suppress the Pun phenotype, suggesting the regulation of a network of miR-51 family target mRNAs is important in the maintenance of pharyngeal attachment.

(1) Miska, E. A., Alvarez-Saavedra, E. A., Abbott, A. L., Lau, N. P., Hellman, A. B., McGonagle, S., Bartel, D. P., Ambros, V. R., Horvitz, H. R.–PLoS Genetics, 2007)
(2) Portereiko, M.F., Mango, S.E.–Developmental Biology (2001).

930C

Analysis of protein-RNA interactions provides insights into miRNA function and biogenesis. **Giovanni Stefani**, Frank Slack. Dept MCDB, Yale Univ, New Haven, CT.

Protein-RNA interactions play a critical role in RNA silencing pathways. MiRNAs regulate gene expression by directing the miRISC, a multi-protein effector complex, to their mRNA targets. Analogously, siRNAs and piRNAs direct specific protein effector complexes to their regulatory targets. Furthermore, interactions between proteins and RNA precursors exert important regulatory effects on various steps of small RNAs biogenesis and maturation. In an effort to gain a better understanding of miRNA functions and biogenesis, we co-purified RNAs cross-linked in vivo to regulatory proteins in nematodes. By co-purifying mRNAs with Alg-1, an essential component of the miRISC in worms, we aim to identify functional targets of miRNAs, and to characterize the modalities of miRNA-target mRNA interactions. Similarly, we are analyzing the pool of RNAs co-purifying with proposed regulators of miRNA biogenesis, such as lin-28, in an effort to fully describe the pool of their regulated targets.

931A

MicroRNA-mediated translation repression and deadenylation in *C.elegans* embryos. **Edlyn Wu**, Mathieu Flamand, Caroline Thivierge, Marc Fabian, Géraldine Mathonnet, Nahum Sonenberg, Thomas Duchaine. Department of Biochemistry, Goodman Cancer Center, McGill University, Montreal, Quebec, Canada.

MicroRNAs (miRNAs) are small RNAs that play a pivotal role in post-transcriptional gene regulation. Most commonly in animals, these regulatory RNAs target mRNAs by imperfectly binding to complementary sites in 3' untranslated regions (3'UTR), thereby affecting the translation of the targets, or reducing their stability. Despite the significant roles miRNAs play in various biological processes, the mechanistic details of how they regulate gene expression remain unclear. We present here the first *in vitro* translation system derived from *C.elegans* embryos that recapitulates cap- and polyA-dependent translation as well as miRNA-dependent silencing. Using luciferase reporters fused to an artificial 3'UTR containing sites complementary to the maternally contributed *mir-35* miRNA family, we observed up to 70% inhibition by *mir-35* over a 3-hour translation time-course. Upon addition of 2'-O-methyl oligonucleotide inhibitors for the endogenous *mir-35* family, translation of our reporters was restored. To understand how this inhibition occurred, we examined the fate of our reporter mRNAs. Inhibited mRNA reporters became fully deadenylated within the first 60 minutes of incubation with our cell-free extract, while a reporter bearing a mutation in the region complementary to the miRNA seed was not susceptible to inhibition or deadenylation. MiRNA-mediated deadenylation is independent of the translation of the targets as neither ApppN capping of the transcripts, nor the presence of the translation inhibitor cycloheximide inhibited deadenylation. We will describe our efforts to screen for endogenous *miR-35* targets via deadenylation and translation inhibition assays. We will further define the genetic requirements for translation repression and processing of target mRNAs by testing extracts that are RNAi-depleted. Our findings indicate that deadenylation plays a prominent role in miRNA-mediated control of gene expression in the *C.elegans* embryo.

932B

Inhibiting miRNA in C. elegans using a potent and selective antisense reagent. **G. Zheng**, W. Li. Departments of Cell Biology and of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX.

Antisense reagents is a useful reverse genetics tool for studying gene function, yet no antisense reagents are available to reliably inhibit microRNAs (miRNAs) in the nematode C. elegans. We have developed a new class of fluorescently labeled antisense reagents to inhibit miRNAs in worms. Using metabolically stable oligoribonucleotides and biocompatible carriers, we have developed novel oligo bioconjugates that can be conveniently introduced into the germline of adult hermaphrodites. The antisense reagent is transmitted to the progeny of injected worms to inhibit miRNAs in different tissues including nervous systems with high efficiency, and they selectively inhibit single miRNAs in a sequence specific manner without affecting other miRNAs sharing similar sequences. Examples are presented of using this new class of antisense reagents to block lin-4, let-7 and lsy-6 to produce the expected loss of function phenotypes. Finally, we show that these reagents can be used combinatorially to inhibit more than one miRNAs at a time, thus offering a convenient approach to study miRNAs with overlapping functions.

933C

small RNA profiling in the C. elegans germline and germ cells. **Arun Prasad Manoharan**[1], Ting Han[1], Vishal Khivansara[1], Colin Fitzpatrick[2], Martin Hirst[3], Marco Marra[3], Diana Chu[2], John Kim[1]. 1) Life Sciences Institute, Ann Arbor, MI; 2) Department of Biology, San Francisco State University, San Francisco, CA; 3) Genome Sciences Centre, British Columbia Cancer Center, Vancouver, BC, Canada.

To identify classes of small RNAs expressed in the germline and germ cells, we performed a genome-wide analysis of the small RNA transcriptome in C. elegans. Small RNAs play central roles in regulating germline development. Mutations affecting various small RNA pathways are frequently associated with the loss of fertility. To uncover small RNAs enriched in the germline and germ cells, we sequenced the small RNAs expressed in N2 worms, the *glp-4(bn2)* temperature-sensitive germline mutant, purified sperm from *him-8* and purified oocytes from *fer-1*, and in N2 embryos. By high-throughput deep sequencing, using the Solexa (Illumina) and 454 (Roche) platforms, we generated over 14 million sequence reads that map perfectly to the C. elegans genome.

Comparative analysis allowed us to determine enrichment of particular small RNAs to different cell types. For example, comparing the expression of small RNAs in N2 vs. *glp-4* identified 25 miRNAs that were enriched 5-fold or higher in the germline and 19 miRNAs that were enriched in the soma. Comparisons between oocyte and sperm samples identified 49 miRNAs that were enriched 5-fold or higher in oocytes but only 3 miRNAs that were enriched in sperm. Computational analysis indicates that the putative miRNA targets are also enriched in these particular tissues or cell types.

In addition to comparative analysis of known classes of small RNAs, we have identified 70 potentially novel microRNAs as well as additional germline-expressed 21U RNAs (1-4), many of which we validated by northern blot analysis and a PCR-amplified sequencing method. Finally, deep sequencing revealed a class of endogenous siRNAs, the 26G RNAs, which are enriched in sperm, oocytes, and embryos (discussed in a separate abstract.). We are currently investigating a select number of small RNAs for further analysis. Our deep sequencing of the small RNA transcriptome may provide a useful resource for future studies of gene regulation mediated by different classes of small RNAs in the germline.

•1.Ruby, J.G. et al., Cell (2006) 127:1193-1207. •2.Batista, P.J. et al., Mol. Cell (2008) 31: 67-78. •3.Das, P.P. et al., Mol Cell (2008) 31: 79-90. •4.Wang, G. and Reinke, V., Curr. Biol. (2008) 18: 861-867.

934A

Characterization of the small RNA populations of *C. briggsae* and natural isolates of *C. elegans*. **Pedro J. Batista**[1], Elaine M. Youngman[1], Molly C. Hammell[1], Weifeng Gu[1], Masaki Shirayama[1], Craig C. Mello[1,2]. 1) Program Molecular Medicine, Univ Massachusetts Med Sch, Worcester, MA; 2) Howard Hughes Medical Institute.

Small-RNA-mediated pathways are involved in a myriad of processes that regulate gene expression. In *Caenorhabditis elegans* several classes of endogenous small RNAs have been identified. At least two of these classes–the Piwi-associated small RNAs (21U-RNAs) and the worm argonaute (WAGO)-associated small RNAs (22G-RNAs)–are expressed in and essential for the proper development and function of the germline. However the biogenesis and functions of these small RNA classes remain almost entirely mysterious. We have taken a phylogenetic approach to explore small RNA biogenesis and function in Caenorhabditis. We have sequenced small RNA libraries generated from wild isolates of *C. elegans* (AB1, KR314, JU258, RW7000 and CB4856), as well as from the related species *Caenorhabditis briggsae*. As expected, the *C. elegans* wild strains exhibited globally similar levels for the various known classes of small RNAs. However, substantial variations from the N2 levels were observed at fewer than 10% of the 22G-RNA-generating loci, and at fewer than 15% of the 21U loci. In some cases one or more wild isolates exhibited a complete lack of specific 22G or 21U species that are abundant in N2. Our analysis so far suggests that these changes rarely reflect deletions that remove the corresponding sequence, although in many cases there are small insertions or deletions in nearby regions. For those cases where the corresponding regions remain structurally intact we are now searching by DNA sequencing in the wild isolates for alterations that might underlie the absence or acquisition of 22G or 21U-RNA species. Small RNAs with the hallmarks of both 22G- and 21U-RNAs are present in *C. briggsae*, as are the Argonautes and other factors involved in their biogenesis. As in *C. elegans*, 22G-RNAs are the most abundant small RNAs in *C. briggsae*. The *C. briggsae* 21U-RNAs are highly divergent but associated to the same upstream motif originally characterized in *C. elegans*, and like their *C. elegans* counterparts begin predominanty with a U and are modified at the 3' end. Curiously, while the vast majority of 21U-RNAs in *C. elegans* arise from 2 clusters on chromosome IV, in *C. briggsae* these small RNAs are generated from at least 3 clusters: 2 on chromosome IV and 1 on chromosome I. Ultimately, we believe this examination of small RNA populations in both closely- and distantly-related nematode strains will provide important insight into the largely mysterious mechanisms that underlie the biogenesis and function of these small RNA pathways.

935B

Isolation of mutant animals expressing novel endogenous small RNAs. **Beth Buckley**, Shouhong Guang, Scott Kennedy. University of Wisconsin-Madison, Madison, WI.

Small RNAs are crucial regulators of gene expression in most eukaryotes. In *C. elegans,* endogenous short interfering RNAs (siRNAs) regulate the expression level of cellular mRNAs. Little is known about the biogenesis and regulation of these endogenous siRNAs. In the presence of endogenous siRNAs, the Argonaute protein NRDE-3, localizes to the nucleus, while in their absence NRDE-3 localizes to the cytoplasm. The response of NRDE-3 to siRNA abundance is, at least somewhat, quantitative and linear. Thus, NRDE-3 sub-cellular localization can be thought of as an "siRNA thermometer" reading out cellular siRNA abundance. We used this "siRNA thermometer" in conjunction with forward genetics to identify factors that alter the production and/or regulation of endogenous small RNAs.

Our screen recovered six mutant alleles fulfilling these criteria. We have begun a preliminary characterization of one of these mutant alleles, termed *gg135*. *gg135* animals express small RNAs that are anti-sense to rRNAs. Eukaryotic genomes encode multiple rDNA genes, but typically only a subset of these genes are expressed in a given cell; the rest are silenced via histone modifications frequently associated with siRNA-mediated heterochromatin formation. Our preliminary characterization of *gg135* establishes that small RNAs anti-sense to rRNAs can be expressed in metazoan cells and suggests that *gg135* encodes a mutant allele of a gene that normally functions to limit the scope, or duration, of rDNA silencing. Alternatively, we may have disrupted normal rRNA processing leading to aberrant entry of rRNAs into the RNAi pathway.

We have now shown that at least three additional mutant animals isolated from our screen produce small RNAs that are ~22nt in length. The sequence of these small RNAs has not yet been determined. Interestingly, the small RNA expression phenotypes of the mutant animals we recovered are cold sensitive; novel small RNAs are observed at 15C, but not at 20C. In addition, these animals exhibit cold sensitive sterility phenotypes, suggesting that the expression of small RNAs that target rRNAs (and potentially other cellular RNAs) is detrimental to viability. I am currently characterizing the cold sensitive growth defects associated with these mutant animals, identifying the classes of small RNAs present in my six mutant strains, and attempting to clone the genes responsible for these phenotypes.

936C

A forward genetic screen identifies *nuclear RNAi defective-4* (*nrde-4*). **Kirk Benjamin Burkhart**, Beth Buckley, Scott Kennedy. Genetics, Univ Wisconsin, Madison, WI.

Small RNA-guided silencing complexes regulate gene expression in a wide range of biological processes. Many of these silencing complexes act within the nucleus. We conducted a forward genetic screen to identify factors required for RNAi in the nuclei of *Caenorhabditis elegans* [1]. This screen identified three nuclear RNAi factors termed *nrde-1*, *nrde-2*, and *nrde-3*. *nrde-1* encodes a novel protein of unknown function. *nrde-2* encodes a conserved protein of unknown function, and *nrde-3* encodes an Argonaute protein with a nuclear localization signal. All three proteins localize to the nucleus, and are recruited to pre-mRNAs that have been targeted by RNAi. I hypothesized that additional cellular factors function with *nrde-1*, *nrde-2*, and *nrde-3* in nuclear RNAi. In order to identify these factors, I modified the genetic screen that identified *nrde-1*, *nrde-2*, and *nrde-3*. My screen recovered eight new nrde alleles that define at least four novel nrde genes. Five of the eight new nrde alleles define the complementation group *nrde-4*. *nrde-4* mutants exhibit the same Nrde phenotype as *nrde-1(-)*, *nrde-2(-)*, and *nrde-3(-)* animals. In addition to this Nrde phenotype, *nrde-1*, *nrde-2*, and *nrde-4* mutant animals have reduced brood sizes compared to wild type animals, indicating that these gene products are important for fertility. I have mapped *nrde-4* to a 0.03 cM interval containing 67 genes. Progress towards identifying and characterizing nrde-4 will be reported. 1. Guang S. et al. *Science*. 2008 Jul 25;321(5888):537-41.

937A

PIR-1 is a 5' RNA phosphatase that interacts with Dicer and is essential for *C. elegans* development. **Daniel Chaves**[1,2], James Moresco[3], Weifeng Gu[1], Shohei Mitani[4], John Yates III[3], Craig Mello[1,5]. 1) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester MA, USA; 2) Institute of Molecular Medicine, University of Lisbon, Portugal; 3) Department of Chemical Physiology, The Scripps Research Institute, La Jolla CA, USA; 4) 4 Department of Physiology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan; 5) Howard Hughes Medical Institute.

Proteomics screens have identified PIR-1 as a major component of *C. elegans* Dicer complexes (Duchaine *et al.*, 2006). PIR-1 is strongly conserved in metazoans and is related to the phosphatase domain of the mRNA capping enzyme. Human PIR1 sequentially removes the 5'-end γ- and β-phosphates from triphosphorylated RNA molecules in vitro, leaving a 5'-monophosphate (Yuan *et al.*, 1998; Deshpande *et al.*, 1999). A recent study has established human PIR1 as a p53 transcription factor target, and suggests that changes in PIR1 levels affect cell proliferation (Helin *et al.*, 2008). *C. elegans pir-1* deletion mutants are sterile and arrest development as young adults. Their germline is smaller than normal and exhibits no signs of oogenesis. Malformed vulvae and occasional bursting also suggest a role in somatic development. Despite the severity of the phenotype, these animals remain active for the duration of a normal life span. These defects are fully rescued by GFP and 3xFlag fusions of *pir-1*. PIR-1::GFP is expressed in all tissues throughout development where it localizes in nuclei. Immunoprecipitation of these functional PIR-1 fusion proteins confirmed Dicer as a robust interactor and, through a combination of Western blotting and MudPIT proteomics, led to the identification of several other RNAi-related interactors. These novel interactions are now under further investigation. Northern blotting and deep sequencing experiments suggest that arrested *pir-1* animals are wild-type for all known endogenous small RNA pathways, including Dicer-dependent pathways such as miRNA biogenesis. Exogenously triggered RNAi is also unaffected in *pir-1* mutants, as exo-siRNAs can be detected and are accompanied by downregulation of their target mRNAs. Furthermore, these small RNAs have the same 5' phosphorylation status as those of wild-type animals. Current efforts are centered on exploring whether and how PIR-1 functions in concert with Dicer to promote development, perhaps through a hitherto uncharacterized small RNA pathway.

938B

Negative regulation of small RNA-mediated gene silencing pathways. **Sylvia E J Fischer**, Qi Pan, Gary Ruvkun. Dept Molecular Biol, Massachusetts General Hosp, Boston, MA.

Small RNAs in *C. elegans* regulate the expression of endogenous genes, and silence transposons and viruses. Several types of small RNAs have been described in *C. elegans*, each acting in similar but distinct pathways that serve different functions: whereas microRNAs silence endogenous genes, short interfering RNAs (siRNAs) silence transposons and viruses. Exogenous RNA interference (RNAi), transgene silencing and silencing of viruses, largely depend on the same factors, suggesting that the endogenous function of RNAi is an anti-viral response, that can be studied using the surrogate transgene silencing phenotype.

The molecular mechanisms of biogenesis and action as well as the function of small RNAs are only partially understood. It is apparent that the extended *C. elegans* Argonaute family plays a central role in various steps in small RNA mediated gene silencing pathways, with individual Argonaute proteins showing specificity for different types of small RNAs. On the other hand, the small RNA pathways seem to compete for shared, limiting components: several factors that act in an endogenous RNAi pathway where shown to negatively regulate the exogenous RNAi pathway.

To identify negative regulators of the RNAi pathway in *C. elegans* we performed a genome-wide screen for enhanced transgene silencing. To include negative regulators of microRNA activity, we appended a microRNA-regulated 3'UTR to the transgene. We identified 100 candidate small RNA pathway genes that based on mutant phenotypes and secondary RNAi assays, include negative regulators of exogenous RNAi, positive factors in the endogenous or exogenous RNAi pathway, and microRNA pathway factors. In addition, we identified factors that affect transgene silencing but do not act in the RNAi and microRNA pathways, such as the *adr* genes. Our findings show extensive interactions between small RNA pathways, with multiple genes differentially affecting specific pathways.

939C

SID-5 is an endosome-associated protein required for efficient uptake and intercellular spreading of RNAi. **Andrea Hinas**, Amanda J. Wright, Craig P. Hunter. Dept. of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

RNA interference (RNAi), sequence-specific gene silencing induced by double-stranded RNA (dsRNA), is highly efficient in *C. elegans*. Remarkably, an RNAi response initiated in one cell can also spread to distant cells and tissues and to the progeny, a phenomenon known as systemic RNAi. Systemic silencing in worms can be triggered by exposure to environmental dsRNA by direct soaking or feeding on bacteria expressing dsRNA, as well as by injection or transgenic expression of dsRNA. Several genes required for systemic RNAi have been identified previously. For example, the broadly expressed *sid-1* (systemic RNAi defective) encodes a dsRNA channel that is required for RNAi transport into cells and is conserved in most animals, including all vertebrates. Conversely, *sid-2* encodes a nematode-specific transmembrane protein that localizes to the luminal membrane of the intestine and is responsible for uptake of RNAi triggers from the environment into the intestine, but not for further transport within the animal. A number of endosomal genes have also been implicated in uptake and/or spreading of RNAi, although their role has not yet been further investigated.

Here we describe the characterization of *sid-5*, a novel gene in the *C. elegans* systemic RNAi pathway. *sid-5* is predicted to encode a small protein with a single transmembrane domain and is not conserved beyond nematodes. It is required for an efficient response to environmental RNAi targeting genes expressed in all tissues tested including the intestine, body-wall muscle, hypodermis and germline. This inability of *sid-5* mutants to silence target genes in the intestine in response to environmental RNAi suggests that *sid-5* facilitates uptake of dsRNA from the intestinal lumen. Furthermore, *sid-5* mutants are defective in silencing body-wall muscle GFP in response to a hairpin dsRNA expressed in the pharynx. Over expression of wild-type *sid-5* in body-wall muscle cells restores this silencing, suggesting that *sid-5* (like *sid-1*) also is required for import of RNAi into body-wall muscle cells. SID-5::GFP reporter fusions and immunohistochemistry using antibodies against endogenous SID-5 show that SID-5 is expressed in most, if not all, cell types. Subcellularly, SID-5 localizes to cytoplasmic foci that also contain known endosomal proteins. Together, these results indicate that SID-5 functions in endosomes to import extracellular dsRNA or a processed silencing signal. Whether SID-5 functions directly in this transport or indirectly, for example, to localize other dsRNA receptors remains to be determined.

940A

C. elegans tissues do not require RNA interference to generate and export silencing signals derived from expressed RNAs. **Antony M. Jose**, Carlo Garcia, Jessica J. Smith, Craig P. Hunter. Molec & Cell Biol Dept, Harvard Univ, Cambridge, MA.

Double-stranded RNA (dsRNA) triggers RNA interference (RNAi) to silence genes of matching sequence. RNAi-like mechanisms underlie key cellular processes such as heterochromatin formation, regulation of gene expression, and silencing of repetitive DNA. In some animals, when exogenous dsRNA is introduced into a cell, the resultant silencing is transported to other cells. However, numerous dsRNAs and hairpin RNAs (hpRNAs) are transcribed from animal genomes but whether expressed RNAs and endogenous RNAi-like mechanisms generate mobile silencing signals is unknown.

Studies in *C. elegans* have shown that the conserved dsRNA channel Systemic RNAi Defective-1 (SID-1) is required for the import of transported silencing signals. We found that the efficient silencing of multicopy transgenes requires SID-1, suggesting that transgene silencing in one cell produces mobile silencing signals that function to initiate and/or maintain transgene silencing in another cell. Further, the tissue-specific expression of RNAi triggers resulted in the transport of silencing from all tested tissues to other tissues, consistent with expressed RNAi triggers generating mobile silencing signals. Although import through SID-1 suggests that mobile silencing signals are likely forms of dsRNA, their precise identity and biogenesis are unknown.

We reasoned that proteins that act on dsRNA for RNAi within a cell may modify dsRNA or other dsRNA-derived RNAs to generate mobile silencing signals. Therefore, we examined the role of RNAi Defective-1 (RDE-1), an Argonaute protein required for RNAi in C. elegans, in the export of mobile silencing signals. In *rde-1(-)* animals, when *gfp*-dsRNA was expressed in the pharynx, no silencing of GFP expression was detected in either the pharynx or the body wall muscles. However, when *rde-1(+)* was expressed specifically in the body wall muscles of these animals, *gfp* expression was silenced only in that tissue. Consistent with the export of mobile silencing signals from an *rde-1(-)* pharynx, GFP expression in the pharynx remained unaffected. Thus, RNAi-mediated degradation of target mRNA is not required for the generation and export of mobile silencing signals derived from transgenically expressed RNAi triggers.

We are systematically testing additional components and regulators of the RNAi pathway for possible roles in the generation of mobile silencing signals.

941B

The nuclear RNAi pathway promotes off-target gene silencing. **Laura Opperman**, Shouhong Guang, Morgan Sell, Kirk Burkhart, Scott Kennedy. Genetics, University of Wisconsin-Madison, Madison, WI.

RNA interference (RNAi) mediates gene silencing via small interfering RNAs (siRNAs), which base pair with target RNAs to elicit silencing. Current models postulate that near perfect siRNA/mRNA complementarity is required to elicit silencing. Exposure of eukaryotic cells to dsRNAs frequently results in unintended silencing of RNA molecules exhibiting <100% sequence homology to the silencing trigger; a process termed RNAi off-target effects. These off-target effects are of major concern during the use of RNAi as a gene discovery tool and in human therapeutics.

eri-1(-) animals exhibit enhanced sensitivity to dsRNAs (1). In addition, loss of ERI-1 promotes RNAi off-target effects. For instance, animals harboring a null allele of the *dpy-13* gene exhibit a dumpy phenotype. Surprisingly, RNAi targeting the *dpy-13* gene in *eri-1(-)* animals triggers a dumpy phenotype dramatically more severe (super dumpy) than wild-type animals exposed to *dpy-13* RNAi and the null phenotype of *dpy-13* (2). In addition, in response to *dpy-13* RNAi, animals harboring a large deletion within the *dpy-13* gene exhibit a super dumpy phenotype even when RNAi is directed towards sequences absent in the *dpy-13* deletion strain. These data demonstrate that the super dumpy phenotype, elicited by *dpy-13* RNAi in *eri-1(-)* animals, results from off-target silencing of nucleic acid sequences encoded outside the *dpy-13* locus.

These results have provided us with a venue to address the genetics of RNAi off-target effects. NRDE-1/2/3/4 are required for RNAi-based silencing of nuclear-localized RNAs (3). Interestingly, *nrde-1/2/3/4* mutant animals do not exhibit RNAi off-target effects; they fail to exhibit a super dumpy phenotype in response to *dpy-13* RNAi. *nrde-3* encodes a secondary Argonaute (Ago). Strains harboring deletion alleles of five other secondary Ago proteins retain the ability to initiate off-target silencing. These data suggest that RNAi off-target effects may occur primarily in the nucleus, and hint that the nuclear RNAi apparatus may be sensitized, by an unknown mechanism, towards silencing of repetitive DNA elements. We hypothesize that the off-target effects may not be an obligatory side effect of RNAi but, rather, may represent an intentional and genetically programmed component of nuclear RNAi responses in metazoans. (1) Nature. 2004. 427(6975):645-9. (2) Nature. 2005. 436(7050):593-7. (3) Science. 2008. 321(5888):537-41.

942C

Tissue-specific requirements for Eri factors in endogenous RNAi processes. **Derek M Pavelec**[1], Jennifer Lachowiec[1], Thomas F Duchaine[2], Harold E Smith[3], Scott Kennedy[1]. 1) University of Wisconsin, Madison, WI; 2) McGill University, Quebec, Canada; 3) University of Maryland Biotechnology Institute, Rockville, MD.

RNA interference (RNAi) is a fundamental process conserved in metazoans. We are interested in understanding the endogenous biological functions of the RNAi machinery. In order to further our understanding of RNAi, we are conducting genetic screens in the model organism *C. elegans* to identify components of the endogenous RNAi machinery. Animals carrying mutations in endogenous RNAi genes exhibit enhanced RNAi (Eri) phenotypes following exposure to exogenous dsRNA. Previous genetic screens have identified a series of factors termed ERI-1, RRF-3, ERI-3, and ERI-5 that exist in a protein complex with the enzyme Dicer (DCR-1). We have conducted additional screens that have identified ERGO-1, ERI-9, and ERI-11 as novel endogenous RNAi factors. *eri-1*, *rrf-3*, *eri-3*, *eri-5*, or *dcr-1* mutations result in temperature-sensitive, sperm-specific sterility and increased occurrence of male progeny (Him phenotype). Interestingly, *ergo-1*, *eri-9*, and *eri-11* deficient animals do not share these germline pleiotropies. Consequently, we have subdivided the Eri genes into two classes based on the presence (class I) or absence (class II) of these germline phenotypes. Finally, class I animals, but not class II animal, are defective for endogenous RNAi processes in the sperm-producing germline, suggesting that class I Eri dependent small RNAs may function to regulate sperm development. We hypothesize that class I Eri factors represent a core complex which is essential for endogenous RNAi in all tissues while class II Eri factors function to regulate gene expression outside the male germline.

943A

Argonaute proteins possessing mRNA cleavage activity in *C. elegans*. **Hiroaki Tabara**[1,2], Takahiro Asanuma[1], Yoshito Ogawa[1], Megumi Mochizuki[1]. 1) Graduate School of Medicine, Kyoto University, Japan; 2) JST, PREST.

In the RNA interference (RNAi) pathway, small interfering RNAs (siRNAs) and Argonaute proteins cooperatively play important roles. Primary siRNAs bearing monophosphorylated 5' ends are produced from trigger dsRNAs by an RNaseIII-related enzyme called Dicer. In *C. elegans*, secondary siRNAs bearing triphosphorylated 5' ends are also produced by RNA-dependent RNA polymerases, which act on target mRNAs. As a result of *in vitro* analyses using a cell-free assay system prepared from *C. elegans*, we previously showed that secondary-type siRNAs induce a prominent Slicer activity to cleave target mRNAs far more effectively than primary-type siRNAs. An Argonaute protein, CSR-1, is responsible for the Slicer activity induced by secondary-type siRNAs. Monophosphorylated siRNAs also induce a weak mRNA cleavage activity in the cell-free system, but the factor responsible for the weak Slicer activity was not clear.

CSR-1 in cell lysates migrates to high-molecular-weight fractions by gel filtration. Somewhat similarly, recent fluorescent immunostaining experiments have shown that P-granules in germ cells and certain granules in somatic cells are intensely stained with anti-CSR-1 antibodies; non-granular regions of cytoplasm are also moderately stained. Using the same antibodies, we are now trying to examine interactions between CSR-1 and endogenous siRNAs.

On the other hand, we biochemically tested various Argonaute mutants, and found that monophosphorylated siRNAs fail to induce a proper mRNA-cleavage activity in cell lysates prepared from a mutant strain, which corresponds to a 100-kDa Argonaute protein (p100). This Argonaute-mutant strain is homozygous-viable and known to show a weak RNAi-deficient phenotype. We made a recombinant protein of p100 and further examined its activity. The recombinant p100 was able to show an mRNA cleavage activity in conjunction not only with monophosphorylated siRNAs but also with triphosphorylated siRNAs. Several experiments are in progress to investigate the position of p100 in the RNAi pathway.

944B

A two-step RdRP pathway for the Biogenesis of Endogenous Small RNAs. **Jessica J. Vasale**[1], Darryl Conte Jr.[1], Weifeng Gu[1], Craig C. Mello[1,2]. 1) University of Massachusetts Medical School, Worcester, MA; 2) Howard Hughes Medical Institute.

Several classes of Argonaute-associated small RNAs have been shown to play an important role in gene regulation. These include micro RNAs (miRNAs), small interfering RNAs (siRNAs) and Piwi interacting RNAs (piRNAs). Recently, we have characterized a class of 22-nucleotide small RNAs, termed 22G-RNAs, that are expressed abundantly in the germline of *C. elegans* (see Gu et al.). RNA-dependent RNA polymerase (RdRP) proteins are required for the *de novo* synthesis of 22G-RNAs, which bear a 5' tri-phosphate and are thought to be loaded directly onto Argonaute proteins without further processing. We noticed that several loci (termed 22G/26-RNA loci) are targeted by both 22G-RNAs and by a second 26-nucleotide RNA species. Others have shown that the 26-nt RNAs are likely to be 5' mono-phosphorylated. Of the four RdRPs in *C. elegans*, RRF-1 and EGO-1 are required for both RNAi and the accumulation of essentially all endogenous 22G-RNA species. RRF-3 was identified as a Dicer interacting protein and as a component of Eri-pathway, an endogenous RNAi pathway that competes with the exogenous (exo-) RNAi pathway. The function of the remaining RdRP, RRF-2, is still unknown. RRF-3 and other Eri-pathway factors target a number of endogenous loci for silencing. We have found that 22G/26-RNA loci are targets of the Eri pathway and thus depend on both Dicer and RRF-3 for silencing. Interestingly, both the 26 nt and 22G-RNA species were dependent on RRF-3, while a second RdRP (RRF-1, EGO-1 or both, depending on the locus) was required for the production of the 22G-RNA species at these loci. Given the mono-phosphorylated nature of the 26 nt RNA and its dependence on both RRF-3 and Dicer, we hypothesize that these small RNAs arise from the Dicer-dependent processing of RRF-3 dsRNA products. We speculate that these 26 nt Dicer products are loaded into the Eri-pathway Argonaute ERGO-1, which cleaves an mRNA target and leads to the recruitment of RRF-1 or EGO-1. The second RdRP step produces the 22G-RNAs that are loaded onto members of the WAGO family of secondary Argonautes to mediate silencing. These findings indicate that worm RdRPs function with Dicer or independently to produce small RNAs. Why the Eri-dependent Dicer products are 26 nt long, while other Dicer products such as miRNAs are only ~22 nt in length remains a mystery.

945C

Genetic characterization of *rde-10* in RNAi and transgene silencing pathways. **Huan Yang**[1,2], Ho Yi Mak[1,2]. 1) Stowers Institute for Medical Research, Kansas City, MO; 2) University of Kansas Medical Center, Kansas City, KS.

We identified *rde-10*, a new gene that is required for RNAi and transgene silencing. In search for transgenes that allow monitoring of intracellular lipid droplets, we unexpectedly found that a *vha-6p::atgl-1::gfp* transgene was reproducibly silenced in every generation from L2. We screened for mutants that abolish such silencing and isolated more than 10 alleles. Among these, one is a new allele of *rde-1*. Genetic complementation and molecular cloning indicated that we also recovered three alleles that defined a new gene *rde-10*. RDE-10 appears to have no obvious homology to proteins outside the *Caenorhabditis* genus. Nevertheless, RDE-10 is strictly required for RNAi in multiple tissues. And similar to *rde-1*, *rde-10* acts antagonistically to *eri-1* and *eri-3* for transgene silencing. To further explore the molecular function of RDE-10 and its interaction with other small RNA pathway components, we are conducting proteomic analysis on RDE-10 associated proteins.

946A

rde-10 is required for efficient RNAi. **Chi Zhang**[1], Huan Yang[2,3], Ho Yi Mak[2,3], Gary Ruvkun[1]. 1) Department of Molecular Biolgy, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA; 2) The Stowers Institute for Medical Research, Kansas City, MO; 3) 3) University of Kansas Medical Center, Kansas City, KS.

To identify new genes required for RNAi as well as for the trafficking of the silencing signals, we established a reporter strain that allows easy visualization of an efficient RNAi response. We generated constructs that express *gfp* hairpin dsRNAs under the control of neuron-specific promoters (e.g., *snb-1* or *ric-19*). We then introduced these constructs into *sur-5::gfp* transgenic worms expressing GFP in most somatic cells. Although the neuronally expressed dsRNAs do not affect GFP fusion gene expression cell-autonomously, they potently silence GFP expression in intestine, muscle and hypodermal cells in wild type animals. We carried out a large-scale ethyl methanesulfonate (EMS) mutagenesis screen for mutations that reanimate GFP expression in the somatic tissues.

From this screen we identified *rde-10*, a gene that is conserved in the Caenorhabditis genus but not in other organisms. The *rde-10* mutant is resistant to feeding dsRNAs targeting many genes expressed in either germline or somatic tissues (e.g. *pos-1*, *elt-2*, *lin-29* and *nhr-23*). However, this mutant is not completely deficient in RNAi; it is still sensitive to feeding *unc-22* dsRNA, as well as injected *pos-1* dsRNA. Similar to *rde-1* and *rde-4*, and in contrast to *mutators*, *rde-10* mutations do not cause other phenotypic abnormalities. The mutant has normal brood size at various growth temperatures, normal germline cytology, and does not have high incidence of chromosome loss during meiosis. In addition to its role in the exogenous RNAi pathway, *rde-10* is also required for the biogenesis and/or stability of certain classes of endogenous small RNAs. For example, the level of X-cluster tiny non-coding RNAs derived from a unique region on the X chromosome, is significantly reduced in the *rde-10* mutant.

rde-10 function is being further characterized using a combination of genetic, molecular, cellular and biochemical approaches. Studies are underway to determine whether *rde-10* is required for the biogenesis or activity of small interference RNAs (siRNAs) derived from the exogenous long dsRNA triggers. We are conducting experiments to identify its interacting protein factors and associated small RNAs. We will also explore how *rde-10* mutations affect endogenous small RNA levels by Solexa deep sequencing technology.

947B

Characterization of the Transposon Silencing Machinery. **Carolyn M. Phillips**[1,2], Gary Ruvkun[1,2]. 1) Molecular Biology Department, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Small RNA-mediated gene silencing is a conserved mechanism used by nearly all eukaryotes as a way to regulate gene expression at both the transcriptional and post-transcriptional level. The double-strand RNA can be delivered from outside the cell or can be generated from endogenous transcription of coding and non-coding genomic loci. Some processes known to be regulated by RNAi include transposon silencing, defense against viral infection, and maintenance of heterochromatin. Transposon silencing is an important mechanism to prevent DNA damage and subsequent mutations in the germline. A subset of the genes shown to be involved in exogenous RNAi have also been shown to be involved in transposon silencing in the germline, including *mut-2, mut-7, mut-14, mut-15, mut-16,* and *rde-2*. When these genes are mutated, transposons can be excised, leaving a double-strand break (DSBs), which must be repaired by the cellular machinery, either through homologous recombination or non-homologous end joining. I am currently developing a strategy to visualize DSBs in the germline generated by unregulated transposons in the transposon silencing mutants. This assay will allow me to perform a small-scale cytological screen for new genes in the transposon silencing pathway. Additionally, I am generating fluorescently tagged proteins to perform localization analysis of both new and previously characterized genes in this pathway. Localization may identify particular cells where RNA based immunity is important or sub-cellular structures to which these proteins concentrate. Finally, to identify direct binding partners of these proteins and potentially identify additional components of the transposon silencing machinery, I will biochemically purify the components of this pathway. The tagged constructs that I am generating for cytology will include a modified version of the Tandem Affinity Purification (TAP) tag generated by Cheesman *et al.* (2004), which includes a fluorescent tag followed by a TEV protease cleavage site and the S peptide domain and is referred to as the Localization and Affinity Purification (LAP) tag. It allows for both visualization of the protein and its affinity purification. Interacting proteins will be identified by mass spectrometry. Any components identified through this strategy can be further characterized by mutational and localization analysis. These experiments will examine the relationship between the RNAi pathway and the maintenance of genome integrity, and further elucidate the mechanisms of transposon silencing in the germline.

948C

An RNAi-based screen to identify new genes involved in systemic RNAi in *C. elegans*. **Jennifer Whangbo**[1,2,3], Aidan Porter[1,3], Nina Rajpurohit[3], Craig Hunter[3]. 1) Pediatric Hematology/Oncology, Children's Hospital Boston, Boston, MA 02115; 2) Dana-Farber Cancer Institute, Boston, MA 02115; 3) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138.

A remarkable property of RNA interference (RNAi) in *C. elegans* is its systemic nature: silencing signals can cross cellular boundaries to spread silencing to cells and tissues that did not encounter the initiating dsRNA. Forward genetic screens have identified several components required for this process, known as systemic RNAi. One of these genes, *sid-1*, encodes a widely conserved transmembrane protein that transports double-stranded RNA (dsRNA). Recent reports demonstrate that mouse and human SID-1 proteins are important for the transport of dsRNA into cells, indicating that the mechanisms for transporting RNA-mediated silencing signals in nematodes are likely to be relevant for dsRNA transport mechanisms in mammals. Although forward genetic screens have identified a number of *sid* (*s*ystemic RNA*i d*efective) genes, these studies were biased against the discovery of mutations that also cause lethality, sterility, and slow growth. Thus, additional components of the systemic RNAi machinery presumably await identification. We have devised a two-stage reverse genetic approach using RNAi feeding libraries to first knockdown individual genes and then assaying whether the treated animals have been rendered resistant to feeding RNAi targeting a germline-expressed gene, *pal-1*. Under this schema, animals rendered Sid by the first feeding step are resistant to *pal-1* RNAi in the second feeding step and produce live progeny. Conversely, animals that remain unaffected by the first feeding step are sensitive to *pal-1* RNAi and produce dead progeny. Using this screening method, we expect to identify genes involved in the following steps: 1) uptake of dsRNA by the intestinal cells, 2) export of dsRNA-derived silencing signals out of the intestinal cells, and 3) import of the silencing signals into the gonad. Our initial screen of chromosome I yielded 174 candidate genes, among them *rde-2*, a known component of the RNAi pathway. This was expected since our screen does not distinguish between genes required for cell autonomous RNAi and those exclusively required for systemic spread. Our screen failed to detect the other known RNAi pathway gene on chromosome I (*rsd-6*), indicating that this approach will not identify all relevant genes. We are currently narrowing down our candidates with additional rounds of testing and by characterizing systemic RNAi defects in non-germline tissues. Confirmed positive genes are being further tested using available genetic mutants.

949A

A novel alignment-independent motif finding algorithm detects conserved functional regulatory elements responsible for *C. elegans* ribosomal protein coexpression. Donavan T. S. Cheng, **Michael A. Beer**. Biomed Engineering, Johns Hopkins Univ, Baltimore, MD.

Recent data generated by the ENCODE project[Birney, Nature, 2007], and others[McGaughey, Genome Res, 2008], has revealed that sequence alignment algorithms detect functional regulatory DNA sequence elements with low accuracy (~50%). To more accurately detect functional DNA sequence elements, we have developed an alignment-independent Gibbs-sampling based algorithm which uses over-representation and evolutionary conservation equally to detect conserved groups of short DNA regulatory elements in orthologous regions. We have optimized this algorithm on synthetic sequence data, where we have shown that it can identify seeded motif sites with up to 25% higher sensitivity and accuracy than alignment based approaches[Siddharthan, PLoS Comput Biol, 2005;Sinha, BMC Bioinformatics, 2004;Wang, Bioinformatics, 2003], especially when the compared species are as diverged as *C. elegans*, *remanei*, *briggsae*, and *brenneri*. Our algorithm is also more sensitive in detecting shared regulatory elements in genes which are bound by a known TF[Harbison, Nature, 2004;Li, PLoS Biol, 2008]. Our approach joins similar methods advocating an alignment free approach for assessing conservation[Ward, Bioinformatics, 2008], with the distinction that our algorithm is capable of *de novo* motif discovery without prior knowledge of TF specificity. We have identified four significantly conserved motifs in the ribosomal protein (RP) promoters of *C. elegans*, *remanei*, *briggsae*, and *brenneri*. Using promoter::mCherry reporters, we show that these motifs are necessary for proper RP expression, not only in *C. elegans* but also in *C. briggsae*. While often considered to be housekeeping genes, RPs are tightly regulated temporally and in a tissue specific manner in worms, mice, and in humans, but the pathways and TFs which regulate RP biosynthesis are unknown. The tight regulatory control of RPs is corroborated by recent findings that improper expression of RPs and ribosomal RNA leads to diverse developmental defects: 1) heterozygous RP mutant flies exhibit a "minute" phenotype[Marygold, Genome Biol, 2007], 2) reduced rRNA expression leads to defects in *glp-1/notch* signaling in the C. elegans gonad[Voutev, Dev Biol, 2006], 3) decreased levels of rRNA cause increased apoptosis in the zebrafish CNS and embryonic lethality[Azuma, J Biol Chem, 2006], and 4) decreased levels of RPS3 due to a promoter mutation result in defects in gonadogenesis in flies[Saeboe-Larssen, Genetics, 1998]. We hypothesize that defects in ribosomal protein regulation will have diverse phenotypes in a wide range of translationally challenged cells.

950B

Whole genome sequencing for mutant identification. **Henry R. Bigelow**, Maria Doitsidou, Oliver Hobert. Dept of Biochemistry, Columbia University, New York, NY.

We present whole-genome Deep Sequencing technology using an Illumina® Genome Analyzer II as a means to identify molecular identity of mutant animals. Previously, we performed a 5-day run of a full chip (7 of 8 lanes) on a *C. elegans* mutant strain which produced about 54.5 million mate-paired 36-mer reads, thus approximately 3.9 billion bases or 39x average sequencing depth of the genome (Sarin *et al*. Nat Methods. 2008 Oct;5(10):865-7). Recent improvements in the technology doubled the per-chip yield, making it possible to achieve the required sequencing depth for uncovering mutations by using only 2 lanes of a chip.

We give a practical overview (with some theory) of the technology, present a comparison of alternative protocols that can be used to identify mutations (e.g. paired end vs. single run) and give a detailed account of our experience using this technology to clone mutants retrieved from screens in the lab. Furthermore, we introduce all computational steps needed to convert raw signal intensity to a list of gene-annotated point mutations and indels. We present our web interface, MAQWeb, for running MAQ (Mapping and Assembly with Quality, Li *et al*. Genome Res. 2008 Nov;18(11):1851-8) software and annotating the resulting point mutations and indels by any of the possible mutation types: nongenic, silent, missense, nonstart, premature stop, splice donor, splice acceptor, read-through, frameshift, and inframe. The final output is easily converted to a single Excel spreadsheet of variants to be sorted and filtered as needed. A second output format may be uploaded as custom tracks and viewed in WormBase's Genome Browser alongside any other genomic features.

Due to computational load, MAQWeb is not offered as a world-wide web service. Rather, we provide step by step instructions for local installation on a Linux server for which one or more labs have access, which requires a level of computer technical knowledge and administrative privileges on the server. Once installed, however, it may be used for routine processing of deep sequencing data by bench scientists.

Note: This work will be presented in-depth in the Workshop by the same title.

951C

Comparative Genomics using WormBase. **Michael Han**[1], WormBase Consortium[1,2,3,4,5]. 1) Dept Informatics, Wellcome Trust Sanger Institute, Cambridge, UK; 2) California Institute of Technology, Pasadena, US; 3) Ontario Institute for Cancer Research, Toronto,CA; 4) Washington University, St. Louis, US; 5) Cold Spring Harbour Laboratory, New York, US.

The recent increase in sequenced nematode genomes opened up possibilities to improve WormBase by integrating comparative genomics data. We included information on ortholog disease genes to enhance the role of *C.elegans* as model organism, and annotated the newly sequenced genomes with information derived from *C.elegans*. In addition, better integration into external databases (EnsEMBL Genomes, Inparanoid, TreeFam) and improvements on the WormBase website based on user feedback increase the accessibility of WormBase comparative data.

952A

Comparative Genomics of Aging and Proteostasis. **Ana P. C. Rodrigues**[1], Anna Luan[1], Andrew Dillin[2], Gerard Manning[1]. 1) Ravazi Newman Center for Bioinformatics, Salk Institute, La Jolla, CA 92037; 2) Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037.

A number of transcriptional regulators are known to affect the aging process. For example, in C. elegans, the forkhead transcription factors DAF-16 (FoxO) and PHA-4 (FoxA) are required for the life extensions afforded by reduced IGF signaling and dietary restriction, respectively. It is therefore important to identify their downstream targets/pathways, which ultimately carry out the activities required for lifespan extension. We have determined the expression profiles resulting from manipulation of several such factors, and integrated these with the comparative information provided by five Caenorhabditis genome sequences, to interrogate conserved elements in the promoters of responsive genes. We have developed methods to identify direct and indirect transcriptional targets and putative co-factor binding sites that elucidate promoter architecture and transcriptional programs. We are using this transcriptional and promoter data across several experiments to explore the functions and pathways regulated by these factors, with a particular focus on pathways involved in protein misfolding and the development of amyloid diseases.

953B

GExplore: Multi-gene data mining for worm geneticists. **Harald Hutter**[1], Man-Ping Ng[2], Nansheng Chen[2]. 1) Biological Sciences, Simon Fraser University, Burnaby, BC, Canada; 2) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

The *C. elegans* genome contains some 20,000 genes, the majority of which has not been functionally characterized. Numerous genome wide data sets providing hints for gene function exist, yet mining those data to retrieve potential candidate genes for a particular function remains a challenge. Data most relevant for gene/protein function are–phenotype and expression (if known)–homology to other proteins (which might have been characterized already)–protein domains, which are frequently diagnostic for a biochemical function GExplore provides a simple database interface for worm biologists/geneticists for data mining at the gene/protein function level to help in experimental planning. It combines data downloaded from Wormbase with selected data sets related to gene expression or function, like SAGE data sets containing stage and tissue specific expression profiles. Data are preprocessed and organized for simple searches. The interface allows individual or combinatorial searches for proteins with certain domains, expression in certain tissues and/or certain phenotypes. Genes in a given genetic or physical interval can be listed to provide a convenient way to screen for candidate genes in mapping/cloning experiments. Information about available alleles can be displayed to simplify experimental planning. Literature related to entire lists of genes can be quickly retrieved and surveyed. GExplore operates on a local database and has fast response times, which is essential for exploratory searches. The interface is simple yet flexible enough to accommodate the addition of more data sets in the future. Since it is mainly based on data extracted from Wormbase it can be easily updated as new stable releases become available. GExplore is hosted under: http://genome.sfu.ca/gexplore/.

954C

WormBook Updated and Expanded. **Jane E. Mendel**[1], Qinghua Wang[1], Paul W. Sternberg[1,2], Martin Chalfie[3]. 1) Div Biol, California Inst Technology, Pasadena, CA; 2) Howard Hughes Medical Institute, Pasadena CA; 3) Dept of Biol Science, Columbia Univ., 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. WormBook also includes WormMethods, a collection of protocols for nematode researchers. Wormbook now includes over 140 published chapters; an additional 46 chapters are currently in preparation. WormBook appears in the NCBI Bookshelf and has been indexed in PubMed since the fall of 2007, greatly increasing its visibility in the research community. In 2008, WormBook expanded its discussion forum so that readers could comment on individual chapters. Our goal in making the discussion boards more prominent and easily accessible was to stimulate an open and lively discussion on WormBook topics. At this time we are soliciting revisions for chapters published in 2005 and 2006, and we are also adding introductory chapters to each section. Major upcoming changes to WormBook include the expansion of our coverage of nematodes other than C. elegans. We are currently launching three new sections covering free-living and plant and animal parasitic nematodes. In addition, a new section on using nematodes in teaching is planned. We are also making major changes to the organization and content of WormMethods to make this section more complete, up-to-date and user-friendly. Lastly, WormBook is excited to announce the return on the 'Worm Breeder's Gazette' in a new on-line form. This informal newsletter will be linked from WormBook and will provide a non-reviewed platform for communicating preliminary results, new methods and tips for researchers using C. elegans and other nematodes.

955A

Sequence Curation in WormBase. **Philip Ozersky**[1], Tamberlyn Bieri[1], Darin Blasiar[1], John Spieth[1], Paul Davis[2], Anthony Rogers[2], Gary Williams[2]. 1) The Genome Center at Washington University School of Medicine, St Louis, MO, USA; 2) Wellcome Trust Sanger Institute, Hinxton, United Kingdom.

While *C. elegans* remains WormBase sequence curators' top priority to maintain gene structures and other genomic features, WormBase (http://www.wormbase.org) has expanded its scope of curation to include *C. brenneri*, *C. briggsae*, *C. japonica* and *C. remanei*. Initial gene predictions were created for each species through nGASP (the nematode genome annotation assessment project), resulting in a set of high-quality and consistent gene predictions, which has increased the amount of comparative data available for gene curation. Manual curation on each new species has further improved the initial predictions. Over the last year we've made 1166 changes to *C. elegans* coding gene structures and added 10000 more non-coding RNA genes. Additionally, the modENCODE project and other individual labs are now producing data such as tiling array transcriptome expression levels and short-read high-throughput transcriptome data, which are being used to refine gene models. We have continued to develop tools to automatically detect genomic regions that require curation and to quickly and accurately make the required changes. WormBase encourages researchers to submit their data and provide feedback at wormbase-help@wormbase.org.

956B

ICeE: An Interface for *C. elegans* Experiments. Renaud Julien[1], Frédéric Montañana Sanchis[2], Lisa Matthews[3], **Olivier Zugasti**[2], Jérôme Belougne[2], Jonathan Ewbank[2], Philippe Vaglio[1]. 1) Modul-Bio, 232 Boulevard Sainte Marguerite, 13273 Marseille Cedex 09, FRANCE; 2) CIML, Marseille, FRANCE; 3) Langone Medical Center, NYU, New York.

An increasing number of laboratories are using the COPAS Biosort® to implement high-throughput approaches to tackle diverse biological problems. While providing a powerful tool for generating quantitative data, the utility of the Biosort is currently limited by the absence of resources for data management. We have constructed a simple database, IceE (An Interface for *C. elegans* Experiments) designed to allow easy storage and retrieval of Biosort data for *C. elegans*, but which has a wide potential application for organizing other large datasets. The current version is freely available at: http://www.ciml.univ-mrs.fr/EWBANK_jonathan/software.html. We are now extending this interface to provide an integrated data management solution for high-throughput screens. Upstream of IceE, we will add a laboratory information management system (LIMS), including sample tracking, with barcode identification of 96 well plates. This will be particularly useful for genome-wide RNAi screens. Downstream, we will adapt our genome annotation tool (also freely available at the same web address), to allow projection of results onto the genome browser, within Wormbase. Our aim is to provide a tool that allows seamless data storage and processing capacity for labs conducting large-scale experiments.

957C

Identification and Characterization of Modifiers of the Multivulva Phenotypes in *Caenorhabditis elegans* Ras Mutant. **Nattha Wannissorn**, Andy G. Fraser. Banting and Best Department of Biomedical Research, Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada.

While mutations in ras are found in a high percentage of human tumours, additional mutations are required for progression from transformation to full-blown oncogenesis. Identifying such cooperating mutations is key for understanding the progression in humans. In *C. elegans*, worms homozygous for activating mutations in the ras orthologue *let-60* have multivulva phenotypes (Muv), with approximately 60 percent of *let-60(n1046)* animals being multivulval at 20°C. Therefore, we use this phenotype as the basis for a large-scale RNAi screen to identify either enhancers or suppressors of the *let-60(n1046)* Muv phenotype. Since ras and many cooperating signaling pathways are well conserved between worms and humans, RNAi knockdowns that decrease the percentage of Muv worms are likely to uncover orthologs of protooncogenes, whereas knockdowns that increase the percentage of Muv worms should uncover tumor suppressor gene orthologs. Therefore, we use RNAi screening in *C. elegans* to identify novel cancer associated genes in humans. Rather than carry out genome-scale screens, we focused on two sets of genes to test initially. The first is a set of ~250 chromatin remodeling factors. Many chromatin remodeling factors have been implicated roles in cancer including p105Rb, histone acetyl transferases, and NuRD complex components. In worms, the SynMuv genes repress the vulval fate and they encode nuclear proteins that function principally through chromatin remodeling. Thus chromatin remodeling factors are likely to be enriched in regulators of Ras signaling in both vulval induction and carcinogenesis. We present here our data from screening all 250 chromatin remodeling factors for enhancers or suppressors of the *let-60(n1046)* Muv phenotype. The second set of genes was chosen as orthologues of human genes whose mutation in cancers is being studied in depth by The Cancer Genome Atlas Project. This is a collaborative effort to sequence tumor genomes from tumor samples. In the pilot projects, 623 known cancer-associated genes were selected for gene sequencing and we are screening all orthologues of these genes in the worm for enhancers or suppressors of the *let-60(n1046)* Muv phenotype. Since the cancer genomes may accumulate higher background mutation rates, the presence or the frequency of mutations uncovered by DNA sequencing may not indicate the biological relevance of the mutations themselves. We hope that the studies we are carrying out in the worm will aid assigning biological relevance to the identified mutations in tumours. We will present the results from these screens here.

958A

Syning the CIN. **Sanja Tarailo**, George Chung, Nigel O'Neil, Martin Jones, Zoe Lohn, Jessica McLellan, Phil Hieter, Ann Rose. Department of Medical Genetics, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada.

Chromosome instability (CIN) is a hallmark of many cancer types. Identifying mutations that are synthetic lethal in combination with a CIN phenotype is therefore a promising approach for identifying gene targets for cancer therapeutics. We have previously identified the helicase DOG-1 as the functional ortholog of human FANCJ. When mutated, FANCJ leads to the development of Fanconi Anemia, a hereditary disorder that confers a predisposition to cancer development due to CIN. In *C. elegans*, the *dog-1* mutant also displays a CIN phenotype arising from deletions that initiate in G-rich DNA. Previous work in our lab has revealed that *dog-1* mutants in combination with mutations in the anti-recombinase *rtel-1* are synthetic lethal. We are currently screening for additional gene products that when non-functional result in lethality specifically in the absence of *dog-1*. We have conducted several EMS and X-ray mutagenesis screens designed to assay for somatic cell loss due to synthetic lethality. We have generated 143 potential mutations that we are currently mapping using a combination of traditional mapping techniques and Array Comparative Genomic Hybridization (aCGH). This work will reveal mutations that specifically kill unstable CIN cells in *dog-1* mutant animals. The conserved function of DOG-1 and human FANCJ indicate that the mutated genes we discover are potential therapeutic targets for the specific killing of FANCJ cells in Fanconi Anemia patients. This work is funded by CIHR and MSFHR.

959B

C. elegans as a model system to study environment-genetic interactions of nematodes in grassland ecosystems. **Vinod K Mony**, Joseph D Coolon, Michael A Herman. Division of Biology, Kansas State University, Manhattan, KS.

Nematodes are the most abundant invertebrates in grassland ecosystems and their interactions with soil microbes affect important ecological processes such as decomposition and nutrient recycling. We are studying the genetic responses of native grassland nematodes to environment, focusing on their interactions with bacteria. As native nematodes do not have well-developed genetic resources, we are employing *C elegans* to model their bacterial interactions in laboratory. As a first step, we have isolated bacterial species associated with native grassland soil nematode taxa, *Oscheius sp.* and *Pellioditis sp.* To identify conserved candidate genes we used transcriptomics to identify 204 *C. elegans* genes differentially expressed in response to three soil bacteria: *Bacillus megaterium*, *Pseudomonas sp.*, *Micrococcus luteus*, and *E. coli*). To determine whether these genes were functionally important for the nematode-bacterial interactions, we used available *C. elegans* loss of function mutants (21 in total) to study their responses to the above bacteria. We conducted fitness and pathogenecity tests, which confirmed that many of the identified genes were important for the response to specific bacteria. Furthermore we demonstrated that the degree of differential expression correlated with the degree of phenotypic effect, validating our approach (Coolon et al., submitted). We have continued these studies, focusing on differentially expressed genes involved in metabolism and bacterial defense. Thus far we have tested the responses of *lys-1*, *lys-4*, *lbp-5* and *mtl-1* to *E. coli* and *B megaterium* and found that each were more susceptible than wild type to *E. coli*, but had differential responses to *B. megaterium*. These results suggest that *C. elegans* can be used to explore gene-environment interactions of native nematodes with grassland soil bacteria. Work by many labs has elucidated *C. elegans* innate immunity pathways involved in bacterial defense. In order to understand the role of known *C. elegans* immunity pathways in response to grassland soil bacteria, we conducted pathogenecity assays on mutants of known immunity genes exposed to *B. megaterium*, *Pseudomonas sp.*, *E. coli* and an additional nematode-associated bacteria, *Stenotrophomonas maltophilia*, a ubiquitous bacterium that can cause nosocomial infections in immuno-compromised humans. We observed that the lifespan of *daf-2* mutants, which is extended on all bacteria tested to date, was not extended on *S. maltophilia*. We are currently exploring which other pathways must be involved in this interaction. Thus in addition to modeling native nematode-bacterial interactions, our studies may help to more fully characterize *C. elegans* innate immune responses.

960C

The role of translesion synthesis polymerases in the maintenance of genome stability in *C. elegans*. **Sophie Roerink**[1,2], Marcel Tijsterman[1]. 1) Hubrecht Institute, Utrecht, the Netherlands; 2) University Medical Center Utrecht, the Netherlands.

Replication fork stalling due to base damage in the template DNA strand, threatens the ability of the cell to complete its cell cycle. In addition to conservative repair pathways such as nucleotide excision repair and homologous recombination, mechanisms have been evolved that allow for bypass of the replication block without repair, by specialized translesion synthesis (TLS) polymerases. Although these specialized polymerases perform translesion synthesis with high genetic fidelity on specific lesions, they exhibit high error rates while copying undamaged DNA, leading to their designation as error-prone polymerases. In the *C. elegans* genome, homologues for the Y-family TLS polymerases Polη, Polκ and Rev1 and the B-family member Rev3 have been found. We isolated functional knockouts of *polh-1*, *polk-1* and *rev-1* in *C. elegans*. We found that *polh-1* and *rev-1* mutants are hypersensitive to various sources of DNA damage such as cisplatin and UV-irradiation. In addition, we unexpectedly observed a γ -irradiation hypersensitivity phenotype suggesting that POLH-1 and REV-1 may also have a role in repair of DNA breaks. We found that POLH-1 and POLK-1 can act redundantly in protection against damage caused by the alkylating agent methyl methane sulfonate (MMS): while *polh-1* and *polk-1* mutant animals are both moderately sensitive to MMS, a double mutant is extremely sensitive. RNAi screens in mutant versus wildtype backgrounds on a sublethal dose of MMS enable us to identify more genes involved in TLS mediated protection against alkylating damage. Another advantage of using self-fertilizing hermaphroditic *C. elegans* is the ability to determine the role of *polk-1*, *polh-1* and *rev-1* in genome protection and/or evolution in an unbiased way, by growing successive generations and determining the forward mutation frequency by genome-wide sequencing technologies, which we are currently undertaking.

961A

Towards making a Functional Map for splicing-related genes in *C. elegans*. **Julian Ceron**[1], Monica Ferrer[2], Laura Fontrodona[1], Karine Rebora[3], Leo Gugignard[3], Denis Dupuy[3], Bob Jhonsen[4], Dave Baillie[4], Simo Schwartz[2]. 1) Cancer and Human Molecular Genetics, Bellvitge Institute for Biomedical Research, l'Hospitalet de Llobregat, Barcelona, Spain; 2) CIBBIM-Nanomedicine, Vall d'Hebron University Hospital, Barcelona, Spain; 3) Genome Regulation and Evolution, Institut Europeen de Chimie et Biologie, Pessac, Bordeaux, France; 4) Molecular Biology and Biochemistry, Simon Fraser University, British Columbia, Canada.

We intend to build a functional map for splicing-related genes completing and revising previous genomics information with high-quality functional data. Genes implicated in splicing may have multiple and diverse cellular functions but these genes commonly are essential for the animals viability therefore making it difficult to unravel their mode of action. To overcome this difficulty we will combine the information obtained by RNAi and mutant phenotypes (Phenome), expression patterns (Localizome) and protein-protein interactions (Interactome) to uncover gene functions. As an initial step towards building a functional network for 192 splicing-related components in *C. elegans*, we are doing experiments with the 18 Sm and Sm-like gene family members. We have created RNAi clones for some of these genes and we are building a Phenome map based on numerous RNAi assays. By PCR stitching we made promoter::GFP constructs for all family members, and all of them gave expression as transgenes. These 18 reporters are being carefully analyzed by microscopy and Chronograms profiling. Preliminary results from these Phenome and Localizome studies point towards a variety of functions among Sm and Sm-like family members. Moreover, we are currently cloning ORFs and gene fragments into a bait vector to perform Yeast Two Hybrid screens that should improve and expand the Interactome Map for these genes. All these information will be integrated by using bioinformatics tools to build a pilot Functional Map for splicing-related genes. Due to the high levels of sequence conservation of splicing-related genes through evolution, we believe that the existence of high-confidence functional maps for splicing-related components in *C. elegans* will shed-light into the complexity of splicing-related mechanisms.

962B

Functional Genomics of Heme Homeostasis by RNA Interference. **Scott Matthew Severance**[1], Tamika Samuel[1], Jason Sinclair[1], Iqbal Hamza[1,2]. 1) Dept. of Animal & Avian Sciences, University of Maryland, College Park, MD; 2) Dept. of Cell Biology & Molecular Genetics, University of Maryland, College Park, MD.

It is thought that as much as 80% of the world's population is iron-deficient, making nutritional iron deficiency the world's most common nutritional disorder. Considerable evidence exists to support the idea that heme is an essential bioavailable source of iron in humans, but the pathways for heme absorption and utilization are poorly understood. Heme (iron-protoporphyrin IX) also serves as a prosthetic group in proteins which play a key role in diverse biological processes such as oxygen transport, xenobiotic detoxification, signal transduction, and gene regulation. Since hemes are cytotoxic and insoluble, we hypothesize that specific pathways exist within cells for trafficking heme from the site of synthesis in mitochondria to various intracellular destinations for incorporation into apo-hemoproteins. We have demonstrated that *C. elegans* is absolutely dependent on heme to sustain metabolic processes because it lacks the ability to synthesize heme. *C. elegans*, therefore, represents a unique animal model to identify the genetic and cellular pathways for heme transport because they allow external control of heme transport pathways not permissible in other organisms. We have synthesized a transgenic "heme-sensor" strain in *C. elegans* that expresses green fluorescent protein (GFP) under the control of a heme-responsive gene promoter. In the sensor strain, GFP fluorescence increases under low heme and decreases when the concentration of heme is elevated. We used this strain in a genome-wide, functional reverse genetic RNAi screen using the clones available in the Ahringer and Vidal feeding libraries. Changes in GFP fluorescence were monitored as a function of heme levels and gene knock-downs. We have identified several novel genes which, based on the altered GFP levels they effect, play a role in organismal heme homeostasis. Characterizing these genes simultaneously in worms, yeast, zebrafish, and mammalian cells will provide new mechanistic insights into heme homeostasis in mammals. Furthermore, information gleaned from this study may aid in the development of heme-based nutritional interventions for human iron deficiency and permit identification of novel drug targets for parasitic worm infestations which exacerbate human iron deficiency.

963C

Metazoan operons accelerate transcription and recovery rates. **Alon Zaslaver**[1], Ryan Baugh[1,2], Paul Sternberg[1]. 1) Howard Hughes Medical Institute and California Institute of Technology, Division of Biology, 1200 E. California Blvd., Pasadena, California 91125. USA; 2) Present address–Department of Biology, Duke University, Durham, NC 27708, USA.

Existing theories efficiently explain why operons are advantageous in prokaryotes, but their emergence in metazoans is still an enigma. We present a combination of genomic meta-analysis, experiment and theory to explain how operons could be adaptive during metazoan evolution. Focusing first on *C. elegans*, we show that operon genes, typically consisted of growth genes, are significantly up-regulated during recovery from multiple growth arrested states, and that this expression pattern is anti-correlated to the expression pattern of non-operon genes. In addition, we find that transcriptional resources are initially limited during arrest recovery, and that recovering worms are extremely sensitive to any additional limitation in transcriptional resources. By clustering growth genes into operons, fewer promoters compete for limited transcriptional machinery, effectively increasing the concentration of transcriptional resources and accelerating growth during recovery. A simple mathematical model of transcription dynamics reveals how a moderate increase in transcriptional resources can lead to a substantial enhancement in transcription rate and recovery. We find evidence for this design principle in different nematodes (*e.g., Pristionchus pacificus* and *Brugia malayi*) as well as in the chordate *Ciona intestinalis*. As recovery from a growth arrested state into a fast growing state is a physiological feature shared by many metazoans, operons could evolve as an evolutionary solution to facilitate these processes.

964A

Towards a global quantitative epistasis map. **Weiwei Zhong**[1], Paul Sternberg[2]. 1) Biochemistry and Cell Biology, Rice University, Houston, TX; 2) HHMI and Biology Division, California Institute of Technology.

Epistatic analyses reveal how complex traits are controlled by multiple genes and pathways. To enable large-scale epistasis mapping, we developed a high-throughput pipeline for predicting and testing of genetic interactions in C. elegans. The first component of the pipeline is a computational system (www.GeneOrienteer.org) that generates genome-wide probabilistic predictions of genetic interactions by integrating expression, phenotype, interaction, and function data from multiple species. This component functions to generate hypotheses and prioritize test candidates. The second component of the pipeline is an automatic phenotyping system using computer vision to provide quantitative measurements of nematode phenotypes. This component facilitates high-speed experimental testing of genetic interactions. We applied the pipeline to test a number of genes on body size regulation. We were able to correctly reconstruct known interactions among cuticle collagens and TGFβ pathway genes. We also tested a set of genes in the Go and Gq signaling pathways using movement as a phenotypic readout. Again, our system successfully identified known epistatic relationships among these genes. In both cases, we were able to detect new components in these pathways. We plan to extend the study to genome scale to generate a global, multi-phenotypic, quantitative epistasis map. We are also in the process of constructing a database for public access of our experimental data.

965B

Reannotating the C. briggsae genome using genBlastG, a new homology-based gene finder. **Jeffrey Shih Chieh Chu**[1], Rong She[2], Ke Wang[2], Nansheng Chen[1]. 1) Dept MBB, SFU, Burnaby, BC, Canada; 2) Dept Computing Science, SFU, Burnaby, BC, Canada.

Since the publication of the C. briggsae genome annotation in 2003 [1], not much improvement has been done, although accumulating evidence suggests that many gene models are inaccurately predicted or missing. In this project, we have reannotated the C. briggsae genome, exploiting the much improved C. elegans genome annotation (using WS195, compared to WS77 annotation used for the original C. briggsae annotation), as well as a new homology-based gene finder we have developed, genBlastG. genBlastG builds on our recently published program genBlastA [2] and takes as input a query protein sequences and a genome sequence that will be annotated to produce all homologous gene models. Our analysis suggests that genBlastA outperforms GeneWise in both processing time (on average genBlastG runs ~50 times faster than GeneWise) and accuracy. We applied genBlastG to reannotate the C. briggsae genome. Our preliminary results from genBlastG produced 16,954 homologous models with the majority (11,235) matching well with the current WormBase annotation. However, a significant number (4,828) of genBlastG models exhibit better percent identity (PID) to the query protein sequence, the C. elegans query sequences. Thus, genBlastG models shows better homology to C. elegans models for many genes. In addition to better homology, our predictions also points out 261 WormBase models that should be split and 298 pairs of models should be merged. As an example of a model that should be merged, we found that CBG14800 and CBG14801 may actually be one gene that's orthologous to C54G7.3a. CBG14801 may only represent the shorter isoform that's orthologous to C54G7.3b. As an example of a model that should be split, we found that CBG00366 consists of orthologs from ZK550.3 and ZK550.4. In this presentation, I will summarize all improvement suggested by genBlastG. genBlastG will also be applied to predict gene models in other Caenorhabditis species.

1.Stein, L.D., et al., (2003). The genome sequence of Caenorhabditis briggsae: a platform for comparative genomics. PLoS Biol 1: E45.
2.She, R., et al., (2009). GenBlastA: enabling BLAST to identify homologous gene sequences. Genome Res 19: 143-9.

966C

A rich diversity of 3' UTR isoforms revealed by deep sequencing. **Ting Han**[1], Arun Prasad Manoharan[1], Pascal Bouffard[2], Tim Harkins[2], John Kim[1]. 1) The Department of Human Genetics and the Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109; 2) 454/Roche Applied Science, Indianapolis, IN 46250.

MicroRNAs are known trans-regulatory factors that bind to the 3' untranslated regions (3' UTR) of target mRNAs (1). A major challenge in studying microRNA-mediated gene regulation is identifying the target genes. To date, nearly all genome-wide predictions of microRNA targets have employed arbitrary assignments of 3'UTR lengths (2-4). However, of the 20,036 protein coding genes in the *C. elegans* genome, less than 50% (9,436) have any empirically defined 3' UTRs and less than 10% have more than one annotated 3'UTR isoform. In this study, we devised a method to capture the 3' polyadenylated ends of mRNA transcripts and applied 454 (Roche) deep sequencing technology to annotate the 3'UTRs for ~12,000 (60%) genes in the *C. elegans* genome. We constructed 3' UTR cDNA libraries for the major developmental stages of C. elegans: embryo, L1-L4, adult (hermaphrodite and male), and the dauer stage. 454 pyrosequencing of these libraries generated ~2.2 million sequences matching the genome. These sequences not only validate ~70% of the Wormbase 3'UTR annotations but also identify many novel 3'UTR isoforms. In addition, we identified 3'UTRs for ~2,000 genes with no previous 3'UTR annotation. Notably, of the 12,000 genes in our datasets, ~50% (~6000) have multiple 3' UTR isoforms and ~30% (~4000) have at least one developmental stage-specific 3' UTR isoform. By incorporating our experimentally validated 3'UTRs, we refined microRNA target predictions with significant reduction of potentially false positive rates. Importantly, our findings support the notion that gaining or losing microRNA-mediated regulation may be accomplished though alternative 3'UTR isoforms. Taken together, our study provides a significant increase in the genomic annotation of 3'UTRs and suggests a much more prevalent role of 3'UTR isoforms in the developmental regulation of gene expression than previously appreciated.

Reference:

1.Wightman, B. et al., Cell 75: 855 (1993).
2.Rajewsky, N., Nature Genetics 38, Suppl:S8 (2006).
3.Lall et al., Curr Biol 16, 460 (2006).
4.Grimson et al., Mol Cell 27, 91 (2007).

967A

Motif composition and evolution of the core promoter. **Uladzislau Hryshkevich**, Itai Yanai. Faculty of Biology, Technion Israel Institute of Technology, Haifa, Israel.

Even before specific transcription factors can begin to modulate gene expression, the initiation of transcription is crucially dependent upon the properties of core promoter sequence. While many short motifs such as the initiator motif and the TATA-box are known to occupy this region, a surprising level of variation is present across a genome's core promoters. We hypothesized the existence of a code of short motifs distinguishing the functional properties of core promoters. Here we characterize 12 regulatory elements located in *C. elegans* core promoters. We found that these cluster into two mutually exclusive groups of motifs (Core1 and Core2). For example the initiator motif and T-repeats (with a consensus of TTTTTCCTCCATTTTT) from the Core1 group do not co-occur with the TATA-box and purine-repeats ($(R)_{12}$), of the second group. Invoking gene expression data across embryonic development, we asked whether different core promoter motifs correlate with different expression levels. We found that genes with elements from the first group show significantly elevated expression level. For example genes with the initiator motif exhibit strong expression levels (z-score = 35.7), while genes with motifs from the second group such as the TATA-box show low expression (z-score = -1.7). Our analysis enables us to examine the evolution of motif architecture in the core promoter across different species. Such an analysis of the dynamics of motif composition may lead to an understanding of the tuning of gene expression level by motif combinatorics of the core promoter.

968B

WormNet version 2: An Improved Gene Network of C. elegans predicts Genetic Interactions among Disease-related Genes. **Insuk Lee**[1], Andrew Fraser[2], Edward Marcotte[3], Ben Lehner[4]. 1) Department of Biotechnology, College of Life science and Biotechnology, Yonsei University; 2) Banting and Best department of Medical Research University of Toronto, Canada; 3) Center for Systems and Synthetic Biology, University of Texas at Austin, TX, USA; 4) EMBL-CRG Systems Biology Research Unit and ICREA, Centre for Genomic Regulation, UPF, Barcelona 08003, Spain.

C. elegans (worm) is a powerful model organism for Systems Biology of multi-cellular metazoans, such as human, and recently genome-scale functional network for worm genes, WormNet, has been developed and was proved to be highly predictive loss-of-function phenotypes (1). Here, we present WormNet version 2, covering ~75% of the worm genes encoding proteins (15,038 worm genes) with about 1 million functional links between genes. To construct WormNet version 2, we integrated 21 heterogeneous genomics data sets (mRNA expression profiles by cDNA microarray, text-mining of scientific literature by co-citation, yeast 2 hybrid analyses, gene neighbors, phylogenetic profiles, genetic interactions, and conserved gene interactions transferred from yeast/fly/human). Various computational validations imply improved prediction ability for many loss-of-function phenotypes. Here, we used the network to predict novel genetic interactions among disease related genes in worm. From earlier experimental observation from large-scale genetic interaction screening (2), we found enrichment of genetic interactions between pathways. Assuming epistatic interactions between pathways, we predicted new genetic interactors to several disease genes. The new genetic interactors were predicted by guilt-by-association approach. Cross-validation analysis shows enriched connectivity among genes that genetically interact with each disease related gene. The experimental validations using RNAi experiments are under process and will be present in the meeting. Only about 5% of human diseases in developed country are monogenic. Therefore, understand of genetic interaction among disease related genes is critical in development of therapeutic methods for polygenic common diseases such as cancer and diabetes. Network-guided genetic interaction screening would allow construction of disease related pathway map with reduced cost and time in a model organism, C. elegans, and will shed light on understanding of human disease mechanisms in the future. **Reference** 1.Nature Genetics 40: 181 (2008) 2.Nature Genetics 38: 896 (2006).

969C

Ultra-high throughput sequencing of the genome and transcriptome of *Caenorhabditis* sp. 3 PS1010. **Ali Mortazavi**[1], Erich M. Schwarz[1], Brian Williams[1], Lorian Schaeffer[1], Barbara Wold[1], Paul Sternberg[1,2]. 1) Biology Division, Caltech, Pasadena, CA; 2) Howard Hughes Medical Institute, Pasadena, CA.

The *de novo* sequencing of nematode genomes has been an arduous process that involves large-scale projects working over multi-year time scales to sequence and annotate genomes. The recent advent of ultra-high throughput sequencers that are moving towards the $1,000 human genome foreshadow the coming of the "$50 worm genome" for sequencing reagents, which will afford a much larger scale whole-genome survey of the nematode phylum. In order to develop tools to analyze and annotate nematode genomes of interest using ultra-high-throughput technology, we have sequenced the genome and the transcriptome of *Caenorhabditis* sp. 3 PS1010 using 2x75 bp reads produced on an Illumina GAII. We have been able to compare our genomic DNA and cDNA sequence data to 417 kb of high-quality, annotated contigs built using traditional sanger sequencing of PS1010 fosmids. We assembled 49 million paired reads into 65.5 Mb using the Velvet short read assembler with an N50 of 1.1 kb which achieved 95% coverage of our PS1010 contigs, for which the gene-prediction program AUGUSTUS predicted ~30,000 protein-coding genes or segments of genes. We also sequenced a pool of mixed-stage, polyA-selected RNA with over 26 million mappable reads (including 3.7 million splice-crossing reads), and found that we observed reliable signal of at least 1 or more Reads Per Kb per Million (RPKM) over 75% of the 108,000 AUGUSTUS-predicted exons; this includes developmental control genes expected to be expressed at low levels, such as *lin-3* and *lin-11*. By taking advantage of the paired-end RNA-seq reads, we were able to further improve our assembly using RNA-reads spanning contigs and thus increase our N50 to 1.6 kb. The combination of ultra-high throughput sequencing of genomic DNA and of the transcriptome along with their complementary assembly provides a straightforward path for the further analysis of key species in the nematode phylum.

970A

Filling in the gaps of a C. elegans fosmid library. **Jaryn Perkins**[1,2], Donald G. Moerman[1,2]. 1) Michael Smith Laboratories, Univ British Columbia, Vancouver, BC, Canada; 2) Department of Zoology, University of British Columbia, B.C. Vancouver,Canada.

The fosmid genomic library produced and sequenced by the Moerman laboratory and the Genome Sciences Center, in Vancouver, was created to provide a resource for genome wide studies (IWM 2005 Abstract 1299B). It is meant to supplement, and in some cases replace, older cosmid libraries. With 15,744 clones characterized, the inserts should theoretically contain 5x coverage of the Caenorhabditis elegans genome. However, the mapping of paired end reads display gaps in the sequence that correspond to areas of highly repetitive sequence, possibly suggesting mismapping in these regions. The 12,481 clones, which aligned contiguously, cover approximately 80% of the genomic sequence, and account for 90% of genes within one or more fosmids. To increase the usefulness of the resource to the community we have begun PCR screening the 3,263 ambiguously mapped clones for the presence, using internal primer sets, of the missing genes. Initially we are screening for transcription factors, as 197 of the 936 annotated genes are not represented among the mapped fosmids. Completion of this large and important functional gene family will provide a benchmark for completion of gapped sequences. Initial positive hits for TF genes in the screen provides evidence for the hypothesis that the gaps are caused by ambiguous mapping of some clones, and suggest that a large portion of genes currently missing from contiguous coverage can be found in the remaining ambiguously mapped clones. From the initial 56 transcription factors screened, we have observed amplicons of correct size for 43. These results demonstrate that screening rather than de novo library production may be a cost effective way to complete gapped sequences, as any new clones may be susceptible to ambiguity in the same regions. Using this approach, we hope to close many of the 400 gaps in the current fosmid coverage of the genome.

971B

*Mos*LIB: A PCR-screenable *Mos1* insertion library. **Valérie J P Robert**, Jean-Louis Bessereau. ENS-Dept Biol, Inserm, Paris, France.

*Mos*TIC (for *Mos1* excision transgene-instructed gene conversion)[1] provides a means to engineer the *Caenorhabditis elegans* genome. In *Mos*TIC, transgene-instructed gene conversion occurs during the repair of a DNA double-strand break generated by the excision of a pre-existing *Mos1* insertion located in the genomic region to engineer. *Mos1* is a DNA transposon isolated in *Drosophila mauritiana*. In *C. elegans*, expression of the Mos transposase is sufficient to induce the mobilization of *Mos1* from an extrachromosomal array into the genome[2]. Such genomic insertions are indispensable reagents to initiate *Mos*TIC.

Here, we present the development and the characterization of *Mos*LIB, a *Mos1* insertion library consisting of 40,000 independent clonal strains containing *Mos1* insertions[3]. *Mos*LIB is made of 719 pools of 48 to 72 clonal strains. Each pool is represented by a duplicated frozen nematode stock and the corresponding genomic DNA. We estimated that at least 80,000 *Mos1* insertions are present in *Mos*LIB, 65 percent of them being close enough to coding regions to be valuable reagents to engineer genes by *Mos*TIC.

To screen *Mos*LIB, we first perform a single PCR on the DNA stock with one primer in *Mos1* and one specific primer designed in the genomic region of interest. Once a positive signal is obtained and its identity confirmed by sequencing, we thaw one tube of the corresponding nematode stock and we make 20 to 40 pools of 20 animals which survived freezing. These pools are screened by PCR for the presence of animals carrying the insertion of interest and rounds of sib-selection are made until a single homozygous animal carrying the insertion of interest is isolated.

To optimize the screening of *Mos*LIB, we now aim at using high-throughtput sequencing technologies to map the *Mos1* insertions present in *Mos*LIB.

We would like to thank Y. Duverger, J. Ewbank and members of the Bessereau's lab for their help in the making of the library.

1.Robert, V. and Bessereau, J. L. Embo J 26, 170-83 (2007). 2.Bessereau, J. L., Wright, A., Williams, D. C., Schuske, K., Davis, M. W. and Jorgensen, E. M. Nature 413, 70-4 (2001). 3.Duverger, Y., Belougne, J., Scaglione, S., Brandli, D., Beclin, C. and Ewbank, J. J. Nucleic Acids Res 35, e11 (2007).

972C

Characterization of the C. elegans ionome. **Tamika Samuel**[1], Brett Lahner[2], David Salt[2], Iqbal Hamza[1]. 1) Department of Animal and Avian Sciences, University of Maryland, College Park, MD; 2) Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907, USA.

The ionome is defined as the complete elemental compositions of an organism, including essential mineral nutrients and trace elements. Ionomics, the study of the ionome, is utilized to uncover the genes and gene networks that regulate the ionome and the physiological stimuli that affect these networks. In ionomics, high throughput elemental analysis is integrated with genetics, genomics, and bioinformatics to gain a comprehensive picture of the ionome of an organism. Because of its invariant and precise somatic cell lineages, C. elegans is an excellent experimental model system to apply the concept of ionomics to gene networks within an intact animal. Wild-type N2 worms were grown axenically in modified CeHR liquid medium to mid L4 stage, collected on cellulose filters, and dried before analysis by inductively coupled plasma-mass spectrometry (ICP-MS) at the Purdue Ionomics Center. Using this approach, we have been able to precisely quantify the concentration of P, Ca, K, Mg, Cu, Fe, Zn, Mn, Co, Ni, B, Se, Mo, Na, As, and Cd in C. elegans. Our ultimate goals are to superimpose the ionome network with the regulatory circuit which defines developmental processes in an animal by combining functional ionomics with genome-wide RNAi screens.

973A

Genome and transcriptome analysis by the next-generation sequencer. **Tadasu Shin-i**[1], Hiroshi Kagoshima[1], Yoshiki Andachi[1], Kazuko Ohishi[1], Atsushi Toyoda[2], Asao Fujiyama[2], Yutaka Suzuki[3], Sumio Sugano[3], Yuji Kohara[1]. 1) Genome Biol Lab., National Inst. Genetics, Mishima, Japan; 2) Comparative Genomics Lab., National Inst. Genetics, Mishima, Japan; 3) Graduate School of Frontier Sciences, Univ. Tokyo, Kashiwa, Japan.

We have been analyzing the gene expression patterns of the nematode *C.elegans* systematically by EST sequencing and whole mount in situ hybridization. All the results are integrated with the genome map based at NEXTDB http://nematode.lab.nig.ac.jp. As an extension of the project we performed whole transcriptome analysis using the SOLEXA sequencer. Poly-A RNA were prepared from 4 samples; embryo, larvae, and adult of wild type hermaphrodites, and mixed stage population of male rich worms and then converted to double-stranded cDNA using the random hexamers primers. The resulting cDNA were subjected to the SOLEXA sequencer, and then the short reads (effective length of 32 bases) were mapped onto the *C.elegans* genome by the ELAND and our newly developed mapping program that allowed a gap to detect the reads from exon-intron junctions. As to the 5' end, splice-leader capped sequences were detected and mapped. These data are integrated in NEXTDB and can be viewed as to exon structure, expression level, developmental stages, sex and so on.

In parallel, we tried the *de novo* assembling of the transcripts using the total 1Gb reads from all the samples by the VELVET assembler which was developed at EBI, and obtained total 36,000 contigs with maximum length of 5 kb and N50 of 450 bases. Although an alternatively spliced region should be assembled to a branch structure, this assembler generates the branches and the trunk as separate contigs, which may cause the relatively short contigs. However, even with such short contigs we have made many corrections on the gene models, e.g., exon structure and splicing patterns.

We are also conducting the genome sequencing of the other nematode *Diploscapter coronatus* in collaboration with Dr. Einhard Schierenberg at Univ. of Koeln; the nematode shows an interesting cell cleavage and arrangement pattern to gastrulation stage. Genomic DNA of the nematode was analyzed by the SOLEXA sequencer and 6Gb paired-end reads (each length of 36 bases) were obtained. They are being assembled in a hybrid manner together with low coverage Sanger shotgun reads. We have already done EST analysis of the nematode and identified 10,000 unique genes in which 5,800 were found to have homologs or orthologs to C.elegans. We will combine these genome and EST data.

974B

Genome-wide evidence for genetic robustness of the alternative splicing machinery in C. elegans. **L. Basten Snoek**[1], Yang Li[2], Rainer Breitling[2], Joost A.G. Riksen[1], Ritsert Jansen[2], Jan E. Kammenga[1]. 1) Nematology, Wageningen University, Wageningen, Netherlands; 2) Groningen Bioinformatics Centre, University of Groningen, Groningen, Netherlands.

Alternative splicing is considered a major mechanism for creating multicellular diversity from a limited repertoire of genes. Here, we studied genetic variation in alternative splicing patterns in a recombinant inbred population of C. elegans, using whole-genome tiling arrays. This experiment allowed us to detect heritable differences in gene expression with exquisite sensitivity and resolution. Nonetheless, we find only a relatively small number of examples of heritable variation in alternative splicing patterns. This is in striking contrast to earlier observations in humans, which showed much less genetic robustness. This observation points to a profound difference in the regulation of the alternative splicing machinery, which parallels the differences in cellular diversity and developmental flexibility in the two species. Nevertheless some striking examples were found of heritable variation in alternative splicing patterns. We detected 382 genes with substantial heritable variation for at least one exon of which the large majority of eQTLs lead to a consistent differential expression across all exons of the affected gene. Some cases show evidence for a necessary refinement of existing gene definitions, predominantly by expanding known exons. We will present genome-wide proof for earlier hypotheses that in C. elegans the alternative splicing machinery exhibits a general genetic robustness, and only a minor fraction of genes shows heritable variation in splicing forms and relative abundance. Furthermore we will show some of these genes showing heritable variation in alternative splicing.

975C

Characterization of synteny blocks and comparative analysis of operons in *Caenorhabditis* species. **Ismael A. Vergara**, Nansheng Chen. Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

Accurate detection of synteny blocks is an important task for understanding genome architecture as well as genome expression because these conserved regions could encode essential functionality critical for the fitness of the organism. In this work we apply our newly developed program, OrthoCluster, for identifying and characterizing synteny blocks between the genomes of *Caenorhabditis elegans* and *Caenorhabditis briggsae*. These synteny blocks have enabled us to systematically improve the genome annotation of *C. elegans* and *C. briggsae*, identifying 52 potential novel genes in *C. elegans*, 195 potential new genes in *C. briggsae* as well as 949 novel orthologous relationships. Using this improved dataset, we detected 3,058 perfect synteny blocks between *C. elegans* and *C. briggsae*, with only 288 of these blocks mapping to non-homologous chromosomes in *C. briggsae*. The largest perfect block contains 42 genes and spans 201.2 kbp in chromosome V of *C. elegans*. Additionally, taking advantage of the perfect synteny blocks detected with OrthoCluster, we have examined the conservation of operons between these two genomes. In contrast to previous findings reporting close to 100% (96% and 93.2%) conserved operons between *C. elegans* and *C. briggsae*, we found that only 75.4% of operons are conserved between these two sister species. Operons not conserved are disrupted by different types of genomic structural variations such as translocations, inversions or deletions of one or more orthologous genes in *C. briggsae*. This analysis suggests that operons in *Caenorhabditis* species are more actively evolving than previously thought. This work demonstrates that OrthoCluster can be effectively applied for examining the conservation of any type of eukaryotic genomes and to improve genome annotations based on syntenic information, as long as the correspondence between orthologous genomic features can be established. In the future, this analysis will be extended to include the recently sequenced genomes of *C. remanei*, *C. brenneri* and *C. japonica*. OrthoCluster is accessible at http://genome.sfu.ca/orthoclusterdb/.

976A

The Tol1 element of the medaka fish, a member of the hAT transposable element family, jumps in Caenorhabditis elegans. **K. Kodama**[1], S. Takagi[1], A. Koga[2]. 1) Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya, Japan; 2) Primate Research Institute, Kyoto University, Aichi, Japan.

Transposons have been served as useful tools for mutagenesis or gene/enhancer trap experiments in model organisms. In C. elegans, the Mos1 element of the fruitfly Drosophila mauritiana has been used for the mutagenesis. However, it has not been used for gene/enhancer trap experiments, because the Mos1 element containing a DNA fragment longer than 2kb cannot be mobilized in C. elegans cells. To overcome the problem of limited cargo capacity, we attemped to apply the Tol1 element, a member of the hAT transposable element family isolated from the medaka fish, to the C. elegans transgenesis. We have generated a transgenic line carrying an extrachromosomal array containing both the Tol1 element and the Tol1 transposase under the control of a heat-shock promoter. We confirmed that Tol1 elements were excised from the array and then inserted into the C. elegans genome after heat-shock treatments. This is the first evidence that a member of the hAT transposable element family native to a deuterostome animal is transposed in cells of a protostome animal, which is interesting in respect of the evolution of transposons. The Tol1 element used in our experiments contained a DNA fragment of about 2kb long, indicating that it can be used for insertion of a larger DNA fragment into C. elegans genome compared to the Mos1 element. We are now trying to generate transgenic lines which express the Tol1 transposase in the germ line cells.

977B

Regulation of cytosolic muscle protein degradation by *unc* genes. **Freya Shephard**[1], Ademola A Adenle[1], Susann Lehmann[1], Lew Jacobson[2], Nate Szewczyk[1]. 1) School of Graduate Entry Medicine & Health, University of Nottingham, Derby City General Hospital, Derby, DE22 3DT, UK; 2) Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA15260, USA.

Over more than 10 years we have developed *C. elegans* as a model for identifying genes involved in the regulation of cytosolic muscle protein degradation. We now know the regulation of cytosolic muscle protein degradation is complex with at least five extra-muscular signals regulating at least three distinct proteolytic mechanisms. The complex nature of this regulation makes sense teleologically, but begs the question: how complex is the regulation? As a genomic model, *C. elegans* allows us to begin to address this.

Roughly 1750 genes have been reported to give an Unc phenotype when targeted by RNAi and 118 when targeted by mutation. Our past work suggests that *unc* genes may represent 25% of the genes which regulate cytosolic muscle protein degradation (6 of 25 genes identified from 100 mutant strains) and that as many as 20% of *unc* genes may do so (6 of 30 *unc* mutants).

We selected the 118 mutationally identified *unc* genes as a starting point for understanding the genomic regulation of cytosolic muscle protein degradation. We employed RNAi feeding vectors (85 from the Ahringer RNAi library), mutants and our established transgenic lines and methods in these studies. As of 1-Feb-2009 we have examined 66 *unc* genes, 17 by mutation and RNAi, 16 by mutation only, and 33 by RNAi only. Similar to our past studies using mutants alone we find that as many as 33% of *unc* genes (22 of 66) may act to inhibit muscle protein degradation. Of the 17 genes examined by mutation and RNAi, 12 (70%) provide identical results by both methods. However, the cases where there is not concordance point to the limitations of RNAi as an approach and identify areas of caution in applying an RNAi only approach (for example two of the five cases of discordance appear to result from RNAi producing a loss-of-function while the mutant allele tested produced a presumptive gain-of-function).

Together our results suggest that RNAi against the RNAi identified *unc* genes may be productive in widening our understanding of the genomic regulation of cytosolic muscle protein degradation. Ongoing work aims to determine if the newly identified genes regulate cytosolic muscle protein degradation via established or new regulatory pathways/mechanisms.

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978C

Identification of Mutations in *Caenorhabditis elegans* That Cause Resistance to High Levels of Dietary Zinc and Analysis Using a Genomewide Map of Single Nucleotide Polymorphisms Scored by Pyrosequencing. **John Thomas Murphy**[1], Janelle J. Bruinsma[2], Daniel L. Schneider[1], Diana E. Davis[1], Brinda L. Armstead[1], Kerry Kornfeld[1]. 1) Washington University in St. Louis, Department of Developmental Biology, 660 S. Euclid St. St Louis, MO 63110; 2) EMD Chemicals, Madison, WI 53719.

Zinc plays many critical roles in biological systems: zinc bound to proteins has structural and catalytic functions, and zinc is proposed to act as a signaling molecule. Because zinc deficiency and excess result in toxicity, animals have evolved sophisticated mechanisms for zinc metabolism and homeostasis. However, these mechanisms remain poorly defined. To identify genes involved in zinc metabolism, we conducted a forward genetic screen for chemically induced mutations that cause *Caenorhabditis elegans* to be resistant to high levels of dietary zinc. Nineteen mutations that confer significant resistance to supplemental dietary zinc were identified. To determine the map positions of these mutations, we developed a genomewide map of single nucleotide polymorphisms (SNPs) that can be scored by the high-throughput method of DNA pyrosequencing. This map was used to determine the approximate chromosomal position of each mutation, and the accuracy of this approach was verified by conducting three-factor mapping experiments with mutations that cause visible phenotypes. This is a generally applicable mapping approach that can be used to position a wide variety of *C. elegans* mutations. The mapping experiments demonstrate that the 19 mutations identify at least three genes that, when mutated, confer resistance to toxicity caused by supplemental dietary zinc. It is notable that some zinc metabolism genes function in the metabolism of other metals. Preliminary genetic analysis with physiologically relevant metals such as iron, copper and selenium has confirmed that the mutations are specific to zinc metabolism. This research fills a critical need in the field of metal research, since there is a lack of understanding of how organisms cope with high levels of dietary zinc. These genes are likely to be involved in zinc metabolism, and the analysis of these genes will provide insights into mechanisms of zinc toxicity.

979A

A high-throughput platform for quantitative analysis of RNAi phenotypes in C. elegans. **Ilyass ZNIBER**, Karine REBORA, Leo GUIGNARD, Denis DUPUY. Genome Regulation and evolution, Institut Europeen de Chimie et Biologie (Bordeaux), Pessac, France.

Genome scale RNAi studies are generating a wealth of information on the phenotypic effects of gene depletion in C. elegans. However, for about 90% of genes no physiological effect could be observed in the initial visual screens. The use of RNAi sensitised mutant strains and fluorescent reporters allowed to uncover phenotypes for an additional 5 % of the gene tested. Provided we used the appropriate fluorescent reporter construct, it is likely that it would be possible to observe a modifying phenotype for the vast majority of genes with otherwise undetectable physiological effect on the animal. However, the number of RNAi screens to be performed to cover would be unpracticable using traditional microscopy and are extremely challenging for automated image analysis software. Here we present a high throughput platform for screening of fluorescent modulation in C. elegans. We use the COPAS-Profiler to perform multidimensional quantitative analysis on the knocked-down worm populations. This enables us to detect not only variation in growth and fertility but also any modification of the relative expression of up to three fluorescent reporters. We modified the Reflx plate handling system to increase the processing speed. We are currently able to collect quantitative information for up to 1000 worms from each well of a 96-plate in 25 minutes. In this set up we are able to measure, for each individual RNAi experiment, the total number of worms per well, their sizes distribution, as well as collect longitudinal fluorescence expression profiles. Importantly, we are able to compare expression patterns within individual animals, thus providing an endogenous control for stochastic variations in overall expression levels.

980B

In search of inheritance of gene expression states. **Daniel Schott**[1], Itai Yanai[2], Craig Hunter[1]. 1) Molecular & Cellular Biology, Harvard University, Cambridge, MA., USA; 2) Faculty of Biology, Technion–Israel Institute of Technology, Haifa 32000, Israel.

The interaction of the genome with the environment occurs over vastly different timescales: Within minutes or hours individuals show transcriptional responses to new environments, while over many generations populations show adaptive genetic change by natural selection. Adaptation to environmental conditions that vary or recur over the intermediate timescale of only a few generations is still not well understood.

Evidence for intergenerational epigenetic inheritance has been shown in mammals and plants, and Vastenhouw *et al.* (2006) have also shown that RNAi can trigger multigenerational gene silencing in *C. elegans*. However, it is still an open question how general this phenomenon is and whether epigenetic inheritance functions in adaptive responses to ecologically relevant environmental stimuli. In order to address this question on a genome-wide scale, we identified transcripts that continue to be produced at stress-altered levels in animals returned to control conditions. Specifically, we compared mRNA levels in early embryos collected from mothers kept under constant control conditions with embryos collected from mothers reared under moderately stressful conditions and embryos collected from mothers returned the next generation to control culture conditions.

We found that a small but significant number of the gene responses to temperature and pathogenic bacteria persist for at least one generation, suggesting that some memory of environmental stress exists. We are investigating possible mechanisms of inheritance such as heritable chromatin modifications or inheritance of regulatory RNAs acting through parts of the RNAi machinery. Additional mechanisms of inheritance are possible. Examples include self-sustaining positive feedback loops in gene regulatory networks, and changes in the composition of the yolk deposited into oocytes. We also plan to test whether the inherited information has any apparent adaptive value.

981C

Nematode genomic analysis identifies conserved regulatory sequences and gene boundaries. Brandon Barker, **Jim Lund**. Dept of Biology, Univ Kentucky, Lexington, KY.

Ten nematode genomes have been sequenced, two completed and the other eight shotgun sequenced and assembled, representing four of the twelve nematode clades. We have used comparative sequence analysis to identify conserved non-coding sequences and their distribution among these nematodes.

Comparative sequence analysis can identify functional sequence elements by seqeunce conservation. While analysis of a pair of genomes identifies many of these functional elements, additional conserved sequence elements are identified as additional genomes are analyzed and the species range of an element can be used to estimate its age. These conserved sequence elements are often regulatory elements although they are difficult to classify with *in silico* analysis. Analysis of the four sequenced *Caenorhabditis* species identifies extensive conserved non-coding sequence, approximately double the conserved sequence identified comparing a pair of species. *P. pacificus* and *H. glycines* were found to have few putative promoter sequence elements conserved with *C. elegans*, much less than expected based on gene conservation indicating that gene regulation in these species has diverged considerably. In contrast, promoter conservation with *M. hapla* is more extensive than expected.

We have developed web-based software to allow researchers to explore and visualize sequence conservation in the analyzed nematode genomes (http://daf.uky.edu/id_plot/).

Gene regulatory boundaries in the nematode *C. elegans* were identified by examining evolutionary chromosomal recombination events. Conserved sequence elements were linked with particular genes identifying the natural boundaries between genes and the extent of worm promoters.

We have used the set of identified *C. elegans* and *C. briggsae* conserved promoter elements to quantify and analyze promoter complexity. Monte Carlo sampling was used to identify GO and KEGG annotated gene groups that appear to have significantly low or high promoter complexity. Genes annotated as developmental genes and signaling genes especially G-protein coupled receptors and cell-cell signalling genes have high promoter complexity scores. Gene expression in the complete set of published *C. elegans* microarray experiments was analyzed and a strong positive correlation between gene expression variation and promoter complexity was discovered. Genes showing considerable regulation in microarray experiments tend to have complex promoters.

982A

Identification of MAB-22 targets that mediate sensory ray assembly in *C. elegans*. **David C. K. Leung**, Alfred W.H. Chan, King L. Chow. Dept Biol, HKUST, Hong Kong.

Each male tail sensory ray is composed of a structural cell and two neuronal cells embedded within the hypodermal sheath. Previous studies demonstrated that elimination of the structural cells but not the neuronal cells by laser or genetic ablation resulted in a ray-missing phenotype, highlighting the importance of structural cells for ray formation. The *mab-22(bx59)* mutants with an extensive ray-missing phenotype at the non-permissive temperature of 25°C display a failure of the cellular constituent assembly. *mab-22* encodes a T-box containing transcription factor TBX-2 expressed in structural cells. These results suggest that the activity of *mab-22* in structural cells may play an essential role in coordinating ray assembly.

To elucidate the molecular events underlying the ray assembly process, a combination of experimental and *in silico* approaches were adopted to identify the structural cell-specific genes regulated by MAB-22. These candidate genes were subjected to a structural cell-specific *gfp* reporter validation of their roles in the assembly process. Five of these genes were shown to act in ray assembly. Among them, *pqn-47(RNAi)* animals display only ray assembly defect. The *ppqn-47::gfp* transcriptional reporter was expressed in structural cells where *mab-22* acts. Its expression in all 18 structural cells and additional ray cells was noted. To quantify and compare the *pqn-47* activity in the structural cells in WT versus *mab-22(bx59ts)* mutant backgrounds, promoter deletion analysis was initiated to identify the regulatory elements required for the reporter expression in the structural cells. The activity of the so identified element was evaluated in *mab-22(bx59ts)* mutant background to validate its regulation by MAB-22. Paralleling a drop of the *mab-22* activity, the expression level of the *ppqn-47* reporter was also reduced. This observation supports our idea that *pqn-47* is acting downstream of MAB-22 when ray assembly is executed. Further detailed characterization of this structural cell-specific promoter sequence to uncover the MAB-22 binding site(s) will be presented. (This study is supported by the Research Grants Council, Hong Kong).

983B

Genome-wide analysis of the chromosomal distribution of transposable elements in *C. elegans*. **Felipe Avila**, Brad Broadway, Cedric Feschotte, Andre Pires-daSilva. The University of Texas at Arlington, Arlington, TX.

The *C. elegans* genome is around 100 Mb long, and about 12% of it is derived from transposable elements (TEs). There has been a recent growing interest in the possible role of mobile elements in genome regulation and evolutionary plasticity; however, there has been no comprehensive, detailed analysis of the overall *C. elegans* TE distribution and their possible functional roles. In this study, we analyzed the intra and inter-chromosomal distribution pattern of all 156 TE families known in *C. elegans*. We found that more than 12% of these families are over-represented on the X chromosome. The many unique features of the X chromosome, such as dosage compensation, inactivation in the germline and low gene density make it an interesting case study for possible biological relevance of the skewed distribution of these families. Cele45, for example, is a family of Short Interspersed Elements (SINEs) which has 3 times more insertions on the X chromosome than expected. Conversely, we found that 10% of the TE families are completely absent in this chromosome, despite their high copy number (more than 500 copies) in the autosomes. Cele4, a DNA transposon, has 831 copies in the *C. elegans* genome and none of those are found on the X chromosome. It has been previously shown that the distribution pattern of DNA transposons is positively correlated with high recombination rates, which usually occur in the chromosomal arms. In the X chromosome, however, recombination rates are uniform across its length. Intriguingly, we found that the distribution pattern of most of the families (51%) of both DNA and RNA transposons does not correlate with recombination rate on the X chromosome. Thus, other factors must account for the preferential accumulation of these TEs on the arms of the X chromosome.

984C

Automated, high-throughput RNAi and small molecule screening in *C. elegans*. **Annie L. Conery**[1], Eyleen J. O'Rourke[1,2], Terence I. Moy[1,2], Jonah Larkins-Ford[1], Anne E. Carpenter[3], Frederick M. Ausubel[1,2], Gary Ruvkun[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA; 3) Imaging Platform, Broad Institute of MIT and Harvard, Cambridge, MA.

The ability to carry out whole genome RNAi and small molecule screens makes *C. elegans* a compelling model organism to probe a wide range of biological processes. Although there have been successful genome-wide RNAi and small molecule screens in the past, the screening process involved manual manipulations and subjective eye scoring that could introduce variability and represented hours of labor. To make the screening process more powerful and efficient, we endeavored to adapt 2 small-scale screening assays, one looking for regulators of metabolism and another looking for novel anti-infective compounds, into high throughput, large-scale screens. We miniaturized the RNAi and compound screening assays to 96 and 384 well format, respectively, decreasing the volume of the latter to one that is compatible with high throughput chemical screens. A large particle sorter was used to automatically dispense worms into the wells and we adopted a high content screen methodology to produce accurate, quantitative data. Images of the wells at the end points of the assays were acquired using an automated microscope and analyzed using customized image analysis software. In validation of the automated methods, we were able to carry out a pilot RNAi screen and also, a large-scale compound screen of approximately 35,000 small molecules and natural product extracts that led to the discovery of 3 novel classes of anti-infective compounds. In the automation of an RNAi and chemical compound screen, we have developed a scalable and adaptable system that can be applied to a broad range of whole animal screens.

985A

Annotation and analysis of repeats in five *Caenorhabditis* species. **Ismael A. Vergara**[1], Kendrick Fong[1], Erich M. Schwarz[2], David L. Baillie[1], Nansheng Chen[1]. 1) Molec Biol & Biochem, Simon Fraser Univ, Burnaby, BC, Canada; 2) Division of Biology, 156-29, California Institute of Technology, Pasadena, CA 91125.

The presence of repetitive elements explains much of the difference in genome size for different species. In the hermaphroditic species *C. elegans* and *C. briggsae*, it is estimated that 16.5% and 22.4% of their genomes (correspondingly) are composed of different types of repetitive elements. Furthermore, the differences in size between these two genomes is explained mainly by a greater abundance of repetitive elements in *C. briggsae* compared to *C. elegans*. Recently, the genomes of the gonochoristic species *C. remanei*, *C. brenneri* and *C. japonica* were sequenced and no information regarding the content of repetitive elements has been assessed on these species. These three species are particularly interesting given that differences in size of genome sequence is also explained by the presence of heterozygous loci. In this project, we generate a filtered repeat library for each of these five genomes. Given an initial set of repetitive elements obtained with RepeatScout for each species, a methodology is defined and applied in order to remove those repeats that most likely represent ncRNA and gene families. Also, hits of gene models to transposable elements from RepBase were considered at the moment of generating the filtered libraries . The filtered repeat library for each species are further analyzed in order to (i) reduce redundancy within each group of repeats, and (ii) categorize them according to different families of transposable elements and single repeats.

986B

Polymorphic segmental duplications in the nematode *Caenorhabditis elegans*. **Ismael A. Vergara**, Allan K. Mah, Jim C. Huang, Maja Tarailo-Graovac, Robert C. Johnsen, David L. Baillie, Nansheng Chen. Molec Biol & Biochem, Simon Fraser Univ, Burnaby, BC, Canada.

The nematode *Caenorhabditis elegans* was the first multicellular organism to have its genome fully sequenced. Over the last 10 years since the original publication in 1998, the *C. elegans* genome has been scrutinized and the last gaps were filled in November 2002, which present a unique opportunity for examining genome-wide segmental duplications. Here, we performed analysis of the *C. elegans* genome in search for segmental duplications using a new tool—OrthoCluster—we have recently developed. We detected 3,484 duplicated segments—duplicons—ranging in size from 234 bp to 108 kb. The largest pair of duplicons, 108 kb in length each located in the left arm of Chromosome V, was further characterized. They are nearly identical at the DNA level (99.7% identity) and each duplicon contains 26 putative protein coding genes, most of them chemosensory genes. These large duplicons are flanked by nearly-identical *Cemar1* transposable elements, suggesting that this segmental duplication occurred by unequal crossing over enhanced by the presence of these transposons. Furthermore, the junction is located in the intronic region of a gene confirmed by EST data, suggesting that this segmental duplication gave birth to a new gene in *C. elegans*. Genotyping of 76 wild-type N2 strains obtained from different labs in the *C. elegans* community revealed that not all strains contain this duplication. In fact, only 29 strains carry this large segmental duplication, suggesting a very recent duplication event in *C. elegans* genome. This report represents the first demonstration that the *C. elegans* laboratory wild-type N2 strains can acquire large-scale differences.

987C

UNC-53 antagonizes VAB-8 function in posterior migrations of neuronal cell bodies and growth cones. **Amita Pandey**[1], Fred W. Wolf[2], Gian Garriga[1]. 1) Dept Plant & Microbial Biol, Univ California, Berkeley, Berkeley, CA; 2) Ernest Gallo Clinic & Research Center, 5858 Horton St., Suite 200, Emeryville, California 94608, USA.

Nervous system development involves the migrations of neuronal cell bodies and their axons along the dorsal-ventral and anterior-posterior axes of *C. elegans* in response to various navigational cues. The kinesin-like protein VAB-8 is both necessary and sufficient for the posterior migrations of cells and neuronal growth cones. VAB-8 appears to act through the Rac GEF activity of UNC-73 to promote guidance receptor activity that guides these migrations toward the tail [1,2]. While VAB-8 and UNC-73 promote the function of these receptors, we have identified a molecule, UNC-53, that appears to inhibit their function.

UNC-53 is a conserved protein that has an actin binding domain, a SH3 binding domain, a putative microtubule binding domain and an AAA domain. UNC-53 antagonizes VAB-8 function in the ALM and CAN neurons. Ectopic expression of VAB-8 reverses the polarity of the ALM, causing it to extend its anterior process toward the posterior. A reduction in *unc-53* function enhances the frequency of the ALM polarity reversals. Similarly, UNC-53 also suppresses the CAN migration defects of *vab-8* partial loss-of-function but not null mutants.

We found that the mutation identified originally as *enu-1(ev419)* (enhancer of Unc) by Joe Culotti is an allele of *unc-53*. Unlike other *unc-53* alleles, the *ev419* mutation did not cause an Unc phenotype. But like the other alleles, *ev419* enhanced the ALM reversal phenotype and suppressed the CAN migration defects of *vab-8* mutants, suggesting that this allele defines an UNC-53 function that specifically antagonizes VAB-8. The *ev419* mutation affects RNA splicing of the *unc-53* transcript and appears to generate a protein that is lacking 23 amino acids.

unc-53 was identified in a genome-wide RNAi screen for genes involved in endocytosis of yolk protein by oocytes[3]. We also found that *ev419* and other *unc-53* mutants are defective in oocyte endocytosis, suggesting that UNC-53's function in promoting endocytosis antagonizes VAB-8.

[1]Strumpf, N.C and Culotti, J.G. (2007) Nature Neurosci. 10: 161-168. [2]Watari-Goshima, N et al. (2007) Nature Neurosci. 10: 169-176. [3]Balklava, Z. et al. (2007) Nature Cell Biology 9: 1066-1073. Ã.

988A

Investigating PAC-1 asymmetry and the molecular control of radial polarity in the *C. elegans* early embryo. **Dorian C. Anderson**, Jeremy Nance. Department of Developmental Genetics, Skirball Institute and Sackler Program for Biomedical Studies, New York University School of Medicine, New York, NY.

The early embryo polarizes radially during the four-cell stage when the conserved polarity proteins PAR-3, PAR-6 and PKC-3 are asymmetrically positioned to outer (contact-free) surfaces of cells. Radial polarity is directed by the pattern of cell contacts and permits embryonic cells to dynamically regulate cytoskeletal rearrangements that are important for gastrulation. Previously, we showed that the RhoGAP protein PAC-1 (*PA*R-6-at-*c*ontacts) provides a link between cell contacts and PAR-dependent radial polarity by excluding PAR-3, PAR-6, and PKC-3 from the inner (contacted) surfaces of early embryonic cells. PAC-1 is recruited to inner surfaces by cell-cell contact. Our data suggest that PAC-1 utilizes its RhoGAP domain to locally inactivate the Rho GTPase CDC-42, thus excluding PAR-6 from inner surfaces.

We are now investigating how PAC-1 itself is recruited to sites of cell contact. The *pac-1* locus is complex and can produce several different isoforms that contain the RhoGAP domain. Using a combination of RNAi, PAC-1 antibodies, and tagged genomic constructs, we identified the form of PAC-1 that is necessary and sufficient to mediate radial polarity. By expressing fragments of this PAC-1 isoform fused to GFP, we have identified an N-terminal region of PAC-1 that is sufficient to localize to cell contacts. This region contains at least two subdomains that each contribute to PAC-1 localization. We have begun a yeast two-hybrid screen to identify proteins that might link the N-terminus of PAC-1 to cell contacts.

989B

C. elegans Lethal Giant Larvae acts redundantly with PAR-2 to maintain polarity in the early embryo. **Alexander Beatty**, Kenneth Kemphues. Dept MGB, Cornell Univ, Ithaca, NY.

Establishment and maintenance of cell polarity are critical for many developmental and physiological processes. The one-cell *C elegans* embryo serves as a model for studying polarity. Following fertilization, a cue associated with the sperm centrosome triggers a myosin II-dependent contraction of the cortical meshwork asymmetrically towards the future anterior pole. The contraction distributes the highly conserved PDZ proteins PAR-3 and PAR-6, as well as an atypical protein kinase C (PKC-3) to the anterior. The RING-finger protein PAR-2 becomes enriched on the posterior cortex and acts to maintain polarity by preventing these three proteins from returning to the posterior. In addition to the PARs, other proteins, such as the conserved tumor-suppressor Lethal giant larvae (LGL), are required for polarity in many metazoans. In *Drosophila*, LGL is necessary to maintain polarity in epithelial cells of the embryo and is involved in asymmetric cell division of neuroblasts. The mechanism by which LGL functions is not known; however, evidence suggests LGL may regulate non-muscle myosin II or protein trafficking. The *C. elegans* genome contains one gene that is predicted to encode a protein homologous to LGL, but the role of the protein in C. elegans has not yet been studied. We have found that depletion or mutation of the *C. elegans lgl* homologue enhances the embryonic lethality of a *par-2* temperature-sensitive mutant, suggesting LGL contributes to the maintenance of polarity in the early embryo. In addition, similar to PAR-2, GFP-tagged LGL localizes to the posterior of the one-cell embryo following polarity establishment. The asymmetric localization of LGL::GFP depends on PKC-3 as well as PAR-3 and PAR-6. The objective of my future research is to perform a mutational analysis of LGL to gain insight into the mechanism by which the protein functions with PAR-2 to maintain polarity and to determine how LGL is regulated in the early embryo.

990C

Arp2/3-dependent actin nucleation maintains epithelial polarity by regulating adhesion molecules. **Yelena Bernadskaya**, Falshruti Patel, Martha Soto. Dept Pathology & Lab Medicine, UMDNJ/RWJMS, Piscataway, NJ.

During C. elegans morphogenesis the dorsal epidermis must migrate ventrally to enclose the developing embryo. This movement requires dynamic reorganization of actin at the leading edge of migrating cells. Actin dynamics are regulated by the GEX (GTPase/Enhancer of nucleation/actin nucleation eXecution) pathway that consists of Rac1/CED-10, the WAVE/SCAR complex and the Arp2/3 complex. Activation of the GEX pathway results in branched actin nucleation at the membrane that promotes cell protrusion and allows the cell to move. Directional migration of the epidermal cells requires that the cells be polarized in the correct direction. Actin plays a number of roles in epithelial cells to regulate polarity. In the apical intestine actin is a component of the microvilli and the terminal web as well as the belt actin that joins the adherens junctions. Arp2/3-dependent actin nucleation is also thought to facilitate vesicular trafficking that delivers proteins to subcellular locations. We have previously shown that the GEX pathway is required for proper localization of actin to the apical intestine and the basolateral epidermis, the areas that becomes significantly altered in Gex (gut on exterior) mutants. The polarity of these epithelia is properly established in Gex mutants as shown by apical/basal polarity markers. However, as Gex embryos develop there is an apical expansion of the intestinal lumen. This is not due to failure to enclose since Gex embryos that manage to enclose before they die still contain a significantly expanded intestinal lumen. This defect is progressive leading to an increasingly wider lumen as the Gex embryos age. We are testing two mechanisms by which Arp2/3 dependent actin nucleation may contribute to epithelial polarity maintenance: (a) through the regulation of adherens junctions and (b) through the regulation of vesicle trafficking. Loss of the GEX pathway results in altered localization of polarized trafficking molecules and altered shape and positioning of the adherens junctions. We find that levels of adhesion proteins are altered in different Gex backgrounds, suggesting that the functional GEX pathway is required for the proper maintenance of the junctions. This work reveals a previously undescribed role for the GEX pathway in polarity maintenance through the regulation of junctional proteins.

991A

A reaction-diffusion-advection theory for cortical polarization of the *C. elegans* embryo. **Justin S. Bois**[1,2], Philipp Khuc Trong[1,2], Nathan W. Goehring[1], Mirjam Mayer[1,2], Frank Jülicher[2], Ernesto M. Nicola[2], Stephan W. Grill[1,2]. 1) Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany; 2) Max Planck Institute for Physics of Complex Systems, Dresden, Germany.

Cortical polarity in the *C. elegans* zygote is established through dedicated and intricately coupled mechanical and biochemical processes. First, a local down-regulation of myosin activity at the posterior leads to anteriorly directed mechanical flows. These flows then redistribute cortical PAR complexes, which eventually form mutually exclusive anterior and posterior domains and regulate a variety of additional biochemical processes essential for asymmetric cell division. Based on physical first principles, we derived a theoretical description of cortical flows in which myosin activity is controlled by a diffusive regulator. We combine this description with a quantitative model of PAR protein chemistry that essentially relies on cooperative antagonistic interactions between PAR complexes and constant total amounts. Using experimentally measured flow velocities, myosin density, reaction rate constants, and diffusivities, and therefore very few free parameters, this reaction-diffusion-advection theory reliably reproduces *in vivo* observations: anterior-posterior PAR and myosin polarity is established in response to a local down-regulation of myosin activity and is maintained even when cortical flows cease. We furthermore explore the dependence of the system behavior on the interaction rate constants and suggest that this simple coupling between active mechanical stresses and biochemical reactions may represent a unified theoretical framework for translation of local information into global organization.

992B

Dissecting the Centrosome Positioning Pathway. **Jessica L. Feldman**[1,2], James R. Priess[1,2]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute.

Cells, like whole organisms, have an incredible diversity of form. Cell patterning is achieved through the translation of polarizing cues into the specific arrangement of organelles and subcellular structures. Chief among these is the centrosome, the major microtubule organizing center of the cell. Despite being named for its central location in the cell, the centrosome can often occupy asymmetrical positions in differentiated cells, where it can contribute to the formation or function of cell structures such as cilia. During cell state transitions such as wound healing and polarization, centrosomes can also undergo transient repositioning within cells, and the function of this repositioning is not known. In the development of the *C. elegans* intestinal epithelia, centrosomes transiently shift from an anterior or posterior position in the cell to an orthogonal position at the future apical surface. This repositioning occurs at a developmental stage when cells are just beginning to form a polarized epithelium, and lack hallmarks such as apical junctions. We find that, following repositioning, centrosomal proteins become deposited along the apical surface of intestinal cells, suggesting that one function of centrosome repositioning might be to shuttle nucleators of microtubule assembly to the apical surface prior to polarization. We show that the PAR-3/PAR-6/PKC-3 complex localizes apically as has been shown for other epithelia and are exploring the role of the centrosome in this localization. Finally, we are using visual genetic screens to identify genes that are involved in centrosome positioning in epithelial cells, and that link centrosomes to cell polarity.

993C

The role of the centrosomes in the control of cell polarity by the Wnt signaling in *C. elegans*.. **Suhao Han**, Elvis Huarcaya-Najarro, Michael Herman. Division of Biology, Kansas State University, Manhattan, KS.

Multicellular organisms contain a diversity of cell types. One of the major mechanisms responsible for the generation of this cellular diversity are asymmetric cell divisions. A successful asymmetric cell division must be oriented to the body axis, which requires the establishment of the cell polarity. In the *Caenorhabditis elegans* embryo, the centrosomes initiate the establishment of polarity in response to a polarity signal established by the sperm entry point (Cowan and Hyman, 2004). During later embryonic and postembryonic development the polarities of many divisions are controlled by Wnt signaling and the Wnt/PCP pathways, but the role the centrosomes play in these divisions is not clear. Centrosome formation during *C. elegans* embryonic development requires the function of SPD-5, a coiled-coil protein. In *spd-5* mutant embryos, the pericentriolar material (PCM) fails to form, leading to the loss of centrosomes and centrosomal asters, resulting in the loss of embryonic polarity (Hamill et al., 2002). Recently, we discovered that SPD-5 may play a role in the control of postembryonic cell polarities that are also controlled by Wnt signaling. Specifically we found that *spd-5(RNAi)* causes defects in T and B cell polarities. Using SPD-5 antisera, we observed SPD-5 localization in a pattern consistent with centrosomes in many cells in developing larvae. In addition, we observed SPD-5 localization to the cytoplasm of the seam cells. To further investigate the role of SPD-5 in the control of T cell polarity, we have constructed a *gfp::spd-5* fusion protein driven by the SCM (seam cell maker) promoter. Our preliminary results indicate that the localization of the GFP::SPD-5 fusion protein in wild-type animals also consistent with centrosome positions. We are using this GFP::SPD-5 fusion protein as well as genetic experiments to further investigate the molecular and genetic interactions of SPD-5 with Wnt signaling, centrosomal and other cytoskeletal components.

References:
Cowan, C. R., Hyman, A. A., 2004. *Nature*. 431, 92-6.
Hamill, D. R., et al., 2002. *Dev Cell*. 3, 673-84.

994A

The Emerging Role of Ubiquitin-Specific Protease MATH-33 in Polarity of the *Caenorhabditis elegans* Early Embryo. **Richard McCloskey**, Diane G Morton, Wendy A Hoose, Kenneth J Kemphues. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

The study of partitioning defective (PAR) proteins in the nematode model *Caenorhabditis elegans* has been instrumental in elucidating conserved mechanisms of cellular polarity. Recently, our lab has used RNAi feeding as a reverse genetic method to uncover additional genes that may be involved in cellular polarity. We found that depletion of the ubiquitin-specific protease MATH-33 increases embryonic lethality and increases the penetrance of polarity phenotypes in weak *par-1(zu310ts), par-2(it5ts)*, and *par-4(it57ts)* mutants, but does not seem to increase polarity defects in weak *par-3* mutants. Generally, these results indicate that MATH-33 protein functions to support polarity in the one-cell embryo, and that it may specifically function with components that are necessary for maintaining the posterior domain. Furthermore, *math-33* mutation alone appears to subtly affect the distribution of posteriorly localized cellular components such as PAR-2 and P granules, indicating that its activity may contribute to their localization or activity. Lastly, an antibody created against a C-terminal fragment of MATH-33 has shown that MATH-33 is present in embryo cytoplasm, and is enriched in nuclei, indicating that it could be functioning anywhere the one-cell embryo. My future research will continue to explore the mechanism by which these subtle polarity defects arise and, will address whether MATH-33 is responsible for regulating one or more of the PARs directly.

995B

SPAT 1 regulates cell polarity and cell cycle in early embryos. **Anna Noatynska**, Costanza Panbianco, Monica Gotta. Department of Genetic Medicine and Development, University of Geneva Medical School, Geneva, Switzerland.

Asymmetric cell division is the process by which a cell divides to give origin to two daughter cells with different fates. In the worm, the first cell division is asymmetric generating two cells with different size, fate, cell cycle timing and spindle orientation. The highly conserved PAR proteins play a fundamental role in this process. During the division of the 1-cell embryo, PAR proteins are located asymmetrically at the embryonic cortex and define the anterior and posterior axis. Embryos mutant for any par gene fail to establish polarity and die. Despite the fact that PAR proteins are known to orchestrate asymmetric cell division across species, how they regulate polarity and how they are regulated is not fully understood. To identify additional genes required for cell polarity, a systematic RNAi screen for suppressor of the lethal phenotype of a par-2 temperature sensitive mutant was performed. One of the identified suppressors is the spat-1 gene. SPAT-1 (Suppressor of Par-two) is the putative homologue of Drosophila BORA. BORA functions in Drosophila as an activator of Aurora-A, a protein kinase known to play a role in asymmetric cell division and in cell cycle regulation. Depletion of SPAT-1 in *C. elegans* results in polarity and cell cycle defects in the early embryo. By DIC recording, the embryos display meiotic defects, the pronuclei meet centrally and the spindle shows aberrant movements. At the 2-cell stage we observe multiple nuclei and a delay in P1 division. We have generated a SPAT-1::GFP transgenic line. Consistent with the localization of BORA SPAT-1::GFP is cytoplasmic and nuclear. The nuclear localization is cell cycle regulated. At the meeting I will present our initial characterization of SPAT-1's function in *C. elegans*.

996C

Functional relationship of Wnt signaling, MIG-10, UNC-34 and actin cytoskeleton in *C. elegans* Q cell migration. **Guangshuo Ou**, Ron Vale. Cellular and Molecular Pharmacology, HHMI/Univ California, San Francisco, San Francisco, CA.

A big challenge in the cell polarity and migration fields is to forge the link between the extracellular signals that polarize the migration direction and the intracellular machinery that executes the motility response. C. elegans Q neuroblasts and their descendants can be a good model system to address this problem. Previous work suggested that Wnt signaling directs QL and its progeny's posterior migration and directs QR and its progeny to the anterior in C. elegans L1 larva. Work from different organisms or other C. elegans cell types indicated that MIG-10 (lammelipodin homolog) binds to UNC-34 (the sole Enabled/VASP homolog) important for actin dynamics, and MIG-10 and UNC-34 localize at the leading edge of migrating cell. An intriguing hypothesis for Q cell migration directionality can be that Wnt signal polarizes MIG-10 to the front edge of lamella, which further recruits UNC-34 and actin cytoskeleton for migration. We have recently developed fluorescence time-lapse microscopy for C. elegans Q cell migration with spinning disk confocal microscope. We are combining our microscopic methods with Q cell mutants to test this hypothesis. Our Q cell imaging in mig-10 and unc-34 mutants shows that MIG-10 but not UNC-34 is essential for Q cell migration. We developed GFP markers to visualize dynamics of MIG-10, UNC-34 and actin cytoskeleton in migrating Q cells. We find that UNC-34 and actin accumulate at the Q cell leading edges. Surprisingly, we find that MIG-10::YFP initially localizes at the membrane of Q cells, but when Q cells migrate, MIG-10::YFP delocalizes from the membrane. We are performing live animal imaging in mutants to address functional relationship of Wnt signal, MIG-10, UNC-34 and actin cytoskeleton in Q cell migration.

997A

Anterior embryonic polarity is maintained by dynamin. Yuji Nakayama[1,2], Jessica M. Shivas[1], Daniel S. Poole[1], Jennifer M. Kulkoski[1], Jayne M. Squirrell[3], Justin B. Schleede[1], **Ahna R. Skop[1]**. 1) Department of Genetics, University of Wisconsin-Madison, Madison, WI 53706; 2) Department of Molecular Cell Biology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba 260-8675, Japan (current address); 3) Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI 53706.

Cell polarity is crucial for the generation of cell diversity. Recent evidence suggests that the actin cytoskeleton plays a key role in establishment of embryonic polarity, yet the mechanisms that maintain polarity cues in particular membrane domains during development remain unclear. Dynamin, a large GTPase, functions in both endocytosis and actin dynamics. Here, we show that the C. elegans dynamin ortholog, DYN-1, maintains anterior polarity cues and stereotyped spindle pole movements. DYN-1 foci are enriched in the anterior cortex in a manner dependent on the anterior proteins, PAR-6 and PKC-3. Internalization of FM2-10 labeled membrane and formation of actin comets occur primarily from the anterior membrane during polarity maintenance phase, and these are impaired in dyn-1 RNAi-treated embryos. We also show that PAR-6-labeled puncta partially overlap with endocytosed FM dye and early endocytic markers. Our results demonstrate a mechanism for the spatial and temporal regulation of endocytosis in the anterior of the embryo, contributing to the precise localization and maintenance of polarity factors within a dynamic plasma membrane.

998B

PAR-2-dependent mechanisms of polarity maintenance are sufficient to initiate polarity in C. elegans zygotes. **Seth A. Zonies**, Fumio Motegi, Yingsong Hao, Geraldine Seydoux. Dept Molecular Biol & Genetics, Johns Hopkins Medical Sch, Baltimore, MD.

Polarization of the *C. elegans* zygote has been proposed to occur in two successive phases. Polarity is *initiated* by a powerful flow of cortical nonmuscle myosin (NMY-2) and F-actin, which sweeps anterior PARs away from the MTOC during pronuclear migration. During mitosis, polarity is *maintained* by PAR-2, which localizes on the cortex next to the MTOC and prevents myosin and the anterior PARs from flowing back to the posterior (Munro et al 2004, Cuenca et al., 2003). We have obtained evidence that the PAR-2 "maintenance mechanism" is sufficient to initiate polarity when initiation fails.

In a screen for temperature-sensitive polarity mutants, we isolated *ax751*, a partial loss-of-function mutant in *ect-2*. *ect-2* is required for the actomyosin contractility that powers cortical flows during polarity initiation. As expected, *ax751* zygotes fail to develop strong cortical flows during initiation, but unexpectedly still localize PAR-2 to the posterior, and form a myosin cap in the anterior during pronuclear centration. Formation of the myosin cap depends on *par-2*, and correlates with an overall increase in cortical myosin and a dramatic increase in phosphorylation of the myosin light chain in the cytoplasm. Analysis of *ax751; par-2(RNAi)* zygotes suggests that PAR-2 creates the anterior myosin cap by resisting myosin accumulation specifically in the posterior cortex. In *ax751* zygotes, *par-2* is also essential for PAR-3 asymmetry at the cortex and for PIE-1 asymmetry in the cytoplasm.

Together with other experiments in our lab (See abstract by Motegi and Seydoux), our data suggest that polarity in the zygote depends on two redundant mechanisms that reinforce each other. A first mechanism (*par-2*-independent) uses cortical flows to clear myosin and the anterior PARs from the posterior (Munro et al., 2004). A second mechanism depends on loading of PAR-2 in the posterior cortex, which in turn excludes myosin and the anterior PARs. We speculate that this second mechanism may be the primary mechanism used to polarize the P1 blastomere, since P1 polarization occurs in *ax751* embryos but not in par-2 embryos.

999C

Ciliogenesis in the developing C. elegans embryo. **Brian P Piasecki[1,2]**, Jan Burghoorn[1,2], Prasad Phirke[1,2], Elizabeth A. De Stasio[3], Peter Swoboda[1,2]. 1) Biosciences and Nutition, Karolinska Insitute, Stockholm, Sweden; 2) School of Life Sciences, Södertörn University College Huddinge, Sweden; 3) Biology Department, Lawrence University, Appleton, Wisconsin, USA.

Centrioles are dynamic cellular structures, which typically interchange between (i) being components of centrosomes that facilitate faithful chromosome separation in dividing cells, and (ii) developing and maturing into basal bodies that anchor and nucleate the growth of cilia in non-dividing cells. This interchange can be transient, the centriole and with it the cell re-enters the mitotic cell cycle, or permanent, the cell leaves the mitotic cell cycle and terminally differentiates. In Caenorhabditis elegans, centrioles are predominately studied at the one-to-two cell embryonic stage where they retain only 9-singlet microtubules. The only ciliated cells found throughout the entire C. elegans life cycle are contained in a subset of terminally differentiated sensory neurons. These ciliated sensory neurons are unique in that they do not appear to contain bona fide centrioles. Instead, the base of the cilium contains a structure with 9 doublet microtubules termed a transition zone, which in other organisms is known to be a modular addition to the distal end of a centriole that nucleates the growth of a cilium. We are currently investigating whether centriolar structure is retained during the assembly of C. elegans ciliated sensory neurons and is then disassembled/disintegrated, or if the transition zone itself is sufficient for the assembly and maintenance of cilia. For this study, we are constructing transgenic lines carrying a centriole marker, SAS-5::mCherry, and a transition zone and cilia marker, XBX-1::GFP. Expression of these transgenes during embryogenesis will be recorded using 4D (3-dimensions + time) microscopy, which will allow us to determine whether both structures have distinctly different patterns of localization or whether they colocalize during ciliogenesis. Preliminary results from these experiments will be presented.

1000A

UNC-82: An (AMP-independent?) kinase organizing muscle cytoskeleton during development. A. Reedy[1], J. Kintzele[1], S. Hayden[1], H. Qadota[2], G. Benian[2], **P. Hoppe[1]**. 1) Dept Biological Sci, Western Michigan Univ; 2) Dept Pathology, Emory Univ.

unc-82 mutants exhibit defects in the body-wall muscle contractile apparatus and move slowly (Waterston et al. 1980). In previous work we determined that *unc-82* encodes a serine/threonine kinase important for the organization of thick filament proteins myosin and paramyosin and M-line component UNC-89 (obscurin), but not for dense body components and integrin. The timing and nature of the *unc-82* phenotype, which is independent of contractile activity, suggest that *unc-82* mutations prevent cytoskeletal reorganization during growth, but do not affect initial patterning of the contractile apparatus. Although human orthologs ARK5 and SNARK were named for their similarity to AMP-activated kinases, our analyses suggest that UNC-82 is no more closely related to AMPK than it is to the PAR-1, SAD-1, PIG-1, and Kin1/2 protein families. We hypothesize that UNC-82 is part of an AMP-independent signaling pathway important for organization of thick filaments and/or components of the M-line during muscle cell elongation. To better understand the role of *unc-82*, we analyzed mutants for the distributions of components representing different "complexes" at the M-line, which contains many structural and probably signaling components. We found that the membrane-proximal UNC-112, which is closely associated with integrin, was unaffected, and that the LIM proteins UNC-95 and UNC-97 were moderately disorganized. In contrast, UNC-98, a thick filament component, showed large abnormal accumulations of stain, similar to those previously observed with the M-line component UNC-89 (obscurin). These results indicate that proper organization of membrane-distal M-line components, particularly those that directly or indirectly associate with myosin filaments, requires UNC-82 activity, and suggest that UNC-82 targets an M-line or thick filament protein. We also found that removal of phosphorylation sites from body-wall myosins produced protein localization defects like those in *unc-82*, consistent with these sites being direct or indirect targets of UNC-82. Further, the severity of the *unc-82* mutant phenotype was not increased when phosphorylation sites were removed from the M-line proximal isoform, myosin A. In contrast, removal of the homologous phosphorylation sites in paramyosin produced protein localization defects unlike those in *unc-82* mutants, and resulted in synthetic lethality in an *unc-82* mutant background. We propose that at least two partially redundant phosphorylation pathways regulate M-line and thick filament placement during cell elongation, with UNC-82 acting in a myosin pathway and a different kinase acting on paramyosin.

1001B

Characterization of *C. elegans* Filamins. **Ismar Kovacevic**, Jose Orozco, Erin Cram. Biology Department, Northeastern University, Boston, MA.

Filamin is a large, highly versatile structural and signaling scaffold of the cytoskeleton, interacting primarily with filamentous actin and integrins. Filamin is composed of an N-terminal actin-binding domain, and many immunoglobulin (Ig)-like repeats. The C-terminal Ig-like repeats of filamin act as a mechanical sensor by changing conformation under stress, and hence differentially interacting with downstream effectors. The *C. elegans* genome contains five predicted filamin-like genes (WormBase WS199); however, the predicted filamin-like open reading frames (ORFs) lack one or more features characteristic of filamin. Interestingly, the filamin genes are found in two clusters on chromosome IV (ORFs Y66H1B.2, Y66H1B.5, Y66H1B.3) and chromosome X (ORFs C23F12.2, C23F12.1). Our cDNA sequencing suggests that each cluster encodes a full-length, well-conserved filamin gene, as well as additional splice variants including N- and C-terminal truncations.

Our analysis of the filamin mutant *Y66H1B.3 (tm545)* and RNAi treated animals suggest Y66H1B filamin is essential for ovulation in *C. elegans*, and likely acts as a stretch sensor to potentiate dilation of the spermatheca-uterine (sp-ut) valve. Analysis of animals expressing *Y66H1B.3p::GFP* fusion constructs suggests filamin is expressed in the gonadal sheath cells, spermatheca, and uterus. *Y66H1B.3 (tm545)* and Y66H1B RNAi, display a dramatic ovulation defect: oocytes are entrapped in the spermatheca following fertilization due to failure of the sp-ut valve to dilate. The actin cytoskeleton of the spermathecal and uterine cells is disorganized, with predominantly thick, cortical actin filaments. Mosaic analysis of rescued *tm545* animals suggests Y66H1B filamin function is cell-autonomous in the spermatheca. We propose the *C. elegans* reproductive system as a novel, multicellular, myoepithelial model for study of filamin and mechanosensation that will allow genetic and molecular dissection of the mechanism by which cells respond to physical forces.

1002C

Shaping the intestinal tube: Organization of the intermediate filament network by MAP-kinase BMK1/ERK5 homolog SMA-5. **T. Wiesenfahrt**[1,2], H. Gerhardus[1,2], K. Hüsken[1], R. Leube[1], O. Bossinger[1,2]. 1) Institute of Molecular and Cellular Anatomy, RWTH Aachen University, D-52074 Aachen, Germany; 2) Institute of Genetics, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany.

Intermediate filaments (IFs) make up one of the three major fibrous cytoskeletal systems in metazoans. Numerous IF polypeptides are synthesized in cell type-specific combinations suggesting specialized functions. *C. elegans* carries great promise to elucidate the still unresolved mechanisms of IF assembly into complex networks and to determine IF function in a living organism. Intestinal IFs are abundant in the mechanically resilient endotube, a prominent feature of the *C. elegans* intestinal terminal web region. This IF-rich structure brings together all three cytoskeletal filaments that are integrated into a coherent entity by the apical junction thereby completely surrounding and stabilizing the intestinal lumen with its characteristic brush border. To identify factors that are responsible for the formation and positioning of this exquisite structure, IFB-2::CFP reporter strains were subjected to chemical mutagenesis. Among the resulting mutants we found one strain in which the IFB-2::CFP-labelled subapical IF network displays bubble-like protuberances, a phenotype reminiscent to the knock-down of IFC-2 by RNAi (Hüsken et al., 2008). By combined snp-mapping and RNAi-analyses the mutation was localized to the sma-5 gene (Watanabe et al., 2005). sma-5(kc1) encodes a protein that displays an amino acid exchange (R to H, pos. 125) in the catalytic domain. kc1 worms show a reduced body size, produce less progeny and have a shorter mean life span. Larval development of kc1 intestines occur regularly but considerable luminal alterations emerge later becoming manifest and most pronounced in adult worms. Multiple bubble-shaped invaginations protrude into the intestinal cells without affecting junctional integrity or apical domain morphology and composition. This phenotype suggests that the loss of sma-5 compromises intestinal stress resistance, probably by directly interfering with the phosphorylation state of the intestinal IF network.

1003A

Novel Roles of the WAVE/SCAR Complex during Nuclear Migration. **Huajiang Xiong**, Martha C. Soto. Pathology, UMDNJ/RWJMS, Piscataway, NJ 08854.

Dynamic remodeling of the actin cytoskeleton plays an essential role in various cell biological processes such as cell migration and morphogenesis in all organisms. Our laboratory has established that the conserved GEX molecules including the GTPase Rac-1 and the WAVE/SCAR complex stimulate the Arp2/3 complex to nucleate branched actin during C. elegans morphogenesis. In addition, cell migration requires dynamic interplay between filamentous actin (F-actin) and microtubules (MTs). Within a migrating cell, the nucleus migrates along MTs and is repositioned on F-actin. These events require distinct complexes composed of two novel nuclear envelope protein families, KASH and SUN domain proteins. To date, the role of actin nucleators in nuclear migration and positioning remains unknown. Our 4-D imaging of live-embryo undergoing morphogenesis show that depletion of the GEX molecules affects nuclear migration in the dorsal epidermis during dorsal intercalation, demonstrating novel functions of the Arp2/3-dependent actin nucleators. Furthermore, our studies on pronuclear migration in one-cell embryos as well as nuclear migration within the P-cells of larvae revealed that genetic mutation and depletion of the gex genes cause nuclear migration defects. The effect of the Arp2/3-dependent actin nucleators on both embryonic and post-embryonic nuclear movements indicates that actin regulators contribute to nuclear migration throughout development.

1004B

A potential nuclear role for HAM-1 in asymmetric neuroblast division. Pavitra Narasimha[1], Amy Leung[2], Maria Wu[1], **Nancy Hawkins**[1]. 1) Dept Mol. Biol. & Biochem, Simon Fraser Univ, Burnaby, BC, Canada; 2) BC Cancer Agency Research Centre, Vancouver, BC, Canada.

All 302 neurons in *C. elegans* arise through asymmetric division of neuroblasts. To investigate the molecular mechanisms underlying this process, we focused on the characterization of *ham-1*, a gene implicated in many asymmetric neuroblast divisions during embryogenesis. *ham-1* encodes a 414-amino acid protein that contains a winged-helix motif near the N-terminus, a domain that typically mediates DNA binding. When visualized by immunofluorescence, HAM-1 is found at the cell cortex and is localized asymmetrically in many dividing cells during embryogenesis. However, in transgenic embryos expressing a GFP::HAM-1 fusion protein, GFP fluorescence is also detected in the nucleus. When the same transgenic embryos are stained using either anti-GFP or anti- HAM-1 antibodies only localization to the cell cortex is detected. This result suggests that antibody staining is ineffective at detecting nuclear-localized HAM-1 and may explain our inability to detect endogenous HAM-1 in the nucleus. To identify regions of the protein required for localization and function we performed a deletion analysis. For these experiments, GFP was fused to the N-terminus of HAM-1 and expressed under control of the pan-neural *unc-119* promoter. We confirmed that the full-length fusion protein could rescue specific *ham-1* lineage defects. Our results indicate that the N-terminus is essential for cortical localization. In contrast, sequences near the C-terminus are required for nuclear localization; a GFP fusion to the C-terminal half of HAM-1 resided exclusively in the nucleus. We identified two nuclear localization sequences (NLSs) within the C-terminal half of HAM-1. Mutation of both NLSs eliminated nuclear localization, and significantly impaired *ham-1* function. To test if the loss of function was due to lack of HAM-1 nuclear localization, we fused an SV40 NLS to the N-terminus of GFP::HAM-1 with both NLSs mutated to re-target the protein back to the nucleus. Preliminary results show weak nuclear localization and partial rescue of ham-1 mutant defects. To determine if the nuclear export of HAM-1 is regulated, we analyzed the localization of GFP::HAM-1 after knocking down *imb-4* (the *C. elegans* CRM-1 nuclear exportin homologue). *imb-4* RNAi resulted in an increase of GFP::HAM-1 in the nucleus. These results strongly suggest a role for HAM-1 in the nucleus. We propose that the asymmetric localization of HAM-1 at the cell cortex is a mechanism to distribute the protein between daughter cells to mediate a differential transcription program.

1005C

Quantitative measurements and computer simulations of *C. elegans* embryonic cell morphology. **Masashi Fujita**, Shuichi Onami. RIKEN Advanced Science Institute, Yokohama, Japan.

Cells in a developing embryo have various shapes, which imply the diversity of their internal mechanics and external microenvironments. Therefore, cataloging cell shapes and providing mechanical explanations to them will significantly deepen our insight into animal development.

Since quantitative description of cell morphology is a prerequisite for such study, we developed a method for imaging and digitizing embryonic cell shapes. GFP-tagged plasma membrane is recorded using 4D microscopy and processed through semi-automated image analysis. Each cell is color-coded and transformed into a polyhedral model, which is readily applicable to computational analyses. An index of shape skewness was computed for each cell, which captured eccentricity of elongated EMS in the four-cell stage. Interestingly, this analysis also showed that EMS becomes spherical again in the six-cell stage.

The quantitative description of embryo allows us comparison with theoretical predictions by physical models. As the first step, we focused on shape of cell boundary in the two-cell stage embryo. In the case of two bound soap bubbles, the smaller bubble always bulges toward the larger one because of its higher internal pressure. However, embryos do not behave in that way. The boundary between the AB and P1 cells is flat just after the first cleavage, then larger AB bulges toward smaller P1. Theoretically, this can be explained by a gradual increase in cortical tension of AB. Since the major source of cortical tension is considered to be contractile activity of actomyosin, the role of actin filaments was examined using cytoskeletal inhibitors. Treatments with cytochalasin D or latrunculin A showed that actin filaments affect boundary shape but not the sole determinant of it. Other factors are to be explored by further studies.

1006A

Dissection of the LATS kinase pathway in *C. elegans*. **Hanee Lee**. Biological science, IMBG, Seoul national university, Seoul, Korea.

The LATS kinase pathway is one major conserved mechanism governing organ size regulation and cancer development. The fact that some components of the LATS kinase pathway are mutated or hypermethylated in cancer suggests that LATS kinase pathway is important in tumorigenesis. Since most components of LATS kinase pathway are conserved well in *C.elegans*, *C.elegans* is a good model organism to study about LATS kinase pathway. In our previous study, we found that *wts-1* is involved in the cellular polarity of intestinal cell. Loss of *wts-1* causes larval arrest phonotype because of the intestinal defects. In other species such as mammal and fly, LATS kinase is known to act through YAP/yki, the transcription coactivator, but it is possible that YAP is not the only substrate. We tried to find the downstream gene of *wts-1* by using EMS random mutagenesis in *C.elegans*. As a result, we obtained four suppressor lines that suppress larval arrest phenotype of *wts-1*. We are trying to find out which genes are mutated in these lines by SNP mapping. We found that two of them are recessive and the others dominant. One of them is linked at X chromosome. With these data, we will determine which genes act downstream of *wts-1* and it will make advanced understanding about the LATS kinase pathway.

1007B

What is tubulin glutamylation good for? An analysis of glutamylase function in C. elegans. **Nina Peel**, Kevin O'Connell. Laboratory of Biochemistry and Genetics, NIDDK, NIH, Bethesda, MD.

The regulation of microtubule function is crucial to many cellular processes and post-translational tubulin modifications such as tubulin glutamylation have been proposed to be instrumental in regulating microtubule functions. In vitro data suggest that glutamylation influences multiple microtubule-dependent processes such as centriole stability, cilia function, vesicle trafficking and neurite outgrowth. The recent demonstration that the tubulin tyrosine ligase like (TTLL) proteins are responsible for tubulin glutamylation opens the opportunity for the first comprehensive in vivo analysis of the role of glutamylation. C. elegans provides an ideal system for the study of tubulin glutamylation as it possesses fewer TTLLs than other organisms, yet has a proven utility in the study of microtubule dynamics, cell division and development. Moreover the TTLL proteins show a high degree of conservation with their human homologues. C. elegans possesses 6 members of the TTLL protein family including two glutamylation-initiating enzymes, TTLL-4 and TTLL-5. Both TTLL-4 and TTLL-5 are expressed in the C. elegans embryo and similar to homologues, localize to the nucleus. We have identified worms mutant for both ttll-4 and ttll-5, which we are using to assess the function of tubulin glutamylation in cell division and development. Using glutamylation-specific antibodies we find that ttll-4; ttll-5 double mutant worms lack glutmaylated tubulin. These worms are however homozygous viable and fertile and show no overt phenotypes. We did not uncover any alteration of microtubule stability or dynamics in cold-depolymerisation and microtubule-regrowth assays. Furthermore, fixed and live analyses show that centriole duplication and cell division are unaffected by the loss of tubulin glutamylation. A dye-filling assay showed the presence of functional cilia. A number of roles for tubulin glutamylation have been proposed, but in the absence of rigorous genetic analysis the physiological significance of this modification has remained unclear. Our data strongly indicate that despite the high level of conservation of the glutamylase enzymes, glutamylation does not play a role in essential processes such as cell division, centriole stability or sensory function. We speculate that glutamylation may instead have a redundant role which we will address by using glutamylase deficient worms in enhancer screens.

1008C

The role of AIR-1 in polarity establishment. **Sabina Sanegre Sans**, Carrie R. Cowan. Research Institute of Molecular Pathology, Vienna, Austria.

The Aurora A kinase AIR-1 is both a negative and positive regulator of cortical domain formation during polarity establishment. Polarity establishment in one-cell C. *elegans* embryos depends on the centrosomes provided by the sperm. The molecular mechanism by which the centrosomes break the symmetry to establish the anterior and posterior cortical domains remains unknown. The conserved Aurora A kinase AIR-1 has been shown to be essential for polarity establishment. In one-cell embryos, AIR-1 is also required for centrosome assembly, cell cycle timing, nuclear envelope breakdown, spindle microtubule dynamics and cytokinesis furrow specification. We are investigating how AIR-1 regulates polarity establishment and specifically whether AIR-1's role in polarization can be distinguished from its other roles in the cell. Time-lapse imaging of air-1 (RNAi) embryos suggests that AIR-1 is required for two aspects of polarity establishment: first, as a negative regulator of meiotic cortical domain expansion, and second, as a positive regulator of posterior domain expansion. These phenotypes are distinct from centrosome-defective mutants, such as spd-2 and spd-5, suggesting that AIR-1 does not function in polarity establishment strictly through a role in centrosome assembly. We are now trying to determine if AIR-1's polarization roles rely on the same molecular targets through a combination of genetics, time-lapse imaging, and biochemistry.

1009A

Identification of γ-tubulin complexes in *C. elegans* embryos. **Masahiro Terasawa**, Asako Sugimoto. RIKEN Center for Developmental Biology, Kobe, Japan.

γ-tubulin mainly localizes at centrosomes and play key roles in microtubule nucleation in eukaryotic cells. In many metazoans, γ-tubulin forms two types of γ-tubulin complexes, termed the γ-tubulin small complex (γTuSC) and the γ-tubulin ring complex (γTuRC), the latter of which consists of multiple γTuSCs and additional subunits. In *C. elegans*, the *tbg-1* gene encodes γ-tubulin (Bobinnec et al., 2000; Strome et al., 2001; Hannak et al., 2002), and *gip-1* and *gip-2* encode the homologs of the components of γTuSC, Dgrip91/GCP3/Spc98p and Dgrip84/GCP2/Spc97p, respectively (Hannak et al., 2002). However, no clear homologs for the γTuRC-specfic components (Dgrip75/GCP4, Dgrip128/GCP5, Dgrip163/GCP6, and Dgrip71WD/GCP-WD) have been identified based on the sequence similarity, thus it is unclear whether a γTuRC-like complex is present in *C. elegans*. To clarify the composition of the γ-tubulin complexes and their function in microtubule nucleation in *C. elegans*, we aimed to purify γ-tubulin complexes from *C. elegans* embryos. We established integrant lines that express the FLAG-tagged TBG-1 protein and purified the TBG-1-associating proteins by immunoprecipitation. Mass spectrometric analysis revealed that GIP-1 and GIP-2 were included in the co-purified proteins, demonstrating that γ-tubulin forms a complex with GIP-1 and GIP-2 in vivo, possibly as γTuSC. We are currently analyzing other proteins co-purified with TBG-1, and will ask whether or not a γTuRC-like complex exists in *C. elegans*.

1010B

Finding Regulators of Cadherin-Independent Epithelialization. **Stephen E. Von Stetina**, Susan E. Mango. Dept. Onc. Sci., Huntsman Cancer Inst, Univ. of Utah, Salt Lake City, UT.

How are polarized epithelia established and maintained? This question is of critical importance, as the loss of epithelial polarity is associated with metastasis*(1)*. There are many well-studied protein complexes that lie in specific membrane compartments with roles integral to the epithelial cell. The E-cadherin-containing adherens junction serves to link neighboring epithelial cells together while the more basal tight junction functions to separate the apical and basolateral surfaces. For some cells, E-cadherin is the major initiator of cell polarity and epithelium formation via cell-cell adhesion*(2)*. However, recent studies have discovered E-cadherin independent polarity pathways*(3-6)*. *C. elegans* offers a powerful system to study this cadherin-independent mechanism, as E-cadherin is dispensible for the initiation of epithelial polarity in nematodes*(4)*.

We study cadherin-independent epithelium formation during pharynx development. Nine pharyngeal arcade cells undergo a mesenchymal-to-epithelial transition to link the pharynx to the outer epidermis*(7)*. Ablation of the arcade cells results in a Pharynx unattached (Pun) phenotype, in which the pharynx fails to connect to the epidermis*(7)*. Pun animals die as they are unable to eat. Our lab has undertaken a genetic screen for Pun mutants that fail to form the arcade cell epithelium (Portereiko and Mango, unpublished). This screen revealed that loss of the central-spindlin component ZEN-4/MKLP1 induces a Pun phenotype because the arcade cells fail to polarize*(8)*. We are currently studying where and when ZEN-4 is needed for arcade cell polarization. We have also undertaken a structure/function analysis of this mitotic kinesin in order to elucidate its role in epithelialization. In addition, we are in the process of cloning several mutants that were isolated in the Pun mutagenesis screen.

(1). J. M. Lee, S. Dedhar, R. Kalluri, E. W. Thompson, J Cell Biol 172, 973 (Mar 27, 2006). *(2)*. L. N. Nejsum, W. J. Nelson, J Cell Biol 178, 323 (Jul 16, 2007). *(3)*. A. F. Baas et al., Cell 116, 457 (Feb 6, 2004). *(4)*. M. Costa et al., J Cell Biol 141, 297 (Apr 6, 1998). *(5)*. T. J. Harris, M. Peifer, J Cell Biol 167, 135 (Oct 11, 2004). *(6)*. W. B. Raich, C. Agbunag, J. Hardin, Curr Biol 9, 1139 (Oct 21, 1999). *(7)*. M. F. Portereiko, S. E. Mango, Dev Biol 233, 482 (May 15, 2001). *(8)*. M. F. Portereiko, J. Saam, S. E. Mango, Curr Biol 14, 932 (Jun 8, 2004).

1011C
Mapping the protein interaction network that controls cell polarity. **Mike Boxem**. Developmental Biology, Utrecht University, Utrecht, Netherlands.

Interactions between proteins are a key component of most or all biological processes. A key challenge in biology is to generate comprehensive and accurate maps (interactomes) of all possible protein interactions in an organism. This will require iterative rounds of interaction mapping using complementary technologies, as well as technological improvements to the approaches used. For example, we recently developed a novel yeast two-hybrid approach that adds a new level of detail to interaction maps by defining interaction domains(1).

Currently, I am working to generate an interaction map of proteins involved in controlling cell polarity in C. elegans to improve our understanding of the molecular mechanisms that establish and maintain cell polarity in multicellular organisms. I will combine two fundamentally different interaction mapping techniques: the yeast two-hybrid system (Y2H) and affinity purification/mass spectrometry (AP/MS). This will provide more detail by identifying both direct interactions between pairs of proteins by Y2H, and the composition of protein complexes by AP/MS. Moreover, interactions missed by one technology may be detected by the other, leading to a more complete interaction map.

I will integrate the physical interactions with phenotypic characterizations. To this end I will systematically characterize the interaction network in vivo using two distinct models of polarity: asymmetric division of the one-cell embryo, and stem-cell-like divisions of a multicellular epithelium (in collaboration with M. Wildwater and S. van den Heuvel).

1. M. Boxem, Z. Maliga, N. Klitgord, N. Li, I. Lemmens, M. Mana, L. de Lichtervelde, J. D. Mul, D. van de Peut, M. Devos, N. Simonis, M. A. Yildirim, M. Cokol, H. L. Kao, A. S. de Smet, H. Wang, A. L. Schlaitz, T. Hao, S. Milstein, C. Fan, M. Tipsword, K. Drew, M. Galli, K. Rhrissorrakrai, D. Drechsel, D. Koller, F. P. Roth, L. M. Iakoucheva, A. K. Dunker, R. Bonneau, K. C. Gunsalus, D. E. Hill, F. Piano, J. Tavernier, S. van den Heuvel, A. A. Hyman, and M. Vidal, A protein domain-based interactome network for *C. elegans* early embryogenesis. **Cell, 2008**. 134(3): p. 534-545.

1012A
Paxillin and its role in pharyngeal and body wall muscle of *C. elegans*. **Adam D. Warner**[1], Hiroshi Qadota[2], Barbara Meissner[1], Guy M. Benian[2], Don Moerman[1]. 1) Cell Biol Group–Zoology, Univ British Columbia, Vancouver BC, Canada; 2) Dept. of Pathology, Emory University, Atlanta GA, USA.

Attachment of actin and myosin filaments to dense bodies and M-lines, respectively, in body wall muscle is necessary to convert the force generated by sliding myofilaments into movement of the animal. Likewise in pharyngeal muscle, attachment of actin to adhesion structures in the sarcolemma membrane is key for establishing the ability of a pharyngeal muscle cell to contract. Accordingly, worm muscle attachment complexes contain many of the same protein components as vertebrate focal adhesion complexes that rely on anchoring of actin filaments for movement of migrating cells over the extracellular matrix. One of the major focal adhesion components, paxillin, had previously not been identified in the worm as a full-length protein. Here we describe work that demonstrates such a protein is present in the worm, plays an important role in muscle, and is homologous to paxillin in humans and other species.

We have found that a paxillin homolog is encoded by the gene, *pxl-1*. Originally, analysis of genomic sequence data suggested that *C. elegans* did not have a full-length paxillin homolog. However, we have found that two incorrectly annotated adjacent genes are in fact one gene and together code for a full-length paxillin. PXL-1 contains the four C-terminal LIM domains that are conserved in paxillin across all species, as well as three of the five LD motifs found in the N-terminal half of most paxillins. Our analysis demonstrates that paxillin (*pxl-1*) plays a significant role in *C. elegans* muscle. First, we have found that in body wall muscle, both antibodies towards PXL-1 and a full length GFP translational fusion localize strongly to dense bodies and adhesion plaques, and weakly to M-lines. Paxillin also localizes to ring shaped structures in the sarcolemma of pharyngeal muscle, which appear to correspond to sites of actin attachment. Secondly, a *pxl-1* gene knockout (*ok1483*) provided by the *C. elegans* Gene Knockout Consortium leads to L1 arrested animals with hindered movement, and paralyzed pharyngeal muscles. Paralysis of the pharyngeal muscle inevitably leads to starvation of the mutant worms due to an inability to feed. Lastly, yeast two-hybrid experiments, confirmed by biochemical assays using purified proteins, has shown interactions between PXL-1 and other dense body and M-line proteins including DEB-1/vinculin, UNC-95, UIG-1, LIM-8, and UNC-96. We are continuing to characterize the role that *pxl-1* plays in pharyngeal and body wall muscle in the worm.

1013B
Insights from Myosin's Nether Regions. Bryne Ulmschneider, Rachel Stewart, **Taylor Allen**. Dept Biol, Oberlin Col, Oberlin, OH.

Motoring of myosin along filamentous actin underlies many forms of cellular motility, ranging from vesicular shuttling to cytokinesis. Among these diverse manifestations of myosin's activity, muscular contraction holds the greatest promise for illuminating the mechanism by which myosin converts chemical energy into mechanical energy. Research with muscle has allowed the definition of a well-conserved core motor domain, corresponding to residues 83-787 of UNC-54/myosin B. Two regions of myosin often neglected because of their sequence variability flank the core motor domain and drew our attention: 1) the N-terminal ~80 residues, which in crystallographic analyses of myosin from other organisms form a structure making little contact with the motor domain; and 2) the junction between the myosin head, defined largely by the motor domain, and the myosin rod, which permits self-assembly into filaments in striated muscle. Reversion analysis with *unc-54* alleles *e569* and *e1152* was performed with the goal of honing in on the roles and interactions of these two less-studied regions of myosin. Worms homozygous for *e1152* are severely paralyzed, a phenotype attributed to impaired assembly of myosin into filaments as a result of the double mutation G852R and K853M (Dibb *et al. J. Mol. Biol.* **183**: 543-551, 1985). Seven suppressors of *e1152* were isolated following ENU-mutagenesis of ~10^5 haploid genomes, and each partially restores movement and egg-laying. One suppressor is tightly linked to the original mutation, and of the remaining suppressors, one has been mapped to the vicinity of *unc-96*, at the far left of LG *X*. Further characterization should define interactions made by the head-rod junction of myosin to promote assembly of the filament. Like *e1152* mutants, worms homozygous for *e569* are severely paralyzed, and the allele is fully penetrant. Sequencing revealed a nonsense mutation (Q23stop), a presumed null. EMS-mutagenesis of ~$5x10^4$ haploid genomes yielded one suppressor, which because of its tight linkage was sequenced and shown to alter the 3' splice site located four nucleotides 5' to the *e569* mutation: UUUCAG/ altered to UUUCAA/. Amplification of *unc-54* cDNA from suppressed worms identified two distinct transcripts, each shorter than wild type. Both transcripts show use of nearby AG sites for splicing, yielding in-frame deletions of 4 and 9 codons that encompass the *e569* nonsense mutation. These deletions, although allowing myosin to be synthesized, highlight the hitherto neglected importance of the N-terminal region of myosin: worms expressing myosin with the small N-terminal deletions move and lay eggs appreciably less readily than do wild types.

1014C

Paths of Communication among Myosin's Actin-binding Site, Catalytic Site, and Lever Arm. Conor Doss[1], Katherine Erickson[1], Kate Chenault[1], Lisa Goddard[1], Don Moerman[2], **Taylor Allen**[1]. 1) Dept Biol, Oberlin Col, Oberlin, OH; 2) Dept Zool, UBC, Vancouver, BC.

Myosin, a molecular motor involved in diverse facets of cellular motility, from membrane trafficking to muscular contraction, has three essential regions: a catalytic site that hydrolyses ATP, a lever arm that amplifies motions begun at the catalytic site, and an actin-binding portion. Ideas on communication among these three sites during myosin's powerstroke have matured greatly through crystallography and are ripe for testing *in vivo*. Among the few approaches for doing so, reversion analysis, in which pairs of compensating mutations are identified, is particularly powerful. Suppression of one missense mutation by another reveals an interaction at the amino acid level, either direct or indirect, long-lived or fleeting; thus, suppression can discern interactions underlying dynamic or strain-dependent states of myosin, which elude capture in crystallographic work. Random mutagenesis with EMS and ENU was used to induce suppressors of UNC-54/myosin B mutation E524K (allele *st132*), 4 residues N-terminal to the helix-turn-helix motif believed to form the center of myosin's interface with actin. Worms with E524K alone display disorganized A-bands and have a paralysis that worsens with increasing temperature. Thermodynamically, the heat-sensitivity suggests loss of a salt-bridge. The comparable residue in other myosins forms in the post-powerstroke crystallographic structures, but not in the pre-powerstroke one, a salt-bridge with a lysine (=K483 of UNC-54) on the so-called relay helix that runs through the actin-binding region and links indirectly with the start of the lever arm. Thus, in the paralyzed worms, electrostatic repulsion between E524K and K483 potentially destabilizes interactions between the relay helix and the actin-binding interface, thereby hindering the powerstroke. Twenty independent lines of suppressed worms were recovered from a screen of 10^6 mutagenized haploid genomes, and the suppressors mapped to seven residues: V187I, near the P-loop of the catalytic site; E524K→E/T, L547F, A548V, and M579I/L/V, in the actin-binding domain; C712Y, on the SH1-helix; and D724N, in the converter domain, adjacent to the lever arm. The suppressors are well positioned to modify either myosin's interface with actin or motions of the relay helix, thereby promoting the powerstroke. Interestingly, the abnormally high propulsive velocity of M579I/L suppressed worms makes sense only if the contractile force of these worms exceeds that of the wild type. For the other suppressed strains, changes of undulatory frequency and amplitude can account for non-wild-type velocity.

1015A

CSN-5, a Component of the COP9 Signalosome Complex, Regulates the Levels of UNC-96 and UNC-98, two Components of M-lines in *C. elegans* muscle. Rachel K. Miller, Hiroshi Qadota, Thomas J. Stark, Kristina B. Mercer, Tesheka S. Wortham, **Guy M. Benian**. Dept Pathology, Emory Univ, Atlanta, GA.

UNC-98 and UNC-96 are two small proteins that localize to the M-lines in body wall muscle. Mutants of each gene have a similar and characteristic phenotype: by polarized light microscopy, each shows disorganization of myofibrils and birefringent needles at the ends of the muscle cells. There is genetic and biochemical evidence that UNC-98 and UNC-96 interact with each other (Mercer et al. 2006), and with paramyosin (Mercer et al. 2006; Miller et al. 2008). *unc-96* mutants contain discrete accumulations of UNC-98 protein, and *unc-98* mutants contain accumulations of UNC-96 protein. Moreover, in both *unc-98* and *unc-96* mutants, paramyosin is localized both normally to A-bands and abnormally in accumulations. By western blot, in the absence of paramyosin, UNC-98 is diminished, whereas in paramyosin missense mutants, UNC-98 is increased. To explain this and other data, we have proposed a model in which UNC-98 and UNC-96 act as chaperones to promote the incorporation of paramyosin into thick filaments (Miller et al. 2008).

We now report that, unexpectedly, both UNC-98 and UNC-96 interact with CSN-5, a component of the conserved COP9 signalosome which has been implicated in a wide variety of functions usually linked to ubiquitin-mediated proteolysis. The interactions were initially found by screening of a yeast 2-hybrid library, and then confirmed by biochemical methods. Anti-CSN-5 antibody co-localized with paramyosin at A-bands in wild type, and co-localized with accumulations of paramyosin in *unc-98*, *unc-96*, and *unc-15* mutants. Double knock down of *csn-5* and the homologous *csn-6* could slightly suppress the *unc-96* mutant phenotype. In the double knock down of *csn-5* and *csn-6*, the levels of UNC-98 protein were greatly increased and the levels of UNC-96 protein were slightly reduced, suggesting that CSN-5 promotes the degradation of UNC-98 and that CSN-5 stabilizes UNC-96. In *unc-15* and *unc-96* mutants, CSN-5 protein was reduced, implying the existence of feed back regulation from myofibril proteins to CSN-5 protein levels. Our results are the first to implicate CSN-5 or the COP9 signalosome in myofibrillar organization or function.

1016B

Molecular Genetic Analysis of *unc-100*, a Gene Important for Normal Myofibril Organization in *C. elegans*. Tesheka S. Wortham[1], Hiroshi Qadota[1], Karissa N. McClinic[2], Kristina B. Mercer[1], Steven W. L'Hernault[2], **Guy M. Benian**[1]. 1) Dept Pathology, Emory Univ, Atlanta, GA; 2) Dept Biology, Emory Univ, Atlanta, GA.

Mutations in the gene *unc-100* result in animals showing reduced motility and disorganization of myofibrils. *unc-100* was identified in a motility-requiring selection and polarized light screen for defects in muscle function and structure (Zengel & Epstein, 1980). Currently, there are two alleles available for our characterization of *unc-100*, namely *su115* (from the CGC) and *su149* (kindly provided by Pam Hoppe). By polarized light microscopy, pharyngeal muscle appears normal, but the body wall muscle cells have myofibrils with an overall reduction in birefringence and disorganization in the patterning of A and I-bands. Interestingly, the polarized light defect of *su115*, but not *su149*, is suppressed by growth at 15°C. Through the use of a "swimming" assay, we determined that both alleles are slower moving than wild type, with *su149* showing the most dramatic defect—*su149* L4 or adults are ~65% slower than wild type. Brood sizes of each mutant allele are reduced by 50% as compared to wild type. The somatic gonad shows an abnormal morphology that is probably due to a defect in distal tip cell migration, which could explain the observed reduction in brood sizes. Staining of *unc-100* mutant muscle with antibodies to a variety of known sarcomeric proteins revealed disruption of thick filaments, M-lines and dense bodies; this disruption is more severe in the muscle of *su149* animals. Electron microscopy of each mutant allele shows missing M-lines, broken dense bodies that are often detached from the cell membrane, and a lack of defined A- and I-bands. Although the current genetic map position on WormBase for *unc-100* is I:22.75+/-1.190 cM, we used three-factor and SNP mapping to place *unc-100* on chromosome I between 1.87 and 2.26 cM which contains 17 overlapping cosmids. In transgenic rescue experiments, injecting the cosmids in sets of three, we were able to obtain rescue of *unc-100* with the three cosmids, ZK524, T28F4 and C26C6. We are currently performing single cosmid rescue experiments to determine which of the three cosmids *unc-100* lies on. We will then conduct RNAi with each gene predicted on the single cosmid to determine if we can phenocopy *unc-100*. We also plan to determine the mutation sites of both alleles and perform transgenic rescue using a single predicted gene. Once the *unc-100* gene has been identified, we will develop anti-UNC-100 antibodies to localize UNC-100 protein in muscle, and use the protein to screen an extensive yeast 2-hybrid bookshelf of M-line, dense body and thick filament components, to find interacting proteins.

1017C

Twitchin Kinase Interacts with a Conserved MAP Kinase Activated Protein Kinase in *C. elegans* Muscle. **Miho Furukawa**, Hiroshi Qadota, Tesheka S. Wortham, Guy M. Benian. Dept Pathology, Emory University, Atlanta, GA.

Sarcomeres of probably all animals contain a number of extraordinarily large polypeptides (700 kDa–4 MDa) composed of multiple Ig and Fn3 domains, one or two protein kinase domains, and in some cases, elastic regions. *C. elegans* muscle contains three such giant proteins: twitchin (754 kDa) in the polar regions of the A-band, UNC-89 (up to 900 kDa) in the middle of the A-band, and TTN-1 (2.2 MDa) in the I-band. Twitchin is encoded by *unc-22*, which has a characteristic mutant phenotype: "twitching" of the worm's surface that originates from the underlying muscle. When wild type animals are exposed to the acetylcholine agonist nicotine, they become paralyzed, whereas, when *unc-22* mutants twitch violently. The protein kinase domain shows protein kinase activity in vitro and is autoinhibited by the 60 residues C-terminal of the catalytic core. Recent molecular dynamics simulations and AFM experiments suggest that this normally autoinhibited kinase is activated by small forces that occur during muscle contraction/relaxation cycles (Greene et al. 2008). Despite this information, substrates or binding partners for the twitchin kinase domain were unknown.

We screened a yeast 2-hybrid library using as bait, Ig25-Fn31-kinase of twitchin, and recovered clones representing K08F8.1. K08F8.1 encodes a protein homologous to mammalian MAP kinase activated protein kinase 2. There are, in fact, 3 MAPKAP kinase 2 homologs in *C. elegans*: K08F8.1, MAK-2 and MNK-1. Homology among the human and worm proteins resides only within the kinase domains; in this region, K08F8.1 is 53% identical to human MAPKAP kinase 2. SAGE data on WormBase indicates that only K08F8.1 is highly expressed in body wall muscle. Yeast 2-hybrid domain mapping shows that the twitchin / K08F8.1 interaction: (1) requires only the kinase domain of twitchin; and (2) residues 60-405 (essentially the kinase domain) of K08F8.1 are minimally required. Comparable regions of TTN-1 and UNC-89 do not interact with K08F8.1, and preliminarily, MAK-2 and MNK-1 fail to interact with twitchin. Luckily, two independently generated intragenic deletions of K08F8.1, ok2987 and tm3455, are available. By swimming assay, the motility of ok2987 is 14% lower than wild type. Significantly, both ok2987 and tm3455 are hypersensitive to nicotine (they become paralyzed at a faster rate than wild type). The abnormal response to nicotine suggests that K08F8.1 acts in the same pathway as twitchin.

1018A

UNC-112 is a novel interactor with the cytoplasmic tail of PAT-3/β-integrin in *C. elegans* muscle. **Hiroshi Qadota**[1,2], Guy M. Benian[1], Don Moerman[2]. 1) Dept Pathology, Emory Univ, Atlanta, GA; 2) Dept Zoology, Univ of British Columbia, VC, Canada.

Myofilaments within *C. elegan*s body wall muscle cells are organized and anchored to the muscle cell membrane by dense bodies and M-lines. These structures are both analogous and homologous to vertebrate focal adhesion plaques. To clarify the molecular mechanisms regulating these integrin mediated attachment structures, we are investigating protein-protein interactions between integrin-associated proteins using a two hybrid approach.

From the yeast two hybrid screening with PAT-3/β-integrin cytoplasmic tail, we identified UNC-112, a FERM family protein located at dense bodies and M-lines. From the data of domain mapping experiments, we showed that binding of UNC-112 to PAT-3 cytoplasmic tail requires full-length UNC-112. We confirmed this interaction by GST-PAT-3 cytoplasmic tail pulldown of UNC-112 from a yeast lysate expressing HA-tagged UNC-112 or a *C. elegans* lysate. In embryos, it has been reported that PAT-3 is essential for UNC-112 localization to attachment structures (Rogalski et al., 2000 JCB 150: 253-264). We also showed that in adult muscle cells *pat-3* loss-of-function causes diffuse localization of UNC-112 by using an attenuated RNAi method (Qadota et al., 2008 JMB 383: 747-752).

We already reported that the UNC-112 N-terminal half binds to PAT-4/ILK. To clarify the function of UNC-112 C-terminal half, we screened a yeast two hybrid library to look for binding proteins to UNC-112 C-terminal half and isolated cDNA clones of UNC-112 lacking the C-terminal region. We have confirmed the interaction between UNC-112 N-terminus and UNC-112 C-terminus using purified proteins. This interaction suggests a comformational change of UNC-112 between closed and open states. We hypothesize that this UNC-112 conformational change might be regulated by binding of PAT-4 to UNC-112 N-terminus, resulting in ability to bind to the PAT-3 cytoplasmic tail. This hypothesis is supported by a previous report that in *pat-4* mutant embryos UNC-112 cannot localize at attachment structures (Mackinnon et al., 2002 Current Biology 12: 787-797). We are conducting *in vitro* and *in vivo* experiments to obtain more evidence to prove this hypothesis.

1019B

Screen for Interacting Molecules for the Region of UNC-89 Containing Two Protein Kinase Domains. **Kristy J. Wilson**, Hiroshi Qadota, Guy M. Benian. Dept Pathology, Emory Univ, Atlanta, GA.

unc-89 mutants display disorganized myofibrils, especially at the A-band, and usually lack M-lines. *unc-89* is a complex gene: through the use of three promoters and alternative splicing, at least 6 major polypeptides are generated, ranging in size from 156,000 to 900,000 Da. The largest of these isoforms consists of 52 Ig domains, 2 Fn3 domains, a triplet of SH3, DH and PH domains near their N-termini, and two protein kinase domains (called PK1 and PK2) near their C-termini. We have demonstrated that the DH/PH region of UNC-89 has guanine nucleotide exchange factor activity towards RHO-1 (Qadota et al. JMB 383: 747-752, 2008). Antibodies generated to three different regions of UNC-89 localize the protein to M-lines. The human homolog of UNC-89 is called obscurin.

Although there are many mutant alleles of *unc-89* available, given the size of the protein and its gene (>60 kb), it is difficult to use these mutants to characterize the function of each domain. Thus, we have taken the approach of identifying and learning the functions of the binding partners of UNC-89 domains as a way to gain insight into the function of UNC-89. We have shown that the two protein kinase domains of UNC-89 interact with SCPL-1, which contains a CTD type phosphatase domain (Qadota et al. Mol. Biol. Cell 19: 2424-2432, 2008). Antibody staining revealed that SCPL-1 co-localizes with UNC-89 at the M-line. Transient overexpression of SCPL-1 disrupts the organization of UNC-89 (Xiong et al. JMB 386: 976-988, 2009). In addition, *scpl-1(RNAi)* results in a defect in the function of egg-laying muscles. Most recently, we have found that SCPL-1 also interacts with LIM-9 (human homolog called FHL), a protein that we first discovered as an interactor of UNC-97 (PINCH) and UNC-96, components of an M-line costamere in nematode muscle (Qadota et al. Mol. Biol. Cell 18: 4317-4326, 2007). We have also demonstrated that LIM-9 can interact with UNC-89 through its first kinase domain and a portion of unique sequence lying between the two kinase domains. In fact, we have demonstrated a ternary complex between the two protein kinase regions and SCPL-1.

To understand how the kinase containing region of UNC-89 is localized in the sarcomere and regulated, we have begun yeast 2-hybrid screens using portions of UNC-89 immediately upstream of PK1 (Ig48-51—Fn1—Ig52) and PK2 (1/3 interkinase—Ig53—Fn2). So far, we have screened the M-line bookshelf of known M-line components (Qadota et al. 2007) and failed to detect interactors. Therefore, we have begun to screen the yeast 2-hybrid cDNA library RB2.

1020C

UBXN-2 regulates microtubule dependent processes in *C. elegans*. **Elsa Kress**[1], Esther Zanin[2], Françoise Schwager[1], Monica Gotta[1]. 1) Department of Genetic Medicine and Development, University of Geneva Medical School, 1 rue Michel Servet, 1211, Geneva 4, Switzerland; 2) Department of Cellular and Molecular Medicine, Ludwig Institute for Cancer Research, University of California, San Diego, La Jolla, CA 92093, USA.

Spindle positioning is essential for the proper segregation of cell-fate determinants during asymmetric cell division and for cellular arrangements during development. The interaction of astral microtubules with the cell cortex, which relies on microtubule dynamics and on its regulators, plays a major role in spindle positioning. The conserved AAA ATPase Cdc48/p97 is a key regulator of several cellular processes, including events in mitosis. Association with different cofactors confers functional specificity. In mammalian cells, together with its p47 cofactor, cdc48/p97 is involved in biogenesis of endoplasmic reticulum and Golgi apparatus, and in post-mitotic reformation of Golgi stacks. We find that depletion of UBXN-2, the *C. elegans* putative homologue of the p47 cofactor, results in a variety of defects in microtubule dependent processes, including aberrant positioning of the mitotic spindle in the one-cell embryo. In *ubxn-2(RNAi)* embryos the pronuclei meet more posteriorly and do not fully centrate, resulting in a more posterior spindle at cytokinesis. In about half of the embryos the spindle elongates transversely to the polarity axis. We are currently investigating whereas the defects observed in *ubxn-2(RNAi)* embryos are linked to modification of microtubule dynamics or of microtubules associated proteins. I will present our progress towards understanding how UBXN-2 regulates spindle positioning in the embryo.

1021A

A NEW ROLE FOR B-TYPE CYCLINS IN POLARITY AND ASYMMETRIC CELL DIVISION OF THE EARLY EMBRYO. **Alexia Rabilotta**[1], Jean-Claude Labbé[1,2]. 1) Cell Division and Differentiation Laboratory, Institute for Research in Immunology and Cancer, and; 2) Department of Pathology and Cell Biology, Université de Montréal, Québec, Canada.

We are using the *C. elegans* embryo in order to study the molecular mechanisms underlying the establishment and maintenance of cell polarity. In the zygote, the establishment of an antero-posterior axis of polarity depends on contractility of the actomyosin cortex and on asymmetric localization of PAR proteins. During polarization, PAR proteins localize asymmetrically in two mutually exclusive cortical groups: the anterior PAR-3/PAR-6/PKC-3 complex and the posterior group consisting of PAR-2 and PAR-1. The absence of any PAR protein leads to a loss of polarity and to embryonic lethality. Indeed, *par-2* mutant embryos undergo a first symmetric division with a central mitotic spindle. They also have a second symmetric and synchronic division between the two daughter cells AB and P1. A genome-wide RNAi screen previously identified two B-type cyclins, *cyb-2.1* and *cyb-2.2*, as suppressors of the lethality caused by the loss of PAR-2. The goal of this project is to characterize the role of these cyclins in cell polarity.

cyb-2.1 and *cyb-2.2* are 97% identical in their nucleotide sequence and the RNAi construct against either gene that was used in the screen cannot discriminate between the two genes. To determine if *cyb-2.1* and *cyb-2.2* are redundant for their role in polarity, we generated double mutants between *par-2(it5ts)* and either gene. We found that only a mutation in *cyb-2.1* could suppress PAR-2 lethality, indicating that the role of these two B-type cyclins in embryonic polarization may not be redundant. A phenotypic analysis revealed that some of the polarity defects associated with the loss of PAR-2, such as the loss of asynchronic second division, are suppressed in *par-2(it5ts); cyb-2.1(tm2027)* mutants. Furthermore, disrupting B-type cyclin function also caused early defects in spindle positioning in the early embryo. Interestingly, we observed that the distribution of PAR-6 was more anterior in *cyb-2.1(tm2027)* mutants compared to control embryos. These results suggest a possible mechanism of action for CYB-2.1 in regulating polarity establishment, for instance by modulating the contractility of the actomyosin cortex. This will be studied in detail by quantifying the dynamics of actomyosin contractility during polarization of *cyb-2.1(tm2027)* mutant embryos. Our results reveal a novel role for B-type cyclins in C. elegans embryonic polarity, which could underscore a more general functional link between cell cycle regulation and asymmetric cell division in polarized cells.

1022B

A proteomic approach reveals putative phosphorylation sites in the Major Sperm Protein from *C. elegans*. **JJ Fraire-Zamora**, RA Cardullo. Dept Biol, Univ California, Riverside, CA.

Nematode spermatozoa are amoeboid cells that lack an actin-based cytoskeleton, instead, they extend a pseudopod composed of filaments from a unique protein known as the Major Sperm Protein (MSP). MSP constitutes 40% of the total soluble proteins in the sperm cell and, in addition to its role in pseudopod extension, it has also been implicated in oocyte maturation and ovulation. However, to date, there is no evidence of direct involvement of MSP as a target of intracellular signaling pathways that lead to pseudopod extension. Additionally, no direct phosphorylation of MSP has been demonstrated in nematode sperm. Using a large-scale sperm isolation method, we show evidence for MSP phosphorylation in *C. elegans* spermatozoa through immunoblot detection coupled to Mass Spectrometry identification. Analysis of the resulting sequences predict putative phosphorylation sites suggesting a direct role of *C. elegans* MSP as an intracellular signaling protein involved in the cytoskeletal dynamics during sperm activation leading to pseudopod extension.

1023C

LET-99, a novel G protein regulator for the first asymmetric division- and beyond? Lori Krueger, Dae Hwi Park, Jui-Ching Wu, Max Vridine, **Lesilee Rose**. Dept Molec & Cellular Biol, Univ California, Davis, Davis, CA.

Precise positioning of the mitotic spindle is essential for asymmetric divisions and for cellular arrangements important for cell signaling, patterning, and morphogenesis. In several systems, a conserved group of PAR proteins and Gα protein signaling components are asymmetrically localized and regulate spindle positioning. We previously showed that LET-99, a DEPDC1 family protein, acts downstream of PAR polarity cues during asymmetric division in the one-cell C. elegans embryo. LET-99 is required for the nuclear rotation events that orient the spindle onto the anterior-posterior axis, and for the asymmetric metaphase/anaphase spindle displacement movements that result in unequal division. LET-99 is asymmetrically localized to the cortex by the PAR proteins with the highest levels in a lateral posterior band. LET-99 then inhibits the cortical localization of the positive regulators of G protein signaling, GPR and LIN-5. LET-99 and other PAR cues together result in a dynamic distribution of GPR/LIN-5 in which GPR/LIN-5 are first enriched at the anterior cortex during nuclear rotation, and then at the posterior cortex during spindle displacement; the lowest levels of GPR/LIN-5 are always at the LET-99 band region. Using spindle severing, single centrosome movement assays, and centrosome fragmentation, we show that both the anterior and posterior cortices generate more pulling forces than the lateral posterior region corresponding to the LET-99 band. Together these observations suggest a model for spindle displacement in which the steep gradient of GPR/LIN-5 at the posterior results in an asymmetry of forces acting on the posterior spindle pole, such that low forces laterally produce more efficient posterior-directed movement. Using in vitro pull down assays and the yeast two-hybrid system, we find that LET-99 can bind to Gα. These results and immunolocalization studies suggest that LET-99 may inhibit GPR localization by directly competing for binding to Gα at the cortex. In addition, LET-99 inhibits GPR localization during the asymmetric division of the EMS cell, which is regulated by cell signaling independently of the PAR proteins. Mutations in *let-99* also affect spindle position in the AB lineage. Thus, LET-99, Gα and GPR/LIN-5 may form a regulatory cassette that can respond to various developmental cues. We are currently examining the localization of LET-99 in other cells beyond the one-cell stage, and using a temperature sensitive allele to investigate LET-99's role in other developmental processes in the embryo.

1024A

Internalization of primordial germ cells during *C. elegans* gastrulation. **Daisuke Chihara**, Jeremy Nance. Skirball Institute, NYU, New York, NY.

Gastrulation in *C. elegans* begins when the endodermal precursor cells constrict their apical surfaces and ingress into the interior of the embryo. Subsequently, mesodermal cells and the two primordial germ cells (PGCs) ingress in a spatial and temporal sequence that is highly orchestrated. Proper ingression of endodermal precursor cells requires zygotic transcription and a PAR protein-mediated polarity that distinguishes the contacted and contact-free surfaces of the ingressing cells. By contrast, embryonic gene expression has not been observed in the PGCs at the time that they ingress, and these cells do not develop the contact/contact-free asymmetry of PAR proteins that polarizes somatic cells. Therefore somatic cells and the PGCs might utilize distinct mechanisms to ingress during gastrulation.

We hypothesized that forces exerted by surrounding cells might internalize the PGCs. Using time-lapse videomicroscopy, we first identified ingressing somatic cells cells that make contact with ingressing PGCs. Ingressing descendants of the MS and D lineages contacted the PGCs on the surface of the embryo, and endodermal precursor cells, which ingressed earlier during gastrulation, contacted the PGCs from below. We used laser operation to determine which of these cells is important for PGC ingression. Our results suggest that PGC ingressions can occur when ingression of the flanking MS or D descendants is blocked by laser-killing of these cells, but PGCs do not ingress if the endodermal precursors are killed. In addition, the PGCs do not ingress in *end-1 end-3* double mutant embryos, in which endodermal cells are born in their normal position but do not ingress properly and do not form endoderm. We imaged embryos expressing transgenes that label PGCs and endodermal precursor cells and observed that these cells maintain a close association prior to, during, and after PGC ingression. Our experiments suggest that signals or forces from the endodermal precursor cells are critical for ingression of the PGCs.

1025B

PVF-1, a *C. elegans* homologue of VEGF, functions in male ray positioning. **Gratien Dalpe**[1], Marina Tarsitano[2], Graziella Persico[2], James D. McGhee[3], Hong Zheng[1], Lijia W. Zhang[1], Joseph Culotti[1]. 1) Samuel Lunenfeld Research Institute, Toronto, ON, Canada M5G 1X5; 2) Institute of Genetics and Biophysics "A. Buzzati-Traverso", CNR, Naples, Italy; 3) 3Department of Biochemistry and Molecular Biology, Genes and Development Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

VEGF is required for both proliferation and chemotaxis of endothelial cells. Although an extensive amount of work describes VEGF's role in proliferation, its cell migration function is still not understood. In order to understand VEGF's migration function, we analyzed its single *C. elegans* homologue PVF-1. We have previously shown that PVF-1 is a secreted protein that has similar biochemical properties to VEGF (Tarsitano et al., 2006). PVF-1 has the ability to bind to VEGFr1 and VEGFr2 and to induce capillary tube formation in HUVEC and neovascularization in chicken embryos. In order to determine the function of PVF-1 in *C. elegans* we isolated a deletion mutant allele *pvf-1 (ev763)* that is predicted to be a molecular null. We find that *pvf-1 (ev763)* mutants do not have any obvious phenotypes. However, *pvf-1* mutations enhance the ray displacement phenotype observed in *semaphorin-1a/b* and *plexin-1* mutants (Dalpe et al., 2004; Ginzburg et al., 2002) suggesting PVF-1 has a guidance function in a pathway parallel to Semaphorin-1 mediated signaling. Moreover, using GFP reporter constructs and an antibody raised against PVF-1, we find PVF-1 expression in body wall muscles. Transgenic experiments using a *myo-3* promoter driven *pvf-1* cDNA rescues the ray positioning phenotype observed in *pvf-1;plx-1* double mutants, confirming that body wall muscle expression is sufficient for its function. However, we also obtained similar rescues with constructs using an *hsp-16/2* or a *lin-44* promoter driving *pvf-1* cDNA expression, suggesting that PVF-1 is not an instructive guidance cue for male ray positioning. In the end, we find that a *myo-3* promoter driven mouse $vegf_{164}$ cDNA can perfectly rescue the male ray positioning phenotype of *pvf-1;plx-1* double mutants, indicating a functional evolutionary conservation between PVF-1 and mammalian VEGF. All together, the data indicate that PVF-1 is a *C. elegans* VEGF homologue that functions in male ray cell positioning. Body wall muscle secreted PVF-1 guides ray positioning by functioning in a signaling pathway parallel to the Semaphorin-1/Plexin-1 system although these data do not exclude the possibility that it also functions in the Semaphorin-1 mediated signaling pathway. This study suggests that *C. elegans* could provide a simpler model system for investigating the role of VEGF in cell migration.

1026C

Mechanistic study of CACN-1, a novel regulator of cell migration. **Mouna Ibourk**, Erin Cram. Biology, Northeastern University, Boston, MA.

Cell migration is of fundamental importance, essential for embryonic development and tissue and organ morphogenesis in all animals. CACN-1 is a novel and well-conserved protein that is required for distal tip cell migration and gonad morphogenesis in the C. elegans nematode worm. Previous studies have identified proteins that interact in complexes to control cell behaviors such as cell migration. In our study, we conducted a genome wide yeast two hybrid screen to identify specific proteins that interact with CACN-1 in vivo. We have identified several possible interactors of CACN-1, including UNC-15/paramyosin and MIG-5/Dishevelled. UNC-15 and MIG-5 will now be further tested by RNAi to determine if they are required for DTC migration. Finally, the interaction between CACN-1 and the candidate proteins will be confirmed biochemically using a GST pull-down experiment. Results from this study will improve our understanding of the fundamental regulation of cell migration during animal development and provide new and important insights into the mechanisms of cell migration in pathologic conditions such as metastatic cancer.

1027A

nhr-67/Tailless regulates a stage-specific program of linker cell migration during male gonadogenesis. **Mihoko Kato**, Paul Sternberg. HHMI, Division of Biology, California Institute of Technology, Pasadena, CA.

Cell migrations are common events during organogenesis, yet little is known about how migration is temporally coordinated with organ development. The linker cell (LC), an individual, male-specific cell, leads the long-range migration of the developing male gonad from the early L2 stage to the mid-L4 stage, as it travels along the bodywall and executes turns to connect the gonad to the developing proctodeum. We have found that *nhr-67* regulates one temporal subset of changes in the LC from the early L3 to the mid-L4 larval stage. Gonad migration up to the early L3 stage is normal in *nhr-67*(RNAi) males, but is subsequently much slower than in wild-type males. The migrating LC changes its position, gene expression, and cell shape in wild-type males during the L3 and L4 stages; *nhr-67* is required for each of these changes to occur at their normal time. Specifically, *nhr-67* is required to inhibit *unc-5*/netrin receptor expression in the LC in the mid-L3 stage, and to activate *zmp-1*/zinc metalloprotease expression in the L4 stage. In *nhr-67*(RNAi) animals, *unc-5* continues to be expressed after the L3 stage, resulting in a delayed second LC turn, which requires the downregulation of *unc-5*; meanwhile, *zmp-1* is not expressed in L4 stage LCs. The LC normally changes from a round shape in L3 larvae to increasingly polarized arrowhead shapes in L4 larvae, but it remains round throughout most of the L4 stage in *nhr-67*(RNAi) animals. These LC changes are not induced by spatially restricted cues at the L4 stage LC position. In *nhr-67*(RNAi) animals, however, the LC undergoes late L4 stage changes normally, including developing into an extremely polarized shape and undergoing cell death. Also, genes that are expressed in L3 and L4 larvae throughout LC migration (such as *lag-2*, *him-4*, *gon-1*, and *mig-2*) have unchanged expression in *nhr-67*(RNAi) larvae. We thus propose that LC migration consists of a basal migration program and stage-specific modifiers, like *nhr-67* and at least one other potential transcription factor, which provide temporally appropriate programs.

1028B

The *C.elegans* spectraplakin *mig-31* regulates nuclear migration in distal tip cells. **H-S. Kim**[1], R. Murakami[2,3], K. Tamai[2], K. Ohkura[2], M. Labouesse[4], H. Sakamoto[3], K. Nishiwaki[1]. 1) Kwansei-Gakuin Univ, Sanda, Hyogo, Japan; 2) RIKEN CDB, Kobe, Japan; 3) Kobe Univ, Kobe, Japan; 4) IGBMC, CNRS/INSERM/ULP, France.

In *C.elegans*, U-shaped hermaphrodite gonads are formed by directed migration of gonadal distal tip cells (DTCs). Cytoskeletal regulation has been known as one of the important processes to regulate DTC migration, but its molecular mechanism remains elusive. The *mig-31* gene encodes VAB-10 spectraplakins, cytoskeletal linker proteins, and *mig-31(tk27)* mutants exhibit abnormal gonad formation caused by defective DTC migration. Complementation tests and western blot analysis revealed that abnormal gonad formation of *mig-31 (tk27)* mutant was due to the lack of DTC-specific VAB-10B isoform(s), named MIG-31. Immunohystochemical experiments revealed that MIG-31 localize to the plasma membrane of DTCs. When wild type DTCs turn dorsally, the rotation of migratory axis from anteroposterior to dorsoventral is preceded by nuclear migration within DTCs. Interestingly, however, both *mig-31(tk27)* mutation and RNAi-knockdown of *mig-31* inhibited this nuclear migration. Although DTCs appeared to be attracted dorsally by the UNC-6 mediated guidance system even in the *mig-31* knockdown animals, their nuclei stayed at their anterior or posterior ends rather than were relocated to the dorsal side as in the wild type, suggesting that MIG-31 may be required for a directional switching of DTCs. Furthermore, we found that both actin and microtubule cytoskeleton formed a transient spherical structure within the wild type DTCs during dorsal turn, presumably as a result of a cytoskeletal remodeling. In *mig-31(tk27)* mutant, this structure was also detectable, but both cytoskeletons seemed to be disorganized, suggesting that the interactions between MIG-31 and cytoskeletons in DTCs are affected. Taken together, we propose that MIG-31 may play a role in linking cytoskeletons to the plasma membrane of DTCs, which is required for cytoskeleton-based nuclear migration process within DTCs.

1029C

Effect of Folic Acid on Cell Migration During Hypodermal Morphogenesis. **Chelle King Porter**, Timothy Walston. Biology, Truman State University, Kirksville, MO.

In *C. elegans*, dorsal intercalation of the hypodermis during embryonic morphogenesis resembles a simplified version of the morphogenetic process called convergent extension, which narrows tissue along one axis to elongate it along another. In vertebrates, failure of convergent extension often results in neural tube defects (NTDs). Since NTDs in mammals are decreased when periconceptional levels of folate are increased, we are examining the effects of folic acid on hypodermal morphogenesis in *C. elegans*. To analyze this problem, we are utilizing several approaches to remove folate from *C. elegans* diet. We created and evaluated multiple axenic media to determine the best substrate for culturing *C. elegans* with differential nutrient requirements during embryogenesis. Additionally, we have used folate-deficient *E. coli* as a food source and plan to assess the role of valproic acid, a known enhancer of neural tube defects, in cell migration. We are continuing to explore genetic and nutritional methods to enable us to examine the roles of folate on hypodermal morphogenesis in *C. elegans*.

1030A

Regulation of distal tip cell (DTC) turning during gonadogenesis in *Caenorhabditis elegans*. **Maria Martynovsky**, Jean E. Schwarzbauer. Dept Molecular Biol, Princeton Univ, Princeton, NJ.

In *C. elegans* gonadogenesis, the final U-shape of the two mirror-image gonad arms is determined by migration of the distal tip cells (DTCs). These somatic cells first migrate in opposite directions on ventral until specific extracellular cues induce turning, first ventral to dorsal and then back toward the midbody region on the dorsal basement membrane. Using time-lapse video microscopy of wild-type nematodes with GFP-tagged DTCs, we have observed dramatic reorientation of the nucleus during DTC turning and additional cell shape changes during movement from ventral to dorsal surface. To genetically dissect the mechanism of DTC turning we are studying *F40F11.2*, a novel gene whose depletion by RNAi results in failure of DTC turning towards midbody region on dorsal. *F40F11.2* is expressed in the gonad primordium and expression continues throughout DTC migration where it acts cell-autonomously to control DTC turning. UNC-6/netrin signaling pathway is partly responsible for inducing the turn of the DTC from ventral to dorsal. A partial loss of gene function in *unc-6 (e78)* mutant causes DTCs to either remain on ventral or reach dorsal and then immediately descend back to ventral. Our results show that the absence of fully functional netrin sensitizes *unc-6(e78)* hermaphrodites to *F40F11.2* RNAi. *F40F11.2* gene depletion by RNAi in *unc-6(e78)* mutants results in a new phenotype: linear ventralized gonad arms. This defect suggests that *F40F11.2* is required to complete the turn back toward the midbody region on the dorsal side. We propose that an intricate interplay between netrin signaling and *F40F11.2* is required for completion of DTC turning.

1031B

Molecular signatures of Q cell migration in C. elegans larva. **Guangshuo Ou**, Ron Vale. Cellular and Molecular Pharmacology, HHMI/Univ California, San Francisco, San Francisco, CA.

Metazoan cell movement has been studied extensively in vitro, but cell migration in living animals is much less well understood. Here, we have developed live animal imaging methods for following the migration of C. elegans Q neuroblasts and their descendants during L1 larval development. Using spinning disk confocal microscopy and Q cell mutants, we examined dynamics of key molecules of cell migration and uncovered molecular signatures of Q cell migration. Each of the Q descendants migrates at different speeds and for distinct distances. Our quantitative GFP imaging show that Q descendants that migrate faster and longer than their sisters up-regulate protein levels of MIG-2, a Rho family GTPase, and/or down-regulate INA-1, an integrin alpha-subunit, during migration. We also show that Q neuroblasts bearing mutations in either MIG-2 or INA-1 migrate at reduced speeds. The migration defect of the mig-2 mutants, but not ina-1, appears to result from a lack of persistent polarization in the direction of cell migration. Thus, MIG-2 and INA-1 function distinctly to control Q neuroblast migration in living C. elegans.

1032C

Arp2/3-dependent actin nucleation is dispensable for microvillar protrusions but is necessary to maintain epithelial organization. **Falshruti B. Patel**, Yelena Bernadskaya, Martha C. Soto. Dept Pathology, UMDNJ/RWJMS, Piscataway, NJ.

Dynamic changes in the actin cytoskeleton are necessary for proper cell migration during morphogenesis. Branched actin nucleation generates the force at the cell membrane required for cellular protrusions. The WAVE/SCAR complex promotes branched actin nucleation through the Arp2/3 complex upon recruitment by activated Rac GTPase. Our studies of mutations with the Gex (gut on the exterior) embryonic phenotype have identified the GEX (GTPase/Enhancer of nucleation/actin nucleation eXecution) pathway, consisting of Rac1/CED-10, the WAVE/SCAR complex and the Arp2/3 complex, as the major regulator of actin nucleation during embryonic morphogenesis. Mutation in any component of the pathway causes 100% embryonic lethality due to failure in cell migration. 4D movies of the migrating epidermal cells in Gex mutants reveal a drop in the number and size of membrane protrusions in the leading cells of the epidermis. Tissue specific markers also show disorganized internal organs. In the intestine the apical lumen expands in embryos depleted of any GEX pathway component and this correlates with a drop in F-actin levels at the apical intestine. We questioned whether the lumen expansion and drop in the actin levels corresponds with loss of microvilli, which are apical protrusive structures. Analysis of the Gex mutant embryos using microvillar specific markers shows localization of the markers to the expanded apical region. We further conducted electron microscopy (EM) analysis. The mutants have microvilli that appear to be approximately the correct size. This suggests that WAVE/SCAR and Arp2/3-dependent actin nucleation is not required for the microvillar protrusions. 4D movies reveal loss of columnar epithelial cell shape and altered packing in the epidermal cells and in the internal organs suggesting defects in epithelial polarity. In the internal organs we find that cells initially establish apical/basal polarity with properly positioned adherens junctions. However, the mutant embryos are unable to maintain the apical/basal organization. As the embryos age there is a progressive expansion of the lumen and basal shift of the adherens junctions. We also observe mislocalization of apical and basal polarity markers in the larvae and adults depleted of WAVE/SCAR or Arp2/3 components over time. This suggests that the WAVE/SCAR and Arp2/3 complexes are continously required for maintenance of polarity during the life of the animal. I will report on an EM analysis of the embryos at the early and late stages of morphogenesis to further test our model that Arp2/3-dependent actin nucleation maintains epithelial polarity during development.

1033A

Investigation of myoblast migration during embryogenesis in Caenorhabditis elegans. **Ryan Viveiros**[1], Harald Hutter[2], Donald Moerman[1]. 1) Dept Zoology, UBC, Vancouver, BC, Canada; 2) Dept of Biological Sciences, SFU, BC, Canada.

C. elegans body wall muscle is formed after a series of well-orchestrated steps. Initially, embryonic muscle cells accumulate under the hypodermal seam cells, at the left and right lateral midline, and shortly thereafter, begin to migrate dorsally and ventrally, to form the final four muscle quadrants present upon hatching. As the myoblasts migrate they are still dividing, as are many other cells in their immediate environment. This means the cell-cell contact of cells during migration is dynamic and can vary from animal to animal. This situation creates an environment where the extracellular matrix (ECM) and cell surface contacts are in constant flux, which begs the questions as to how these cells navigate unerringly to their final destination. Though a number of ECM and cell surface molecules have previously been shown the be involved in cell migration, positioning and attachment, including perlecan, type IV collagens, the NCAM, cadherin and integrin families, and the UNC-6/netrins, previous studies have not shown any of these molecules to affect the early muscle migration. Using RNAi to target ECM components, we found that loss of laminin results in a number of muscle migration defects. Analysis of the laminin knockdown phenotype reveals defects in the aforementioned dorsal and ventral muscle cell migrations, as well as a posterior displacement of the anterior-most ventral muscle cells. Investigation of this posterior displacement has led to the identification of a previously un-described anterior migration event of the embryonic muscle cells, MSapappp, MSapaaap, MSapapap and MSsappapp. This anterior migration is dependent upon the extension of processes from the anterior-most muscle cells, as these processes appear to be absent in the ventral anterior-most cells of the laminin knockdown embryos. Using spinning disk confocal microscopy we observe that the extension of the muscle processes to the anterior coincides with the onset of elongation and is concurrent with the dorsal and ventral migrations. Wildtype analysis has also revealed the persistence of cell-cell contacts between the dorsally and ventrally migrating muscle cells with the degree of contact increasing towards the posterior. These contacts are eventually lost as the embryo elongates. While laminin is required for a subset of these processes, their extension does not require pat-3/beta-integrin, suggesting either this is an integrin independent migratory event, or that there is a yet to be identified beta-integrin. Investigations into what other genes are involved in mediating these anterior migrations are currently underway.

1034B

Analysis of immunogenic proteins in **C. elegans**. **A. Yamakawa**[1], M. Takimoto[1,2], J. Suzuki[1,3], K. Tani[1,4]. 1) Dept Material Sci, Wakayama National Col, Gobo, Japan; 2) Kyocera Co, Shiga, Japan; 3) Sanbo-chem Co, wakayama, Japan; 4) Nagaoka Univ, Nagaoka, Japan.

Toward construction of monoclonal antibody libraries covering *C. elegans* immunogenic proteins, we immunized Balb/c mice with crude extracts from heterogenous population of *C. elegans*. The spleen cells from the immunized mice were fused with P3U1 myeloma cells and the hybridomas producing monoclonal antibodies were synthesized. Up to the present, the 35 kinds monoclonal anti-*C. elegans* antibodies were selected based on the strong response to *C. elegans* crude extracts. To examine the *C. elegans* immunogenic proteins detected by these monoclonal antibodies, following experiments were done. At first, as the result of Western blotting analysis, tentatively 16 antibodies were classified into 4 types (I, II, III and IV). In these 4 types of monoclonal antibodies, the type III monoclonal antibody reacted with several *C. elegans* proteins which were main immunogenic proteins detected by antiserum against *C. elegans*. The next, the antiserum and three types (I, III and IV) of these monoclonal antibodies were applied to the immunohistochemical staining of *C. elegans*. Type I monoclonal antibody stained the both of body and mouth, type III monoclonal antibody stained the both of intestine and a surface of body, and type IV monoclonal antibody stained intestine. On the other hand, antiserum stained the both of intestine and a surface of body, as well as type III monoclonal antibody. These results derived from Western blotting and immunohistochemical analysis suggest that the type III monoclonal antibody is one of the major antibody in antiserum.

1035C

Active Cell Migration in the Early Embryo. Christian Pohl, Michael Tiongson, **Zhirong Bao**. Developmental Biology Program, Mem Sloan-Kettering Cancer Ctr, New York, NY.

It is generally believed that the reproducible cell positions and contacts in the early *C. elegans* embryo are set up through fine regulation of spindle orientation, and no active migration is involved in adjusting cell positions until gastrulation. We have observed dynamic cell shape changes and active migration in as early as the 6-cell stage embryo. Specifically, one of the six cells, ABpl, ruffles and migrates circumferentially toward the ventral side. Consequently, ABpl detaches from its sister, ABpr, and new contact forms between ABar and C. Disrupting the migration prevents the contact between ABar and C, and subsequently, the ABar spindle fails to rotate, a process known to require a Wnt signal from C. Interestingly, ABpr does not ruffle or migrate, even though it is the bilateral equivalent of ABpl. The earliest left-right asymmetry can be observed as the embryo proceeds from the 4-cell to the 6-cell stage, during which the initially left-right aligned spindles of ABa and ABp skew so that the right daughters are more posterior than their sisters. Temporally, the asymmetric ruffling follows immediately. We are now investigating if the asymmetric ruffling is due to the different cell contacts of ABpl and ABpr resulted from the skewed division of ABp, or that certain factors are distributed asymmetrically during the division. Dynamic ruffling behavior continues as the embryo develops. For example, in the next generation of cells, ABplp, ABarp and Ca, threes cells that are adjacent to each other, all ruffle. Thus, our results suggest that the early embryo is more dynamic than previously thought.

1036A

Genetic studies of the embryonic morphogenesis roles of axonal guidance molecules. **Yelena Bernadskaya**, Falshruti Patel, Martha Soto. Dept Pathology & Lab Medicine, UMDNJ/RWJMS, Piscataway, NJ.

C. elegans morphogenesis is a complex process that requires directed cell migration to organize the mass of embryonic cells into discreet tissues and organs. Our lab has identified three mutants, wve-1, gex-2, and gex-3 that completely fail morphogenesis due to the inability of the epidermal cells to migrate ventrally to enclose the embryo. These are mutations in the C. elegans homologs of the WAVE/SCAR actin nucleation enhancing complex. The GEX (GTPase/Enhancer of nucleation/actin nucleation eXecution) pathway, consisting of Rac1/CED-10, the WAVE/SCAR complex and the Arp2/3 complex, promotes branched actin nucleation. The force generated by the formation of the branched actin network is sufficient to produce protrusions at the membrane and results in cell migration. Currently, the extracellular signals that regulate the GEX pathway during morphogenesis are not known. Using a phenotypic screen we have identified extracellular guidance molecules, including UNC-6/Netrin, UNC-40/DCC, SAX-3/Robo, and SLIT-1, as potential upstream regulators of the GEX pathway. These molecules are known to regulate neuronal cell migrations. UNC-40 signaling is required for proper postsynaptic muscle extension and for the proper migration of the Hermaphrodite Specific Neuron (HSN) during larval development. SAX-3/Robo and SLIT-1 are also required in the embryo for proper epidermal enclosure. UNC-40 and SAX-3 have been shown to function upstream of Rac, a component of the GEX pathway. Further, Rac functions upstream of WAVE/SCAR components during neuronal development. Some of these axonal guidance molecules are first expressed during embryonic morphogenesis. We find that null mutations in these genes result in a partially penetrant Gex (gut on the exterior) embryonic phenotype, suggesting these molecules may be functioning in parallel to regulate GEX pathway activation during embryogenesis. We have also observed genetic interactions between these axonal guidance genes and the gex genes. Defects resulting from overexpression of SLIT-1 are suppressed by loss of WAVE/SCAR and Arp2/3 complex components, suggesting that the GEX pathway functions downstream of SAX-3/SLIT-1. We have shown that null mutations of some candidate genes influence the embryonic levels of WVE-1, suggesting a potential regulatory function at the level of WAVE/SCAR abundance. We are now testing the tissue requirements of the WAVE/SCAR complex to further elucidate the signaling architecture of the GEX pathway. This work suggests a function for extracellular axonal guidance molecules in regulating actin dynamics to organize polarized tissue movements during embryonic morphogenesis.

1037B

The extracellular matrix peroxidase peroxidasin / PXN-2 is important for embryonic morphogenesis. **Jennifer R. Gotenstein**[1], Ryann E. Swale[2], Andrew D. Chisholm[1]. 1) Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093; 2) Department of MCD Biology, University of California Santa Cruz.

Embryonic elongation of *C. elegans* is known to involve multiple components of the extracellular matrix. In screens for mutants defective in late stages of elongation, we identified multiple alleles of *pxn-2*, which encodes a member of the peroxidasin family of extracellular matrix peroxidases. Peroxidasin was first identified in conditioned media from cultured Drosophila cells. Peroxidasin-like proteins have been found in all animals and contain an animal peroxidase domain, multiple leucine-rich repeats and Ig domains, and C-terminal motifs that promote promote trimerization. In Drosophila, peroxidasin is made by hemocytes and localizes to basement membranes. However the *in vivo* roles of peroxidasins have not previously been addressed by genetics. We have characterized the embryonic defects of *pxn-2* mutants using 4D microscopy. *pxn-2* null mutants develop normally until the two-fold stage of elongation and then develop bulges and constrictions in the epidermis and arrest at the three-fold stage. *pxn-2* hypomorphic mutants display semi-lethality and Vab phenotypes. *pxn-2* mutant larvae display progressive detachment of body muscles; this can be suppressed by treatment with levamisole. *pxn-2* adults are Egl and show progressive distortion of the pharynx surface. These defects are consistent with a role for PXN-2 in strengthening basement membranes. We find that missense mutations within the PXN-2 peroxidase catalytic domain cause strong loss of function, supporting the idea that peroxidase activity is likely important for these developmental and maintenance roles. PXN-2 has also been identified in RNAi screens for genes involved in synaptic function (Sieburth et al., 2005). We find that *pxn-2* mutants are resistant to Aldicarb, suggesting a role in neuromuscular transmission. However motor neuron axonal and presynaptic morphology appear largely normal in *pxn-2* mutants. We are investigating whether PXN-2 might function in the synaptic cleft. We will also report on our studies of the role of PXN-2 and related ECM pathways in axonal regeneration. *C. elegans* encodes a second peroxidasin, PXN-1. *pxn-1* deletion mutants appear grossly normal. Unexpectedly, we find that loss of function in *pxn-1* partially suppresses *pxn-2* developmental defects. This phenotypic suppression is allele-nonspecific and gene-specific, suggesting an antagonistic relationship between the two peroxidasins. As *pxn-1* and *pxn-2* appear to be expressed by distinct tissues, this antagonistic interaction may occur in the extracellular matrix.

1038C

Mapping and Cloning of *exc-1*. **Kelly A. Grussendorf**, Matthew Buechner. Molecular Biosciences, University of Kansas, Lawrence, KS.

Tubulogenesis involves the formation and regulation of tubule shape and diameter along both the apical (lumenal) and basal sides. Once the tubule shape is formed this structure then needs to be regulated and maintained. The excretory canal provides a simple model to study these processes. The excretory canal is a single-cell tube that is located near the terminal bulb of the pharynx, which extends two hollow processes to the left and right lateral side of the worm, where they bifurcate and extend anteriorly and posteriorly to form an H-shaped structure. Our lab focuses on the set of *exc* genes, which are involved in maintaining the structure of the apical surface of the canal. Mutations in the *exc* genes allow formation of fluid-filled cysts in the lumen of the canal.

Cysts in *exc-1* mutants are often located at the ends of the canals. These cysts vary in size and number; a single cyst can be barely wider than a normal canal or as large as the entire diameter of the worm. This phenotype is similar to that of *exc-5* (encoding the guanine exchange factor FGD; see presentation by B. Mattingly) mutants, which suggests that the two gene products may act together to regulate maintenance of the canal cytoskeleton. *exc-1* is located on the right side of the X chromosome between *jud-4* and *dyn-1*, a region of 282 kb. This region is covered by 10 fosmids containing 31 predicted genes. RNAi of the 31 predicted genes did not phenocopy the *exc-1* mutation in an N2 background. The excretory canal expresses neural markers and, as for neurons, may be resistant to RNAi. We confirmed previous deletion mapping data for this gene. We are continuing knockdown experiments with RNAi in the RNAi-sensitized background *eri-1*. Along with continuing RNAi work, we are attempting to rescue the *exc-1* mutation by means of microinjection of the 10 fosmids.

1039A

The NR4A nuclear receptor NHR-6 functions with Eph receptor signaling to regulate spermatheca morphogenesis. **Anna Kleshayeva**, Chris Gissendanner. University of Louisiana at Monroe, Monroe, LA.

NHR-6, the *C. elegans* ortholog of the NR4A nuclear receptor, is required for the proper development of the spermatheca and spermatheca-uterine valve. NHR-6 has apparent dualistic functions in regulating cell proliferation and cell differentiation during development of this organ system, similar to the cellular functions of its vertebrate homologs. We are interested in identifying NHR-6 signaling pathways, especially those pathways that modulate differential cell proliferation and cell differentiation activities of NHR-6. A previously published genome-wide RNAi interaction screen (Lehner et al, *Nature Genetics*, 2006) identified *vab*-1 as a potential *nhr-6* interacting gene. *vab-1* encodes the *C. elegans* ortholog of the Eph receptor, a receptor tyrosine kinase important in regulating tissue morphogenesis. We have confirmed that *nhr-6* does indeed genetically interact with *vab-1*. *nhr-6 (RNAi); vab-1* double loss of function animals exhibit a synergistic decrease in reproductive fecundity. We confirmed using two cellular markers that the spermatheca exhibits severe morphological defects in the double loss of function animals. *vab-1* loss of function alone also causes a distinct defect in the morphogenesis of the spermatheca distal constriction. The distal spermatheca constriction is important in regulating oocyte ovulation and loss of normal distal morphology leads to phenotypes associated with ovulation defects (oocyte fragmentation and abnormal egg morphology). Interestingly, *nhr-6* RNAi, which is hypomorphic in the N2 background, also displays a similar loss of distal morphogenesis phenotype. In both *vab-1(lof)* and *nhr-6 (RNAi)* animals this distal defect is not associated with cell proliferation defects, confirming a post-proliferation function for these genes. Interaction and single loss of function spermatheca phenotypes for both VAB-1 kinase and ligand-binding mutations are similar to a *vab-1(null)* mutation indicating that both Eph receptor kinase and ligand-binding functions are important for spermatheca development. Analysis of a GFP-tagged VAB-1 receptor revealed that VAB-1 is localized to the spermatheca cell membranes during the L4 stage. *nhr-6(RNAi)* also similarly interacted with *vab-2*. *vab-2* encodes an ephrin, the membrane-bound ligand for the Eph receptor. *in situ* data from NEXTDB indicates that *vab-2* may be expressed in the distal somatic gonad. We are currently generating and analyzing *nhr-6(null); vab-1(null)* and *nhr-6(null); vab-2(null)* double mutants to determine if *nhr-6* and *vab-1/vab-2* function in a parallel or common pathway. Evidence for the latter would identify VAB-1 as a potential modulator of NHR-6 function during a late morphogenesis stage of spermatheca development.

1040B

A screen for additional genes controlling early steps of pharyngeal morphogenesis. **Israel Muro**, David Fay. Dept Molecular Biol, Univ Wyoming, Laramie, WY.

Previous work in our laboratory has revealed coordinate roles for LIN-35/Rb, a C. elegans pocket protein ortholog, UBC-18-ARI-1, a conserved E2-E3 ubiquitin-ligase complex, and PHA-1, a novel gene, in the control an early step of pharyngeal morphogenesis, termed re-orientation. At this stage, the anterior-most cells of the pharyngeal primordium change shape from a radial configuration (with alignment along the rostrocaudal axis) to a parallel orientation, relative to the dorsoventral axis. At the same time the apical-basal polarities of the leading anterior cells shift from 30-90° to align their axes with the dorsoventral axis. These morphological changes ultimately facilitate the formation of a contiguous epithelial tube comprised of pharyngeal cells and cells of the future buccal cavity (mouth). Mis-execution of this step leads to a failure of the pharynx to attach to the buccal cavity (the Pun phenotype, for Pharynx unattached), as well as defects in pharyngeal elongation. To better understand the process of pharyngeal morphogenesis, we have carried out a screen for Pun mutants using a myo-2::GFP reporter. Among the isolated mutants, we have identified four that appear to be specifically defective at the re-orientation step. All four mutants are zygotically lethal and recessive and show a fully penetrant Pun phenotype. Furthermore, pharyngeal cells in these mutants appear to be correctly specified and to undergo normal patterns of differentiation. We are currently mapping these mutations to identify the affected loci. Characterization of these mutations should lead to further insights into the basics mechanisms underlying organ morphogenesis. In addition, this work may provide additional insights into the roles of LIN-35 and associated factors in pharyngeal development.

1041C

THE NOVEL NUCLEAR PROTEIN VAB-23 IS AN ESSENTIAL REGULATOR OF MORPHOGENESIS IN THE EMBRYO AND VULVA. **Mark W. Pellegrino**[1], Robin B. Gasser[2], Frank Sprenger[3], Attila Stetak[4], Alex Hajnal[1]. 1) University of Zurich, Institute of Zoology, Zurich, Switzerland; 2) The University of Melbourne, Department of Veterinary Sciences, Werribee, Victoria, Australia; 3) The University of Cologne, Institute for Genetics, Köln, Germany; 4) The University of Basel, Institute of Psychology, Missionsstrasse 60/62a, Basel, Switzerland.

Tissue morphogenesis, which normally occurs after the cell fates have been specified, is a key step during the formation of functional organs. *C. elegans* is a well-established model to study cell fate execution and the complex signaling pathways controlling morphogenesis during development. The epidermis of the embryo is one of the first tissues to undergo morphogenesis and is essential for both embryonic and post-embryonic development. We have identified the novel nuclear protein VAB-23 as a key regulator of epidermal morphogenesis during embryogenesis. During embryogenesis, VAB-23 acts in a cell non-autonomous manner from the underlining neuroblasts to control the formation of homotypic cell contacts by ventral epidermal cells. In addition, we find that VAB-23 is essential for the formation of epidermal toroids in the hermaphrodite vulva, the organ through which the eggs are laid. Interestingly, VAB-23 represses ectopic cell-cell contacts between cells of non-homologous fates in both the embryo and vulva, suggesting regulation of similar target gene(s). In the vulva, VAB-23 displays a restricted early expression in the primary P6.p lineage and complete downregulation in secondary P5.p and P7.p cells, consistent with our findings that *vab-23* is a downstream transcriptional target gene of the Ras/MAP kinase pathway. Lastly, we provide evidence for direct transcriptional activation of *vab-23* by LIN-39/Hox and its co-factor CEH-20/PBC. Thus, the Ras/MAP kinase pathway and LIN-39/Hox control vulval morphogenesis through the regulation of the novel target gene *vab-23*.

1042A

Identification and characterization of gastrulation control genes in *C. elegans*. **Monica R. Rohrschneider**, Jason Gill, Jeremy Nance. Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, NYU-School of Medicine, New York, NY.

Gastrulation is a critical stage of development, when cells migrate to set up the proper positioning of the germ layers in the embryo. During *C. elegans* gastrulation, the endodermal (E) cells are the first cells to ingress from the surface into the interior of the embryo. Several cell biological mechanisms contribute to gastrulation—cell cycle lengthening, myosin-mediated apical constriction, and migration of neighboring cells (MS and P_4 cells) to cover the ingressing E cells. The GATA transcription factors END-1 and END-3 function redundantly to specify endoderm and are also required for normal E cell ingression. However, little is known about how these two transcription factors trigger gastrulation movements.

Using expression microarrays and transcriptional reporters, we have identified a suite of 14 candidate END-1/-3 effector genes that are expressed in the E cells during gastrulation. Putative null mutations have been isolated in several of these 14 genes. By measuring the E cell cycle rate, the ingression and apical constriction of the E cells, and the migration of the neighboring MS and P_4 cells in mutant embryos, we have begun to determine whether these 14 genes function downstream of END-1/-3 in controlling specific cell biological aspects of gastrulation. Mutations in several of these genes result in delayed or slowed gastrulation phenotypes. For example, *ttr-30* embryos have delays in both ingression and apical constriction of the E cells, but have a normal E cell cycle length. To test the hypothesis that multiple END-1/-3 effectors function together to control gastrulation, we are also making double mutants and testing whether they have more severe gastrulation defects.

1043B

Association Between Glands and Neurons in the Developing Pharynx. **Patty Rohs**, Jeb Gaudet. Genes and Development Research Group, University of Calgary, Calgary, Alberta, Canada.

Cellular growth and morphogenesis are critical processes in the development of a fully functional organ. Our lab has been studying the glands of the *C. elegans* pharynx to better understand the processes that shape these cells. Each of the five gland cells extend a projection along a specific trajectory, but little is known about how the formation of these projections is controlled. Interestingly, gland projections and pharyngeal neurons share trajectories within the organ–in fact, the axons of many pharyngeal neurons are in contact with the gland projections through most of the length of the pharynx. This association of cell projections is reminiscent of the association between other tissues in other organisms–e.g. blood vessels and lymphatic vessels–and raises the possibility that the pharyngeal glands and neurons may guide one another in the developing organ. We are therefore examining the close association between pharyngeal glands and neurons to gain a better understanding of the development and guidance of these cells.

Preliminary evidence suggests that glands are required for guidance of some pharyngeal neurons. Currently, we have focused on the M1 pharyngeal motor neuron. The axon of M1 is in contact with the projection of the g1P gland throughout the anterior half of the pharynx. When g1P is ablated, M1 shows frequent and severe guidance defects. These results suggest that the gland projections may provide a scaffold for the growth and guidance of pharyngeal neurons.

We are currently attempting to clarify the nature of such interactions by examining other gland cells and pharyngeal neurons. For example, we are examining whether ablation of g1P affects another neuron which it contacts, the I3 neuron. In addition, we are testing whether pharyngeal neurons may influence guidance of pharyngeal glands. We are also examining known fasciculation mutants to determine whether these genes are required for the continued contact between g1P and M1.

1044C

Basement membrane protein MIG-6 involved in gonadogenesis is required for localization of MIG-17 and matrix remodelling. **Takehiro Kawano**, Hong Zheng, Joseph Culotti. Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, ON, Canada.

In *C. elegans* hermaphrodites, the two U-shaped gonad 'arms' are formed by the directed migration of distal tip cells (DTCs) during post-embryonic development. We analyzed two phenotypic classes of *mig-6* mutant alleles. Class-*l* mutations [aka class-1], originally described by Hedgecock *et al.*, were found to lower the rate of DTC movement, whereas class-*s* mutations [aka class-2], which was isolated from a screen for dominant DTC migration defects, affect DTC guidance during the second (ventral to dorsal) phase of its migration. We cloned *mig-6* and found that it encodes an extracellular matrix (ECM) protein that is highly related to the secreted multi-component ECM proteins papilin in *Drosophila* and lacunin in *Manduca sexta*. Immunohistochemical analysis showed that MIG-6 localizes on the basement membranes of the gonad, intestine, pharynx, and body wall muscles. The *mig-6* class-*s* and class-*l* mutations affect the function of the two alternatively spliced mRNAs, *mig-6*S and *mig-6*L, which encode MIG-6S and MIG-6L proteins, respectively. Loss of MIG-6L roughly mimics the DTC phenotype of *gon-1/metalloproteinase* mutant. We found that MIG-6L functions cell autonomously in the DTCs to regulate the rate of their migration, while MIG-6S functions cell non-autonomously to guide the phase 2 (ventral to dorsal) DTC migration. The DTC migration defect and the consequent gonad phenotype of mutation in *mig-17*, which encodes a secreted metalloproteinase, is similar to that of *mig-6(s)*. Intergenic non-complementation suggests that *mig-6(s)* and *mig-17* act in the same mechanistic pathway to ensure normal phase 2 DTC guidance. MIG-17::GFP is abnormally localized in *mig-6(s)* mutants, in a way that suggests that MIG-6S is required for retaining MIG-17 on the gonadal and intestinal basement membranes. *emb-9* and *let-2* encode collagen type IV alpha 1 and 2 subunits, respectively, and are major components of the basement membranes in *C. elegans*. *emb-9* RNAi or *emb-9* null heterozygotes suppressed the phase 2 DTC migration defects in *mig-6* class-*s* mutants. A lesser but substantial suppression was also caused by the temperature sensitive *let-2(g37ts)* mutation. These results suggest that collagen IV acts antagonistically to MIG-6 in guiding phase 2 DTC migration possibly by forming over-abundance of normally or abnormally assembled ECM. We propose that MIG-6 regulates distinct aspects of DTC migration by dynamically regulating the abilities of specific proteinases to remodel different basement membranes encountered during sequential phases of DTC movements.

1045A

Functional analysis of conserved tyrosines in βPAT-3 integrin cytoplasmic tail. Jeong Ahn, Lynda Hoang, **Myeongwoo Lee**. Dept Biol, Baylor Univ, Waco, TX.

Integrin is the αβ heterodimeric cell surface receptor for extracellular matrix (ECM) and an excellent model to investigate the function of conserved tyrosine (Y) phosphorylation motif, NPXY. The β integrin possesses two NPXY motifs, Y792 and Y804, in the cytoplasmic tail, and the phosphorylation of NPXY mediates interaction to a protein with phosphotyrosine binding (PTB) domain. Cell culture analyses suggested that Y to phenylalanine (F) mutation caused mild defects while Y to alanine (A) abolished cellular function of β integrins. We characterized the activation of NPXY using βpat-3 integrin of the nematode Caenorhabditis elegans and generated a tyrosine (Y) to glutamate (E) mutant. βpat-3(Y804E), a transgenic mutant carrying a phosphomimetic mutation in the second NPXY motif of the βpat-3 cytoplasmic tail, showed defective muscles, abnormal gonad migration and tail morphology, ineffective mating, and high incidence in male. Some phenotypes of βpat-3(Y804E) parallel those of him-4/hemicentin, an ECM molecule similar to human fibulin-5, leading us to hypothesize that defective him-4 causes phosphorylation in NPXY motifs of βpat-3 cytoplasmic tail. Thus, we introduced the βpat-3 non-phosphorylatable mutation, βpat-3(YYFF), in the him-4 background. Phenotypic analyses of βpat-3(YYFF); him-4 double mutant indicated that βpat-3(YYFF) mutation suppressed ineffective mating, high incidence in male, and egg-laying defects of him-4 males and hermaphrodites. This suggested that one of the functions of him-4/hemicentin is to prevent phosphorylation of βpat-3 NPXY. Further analysis will provide valuable information on the function of NPXY motifs in β integrin regulation and will enable us to interpret integrin signaling in other species.

1046B

mig-18 encodes a novel protein required for directed migration of distal tip cells. Yuko Kitano[1,4], Shinji Ihara[1], Thomas Harbaugh[3], Kohji Ikenishi[4], Gian Garriga[3], **Kiyoji Nishiwaki[1,2]**. 1) RIKEN, CDB; 2) Department of Bioscience, Kwansei-Gakuin University; 3) Molecular and Cell Biology, University of California; 4) Department of Biology, Osaka City University.

Cell migration is an important process in animal development. The migration of gonadal distal tip cells (DTCs) offers an excellent model for migration of epithelial tubes in organogenesis. *mig-18* mutants cause meandering or wandering migration of DTCs during gonad formation and result in misshapen gonad arms. This phenotype is very similar to that observed in mutations in the *mig-17* gene encoding a secreted metalloprotease of the ADAMTS family. We cloned *mig-18* by injection rescue experiments using fosmid genomic clones. *mig-18* was found to corresponds to a predicted gene *F11F1.6*. The predicted protein encoded by *F11F1.6* is a novel protein only well conserved in nematode species, while it is likely to be a secreted protein because of the N-terminal signal peptide and the conserved cysteine motifs. The double mutants between *mig-17(null)* and *mig-18* mutants exhibited phenotypes similar to those in *mg-17(null)* single mutants, suggesting that *mig-18* and *mig-17* function in a common pathway. We examined the localization of MIG-17 in the gonadal basement membrane in *mig-18* mutants. MIG-17-Venus localized clearly as in the wild type background. Thus, MIG-18 is not required for gonadal localization of MIG-17. The *mig-18::Venus* fusion gene was expressed in the body wall muscle cells. MIG-18-Venus appeared to be secreted from the muscle cells and localize to the gonadal basement membrane, a tissue distribution reminiscent to that observed in MIG-17.

1047C

N-glycosylation regulates the activity of the *C. elegans* FGF receptor, EGL-15, *in vivo*. **Urszula M. Polanska**, Laurence Duchesne, Janet C. Harries, David G. Fernig, Tarja Kinnunen. School of Biological Sciences, University of Liverpool, Crown Street, L69 7ZB Liverpool, United Kingdom.

Fibroblast growth factor receptor (FGFR) signalling controls many cellular events during development and in adult homeostasis and therefore must be tightly regulated. Mutations that perturb normal FGFR function result in cancer and in developmental abnormalities such as skeletal and craniofacial malformations in Crouzon's syndrome and hypochondroplasia. The prevailing hypothesis for the pathogenic effects of these mutations is altered cell division and/or differentiation due to excess or constitutive activation of the receptor. In classical FGFR signalling the FGF receptor forms a ternary complex with the FGF ligand and a heparan sulphate proteoglycan (HSPG) co-receptor. The formation of the active signalling complex is regulated at various levels including tissue specific expression of FGFs/FGFRs and alternative splicing of the FGFRs. *N*-glycosylation has been suggested to regulate FGFR signalling as some of the receptor activating mutations in the human FGFRs abolish consensus *N*-glycosylation sites (Winterpacht et. al. 2000, Physiol Genomics 2: 9-12) and biochemical removal of *N*-glycans alters ligand and HS co-receptor binding *in vitro* (Duchesne et. al. 2006, J. Biol. Sci. 281: 27178-27189). We have used the evolutionarily conserved FGF signalling system of *C. elegans* to address the role of *N*-glycosylation for FGFR function *in vivo*. We show that endogenous *C. elegans* FGFR, EGL-15, is *N*-glycosylated *in vivo*. Genetic substitution of conserved consensus *N*-glycosylation sites leads to defects in the maintenance of fluid homeostasis (clear phenotype) and differentiation of sex muscles, both of which are phenotypes associated with excess EGL-15 signalling. Our results demonstrate that *N*-glycans negatively regulate FGFR signalling *in vivo* and support the hypothesis that mutations in *N*-glycosylation sites in human FGFR may lead to inappropriate activation of the receptor.

1048A

TMD-1 / Tropomodulin Regulates Intestinal Lumen Diameter in *C. elegans*. Vincent Cannataro, Malan Silva, Thomas Gallagher, **Elisabeth (Abbi) Cox**. Biology Department, SUNY College at Geneseo, Geneseo, NY.

Tubes are a central architectural feature of many human tissues including glands, the urogenital and gastrointestinal tracts, the respiratory tract and the vascular system. Despite their importance, much remains unknown about the molecular mechanisms involved in tube development. This work explores a new role for tropomodulins in regulation of endothelial tube formation. Tropomodulins are one of the few proteins known to regulate the slow growing ends of actin filaments. The *C. elegans tmd-1* gene encodes two transcripts (*tmd-1a* and *tmd-1b*) that have similar domain structure to mammalian tropomodulins. We have been examining the role of TMD-1 in development of the *C. elegans* intestine, which is a relatively simple, transparent tube that forms by a cord-hollowing mechanism similar to that used by some capillaries and renal tubules. Antibody staining using a rabbit anti-TMD-1 antibody demonstrates that TMD-1 localizes to the luminal surface of the *C. elegans* intestine as it undergoes morphogenesis. Interestingly, embryos homozygous for the *tmd-1(tm724)* allele, which contains a large deletion that affects both transcripts[1], exhibit areas of intestinal lumen diameter that are 65% larger than wild-type. *tmd-1(sf20)* homozygous mutants, which have a premature stop codon affecting both transcripts[2], also exhibit luminal expansions (50% larger than wild-type). Currently, investigations are underway to determine the molecular mechanisms by which TMD-1 regulates intestinal lumen diameter. Possibilities include modulation of actomyosin contractility, cooperation with the actin-spectrin cytoskeleton to provide mechanical strength to the luminal surface, and/or regulation of vesicle trafficking. This work is supported by National Institutes of Health grant R15HD059952. [1] Yamashiro et al. (2008) J. Cell Sci. 121: 3867-77. [2] Stevensen et al. (2007) J. Mol. Bio. 374: 936-50.

1049B

Cellular basis of Ram (RAy Morphology abnormal) phenotype in *C. elegans*. **Agnes K. Y. Hui**, King L. Chow. Biology, HKUST, Hong Kong, Hong Kong.

Organ morphogenesis is an important process during development. The male tail sensory rays of nematode *C. elegans* serve as a good model for the study of morphogenesis, particularly when many *ram* (RAy Morphology abnormal) mutants with lumpy rays are available. Based on their molecular natures, these ram genes can be classified into distinct groups according to the product features, e.g., *ram-1, ram-2, ram-4* encoding male tail-specific collagens; *ram-5* coding for ZP protein; and *mab-7* and *mab-29* encoding EGF-like domain containing proteins. However, the cellular basis of the sensory ray abnormality is not known. To define the cellular basis of the specific ray morphology, cellular, molecular biology and genetic techniques were employed to reveal biological events occurring inside these swollen rays in different *ram* mutants, *ram-2(bx76)*, *ram-2(bx32)*, *ram-5(bx81)* and *mab-7(e1599)*, each representing a subclass of the *ram* genes. Fluorescent subcellular organelle markers revealed expanded ER, mislocalized nuclei, altered nuclear structure, autophagosomes and lysosomes accumulation in the swollen rays in *ram-2(bx76)*, *ram-2(bx32)* and *mab-7(e1599)* mutant animals, while only expanded ER, mislocalized nuclei were observed in *ram-5(bx81)* mutants. The causal-relationship between the observed subcellular defects and the phenotype was tested. Modulation of autophagy level in *ram-2(bx76)*, *ram-2(bx32)* and *mab-7(e1599)* mutants showed differential effects on the ray swelling, suggesting different mechanisms are responsible for the manifestation of Ram phenotype and distinct roles of autophagy in shaping the tissue morphology. In addition, two of the three stress response pathways were found to contribute to the subcellular defects in *ram-2(bx32)*, but not other *ram* mutants. In summary, this study helps classify *ram* genes not by their molecular properties but by their differential biological impacts on subcellular events in the abnormal rays. (The research is funded by Research Grants Council, Hong Kong.).

1050C

Examining the roles of unc-53 and vab-8 in longitudinal migration. **Nancy Marcus**[1,2], Eve Stringham[1]. 1) Dept Biol, Trinity Western Univ, Langley, Canada; 2) Dept Molec Biol & Biochemistry, Simon Fraser Univ, Burnaby, Canada.

In C. elegans, unc-53/NAV-2 (uncoordinated-53/Neuron Navigator-2) is required for both the anterior and posterior migration of specific cells and processes (Stringham, et al., 2002). UNC-53 protein binds F-actin, the SH2SH3 adaptor protein SEM-5/GRB2, and the Abelson-kinase interactor abi-1, implicating UNC-53 in both signal transduction and actin cytoskeleton dynamics (Stringham, et al., 2002, Schmidt et al., 2009). The gene vab-8 (variable abnormal-8) is essential for most posteriorly directed migrations (Wightman et al., 1996). unc-53 and vab-8 mutants display defects within some of the same longitudinally migrating cells including the excretory canals, ALA, and PDE axons (Stringham et al., 2002; Wightman et al., 1996). Misexpression of VAB-8L in the ALM mechanosensory neuron resulted in the normally anteriorly directed axon to be rerouted posteriorly 44% of the time (Wolf et al., 1998). This rerouting was enhanced to 86% in unc-53(n152) mutants (Wolf, 1998). These results suggest that either: (1) UNC-53 negatively regulates VAB-8 or (2) UNC-53 and VAB-8 operate in separate pathways to control migration. The unc-53 allele n166 corresponds to a single base-pair substitution causing a premature stop codon in exon 19 that results in a truncated product. unc-53(n166); vab-8(e1017) mutants display an enhanced anterior and posterior canal extension defect compared to each single null allele, suggesting the genes act in separate pathways in the migration of the excretory canals. The anterior excretory canal outgrowth is severely disrupted or absent in unc-53(n166); vab-8(e1017) worms (truncated in 16% of animals, absent in 84%). The enhanced anterior defects suggest that there may be a role for vab-8 in anterior guidance not previously reported or there may be a secondary effect as a result of enhanced defects in a pioneering process in the head. Analysis of single and double mutants and RNAi studies directed at putative interactors of UNC-53 or VAB-8 revealed a possible role in excretory canal migration for unc-6, sax-3, slt-1, unc-71, and unc-73. unc-6/netrin RNAi resulted in enhanced posterior canal defects in unc-53(n166). The canal extension was further reduced by unc-6/netrin RNAi in unc-53(n166); vab-8(e1017) mutants. Since the receptor unc-40/DCC did not confer this phenotype, we conclude that unc-6/netrin may be working through another receptor such as SAX-3/ROBO, to promote posterior outgrowth of the excretory canals. unc-53(n166)/mIn1; sax-3 (ky123) animals showed truncation of the excretory canals, while sax-3 (ky123) alone exhibited WT canals. Homozygous double mutants showed severe truncation. Interestingly, slt-1(ev740) and slt-1(eh15) showed truncation as in vab-8(e1017) mutants.

1051A

A conditional *C. elegans* selection system for Rac targets in cancer. **Katherine H. Pedone**, Eldon C. Peters, Channing J. Der, David J. Reiner. Department of Pharmacology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

Rac family small GTPases regulate actin organization, cell cycle progression, vesicular trafficking and gene expression. Growing evidence indicates that cancer cells exploit Rac activity to drive tumor growth, invasion and metastasis. We have devised a novel selection scheme in *C. elegans* to identify critical components of Rac effector activity for possible pharmacologic targeting in cancer. This system is based on our finding that constitutively activated CED-10/Rac1 (Q61L missense mutation) in hypodermal cells drives lethal disruption of morphogenesis. We reasoned that second-site mutations that reduce CED-10 function also would decrease CED-10-dependent lethality, creating an unambiguous selection condition for genes required by CED-10/Rac1. Furthermore, titration of CED-10(Q61L) levels to a minimal activity threshold for 100% lethality would sensitize the system to modest drops in CED-10(Q61L) output. To meet these criteria, we developed a conditional expression system for modulation of CED-10(Q61L) levels. We drove hypodermal-specific cDNA expression with a portion of the *lin-26* promoter, and for conditional expression we used an aberrantly long 3'UTR sensitive to Smg system nonsense-mediated mRNA decay. Animals expressing CED-10(Q61L)::UTR^{NMD} in the temperature-sensitive *smg-1(cc546*ts*)* background were mostly viable at 15°C, but were inviable when grown at restrictive temperature (24°C). Incremental increases in temperature resulted in progressive severity of morphogenetic defects and lethality. Control animals exhibited a ten-fold increase in GFP expression when grown at 23°C relative to 15°C. We validated the selection system by introducing mutations in known CED-10/Rac1 effectors. p21-activated kinases (Paks) are the best-characterized Rac effectors in human cells, and we found that CED-10 signals primarily through MAX-2/Pak during distal tip cell migration. At screening temperature (23°C), loss of MAX-2 function suppressed CED-10(Q61L)-dependent lethality to 90%. Partial suppression by MAX-2 indicates that CED-10 uses additional effectors during hypodermal morphogenesis. Loss of the Rac effector PES-7/IQGAP restored low-level viability (0.6%), while no suppression was seen with loss of the Rac effector F46F6.2/PKN or the CED-10 effector UNC-115/AbLIM. A pilot F1 clonal screen of 200 genomes yielded two suppressor alleles that confer 15-20% survival at 23°C. We will study novel CED-10/Rac1 signaling components identified with our screen directly in human cancer cell lines to analyze potential involvement in Rac-dependent signaling and oncogenic growth transformation.

1052B

Lipocalin signaling controls luminal connectivity in the excretory system. **Craig E. Stone**[1], David Hall[2], Meera V. Sundaram[1]. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) *C. elegans* Center for Anatomy, Albert Einstein College of Medicine, Bronx, New York.

Lipocalins are a structurally related family of secreted proteins thought to function as carriers of small lipophilic cargos such as steroids. Lipocalin signaling may act in mammalian kidney tubulogenesis; its mechanism of action is unclear. We have shown that the *C. elegans* lipocalin-related protein LPR-1 is required for luminal connectivity between unicellular tubes in the excretory system. In *lpr-1* mutants, these tubes (the excretory duct and pore cell lumina) are not connected. As a result, the animals can no longer excrete fluid and die either during or before the first larval stage. Transgenic rescue experiments showed that LPR-1 can function cell non-autonomously. We hypothesize that LPR-1 transports or functions directly as a signaling molecule and targets the duct and/or pore cells. *lpr-1* provides a unique genetic model for studying how lipocalin signaling controls tubulogenesis.

Interestingly, we found that *lpr-1* mutant defects are suppressed by hyper-activation of the Ras/ERK signaling pathway. Specifically, *lpr-1* is suppressed by *sos-1(gf)*, *let-60(gf)* or *lin-1* null mutations. *lpr-1* is also suppressed by a heat-shock-induced LIN-3(EGF) transgene, but not by *lin-15* or *let-23(gf)* mutations. We are investigating several possible explanations for this genetic interaction, including that LPR-1 facilitates LIN-3 signaling, or that Ras/ERK signaling up-regulates the expression of other compensating lipocalin genes.

1053C

High throughput screen for genes interacting with *ten-1*. **Ulrike Topf**, Ruth Chiquet-Ehrismann. Friedrich Miescher Institute, Basel, Switzerland.

TEN-1 belongs to the teneurin protein family. Homologues were described in fly, zebra fish, chicken, mouse and man. All teneurin genes encode type II transmembrane proteins consisting of an intracellular domain, a single transmembrane domain and a large extracellular domain which is the most conserved part among the orthologs. Ten-1 is expressed throughout all developmental stages in *C. elegans*, predominantly in developing gonad and neuronal cells. Ten-1 deletion mutants show severe morphological defects causing lethality, sterility and worms bursting through the vulva. Approximately 40% of mutants develop to viable, fertile worms. By RNAi knock-down, *ten-1* was found to be important for germ cell development, epidermal morphogenesis, gonad migration and neuronal pathfinding (Drabikowski et al., 2005). We have recently shown that *ten-1* is important for basement membrane integrity. Genetic interactions of *ten-1* with basement membrane genes, *ina-1*, *dgn-1* and *epi-1* result in synthetic lethal phenotype suggesting that *ten-1* acts together with these genes (Trzebiatowska et al., 2008). However, the mechanism of Ten-1 action remains to be discovered. Interestingly, the intracellular domain of TEN-1 was shown to translocate to the nucleus (Drabikowski et al., 2005). Thus, TEN-1 is suggested to function as modulator of signaling events resulting in transcriptional regulation. Little is known, however, about the cleavage mechanism and possible target genes. To discover genes acting upstream or downstream of *ten-1* we performed a RNAi -based genome-wide screen. We used an RNAi feeding approach to compare the effect of *ten-1* knock down in wild type versus *ten-1* deletion animals. We identified 37 genes that may genetically interact with *ten-1*. Among them, depletion of 4 genes resulted in suppression of the *ten-1* mutant phenotype and knock down of 18 genes enhanced the *ten-1* phenotype. For 15 other genes only wild type animals were affected and no change of the *ten-1* phenotype could be observed. Genes required in neuronal or reproductive systems are enriched in the *ten-1* interaction screen. The genome-wide RNAi screen provides a basis for dissecting the *ten-1* function and placing it in genetic and developmental pathways. Confirmation of genetic interactions will be performed by crossing the *ten-1* mutant into genetic deletion mutants of the candidates identified.

1054A

Two very long chain fatty acid CoA synthetase genes, *acs-20* and *acs-22*, play roles in the surface barrier function in *C. elegans*. **Eriko Kage-Nakadai**[1,3], Hiroyuki Kobuna[1,3], Keiko Gengyo-Ando[1,3], Takao Inoue[2,3], Hiroyuki Arai[2,3], Shohei Mitani[1,3]. 1) Department of Physiology, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan; 2) Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan; 3) CREST, JST.

Very long chain fatty acid (VLCFA) is activated by VLCFA acyl-CoA synthetase (ACSVL) to be degraded or incorporated into a variety of lipids such as glycerolipids, phospholipids, and sphingolipids. In mammals, ACSVL family is composed of six members called fatty acid transport proteins (FATPs) that differ in their tissue expression patterns and physiological roles. In *C. elegans*, two genes *acs-20 (F28D1.9)* and *acs-22 (D1009.1)*, which are highly homologous to ACSVL family, are found in the genome. However, their activities and physiological roles are poorly understood, so far. In this study, we found that the knockout mutant of *acs-20* is defective in the cuticle barrier that prevents penetration of small molecules. *acs-22* mutant alone rarely exhibits the barrier defect, but *acs-20;acs-22* double mutant shows a severely disrupted barrier function. Moreover, the phenotypes of *acs-20* and *acs-20;acs-22* mutants are rescued by *acs-20*, *acs-22* or human *FATP4* transgenes. Finally, we show that the mutants of *acs-20* and *acs-22* show reduced incorporation of exogenous VLCFA into sphingomyelin in living worms. These results indicate that ACS-20 and ACS-22 are ACSVLs playing roles in the barrier function in *C.elegans*.

1055B

Analysis of zinc transporters that regulate zinc metabolism of C. elegans. **Hyun Cheol Roh**, Kerry Kornfeld. Developmental Biology, Washington University in St. Louis, St. Louis, MO.

Zinc is a trace element essential for many biological processes. As zinc is required for the function of a number of proteins and plays a role in signal transduction, zinc deficiency causes a broad range of defects. Excess zinc can also be deleterious because it causes zinc toxicity by replacing other metals required for protein functions. Thus, it is necessary for organisms to have mechanisms for zinc metabolism to achieve an optimal level of zinc. Zinc metabolism is mediated by various proteins including zinc transporters, zinc-binding proteins and zinc-sensing proteins. Zinc transporters, composed of two families, cation diffusion facilitator (CDF/SLC30) and Zrt-, Irt-like protein (ZIP/SLC39), control the distribution and the levels of zinc in cells by exporting and importing cytoplasmic zinc ions across membranes, respectively. Although many genes in zinc metabolism have been identified, the mechanisms of how the genes coordinate to regulate zinc homeostasis in whole organisms and how they are related to physiological functions are not well understood. We found that C. elegans has 14 putative CDF and 14 ZIP proteins by genome sequence analysis. We have been characterizing zinc transporters in zinc metabolism and physiology. We have examined the expression patterns of zinc transporters, the changes of expression in response to the changes in dietary zinc levels, and the functions of zinc transporters by using loss-of-function genetic approaches. We found that CDF-2 is expressed in the membrane of gut granules, specialized lysosomes in the intestine, and it is induced by zinc and other metal ion supplements. By contrast, CDF-1 and SUR-7, previously identified CDF proteins, do not respond to dietary zinc levels. cdf-2 (lf) mutants were sensitive to excess zinc, but in normal condition, these mutants were similar to N2 in life-span and sensitivity to other stresses such as heat shock and oxidative stress. Based on these results, we hypothesize that CDF-2 transports zinc ions into gut granules, which are the site for excess zinc storage. To address this hypothesis, we are trying to visualize zinc ions by using zinc-specific fluorescent dyes. We are also investigating the mechanism by which CDF-2 is induced by high levels of zinc. Previously, MDT-15, a mediator subunit, was reported to play a role in the transcriptional response of cdf-2. We found that knockdown of mdt-15 disrupts CDF-2 induction in response to high levels of zinc. Using the transgenic animals, we will search for new genes that are responsible for the induction of gene expression in response to high levels of zinc.

1056C

Isolation and Culture of Motile *C. elegans* Sex Myoblast Cells for High-resolution Microscopy. **Sihui Zhang**, Jeffrey Kuhn. Biological Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061.

Light microscopy of isolated cells moving on two-dimensional substrates has driven our understanding of the actin motility machinery. Although the amoeba *Dictyostelium discoideum* has been the primary model system for microscopy, its genome is highly redundant, complicating the dissection of the actin-based motility machinery. The nematode *Caenorhabditis elegans* has a more concise genome and offers a wealth of genetic tools, but single-cell observations have been limited to non-motile embryonic primary cells. Somatic cells from larval and adult worms, including the genetically well-characterized motile sex myoblast (SM) cells, have thus far been difficult to culture owing to the collagenase-resistant cuticle. We have successfully isolated somatic cells from L2 worms using a combination of collagenase treatment and RNA interference (RNAi) targeting of DUOX-1 that catalyzes collagen crosslinking. Cells from small-scale preparations adhere well acid-washed uncoated coverslips, or fibronectin- or collagen-coated coverslips. Cell can be cultured for at least 3 days, and have well defined microtubule networks, nuclei, and actin cytoskeletons including stress-fibers. Because the efficiency of this RNAi method was limited, we developed an alternative large-scale method to break L2 worms by random "chopping". Labeling of myoblast (M) lineage with hlh8::GFP-CAAX showed that M lineage cells, which included SM, remain intact and survive culture conditions. Taken together, our attempt to culture SM cells is promising. Using *C. elegans* as a model system of cell motility will bring the wealth of available RNAi and mutant libraries, targeted gene knockouts, *in vivo* imaging, biochemical tractability, and rapidity and ease of worm culture to bear on the dissection of the cell motility machinery in multicellular organisms.

1057A

ATP synthase subunits e and g regulate mitochondrial morphology and physiological properties of *C. elegans*. **Bum Ho Yoo**[1], Ji Young Mun[2], Tae Hoon Lee[1], Sung Sik Han[2], Hyeon-Sook Koo[1]. 1) Department of Biochemistry, College of Life Science & Biotechnology, Yonsei University, Seoul 120-749, Republic of Korea; 2) School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea.

Mitochondrial F_1F_0-ATP synthase uses the electrochemical potential across the inner membrane generated by the respiratory chain complex to synthesize ATP. Two subunits, e and g, are not essential for the enzyme function as an ATP synthase but are required for the formation of oligomeric forms of the enzyme. Mitochondria of yeast cells deficient in either subunit e or g were found to have onion-like cristae structure. To determine the function of these subunits in a multicellular organism, we measured phonotypes for knockout and knockdown strains of the homologs in *C. elegans*. There are two genes, *asg-1* and *asg-2*, that encode homologs of yeast ATP synthase subunit g and one gene *R04F11.2* (named *ase-1*) for ATP synthase subunit e. Homozygotes of *asg-1*(*tm3508*) survive to the adult stage but are sterile, while *asg-2*(*tm1472*) homozygotes show normal growth. The homozygotes of *ase-1*(*tm3308*) are embryonic lethal. After RNA*i* of either one of *asg-1*, *asg-2* and *ase-1* in the transgenic strains that express GFP in the mitochondria of hypodermal or muscle cells, disrupted overall morphologies of mitochondria were observed. Cryo-electron microscopy and electron tomography were used to determine the effects of these deficiencies on the ultrastructure of mitochondria in muscle cells. We observed circular cristae in *asg-1*(*tm3508*) mutants, parallel elongated sheets of cristae in *asg-2*(*tm1472*) mutants, and onion-like structure of cristae in *ase-1*(*RNAi*) strain. Either deficiency of subunit g1, g2, or e reduced the levels of ATP and reactive oxygen species, and survival of worms under oxidative stress. These results suggest that ATP synthase subunits e and g regulate the formation of mitochondrial cristae and affect physiological properties of worms.

1058B

The Structure-Function Relationship of LON-2/glypican: Negative Regulator of TGF-β signaling in *Caenorhabditis elegans*. **Suparna Bageshwar**, Tina Gumienny. Dept of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Staion, TX.

TGFβ superfamily signaling involves several ligands, receptors, and downstream signal transducers. Superfamily members (TGFβs) are small regulatory molecules that are secreted into the extracellular matrix prior to activating their receptors. Because TGFβ signaling is critical for development and homeostasis, a variety of defects, disorders, and diseases result from misregulated or altered TGFβ pathway signaling, including cardiovascular defects, Marfan syndrome, fibroses, and cancers. Deciphering how TGFβ signaling is regulated will provide not only a better understanding of how TGFβs are controlled, but may also provide targets for therapeutic intervention. We have shown in *C. elegans* that LON-2/glypican regulates DBL-1/TGFβ signaling and that this interaction is direct. Glypicans (GPCs) are heparan sulfate proteoglycans that are tethered to the extracellular surface of the cell membrane by a glycosylphosphatidylinositol (GPI) link. Besides the heparan sulfation sites and GPI linkage site, GPCs contain 14 conserved cysteine residues and a N-terminal secretion signal. While glypicans regulate signaling by other heparin-binding growth factors such as Hedgehog, FGF, and Wnt, their role in TGFβ signaling has been clearly implicated genetically in other systems but the molecular mechanism has not been established despite recent advances. A challenge for researchers now is to identify the precise molecular processes of glypican regulation of TGFβs. We have generated mutant LON-2 constructs and are using them to investigate how glypican interacts with TGFβ.

1059C

Studies with Functional, Fluorescently Tagged DBL-1/TGFβ. Kathy Beifuss, Robbie Schultz, Christi Parham, **Tina Gumienny**. Molecular & Cellular Medicine, Texas A&M HSC, College Station, TX.

In humans, Transforming Growth Factor beta superfamily (TGFβ) signaling regulates growth by controlling cell division, extracellular matrix deposition and remodeling, and apoptosis. Misregulated TGFβ signaling is evidenced in cancers, fibroses, and overgrowth syndromes. While the intracellular signaling pathway has been extensively dissected, extracellular TGFβ regulation is poorly understood. By elucidating TGFβ's extracellular regulators and their roles, we will reveal how their mutation or misregulation contributes to TGFβ-based pathologies and will identify easily accessible therapeutic targets for TGFβ-based diseases. We have generated a plasmid that contains a copy of the wild-type genomic *dbl-1*/TGFβ tagged with GFP, and have generated strains of *C. elegans* that carry extrachromosomal arrays with this plasmid. Western blotting with anti-GFP antibody confirms the expected tagging and processing of DBL-1. GFP fluorescence is visible in neurons and other cells, consistent with published expression patterns based on transcriptional fusions. We discovered that DBL-1:GFP is localized to puncta within nerve cord processes. Furthermore, animals transgenic for our DBL-1:GFP are longer than normal, showing that the GFP moiety is not interfering with the function of DBL-1 and that the pattern we see in animals is physiologically relevant. We have integrated lines carrying this transgene. We are testing candidates from the *C. elegans* ORFeome-based RNAi library to look for genes required for the expression, secretion, and localization of DBL-1/TGFβ, and will perform a non-biased screen once back-crossing is complete. In addition, we are generating *dbl-1* constructs with photoactivatable and photoswitchable fluorophores to conduct trafficking and protein stability experiments.

1060A

Studying Enhancers of the Nuclear Migration Defects of *unc-83* or *unc-84*. **Yu-Tai Chang**, Daniel Dranow, Dmitry Ratner, Daniel Starr. Molecular & Cellular Biology, University of California, Davis, Davis, CA.

Nuclear migration plays essential roles in mitosis, meiosis, cell migration, and establishment of polarity. In humans, defects in nuclear migration lead to the smooth brain disease, lissencephaly, which causes severe psychomotor retardation. We use P-cells in *C. elegans* to study nuclear migration. P-cell nuclei normally migrate from a lateral position to the ventral cord during L1. Failure of P-cell nuclear migration leads to P-cell death. This causes a loss of ventral neurons and vulva cells, which leads to Unc and Egl phenotypes. Interestingly, P-cell nuclear migration defect is temperature sensitive. P-cell nuclear migration is disrupted in *unc-83* or *unc-84* null animals at 25°C; however, at 15°C, P-cell nuclear migration occurs normally in *unc-83* or *unc-84* null animals. This suggests that an enhancer pathway functions redundantly with the *unc-83*/*unc-84* pathway. We therefore hypothesize that an uncharacterized pathway controlling P-cell nuclear migration is required at 15°C. In previous forward genetic screens, we have successfully isolated multiple "enhancer of the nuclear migration defect of *unc-83* or *unc-84* alleles" (*emu*) alleles at 15°C in *unc-84* null animals in the background of an *unc-84* rescuing array. The phenotypes of nine *emu* lines at 15°C, 20°C and 25°C have been characterized using an UNC-47::GFP, a GABA neuron marker to follow P-cell derived lineages. Compared to *unc-84* null animals, these *emu unc-84* animals have significantly less numbers of GABA neurons. Also, nearly no lateral-side GABA neuron has been observed in these *emu unc-84* animals, suggesting that *emu* pathway does not disrupt the more general P-cell migration. Four *emu* alleles have been backcrossed five times and consistently cause severe defects in P-cell nuclear migration at 15°C. Among these four alleles, three are recessive and one is dominant. The preliminary two-point mapping analyses suggest that these alleles might be located on chromosome II and X. We are currently further mapping *emu* alleles with the goal of eventually determining their molecular identity.

1061B

Control of body size by EGL-4 G-kinase. **S. So**, H. Okutsu, T. Tanaka, K. Miyahara, Y. Ohshima. Department of Applied Life Science, Sojo University, Kumamoto, Japan.

In animals such as *Drosophila* and mouse, growth hormone-insulin and TGFβ signal pathways are known to control body size and organ size (Conlon and Raff, 1999). In *C. elegans*, mutants in the TGFβ/DBL-1 pathway are all small indicating that this pathway is important in body size control (Patterson and Padgett, 2000). We previously isolated many big mutants, and showed that several of them are mutants in the *egl-4* gene encoding a cGMP-dependent protein kinase (G kinase). Genetic interaction studies suggest that EGL-4 inhibits activity of DBL-1/TGFβ pathway for body size control (Hirose et al. 2003). Although DBL-1 and SMA-2 could be substrates of EGL-4, both relation between EGL-4 and the TGFβ pathway and mechanisms of body size control by the TGFβ pathway remain poorly understood.

To determine these issues, we screened for proteins that bind to EGL-4 using yeast two hybrid system, and identified six proteins: Ras homolog (DRN-1), seven transmembrane protein (C52B9.4), CDP diglyceride synthetase ortholog (C33H5.18), collagen related protein (COL-167), transport protein Sec61α subunit homolog (Y57G11C.15) and an unknown protein (Y43F8C.11). *egl-4* gene is known to produce two alternatively spliced variants called PKGa and PKGb, which differ in the promoters and the first exon. We found that the above proteins specifically bind in yeast to the PKGa N-terminal region containing a glycine-rich domain encoded by the PKGa first exon, suggesting an important role of this domain to allow access by the binding proteins. We also found that mutants in the three binding proteins, *drn-1*(*ok400*), C52B9.4(*tm2431*) and *col-167*(*tm3691*) were slightly smaller than wild type, which suggest that these proteins play a role in body size control.

At present, we are trying to purify tagged EGL-4 synthesized in a cell-free protein synthesis system for substrate identification. We are also examining direct interaction in vitro between EGL-4 and the proteins found by the yeast two-hybrid screening. In the process of these works, when we grew worms on *E. coli* HB101, adult worms were found to be larger in volume than those fed on OP50 by about 50%. Although it was known that HB101 food speeds up the growth to adulthood (Shtonda and Avery 2006), our result shows that it also affects the maximum body volume. The worms fed on HB101 have enhanced body size difference depending on the genetic background, resulting in easier identification of body size phenotypes.

1062C

INX-18 and UNC-9 mediate electrical coupling of *C. elegans* body-wall muscle cells. **Qian Ge**[1], Bojun Chen[1], Zeynep F. Altun[2], Manish Rauthan[3], David H. Hall[2], X Z Shawn Xu[3], Zhao-Wen Wang[1]. 1) Department of Neuroscience, University of Connecticut Health Center, Farmington, CT; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI.

Two or more gap junction (GJ) proteins (connexins, pannexins, and innexins) are often coexpressed in the same cells. A poorly resolved question is how different GJ proteins contribute to electrical coupling of the same cells. Analyses of *C. elegans* body-wall muscle might shed light on this question because contributions of different GJ proteins may be evaluated by a combination of electrophysiological and genetic approaches. We previously showed that electrical coupling of the muscle cells is deficient but not absent in a null mutant of the innexin *unc-9*, suggesting that UNC-9 and at least one additional innexin play roles in the coupling. To identify the remaining innexin(s) contributing to the coupling, we analyzed expression patterns for the remaining 24 members of the worm innexin family by expressing promoter::GFP transcriptional fusions. This analysis led to the identification of five more innexins expressed in body-wall muscle cells, including INX-8, INX-9, INX-11, INX-14 and INX-18. Comparisons of junctional currents (I_j), which are through intercellular channels, between wild-type and mutant worms suggest that INX-8, INX-9 and INX-11 do not contribute to the coupling. INX-14 also did not appear to play a role in the coupling because it was not localized to intercellular junctions. In contrast, I_j was significantly reduced in inx-18 mutant and completely absent in *inx-18;unc-9* double mutant, suggesting that INX-18 is an important contributor to the coupling. Immunohistochemistry with worms expressing epitope-tagged INX-18 and UNC-9 in muscle cells showed that INX-18 colocalized with UNC-9 as puncta at intercellular junctions. Nevertheless, INX-18 and UNC-9 localizations were independent, as determined by analyzing localization of one innexin in mutant of the other. *inx-18* mutant exhibited a significant increase in the bending amplitude of locomotion. Because *inx-18* expression was not detected in any neurons important to locomotion, this mutant phenotype suggests that the function of INX-18 in muscle cells is important to generating the normal locomotion waveform. Collectively, our observations suggest that INX-18 and UNC-9 are the most important contributors to electrical coupling of body-wall muscle cells.

1063A

git-1, pix-1 and pak-1 control embryo elongation through induction of myosin light chain phosphorylation in Caenorhabditis elegans. **S. Harel**[1], A.Y. Lee[2], S. Jenna[1]. 1) Chemistry Department, PharmaQÀM, BioMed, Université du Québec à Montréal, Montréal, Québec, Canada; 2) McGill Center for Bioinformatics, School of Computer Sciences, McGill University, Montréal, Québec.

git-1, pix-1 and pak-1 are the orthologs of human GIT (G protein-coupled receptor kinase interactor), PIX (Pak1-interacting exchange factor) and PAK (p21 activated kinase). GIT1 is a GAP (GTPase-activating protein) for the Arf GTPases. PIX is a GEF (guanine-nucleotide exchange factor) for the Rho GTPases Rac and Cdc42 and PAK an effector of these later GTPases. These proteins form a complex shown to control morphology and migration of neuronal and non-neuronal cells in vertebrates mainly through regulation of membrane trafficking, focal adhesion dynamics and actin-myosin-mediated contraction. In C. elegans, GIT/PIX/PAK complex together with the integrins were shown to control cell shape and migration of distal tip cells during gonad morphogenesis. We demonstrate here that git-1, pix-1 and pak-1 control early elongation events during C. elegans embryonic development. Early elongation involves machineries controlling the phosphorylation of the myosin-light chain (MLC), mlc-4, and consequently the contraction of the actin-myosin contractile apparatus (AMCA). The Rho-binding kinase let-502 was shown to promote AMCA contraction through inhibition of the MLC phosphatase activity of mel-11 in lateral epidermal seam cells. let-502/mel-11 elongation pathway was shown to act redundantly with a unknown pathway involving a MLC kinase activity. Interestingly, this parallel pathway involves fem-2, a serine-threonine phosphatase whose closer homolog in mammals PP2c is known to control PAK activity downstream of PIX. Using loss-of function and dominant negative alleles of pix-1, git-1 and pak-1, RNAi treatment, specific chemical inhibitors of MLC phosphorylation regulators and 4D-imaging we showed that reduction or suppression of GIT/PIX/PAK function results to similar elongation defects than observed in let-502 loss-of-function mutants. We also showed that git-1, pix-1 and pak-1 are antagonizing MLC dephosphorylation during elongation. Preliminary data suggest that this function of GIT/PIX/PAK during elongation would involve integrins. We are currently investigating whether this novel pathway acts in parallel to mel-11 and let-502 and if it involves fem-2.

1064B

*mua-1*and *mua-2* are essential for robust epidermal-cuticle attachment. **John Plenefisch**, Sushant Khandekar, Kristen Williams, Stephanie Charles, Lillie Montgomery, Christine Swade. Department of Biological Sciences, The University of Toledo, Toledo, OH.

Hemidesmosome-intermediate filament complexes play an essential role in maintaining adhesion and transmitting force between tissues at sites of mechanical stress. They are prominently observed in the epidermis where muscle force is transmitted across this tissue to the cuticle. Mutations affecting proteins in these complexes have been previously shown to result in failure of the mechanical linkage between muscle and cuticle at varying stages of development. Mutations in *mua-1* and *mua-2* result in postembryonic epidermal-cuticle separation. *mua-1* encodes one of the three members of the Kruppel-like family (KLF) of transcription factors in *C. elegans*. In vertebrates, these proteins are involved in regulating tissue specific gene expression in a wide variety of developmental events, and we predict that MUA-1 is likely to be regulating attachments between epidermis and cuticle in response to developmental cues. *mua-1* mutations also result in prolapse of the uterus through the vulval opening suggesting MUA-1 may also regulate attachment of the uterus to the epidermal seam. A *mua-1*p::*gfp* reporter construct is expressed throughout development in the epidermis, excepting the seam, and at the L4-adult transition in the utse, uv1, uv2, and uv3. Preliminary results show nuclear localization of a MUA-1::GFP translation fusion in epidermal cells, consistent with its predicted regulatory role. We are currently testing whether other proteins expressed at the uterine seam are mis-expressed or their pattern disturbed in *mua-1* mutants.

Positional cloning mapped *mua-2* to a region of DNA covered by the YAC Y66D12. Initial observations suggest that *mua-2* encodes a novel single pass transmembrane receptor, based on weak transgene rescue and the finding of a frame-shift associated with *mua-2(rh174)* DNA. Furthermore, RNAi results in defects similar to those seen in mutant *mua-2* homozygotes, however a clear Mua (muscle detachment) phenotype in RNAi exposed worms was not observed. We are currently sequencing a second *mua-2* allele to confirm the predicted identity, and constructing a rescuing MUA-2::GFP translational fusion reporter to test the predicted epidermal expression. In addition, we identified a second gene in the *C. elegans* genome with extensive sequence similarity to the presumptive *mua-2* gene. We are testing through RNAi analysis in wild-type and in *mua-2* mutant animals whether the protein encoded by this second gene plays a role in cell attachment or interacts with MUA-2.

1065C

Reconstitution of gap junctions by coexpressing UNC-9 and UNC-1 in Neuro-2A cells. **Haiying Zhan**, Bojun Chen, Qian Ge, Xue-Jun Li, Zhao-Wen Wang. University of Connecticut Health Center, farmington, CT.

Gap junctions are intercellular channels that play very important roles in physiology and development. However, much remains to be learned about the regulation and molecular compositions of gap junctions. We use *C. elegans* as a model system to study modulation, functions, and compositions of gap junctions because major structural and functional properties are conserved between invertebrate and vertebrate gap junctions. Our recent study showed that gap junctions formed by the innexin UNC-9 in *C. elegans* body-wall muscle cells and neurons require the stomatin-like protein UNC-1 to function, which challenges the dogma that gap junctions function solely as stand-alone molecules. This study also showed that appending GFP to the carboxyl terminus of UNC-9 enabled the innexin to form functional gap junctions in the absence of UNC-1. These observations led us to hypothesize that UNC-1 interacts with the carboxyl terminal of UNC-9 to modulate gap junction gating. We have started to test this model by expressing UNC-9 and UNC-1 in heterologous expression systems. We first expressed these proteins in Neuro-2A cells and analyzed electrical coupling between neighboring transfected cells using the dual whole-cell voltage clamp technique. Electrical coupling was detected when UNC-9 was coexpressed with UNC-1 but not when UNC-9 was expressed alone. In contrast, INX-18, another innexin expressed in *C. elegans* body-wall muscle, was independent of UNC-1 in inducing electrical coupling of Neuro-2A cells. These observations showed that the modulation of UNC-9-based gap junctions by UNC-1 may be reconstituted in a heterologous expression system, which opens the door for using the heterologous expression system to test our model. The differential dependence of UNC-9 and INX-18 on UNC-1 makes it possible to construct chimeras between these two innexins to facilitate the identification of domains important to UNC-9 gating and regulation.

1066A

Cyclin B3 is necessary for the generation of functional microtubule-kinetochore attachments. **Gary Riefler**, Tokiko Furuta, Jill Schumacher. Dept of Genetics, MD Anderson Cancer Ctr, Houston, TX.

The function of the cell cycle is to generate two genomically identical daughter cells from one predecessor cell. Chromosome duplication and segregation are two complex cell cycle processes that must occur error-free so that chromosome ploidy is maintained. The master regulators of the cell cycle are cyclin-dependent kinases (Cdks) that influence the function of a myriad of proteins via phosphorylation. Experimental evidence accumulated over two decades has confirmed that mitotic Cdk1 is activated by A-, as well as B1- and B2-type cyclins. The cell cycle-dependent function of cyclin B3, however, is less well defined. Here, we show that *C. elegans* CYB-3 is essential for embryonic viability. Although the three other cyclin B isoforms (CYB-1, CYB-2.1, and CYB-2.2) are also required for proper chromosome segregation and embryonic viability, the mitotic abnormalities caused by the combined loss of these three B-type cyclins are distinct from *cyb-3(RNAi)* defects. The depletion of CYB-3 causes chromosome congression defects during prometaphase during the first mitotic division, as well as a block at metaphase that is mediated by the spindle assembly checkpoint. CYB-3 is required for appropriate architecture of the metaphase kinetochore and for the generation of tension between sister chromatid centromeres. These defects appear to be in part due to an alteration in the activity of AIR-2/Aurora B, a conserved and critical regulator of kinetochore-microtubule attachment. Altogether, these data suggest that CYB-3 has a surprising role in influencing kinetochore-microtubule interactions, turning off the spindle assembly checkpoint, and regulating Aurora B. Our future goals are to identify kinetochore targets of CYB-3 and to elucidate the molecular mechanisms by which CYB-3 influences mitotic progression.

1067B

Timing of the First Embryonic Cell Division in *C. elegans*. **Maria L. Begasse**, Anthony Hyman. Max Planck Institute of Cell Biology and Genetics, Dresden, Germany.

The order of events in the somatic cell cycle is thought to be regulated by cyclin dependent kinases while its speed is controlled by metabolism and checkpoint signals. But in *C. elegans* embryos there is little evidence for any checkpoints and the phenotypic changes in an embryonic cell are more complex than in somatic cells. Still, the first mitotic divisions of the *C. elegans* embryo are very consistent in the succession of events and the progression speed at any given temperature. The outstanding problem is how cells choreograph a sequence of morphological changes that ensure proper development while being responsive to a change in their environment.

As ectotherms, nematodes can not produce body heat, leaving them directly affected by the ambient temperature. We found that up to the upper temperature limit of embryonic proliferation at 25°C, the sequence of events is not perturbed although timing is faster with increasing temperature. Rather than studying the molecular details of one specific phenomenon, we take a systems approach to investigate the phenotypic transitions that cells undergo during the cell cycle. As opposed to conventional forward and reverse genetic techniques which induce phenotypes through limiting components, we adjust temperature to globally vary kinetic barriers and observe the effect on the whole system.

These approaches are combined to systematically interrogate the timing of the early embryonic cell cycle using a synthesis of imaging, RNAi and cross-species comparison among related nematodes. We investigate the role of metabolic components and key cell cycle regulators with the aim to identify the link between metabolism and cell cycle regulation.

1068C

The mechanisms of the microtubule-depolymerizing kinesin in regulating MT dynamics in the early *C. elegans* embryos. **X Han**, M Srayko. Biological Sciences, Univ of Alberta, Edmonton, AB, Canada.

Microtubules (MTs) are one of the major cytoskeletal components and they are essential for fundamental cellular processes including mitosis, cytokinesis, and vesicle transport. The temporal and spatial regulation of MT dynamics is key to our understanding of their cellular functions. I am specifically interested in how MT growth is controlled at centrosomes, the epicenters for MT outgrowth in the cell.

Recently, the protein KLP-7 (kinesin-like protein) was implicated in regulating MT outgrowth at the centrosome (Srayko, *et al*., 2005, Schlaitz *et al*., 2007). KLP-7 is a member of MT-depolymerizing proteins. When the KLP-7 protein is removed, twice the number of MTs grow out of the centrosome. Work from the vertebrate homologues suggests that KLP-7 is turned off via phosphorylation by Aurora A kinase (Zhang X. *et al*., 2008), and there is indirect evidence in *C. elegans* to support a mechanism whereby KLP-7 is negatively regulated via the Aurora A kinase (Schlaitz *et al*., 2007). The KLP-7 protein has 13 identifiable Aurora kinase sites, in regions similar to the vertebrate homologues. A major focus of my work is to determine how phosphorylation/dephosphorylation of KLP-7's Aurora kinase sites affects its intracellular location and/or function with respect to MT outgrowth at the centrosome.

We are currently performing a structure-function analysis to determine which Aurora sites are required for KLP-7's intracellular location and its depolymerase activity at the centrosome. Results obtained thus far indicate that mutating one of the C-terminal Aurora sites from serine to glutamic acid (to mimic constitutive phosphorylation) interferes with KLP-7 localization at the centrosomes. Experiments are underway to measure MT outgrowth in embryos expressing different phosphorylated forms of KLP-7. In parallel, we are pursuing the identification and characterization of putative KLP-7-interacting proteins through co-immunoprecipitations and yeast 2-hybrid.

1069A

A genetic screen for suppressors of *rsa-1*. **Karen Lange**[1], Meredith Price[2], Bruce Bowerman[2], Martin Srayko[1]. 1) University of Alberta, Edmonton, Alberta, Canada; 2) Institute of Molecular Biology, University of Oregon, Eugene, OR, USA.

In mitotic cells, the centrosomes are the primary sites of microtubule nucleation and they are essential for mitotic spindle assembly in most animal cells. We are studying a cellular pathway that regulates centrosome-based microtubule outgrowth.

In *C. elegans* the RSA heterotrimeric phosphatase complex (regulator of spindle assembly), is required for robust microtubule outgrowth from the centrosome[1]. RSA-1 is a regulatory subunit that interacts with a PP2A catalytic subunit and a structural subunit termed RSA-2[1]. Embryos depleted of RSA-1 exhibit a severe reduction in microtubule outgrowth from centrosomes and a spindle-collapse phenotype whereby the centrosomes move toward the chromosome mass during spindle assembly. Based on previous immunostaining results, the centrosomes in *rsa-1* mutants appear to have all major components and they are otherwise competent to support microtubule outgrowth. For example, double-depletion of RSA-1 and a microtubule-depolymerizing kinesin KLP-7 rescues the microtubule outgrowth phenotype (ref. 1 and see poster by Han and Srayko). Therefore, RSA-1 could increase microtubule outgrowth by attenuating KLP-7 activity.

In order to find downstream targets or upstream regulators of RSA, we are performing genetic screens for suppressors of *rsa-1(or598)*. *rsa-1(or598ts)* was discovered in a temperature-sensitive maternal-effect lethal screen. The mutant embryos exhibited a spindle assembly defect similar to previously identified *rsa-1* alleles. Furthermore, *or598ts* failed to complement *rsa-1(dd13)*, and sequencing revealed a missense alteration in the coding sequence, D319G. Progeny from *or598/or598* adults exhibit 97% lethality at 25°C but only 20% at 15°C, making this allele ideal for our suppressor screens. *or598* homozygotes are also sterile when shifted to the restrictive temperature as L3s or earlier. This suggests that RSA could also function in the germline; previous work with *rsa-1* used non-conditional alleles and RNAi, which result in embryonic lethality and thus mask the sterility.

To date, we have screened an estimated 10,000 mutagenized haploid genomes. From this initial screen, two candidate suppressors have been identified. Characterization and mapping of the suppressors is in progress.

1. Schlaitz, A.L., *et al*. (2007). *Cell* **128**, 115-127.

1070B

Analysis of DNA replication in *C. elegans* early embryos. **Rémi Sonneville**, Julian Blow, Anton Gartner. Wellcome Trust Centre for Gene Regulation & Expression, Dundee university, UK.

During each cell cycle, DNA must be entirely replicated once, and only once, to ensure genome stability. In eukaryotes, ORC complexes bind DNA at replication origins. At the end of mitosis and early G1 phase, the two licensing factors CDC-6 and CDT-1 recruit the replicative helicase, MCM-2 to MCM-7 complexes (MCMs), to origins. When CDK activity rises, in S phase, MCMs bound by GINS complexes and CDC-45 are activated, unwind DNA and allow single strand binding proteins to recruit the replication machinery at replication forks. Such a step wise regulation ensures that newly synthesised DNA is not re-replicated within the same cell cycle. Furthermore, in order to fully replicate all DNA, replication forks must be distributed uniformly along DNA. The mechanism ensuring that origins are evenly distributed on DNA is not understood yet.

We use time lapse video microscopy to characterise the regulation of DNA replication in *C. elegans* early embryos. We generated transgenic worms expressing GFP-tagged ORC, CDC-6, MCMs, GINS, CDC-45, RPA, and a Primase subunit. We determined their distributions during oocyte meiotic divisions and the first embryonic cell cycle. We found that DNA becomes licensed by binding MCMs from the anaphase of meiosis II and the metaphase of mitosis. RNAi knockdown of *orc-5*, *cdc-6* or *cdt-1* reduces MCMs loading on DNA. As expected, GFP-ORC and GFP-CDC-6 are already bound to DNA at these stages. Interestingly, GFP-ORC and GFP-CDC-6 become excluded from nuclei when nuclear envelope is formed, which may be an important mechanism to prevent licensing during S phase and thus, preventing re-replication of newly synthesized DNA. In addition, we found that when licensing is blocked a “second wave” of ORC binding to DNA occurs during anaphase of meiosis II and mitosis. We speculate that this could be an important mechanism in wild type cells to uniformly distribute origins and replication forks on DNA. Downstream of licensing, we found that all reporters but one appear imported in nuclei, preventing us to determine in live their binding to DNA. In contrast, GFP-CDC-45 is excluded from nuclei in cells that do not replicate DNA (maturating oocytes and early embryos with condensed chromosomes). GFP-CDC-45 localizes to nuclei shortly after exit from mitosis when chromosomes are decondensed and thus, it appears to be an S phase marker. Our ongoing analysis of DNA replication is compatible with models derived from others species and also provides new insights about DNA replication that could be specific to *C. elegans* early embryos (ORC exclusion from nuclei) or may be fundamental mechanisms (a “second wave” of ORC binding unlicensed DNA).

1071C

Analysis of a spindle assembly mutant in *C. elegans*. **Danielle R. Hamill**[1], Marie McNeeley[1], Molly Everett[1], Amy Gearica[1], Sahar Mazhar[1], Meredith Price[2], Bruce Bowerman[2]. 1) Zoology Department, Ohio Wesleyan University, Delaware, OH 43015; 2) Institute of Molecular Biology, University of Oregon, Eugene, OR 97403.

Cell division is a highly conserved and complex process that must be carefully orchestrated. *or452ts* was isolated in a screen for temperature sensitive embryonic lethal cell division mutants. In *or452ts* mutant embryos, bipolar spindles often fail to form. In these mutants DNA localizes around microtubules emanating from a single, often misshapen, centrosome. Staining with centriole markers suggests that a centriole duplication defect may underlie the spindle assembly defect. Using live cell imaging and fluorescence transgenes (including GFP::gamma tubulin), we have noticed centrosome streaking and splitting that is not seen in wild-type embryos. We also have observed the formation of asymmetric spindles. Towards the goal of identifying the gene that is mutated in *or452ts*, we have done meiotic mapping using phenotypic markers and snip-SNPs. We have narrowed the genetic location down to about 0.5 cM around +2.5 on LGIII. This region contains less than 500 kbp of DNA, and there are about 40 predicted genes in this region. In collaboration with Doug Turnbull and Eric Johnson (University of Oregon), we are sequencing the DNA from this region to try to identify the mutation responsible for the *or452ts* mutant phenotype. We believe that characterization of this mutant, and identification of the gene responsible, will provide new insights into the process of mitotic spindle assembly and cell division.

1072A

Cell-size-dependent spindle elongation in the *Caenorhabditis elegans* early embryo. **Yuki Hara**[1,2], Akatsuki Kimura[1,2]. 1) Cell Architecture Laboratory, National Institute of Genetics; 2) The Graduate University for Advanced Studies (SOKENDAI), Mishima, Sizuoka, Japan.

Cell division occurs in cells of various sizes. For appropriate chromosome segregation during anaphase, the mitotic spindle must elongate in a cell-size-dependent manner. Here, we quantified the relationship between spindle elongation and cell size in the *Caenorhabditis elegans* early embryo and propose possible models for cell-size-dependent spindle elongation.

First, we measured the dynamics of spindle elongation quantitatively in cells with various sizes. We found that the extent and speed of spindle elongation are proportional to the cell size. To explore the mechanism underlying the cell-size-dependent spindle elongation, we investigated the role of Gα activity-sensitive force pulling astral microtubules toward the cortex by using RNAi knockdown of *gpr-1/2* (G protein regulator). In the *gpr-1/2* (RNAi) embryos, the spindles failed to fully elongate in cells with various sizes and the speed of spindle elongation was almost constant regardless of the cell size. The results indicated that the Gα activity-sensitive cortical pulling force is involved in the cell-size-dependent spindle elongation. In addition, this quantification demonstrated that Gα activity-insensitive force contributes to the spindle elongation as well.

Next, to obtain insights into the mechanisms of Gα activity-sensitive and -insensitive force, we evaluated possible models through numerical modeling and experiments. A force generator-limited model, which is based on a previous estimation of the number of Gα activity-sensitive force generators [1], reproduced characteristics of the Gα activity-sensitive mechanism. A constant pulling model, which assumes saturated number of weak force generators at the cortex, reproduced the characteristics of the Gα activity-insensitive mechanism. When we performed a simulation using these two models together, the in vivo cell-size-dependent spindle elongation behavior could be explained.

Thus, we propose that these two models act in concert to elongate the mitotic spindle in a cell-size-dependent manner. At this meeting, we will present our recent analyses on the molecular bases and theoretical features of the models.

[1] Grill, S.W., Howard, J., Schaffer, E., Stelzer, E.H., and Hyman, A.A. (2003) *Science*, 301, 518-521.

1073B

Levels of the ubiquitin ligase substrate adaptor MEL-26 are inversely correlated with MEI-1/katanin microtubule severing activity during both meiosis and mitosis. **Jacque-Lynne F. Johnson**[1,3], Chenggang Lu[1,4], Eko Raharjo[1], Karen McNally[2], Francis J. McNally[2], Paul E. Mains[1]. 1) Dept of Biochemistry and Molecular Biology, University of Calgary, Canada; 2) Section of Molecular and Cellular Biology, University of California, Davis, USA; 3) Dept of Molecular Biology and Biochemistry, Simon Fraser University, Canada; 4) Dept of Developmental Biology, Stanford University School of Medicine, USA.

The MEI-1/MEI-2 katanin microtubule-severing complex is required for meiotic spindle formation in *C. elegans*. While essential for meiosis, MEI-1/MEI-2 must be quickly downregulated to prevent interference with formation of the first mitotic spindle. Post-meiotic inactivation of MEI-1 is accomplished by two independent protein degradation pathways. One pathway requires MEL-26 as a substrate-specific adaptor for the CUL-3 based E3 ubiquitin ligase, while a parallel pathway requires phosphorylation of MEI-1 by MBK-2 kinase. MBK-2 mediated degradation of MEI-1 appears to be coupled with meiotic exit after which MEL-26 completes the degradation process. A phosphorylation system involving Protein Phosphatase 4 also acts independently of these degradation systems to regulate meiotic and mitotic MEI-1 activity (Han et al, 2009). The parallel MEL-26 and MBK-2 systems explain how MEI-1 microtubule-severing activity is degraded during mitosis, but how is MEI-1 degradation delayed until its essential meiotic function is complete. Stitzel et al (2006) showed that MBK-2 activity is held in check by EGG-3 until meiosis is completed. Our work reveals that the MEL-26 branch of the MEI-1 degradation pathway is regulated in part by MEL-26 protein accumulation. Affinity-purified antibodies revealed that MEL-26 is present at low (but nonzero) levels until meiosis is complete, after which MEL-26 levels increase substantially, paralleling the increase in post-meiotic MEI-1 degradation. During meiosis, MEL-26 levels are kept low by another type of ubiquitin ligase containing CUL-2, and by an upstream activator of ubiquitin ligase activity, RFL-1. Thus, a cascade of proteolysis, triggered at fertilization, results in the correct timing of MEI-1 activity. However, premature meiotic activity of either MEL-26 or MBK-2 (independently or together) is not sufficient to block spindle formation, indicating that additional factors are rate limiting for the downregulation of MEI-1. While it was initially thought that MEL-26 did not play a role during meiosis, we find the low levels of meiotic MEL-26 have a subtle function, acting to moderate MEI-1 activity in the meiotic spindle. In addition, our results demonstrate that while RFL-1 has additional essential targets, MEI-1 is the only essential target for MEL-26 and possibly for the E3 ubiquitin ligase CUL-3.

1074C

Quantification and theoretical analyses of cell shape predict novel roles of cell surface and contractile ring in cytokinesis. **Hiroshi Koyama**, Akatsuki Kimura. Cell Architecture Lab., National Institute of Genetics, Mishima-shi, Shizuoka-ken, Japan.

Cytokinesis is a mechanical process mediated by the interaction between contractile ring and cell surface mechanics. Conventionally, contractile ring generates contraction force to overcome the resistive force supplied from cell surface mechanics, resulting in furrow ingression. Actin-myosin II network is responsible for the force generation of contractile ring. In contrast, the precise contribution of cell surface mechanics on furrow ingression is not well understood. Here, to obtain insight into the role of cell surface mechanics, we employed theoretical approaches combined with quantitative evaluation of cell shape. In our theoretical model, cell surface mechanics was based on bending elasticity similar to liposome. We examined whether the cell shape observed in *C. elegans* 1 cell stage embryos could be reproduced by this model. We systematically searched the spatial distribution of bending modulus which can reproduce the real cell shape during furrow ingression. Actually, we found such spatial distribution of bending modulus. Surprisingly, the reproduction of the cell shape did not require the contraction force conferred by contractile ring. These results suggest that cell surface mechanics can function to facilitate ingression, which is contrast to the conventional notion that cell surface mechanics provides the resistive force against ingression. We also analyzed the role of contractile ring using our theoretical model, which will be discussed in the meeting. In conclusion, we propose a novel role of cell surface mechanics. Furthermore, our spatial estimation of bending modulus can be useful to predict the status of cytoskeleton and its regulation.

1075A

Aster separation is essential to couple furrow formation to anaphase onset and limit furrowing to a single site. **Lindsay Lewellyn**[1,2], Arshad Desai[1,2], Karen Oegema[1,2]. 1) Cellular and Molecular Medicine, University of California, San Diego, La Jolla, CA; 2) Ludwig Institute for Cancer Research.

Cytokinesis is the final step of mitosis that physically divides a single cell into two daughter cells following chromosome segregation. Signaling from the centrosomal asters and the spindle midzone are coordinately required to promote furrow formation, but the relative contributions of these two signals is not known. Here, we explore the importance of aster separation in promoting furrow formation and ingression, as well as the relationship between aster and midzone-based signaling. In order to test the role of aster separation in cytokinesis, we use depletion of TPXL-1, which shortens the metaphase spindle, thereby delaying aster separation following anaphase onset. This effect that can be reversed by co-inhibition of kinetochore assembly. Using this controlled system, we show that delaying aster separation leads to a similar delay in furrow formation without affecting the subsequent rate of furrow ingression. In contrast to control embryos, when aster separation is delayed, two midzone based signaling complexes–Centralspindlin and the Chromosomal Passenger Complex–are essential for furrow formation. Remarkably, preventing aster separation, by simultaneously inhibiting TPXL-1 and cortical pulling forces on the asters, leads to the coincident formation of multiple furrows. Thus, aster separation is essential to: (1) couple furrow formation to anaphase onset and, (2) limit furrow formation to a unique site.

1076B

A genetic network controlling DNA damage tolerance in early embryos. **Matthew Michael**, Seung-Hwan Kim. Dept Molecular & Cell Biol, Harvard Univ, Cambridge, MA.

Cell cycle progression during early embryogenesis in *C. elegans* is remarkably resistant to DNA damage-imposed delays. Previous studies have shown that even when chromosomes are heavily damaged, the time required to complete DNA replication is not extended, and cell cycle checkpoint pathways are not hyper-stimulated in early embryos. This is by contrast to proliferating cells in the mitotic zone of hermaphrodite gonad, which exhibit checkpoint-dependent cell cycle arrest in response to DNA damage. Our laboratory has defined a genetic network that allows early embryos to tolerate DNA damage, and we have begun to unravel the molecular mechanisms involved. We will show that damage tolerance is controlled by at least two pathways. The first, which requires the *gei-17*, *polh-1*, and *mus-101* genes, functions to promote the efficient replication of damaged chromosomes. The second pathway, which requires *smk-1*/*rad-2* and *pph-4.1*, functions to suppress activation of the *atl-1*/*chk-1* checkpoint by DNA damage. The combined activities of these two pathways results in robust replication despite damage, and an inability of stalled replication forks to trigger checkpoint-dependent delays in cell cycle progression. Previous studies have shown that cell cycle delay during early embryogenesis is incompatible with development, and thus our work explains how the cell cycle timing program in early embryos is protected during a DNA damage response. Recent results on the molecular mechanisms controlling the function of this damage tolerance network will be presented.

1077C

SZY-5, a novel regulator of centrosome duplication, may control centrosomal protein levels post-transcriptionally. **Nicholas B. Miliaras**, Kevin F. O'Connell. Laboratory of Biochemistry & Genetics, NIDDK/NIH, Bethesda, MD.

The centrosome is the primary microtubule-organizing center (MTOC) of most eukaryotic cells and plays an essential role in cell polarity, protein trafficking and the formation of a bipolar mitotic spindle. A pair of centrioles forms the structural core of the centrosome. The centriole pair must duplicate once and only once per cell cycle to ensure a bipolar mitotic spindle and proper chromosome segregation. Many human cancer cells have excess centrosomes, which presumably exacerbates the chromosomal aberrations that occur in tumors. Thus, a better understanding of the centrosome and how it duplicates could help to shed light on the mechanisms of how cells become cancerous. The kinase ZYG-1 is essential for centrosome duplication in C.elegans and functions by recruiting a series of proteins to assemble a new centriole adjacent to the parent one. However, in embryos that lack ZYG-1 activity, this fails to occur, resulting in the assembly of monopolar spindles at the two-cell stage and embryonic lethality. To understand how ZYG-1 activity is regulated, I have been studying the szy-5 (suppressor of zyg-1) gene. The szy-5*Italic Text*(bs7*Italic Text*) mutation rescues the lethality and spindle defect observed in zyg-1*Italic Text*(it25*Italic Text*) embryos. Interestingly, the szy-5*Italic Text*(bs7*Italic Text*) mutation also causes a temperature-sensitive embryonic lethal phenotype of its own. These embryos display cell division defects such as a delayed cell cycle and mis-positioning of the mitotic spindle. Also, novel aggregates frequently form within the cytoplasm of szy-5*Italic Text*(bs7*Italic Text*) embryos. We have found that the szy-5*Italic Text* gene encodes a zinc finger protein, and exhibits cytoplasmic and nuclear localization. An in*Italic Text* vitro*Italic Text* assay suggests that SZY-5 can bind RNA. Quantitative, real-time PCR analysis does not show any significant differences between wild-type and the szy-5*Italic Text*(bs7*Italic Text*) mutant in the levels of mRNAs that encode centrosomal proteins. However, in contrast to the wild type, szy-5*Italic Text*(bs7*Italic Text*) embryos exhibit increased levels of GFP-gγ-tubulin at the centrosome. Preliminary results suggest that the levels of the SPD-2 protein may also be increased at centrosome in szy-5*Italic Text*(bs7*Italic Text*) embryos. Our results suggest a model in which SZY-5 functions post-transcriptionally to regulate the level of proteins at the centrosome.

1078A

Chondroitin Proteoglycans are Required for *C. elegans* Eggshell Assembly. **Sara K. Olson**[1], Thomas Müller-Reichert[2], Karen Oegema[1]. 1) Ludwig Institute for Cancer Research, UC San Diego, La Jolla, CA; 2) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

The nematode eggshell is a rigid, impermeable structure that protects the embryo during early development, and is required for the first embryonic cell division. The essential function of the eggshell makes it an attractive drug target to combat parasitic nematode infection, but little is known about the composition of the eggshell or the process by which it forms. We have identified two genes redundantly required for eggshell formation. *cpg-1/cej-1* and *cpg-2* encode chondroitin proteoglycans, a class of extracellular glycoproteins modified with chondroitin sugar chains. Live imaging of fluorescent strains shows that CPG-1 and CPG-2 are secreted from caveolin-enriched cortical granules during meiosis I, while immuno-electron microscopy experiments show they associate with the inner layer (the permeability barrier) of the eggshell. To better understand the function of chondroitin proteoglycans in eggshell assembly, we depleted CPG-1, CPG-2, and SQV-5 (the enzyme that synthesizes chondroitin) by RNAi. In utero, embryos co-depleted of CPG-1/CPG-2 or chondroitin have identical phenotypes, exhibiting defects in osmotic integrity and early cortical events dependent on the actomyosin contractile network, including polar body extrusion during meiosis I and II, membrane ruffling, pseudocleavage formation, polarity establishment, and cytokinesis. However, when osmotic support is provided to dissected embryos, early embryonic events (including cytokinesis and polarity establishment) are robustly rescued in chondroitin depletions, but only partially rescued in CPG-1/CPG-2 co-depletions. Ultra-structural analysis shows embryos depleted of chondroitin are able to form all three eggshell layers, while embryos co-depleted of CPG-1/CPG-2 fail to form the inner layer. The correlation of structural and functional data suggests that CPG protein cores have a function separable from that of the chondroitin chains during eggshell assembly, and provides evidence for the first structural proteins required for eggshell formation. To expand on this work, we are conducting an RNAi-based screen to identify additional genes required for the enigmatic process of eggshell assembly.

1079B

EMB-1 is a novel protein involved in the metaphase-to-anaphase transition. Diane Shakes[1], **Andy Golden**[2]. 1) Dept. of Biology, College of William and Mary, Williamsburg, VA; 2) Laboratory of Biochemistry and Genetics, NIDDK, NIH, Bethesda, MD.

The Anaphase Promoting Complex (APC) is a multi-subunit E3 ubiquitin ligase that promotes the metaphase-to-anaphase transition during meiotic and mitotic divisions. Temperature-sensitive (ts) mutants in mat-1, mat-2, mat-3, emb-27, and emb-30 arrest as 1-cell embryos, stuck in metaphase of meiosis I. These five genes code for five subunits of the APC. The ts alleles of emb-1 have grabbed our attention because their arrest phenotype is indistinguishable from the APC mutants. Furthermore, genetic doubles constructed between emb-1(hc62) and the APC mutants cannot be maintained at the permissive temperature, a common feature of any APC double mutant. Additionally, suppressors that suppress the APC mutants (Stein et al., 2007) also suppress emb-1. What is EMB-1 you may ask? We mapped emb-1 to a tiny interval on LG III and used RNAi to phenocopy the 1-cell arrest phenotype. Rescue and sequencing confirmed that emb-1 codes for a novel protein with no known homologies outside of Caenorhabditis species. Localization studies are underway. We propose that EMB-1 is a novel subunit or regulator of the APC in C. elegans.

1080C

Analysis of the role of $CRL2^{ZYG-11}$ in *C. elegans* meiosis. **C.S. Heighington**, S. Vasudevan, E.T. Kipreos. University of Georgia, Athens, GA.

Chromosome segregation is essential for organismal viability. A cullin-RING finger ubiquitin ligase complex, $CRL2^{ZYG-11}$, is required for the meiotic metaphase II-to-anaphase II transition in *C. elegans*. Additionally, *zyg-11* mutants have defects in embryonic anterior-posterior polarity, chromosome condensation, cytoplasmic organization, and the turnover of maternally-provided cyclin B1. In an attempt to understand these complex phenotypes we have isolated genetic suppressors of a temperature sensitive *zyg-11* mutant. We are currently positionally cloning the *zyg-11* suppressors using SNP mapping, and will present our findings at the meeting. We anticipate that cloning of the suppressors will identify key components in ZYG-11-mediated developmental pathways or identify critical ZYG-11 substrates.

1081A

Characterization of a Novel Mutation Activating the Spindle Assembly Checkpoint in *C. elegans* . **Alexandra Bezler**[1], Ralf Schnabel[2], Pierre Gönczy[1]. 1) School of Life Sciences, Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland; 2) TU Braunschweig, Germany.

Correct bipolar attachment of sister chromatids is crucial for faithful segregation of the genetic material in mitosis. This process is regulated by the spindle assembly checkpoint (SAC), which inhibits the anaphase promoting complex/cyclosome (APC/C) until proper attachment is achieved. We are characterizing a mutant strain, tentatively named *emb-86(t1565)*, in which embryos exhibit variable spindle defects (multipolar or anastral monopolar) and altered M phase duration. We found that *emb-86(t1565)* mutant sperm contains aberrant centriole numbers and DNA content, likely explaining the observed spindle defects in the resulting embryos. Moreover, we observed that anteroposterior axis specification is defective in *emb-86(t1565)* mutant embryos fertilized by acentrosomal sperm, as anticipated from previous work. Importantly in addition, we found that M phase duration is approximately 5-fold longer in *emb-86(t1565)* mutant embryos with anastral monopolar mitosis than in the wild-type. By contrast, incorrect chromosome attachment in the wild-type causes only a 2-fold delay in M phase. We established that the 5-fold delay in *emb-86(t1565)* is of maternal origin and caused by SAC engagement, since inactivation of MDF-1 or MDF-2 by RNAi rescues proper M phase duration in *emb-86(t1565)* mutant embryos. We mapped the *emb-86(t1565)* locus to a region on the right arm of LGIII comprising 21 annotated genes, including *such-1*, an APC5 related subunit. Previous work has shown that *such-1(h1960)* mutant embryos show a slight M phase delay. Here, we established that monopolar anastral mitosis in *such-1(h1960)* embryos leads to a 5-fold increase in M phase duration, as in *emb-86(t1565)* mutants. Moreover, *emb-86(t1565)* fails to complement *such-1(h1960)* under conditions where the SAC is activated, suggesting that *emb-86(t1565)* is a novel allele of *such-1*. Taken together, compromising the APC/C may cause a SAC dependent M phase delay, in addition to the previously described SAC independent delay. Our unexpected findings raise the possibility that the APC/C may act as negative regulator of the SAC.

1082B

Functional characterization of a novel cullin-based E3-ligase involved in cell cycle progression in *C. elegans*. **Julien Burger**, Jorge Merlet, Bénédicte Richaudeau, Lionel Pintard. Development, Institut Jacques Monod, CNRS, Univ. Paris-VII-Diderot, Paris, France.

The COP9 signalosome (CSN) is an evolutionary conserved macromolecular complex that interacts with Cullin-RING ubiquitin-Ligases (CRLs) and regulates their activity by hydrolyzing cullin-Nedd8 conjugates. In particular, the CSN sequesters inactive $CRL4^{Ddb2}$ ubiquitin-ligase, which rapidly dissociates from the CSN upon DNA damage. Whether other CRLs are similarly sequestered and regulated by the CSN is currently unknown. We have recently defined the protein interaction network of the mammalian CSN using a combination of affinity purification tagging and sensitive tandem mass spectrometry approaches (LC-MS/MS). Notably we identified a small subset of CRLs, which stably interact with the CSN, and thus might be specifically activated by dissociation from the CSN in response to specific cues, such as DNA damage. Consistent with this hypothesis, most of the CSN-interacting CRLs identified in this study appear to be involved in cell cycle progression and DNA metabolism; however, the function of others remains elusive. We have identified worm orthologues of most of these CRLs and we are now using *C. elegans* as a model system to dissect their function during cell cycle progression. In particular, we are currently focusing on a CUL-2-based ubiquitin-ligase that uses F33G12.4/LRR-1 as a substrate-recognition subunit. LRR-1 contains a typical BC/CUL-2 box and binds CRL2 components *in vitro* and *in vivo*. RNAi-mediated depletion of *lrr-1* results in an embryonic lethal phenotype, with severe delays in S-phase, as revealed by time-lapse video-microscopy. Importantly, this delay is entirely suppressed by inactivation of the DNA replication checkpoint, suggesting that the $CRL2^{LRR-1}$ complex might control DNA replication in the early embryo. Consistent with this hypothesis, *lrr-1(RNAi)* treated embryos are hypersensitive to sublethal doses of the replication inhibitor hydroxyurea (HU) and the DNA-damaging agent methyl methanesulfonate (MMS). Experiments are under way to elucidate the function of this ubiquitin-ligase and to identify its critical substrate(s).

1083C

Characterization of the role of TLK-1 and KNL-2 in kinetochore assembly in *C. elegans*. **Jessica M. De Orbeta**[1,2], Jill M. Schumacher[1]. 1) Department of Genetics, UT MD Anderson Cancer Center, Houston, TX; 2) Program in Genes and Development, UT Houston Graduate School of Biomedical Sciences, Houston, TX.

During cell division, chromosome segregation must occur accurately in order to maintain cell viability and proper organismal development. Kinetochore assembly, a prerequisite for the attachment of centromeric DNA to spindle microtubules, is a critical step during chromosome segregation. Failure to assemble the kinetochore disables chromosome attachment to the mitotic spindle, resulting in defective chromosome segregation. The centromeric protein CENP-A, is a component of all eukaryotic centromeres and appears to be the primary epigenetic signal for centromere identity and kinetochore establishment. Recently, it was shown that KNL-2, a Myb-domain protein, is necessary for the loading of CENP-A to the centromere. In *C. elegans*, both *cenp-A(RNAi)* embryos and *knl-2(RNAi)* embryos display a kinetochore null phenotype, with no evidence of kinetochore recruitment or chromosome attachment to mitotic spindle microtubules. In human cells, the KNL-2 homolog, Mis18Bp, is also required for CENP-A loading at the centromere. Although, KNL-2 is required to load CENP-A at the centromere, the mechanism by which KNL-2 is regulated remains unknown. KNL-2 is an unusual Myb- domain protein since it carries a single copy of this domain, whereas the majority of Myb-proteins have two or more domains. This characteristic is also shared by Tki, a Tousled kinase substrate identified in plants. In *C. elegans*, the Tousled-like kinase (TLK-1) is involved in many cell cycle events. Our lab has found that phosphorylation localizes TLK-1 to the kinetochore during metaphase in *C. elegans* embryos. Given these similarities and the localization of TLK-1 and KNL-2 at the kinetochore we hypothesize that KNL-2 is an *in vivo* substrate of TLK-1, and that TLK-1 phosphorylation impacts the function of KNL-2 in kinetochore assembly. We have mapped the phosphorylation site of in KNL-2 *in vitro*. Simultaneously, we performed immunolocalization experiments to determine the sub-cellular relationship between KNL-2 and TLK-1 in *C. elegans* embryos. As expected, pTLK-1 staining is reduced in *knl-2(RNAi)* embryos as compared to controls, suggesting that pTLK-1 is a *bona fide* kinetochore component. Currently, we are raising a phospho-specific antibody to determine when and where KNL-2 is phosphorylated by TLK-1 *in vivo*. Additionally, transgenic animals expressing phospho-mutant versions of KNL-2 are being generated to study the effect of KNL-2 phosphorylation by TLK-1 during the cell cycle. Understanding the effect of KNL-2 phosphorylation in *C. elegans* will help us to elucidate the role of TLK-1 during the cell cycle.

1084A

Untousling the Cell Cycle. **Jason R. Ford**[1,2], Gary M. Riefler[1,2], Zhenbo Han[1], Chih-Chao Yang[2], Jill M. Schumacher[1,2]. 1) Department of Genetics, UT M.D. Anderson Cancer Center, Houston, TX; 2) Program in Genes and Development, UT Houston Graduate School of Biomedical Sciences, Houston, TX.

Proper replication and segregation of DNA are vital for cellular viability. The Aurora kinase family is necessary for a multitude of cellular processes during mitosis. The *C. elegans* homolog of the Aurora B kinase, AIR-2, phosphorylates many fundamental substrates with important roles in mitosis. One substrate of AIR-2 is the Tousled-like kinase, TLK-1, which achieves its maximum activity during S-phase. TLK-1 is a serine/threonine kinase and has been implicated in many cell-cycle processes, including DNA replication and repair, transcription, and chromosome segregation.

Interestingly, we have shown that TLK-1 contributes to chromosome segregation as a substrate activator of the AIR-2 kinase independent of TLK-1 kinase activity. AIR-2-dependent phosphorylation of TLK-1 is detectable at the kinetochore from early prophase to metaphase, suggesting a role for TLK-1 in mitosis. We have demonstrated that knocking down TLK-1 via RNAi in both wild-type and tlk-1Δ backgrounds results in a significant cell cycle delay, most prominently from nuclear envelope breakdown to metaphase. We also show this delay is dependent on the spindle assembly checkpoint (SAC): depletion of MDF-1, a SAC component, results in a strong enhancement of the *tlk-1(RNAi)* phenotype, including multiple centrosomes, abrogation of the *tlk-1(RNAi)*-induced metaphase delay, and more severe chromosome segregation defects.

Few *in vivo* substrates of Tousled-like kinases have been reported. The most studied of them is the chromatin assembly factor Asf; however, the consequence of this phosphorylation is unknown. We have recently found that the *C. elegans* ortholog of the Proliferating Cell Nuclear Antigen (PCNA), PCN-1, is also an *in vitro* substrate of the TLK-1 kinase. PCNA is part of the DNA replication machinery. To specifically analyze the spatial and temporal localization of TLK-1-phosphorylated PCN-1 during the cell cycle in the absence of endogenous PCN-1, we have generated transgenes expressing RNAi-resistant, GFP-tagged wild-type PCN-1, non-phosphorylatable PCN-1, and phosphomimetic PCN-1 that will be introduced into *C. elegans* via Mos single-copy insertion. Based on these observations, we hypothesize that TLK-1 affects cell cycle progression in S-phase by influencing DNA replication and/or repair through phosphorylation of ASF-1 and PCN-1. Furthermore, TLK-1 exhibits a phospho-dependent mitotic function by targeting to the kinetochore where it may affect spindle assembly and chromosome segregation.

1085B

CDC-25.2 regulates intestinal cell proliferation and hyper activity of CDC25.1 can compensate lack of CDC-25.2. **Yong-Uk Lee**, Jiyoung Kim, Ichiro Kawasaki, Yhong-Hee Shim. Dept. of Bioscience and Biotechnology, Bio/Molecular Informatics Center, Konkuk University, Seoul Korea.

There are four *cdc-25* family members, *cdc-25.1, -25.2, -25.3,* and *-25.4* in *C. elegans*. CDC-25s are key cell cycle regulators and essential during development. Single mutation or single RNAi of each gene showed sterility resulting from defects in germ cell proliferation or gametogenesis. However, their soma appeared to be unaffected, suggesting two possibilities: their zygotic expressions are dispensable for the post-embryonic development in soma or unlike in the germ line they are functionally redundant in soma. To investigate these possibilities we examined somatic development of *cdc-25.2(ok597)* mutant hermaphrodites that are sterile because they produce abnormal oocytes. Interestingly, *cdc-25.2(ok597)* showed less number of intestinal nuclei because of defects in E-lineage cell division during embryogenesis, indicating that it has unique function in E-lineage and the other *cdc-25* family members can not compensate for its loss. The functional uniqueness is possibly due to tissue-specific expression of *cdc-25.2* in E-lineage. To test this possibility *cdc-25.1* was ectopically expressed in *cdc-25.2(ok597)* mutant by constructing *cdc-25.1(rr31gf); cdc-25.2(ok592)* double mutants and intestinal cell division was examined. *cdc-25.1(rr31gf); cdc-25.2(ok592)* double mutants showed extra cell division in intestine, indicating that *cdc-25.1* can functionally compensate for the function of *cdc-25.2* in E-lineage. In this study, we showed that *cdc-25.2* has tissue-specific role in E-lineage. We assume that functional specificity of *cdc-25* family members in soma is likely based on the specific control of expression of each member.

1086C

The function of a spindle checkpoint gene *bub-1* in *C. elegans* development. **Min Liu**, Xiangming Wang, Zuoyan Zhu, Qichang Fan. College of LifeScience, Peking University, Beijing.

The serine/threonine kinase BUB1 (Budding Uninhibited by Benzimidazole 1) was originally identified in yeast as a checkpoint protein, based on its mutant incapacity of delaying the cell cycle in response to loss of microtubules. Our understanding of its function is primarily from studies carried out in yeast *S. cerevisae*. It has been shown that it acts at the spindle-check point and regulates the separation of sister chromatids through its downstream molecules. However, its roles in multi-cellular organisms remain unclear. In nematode *C. elegans*, rapid cell divisions primarily occur in embryos and in germline of postembryonic larvae and adults. In addition, a select set of cells undergo a few round of cell division postembryonically. One common phenotype associated with impaired cell division is described as Stu (Sterile and Uncoordinated). We conducted a genetic screen for zygotic mutants that displayed Stu in *C. elegans*. We isolated seven Stu mutants that fell into five complementation groups. We report here that two mutations, *FanWang5* (*fw5*) and *FanWang8* (*fw8*) affect the *bub-1* gene, a homology of the yeast serine/threonine kinase gene *BUB1*. Both mutant alleles *fw5* and *fw8* exhibited variable behavioral defects, including developmental arrest, uncoordination and sterility. The number of postembryonically born neurons in the ventral cord decreased and their axon morphology was abnormal. The decreased number of cells could not be suppressed by a caspase-3 loss-of-function mutant. In addition, *bub-1*(*fw5* and *fw8*) mutants showed widespread effects on postembryonic development in many cell lineages. We found that bub-1 functioned maternally in several developmental lineages at the embryonic stage in *C. elegans*. Studies in yeast have shown that BUB1 functions as a spindle checkpoint protein by regulating the anaphase promoting complex/cyclosome (APC/C). We performed double mutant analysis and observed that *bub-1* genetically interacted with several downstream genes, including *fzy-1*(*h1983*)/*CDC20*, *mat-2*(*ax102*)/*APC-1* and *emb-27*(*g48*) /*APC-6*. Our results demonstrate a conserved role of *bub-1* in cell-cycle regulation and reveal that *C. elegans bub-1* is required both maternally and zygotically. Further, our genetic analysis is consistent with that the function of *bub-1* in *C. elegans* is likely similar to its yeast and mammalian homologue.

1087A

Centrosome elimination during *C. elegans* development. **Yu Lu**, Richard Roy. Dept Biol, McGill Univ, Montreal, PQ, Canada.

Many sexually reproducing organisms must eliminate a pair of centrioles from their gametes prior to the first zygotic division to avoid the formation of a multipolar spindle. In the *C. elegans* germ line, centrosomes are eliminated just prior to oocyte specification and this process requires the activity of the p21/p27-like CDK inhibitor protein *cki-2*, which may function by antagonizing cyclin E/CDK-2. This suggests that changes in CDK activity are required for this process and altering CDK activities may affect proper centrosome elimination. We therefore decided to examine whether CDKs exert these effects through proteins important for centrosome duplication. In addition to oocyte-specific elimination of the centrosome, we found that centrosomes are eliminated from cells undergoing endocycles. The intestinal and hypodermal cells undergo endoreduplication during postembryonic development. Using a SPD-2::GFP reporter to detect the presence of centrosomes in these tissues, we observed that the centrosomes were gradually lost from intestinal nuclei after the L1 stage, while they disappeared from the hypodermal cells at the L2 stage. Using an anti-SPD-2 antibody, we further confirmed that the intestinal cells actively eliminate their centrosomes at the onset of endoreduplication. We are therefore using a candidate gene approach in the intestine while monitoring SPD-2::GFP to address the molecular mechanism underlying this process. Interestingly, SPD-2 possesses multiple canonical CDK phosphorylation sites and we mutated the highly predicted sites to generate SPD-2 variants. Subsequently we examined them in various developmental contexts where centrosome elimination occurs. When we introduced the non-phosphorylable SPD-2 variant we noted extra centrosomes in the intestine, which were visible after the L2 stage, when the centrosomes are normally eliminated, suggesting that the proper phosphorylation of SPD-2 may contribute to timely centrosome elimination in the intestine. We are currently testing phosphomimetic SPD-2 for its effects. Considering that continued CDK-mediated phosphorylation results in centrosome persistence in the germ line, our observation in the intestine suggests that perhaps a second means of triggering centrosome elimination may function in the endocycling gut that is distinct from that during oocyte specification, but may nevertheless require CDK-mediated phosphorylation.

1088B

Involvement of glycogenes in cell division and morphogenesis: . **Kazuya Nomura**[1,2], Kazuko H. Nomura[1,2], Daisuke Murata[1,2], Katsufumi Dejima[1,2], Souhei Mizuguchi[1,2], Keiko-Gengyo Ando[2,3], Shohei Mitani[2,3], Nana Kawasaki[2,4], Katsuko Yamashita[2,5], Hiroko Ideo[2,5], Keiko Fukushima[2,5], Yoshio Hirabayashi[2,6], Yoshikatsu Kanai[2,7], Yeon-Dae Kwon[8], Hisashi Narimatsu[8], Hiroshi Kitagawa[2,9], Yasuhiro Hayashi[1,2], Makoto Ito[1,2]. 1) Kyushu Univ, Fukuoka, Japan; 2) CREST, JST, Saitama, Japan; 3) Dept of Physiology, Tokyo Women's Medical University,Japan; 4) National Inst of Health Sci, Tokyo, Japan; 5) Innovative Res Initiatives, Tokyo Inst of Technology, Yokohama, Japan; 6) Riken Brain Inst, Saitama, Japan; 7) Osaka Univ, Toyonaka, Japan; 8) National Inst of Advanced Industrial Sci and Technology, Japan; 9) Dept of Biochem, Kobe Pharmaceutical Univ, Japan.

Glycogenes are genes essential for synthesis, degradation, modification or recognition of glycoconjugates. Among them are genes of glycosyltransferases involved in synthesis of N-glycans and O-glycans, glycoconjugate degradation enzymes, sulfation-related enzymes, sugar transporters, glycolipid synthesizing and depredating enzymes, GPI-anchor related enzymes as well as various lectins recognizing carbohydrates and related structures. In the present study, to understand the roles of carbohydrates in development and morphogenesis of multicellular organisms, we performed systematic knocking out of glycogenes which are orthologues of human glycogenes. By using sophisticated bioinformatics techniques, about 60% of glycogenes in the worm genome are shown to be orthologues of human glycogenes. For instance, we found 145 predicted orthologues of human glycosyltransferase (GT) genes in the worm genome. In addition to various genes involved in glycosphingolipid or GPI anchor synthesis, we found various sulfation related genes and lectin orthologues in the worm genome. We knocked down all of these gene functions by RNAi and/or TMP/UV deletion mutagenesis and found at least 10% of them are essential and over 30% of these genes show various phenotypes when knocked down or knocked out. Larval lethality and ER stress phenotypes were observed in sulfation related gene KO experiments (PAPS synthase *pps-1* or PAPS transporter genes) and ER stress phenotypes were observed when *hut-1* gene was knocked out. Especially intriguing phenotype was the abnormal cytokinesis in early embryonic division observed in chondroitin related gene KO experiments (chondroitin synthase: *sqv-5*, chondroitin polymerizing factor: *mig-22*/*pfc-1* or *cpg-1* and *cpg-2* double KO). Meiotic division abnormal phenotypes were also observed in chondroitin related gene KO experiments, and we also found that various other glycogenes are involved in progression of meiotic cell cycle. These results indicate that genes involved in glycosylation are playing essential roles in embryonic cell division as well as in meiotic cell division.

1089C

Mechanisms of Q cell asymmetric division in *C. elegans* larva. **Guangshuo Ou**, Ron Vale. Cellular and Molecular Pharmacology, HHMI/Univ California, San Francisco, San Francisco, CA.

Cell divisions in C. elegans embryos have been extensively studied, but these divisions only make 558 somatic cells for the adult. The rest 401 cells are generated by cell divisions in the larva, which we know very little about. Q neuroblasts are the first cells that divide after hatching, and they undergo 3 rounds of asymmetric cell division to generate 3 distinct neurons and 2 apoptotic cells. Their second round division is of our particular interest because it produces one big cell that survives and one small cell that dies by apoptosis, distinct from cell fates of daughter cells in the C. elegans early embryo or the Drosophila neuroblasts. We have recently developed fluorescence time-lapse microscopy to visualize Q cell division with spinning disk confocal microscope. We have also developed GFP/mcherry based cellular markers to label chromosomes, cytoskeleton, centromsomes and membrane of Q cells. By visualizing these markers in dividing Q.a (anterior) and Q.p (posterior) cells, we find that they exploit distinct mechanisms to make two daughter cells of different sizes. For Q.a cell, its spindle positioning and cytokinetic furrow initiation are both in the center of the cell, but the content for two daughter cells are unevenly distributed during cytokinesis. For Q.p cell, its spindle is positioned towards the part that makes the small cell and cytokinesis occurs in the center of its displaced spindle. The furrow is formed by actomyosin ring, and we are visualizing dynamics of GFP labeled actin and non-muscle myosin-II during Q.a/p cytokinesis to better understand the process of asymmetric cell division.

1090A

The protein-phosphatase 4 subunit PPFR-1 is a potential activator of MEI-1/Katanin. José-Eduardo Gomes[1], Bénédicte Richaudeau[1], Etienne Formstecher[2], Xue Han[3], Paul E. Mains[3], **Lionel Pintard**[1]. 1) Institut Jacques Monod, CNRS, Université Paris Diderot, Paris France; 2) HYBRIGENICS, Paris, France; 3) University of Calgary, Alberta, Canada.

Upon fertilization the *C. elegans* oocyte resumes and completes meiosis, and twenty minutes latter the first embryonic mitosis takes place. Despite sharing the same cytoplasm, the meiotic and mitotic spindles are radically different: while the small asterless meiotic spindle assembles close to the cell cortex, the mitotic spindle is large, with robust asters, and is positioned in the center of the embryo. MEI-1/Katanin is a microtubule-severing protein essential for meiotic spindle formation; however its activity is incompatible with the assembly of a fully functional mitotic spindle. In wild-type embryos, MEI-1 is inactivated at the meiosis-to-mitosis transition allowing for mitotic spindle assembly. We have previously established that, after meiosis, a Cullin-3-based E3-ligase targets MEI-1 for ubiquitin-mediated proteasomal degradation. MEI-1 is recruited by the CUL-3-based complex through the adaptor protein MEL-26. In order to identify new components of the pathway, we conducted a Yeast Two-Hybrid screen using MEL-26 as bait. In this screen we identified PPFR-1, a regulatory subunit of the serine/threonine phosphatase PP4 complex. *Loss-of-function* of *ppfr-1*, through both RNA-interference or a putative null allele, suppresses the lethality of ectopic MEI-1 expression during mitosis, either due to *mel-26 loss-of-function* or a to *mei-1 gain-of-function* allele encoding a protein refractory to MEL-26 mediated degradation. This suppression suggests the PP4 phosphatase is an activator of MEI-1. In addition, the *ppfr-1* putative null allele displays a partly penetrant meiosis failure phenotype, again consistent with PP4 being an activator of MEI-1. To test whether PP4 modifies MEI-1 protein in vivo, we performed 2D gels on embryonic extracts; we were able to show that MEI-1 is phosophorylated in vivo and that these modifications are dependent on PPFR-1. Our results also show that PPFR-1 regulates MEI-1 independently of protein degradation. We are currently testing whether PPFR-1 is targeted for degradation by the CUL-3^{MEL-26} ligase at the meiosis-to-mitosis transition.

1091B

Identifying the Network Controlling Cell-Cycle Quiescence During *C. elegans* Vulva Development. **R.M. Saito**, Eleanor Beltz, Jenna Holmen, Joseph Clayton, Sarah Buck. Genetics, Dartmouth Medical School, Hanover, NH.

The mechanisms controlling cell divisions during development are not completely understood. To examine the regulation of cell-cycle entry in a physiologically normal environment, we use *C. elegans* vulva development as a model system. During the first larval stage, six vulval precursor cells (VPCs) arise and immediately undergo temporary cell-cycle arrest. The VPCs remain quiescent until mid-third larval stage when they coordinately resume cell divisions and differentiate as either hypodermal or vulval cells. We previously performed a forward genetic screen to identify genes necessary for this VPC cell-cycle quiescence. We have now completed the first genome-wide screen to identify genes required for cell-cycle quiescence. In addition to the few genes previously characterized, our screen identified approximately fifty genes not previously known to regulate cell-cycle quiescence. For example, the screen identified *cki-2* as necessary for VPC quiescence. In contrast to prior studies suggesting an alternate function for *cki-2*, our detailed analyses support a role for *cki-2* as a canonical cyclin-dependent kinase inhibitor. Our ultimate goal is to integrate *cki-2* and the other newly discovered genes into a comprehensive model describing the regulatory logic of the developmental network.

1092C

The Patched-related protein PTR-2 is required for somatic cytokinesis in *C. elegans*. **Alexander Soloviev**, Olivier Zugasti, Patricia Kuwabara. Biochemistry, University of Bristol, Bristol, United Kingdom.

C. elegans is positioned at an evolutionary cusp with regard to the Hedgehog (Hh) /Patched (Ptc) signalling pathway. The worm lacks an obvious Hh orthologue, but encodes a large number of Ptc-related proteins (Zugasti et al., 2005). Moreover, the Ptc family of proteins has undergone a significant expansion in the worm (Burglin and Kuwabara, 2006). It remains unclear whether the Ptc proteins of *C. elegans* participate in cell-cell signalling; however, we have shown that *ptc-1* plays an essential role in the development of the germ line. Mutants lacking *ptc-1* are sterile because of a defect in cytokinesis (Kuwabara et al., 2000). In order to determine whether the PTC proteins play more global role in cytokinesis, we sought to determine whether the absence of any member of this family disrupted somatic cytokinesis. To this end, we identified the *ptr-2 (ptc-related)* gene. We have shown that *ptr-2*(RNAi) causes embryos to arrest due to a cytokinesis defect that results in the formation of multinucleated blastomeres. In the most severe cases, the embryos arrest at the 1-cell stage of development. Time-lapse recordings of *ptr-2*(RNAi) embryos reveal that a knockdown of *ptr-2* activity affects multiple aspects of cell division, including chromosome condensation/segregation, spindle positioning and cleavage furrow formation (Zugasti et al., 2005). Given that there are a large number of ptc and ptr genes in the worm, we felt that it was important to establish that the *ptr-2*(RNAi) defects were not due to off target effects. We show that a mutation in *ptr-2* gives rise to an embryonic cytokinesis defect, although the mutant phenotype is not identical to that observed by RNAi. To understand the basis of the multiple defects caused by the knockdown of *ptr-2*, we have also examined the intracellular localisation of PTR-2 protein using an anti-PTR antibody. We will show that PTR-2 is dynamically distributed in vesicles throughout the cell-cycle and discuss the role of the cytoskeleton in PTR-2 localisation.

1093A

How Does the Cell Control the Size of Microtubule Organizing Center? **Mi Hye Song**, Kevin O"Connell. Lab Biochem/Genetics, MSC 0830, NIDDK/NIH, Bethesda, MD.

Microtubules form highly organized and polarized arrays. In proliferating cells, these arrays undergo cell cycle-dependent reorganization to establish bipolar spindles during mitosis. Microtubule rearrangements are largely under the control of the centrosome, the primary microtubule-organizing center. Centrosomes comprise a pair of centrioles surrounded by a mass of pericentriolar material (PCM), and undergo dynamic changes in both size and number during the cell cycle. While many components of the centrosome have been identified, how the cell regulates assembly of centrosomes remains unclear. Recently we identified SZY-20 (Suppressor of ZYG-1) that limits centrosome size by negatively regulating the Plk4-related kinase ZYG-1 at the centrosome (Song et al., Dev. Cell 15: 901-912 2008). We would like to understand how SZY-20 antagonizes ZYG-1 to influence centrosome size. Previously, we have shown SZY-20 contains conserved domains (SUZ and SUZ-C) found in a number of known RNA-binding proteins and that mutation of these domains disrupts both *in vitro* RNA-binding and the control of centrosome size. SZY-20 might regulate centrosome size through the RNA-binding role. To address this, we have sought to identify RNAs and proteins that interact with SZY-20. We have immunoprecipitated (IP) endogenous SZY-20 from wild-type embryonic extracts, and analyzed co-precipitating factors by mass spectrometry (MS) for proteins, and by high-throughput Illumina sequencing for RNAs. Using this approach, we have identified a large number of proteins that co-precipitate with SZY-20, including factors involved in RNA metabolism, protein synthesis and degradation, and nuclear-cytoplasmic transport. We are currently using RNAi and mutant alleles to determine which of these factors are required for embryonic viability, cell cycle and centrosome function. From this analysis, a subset of factors will be subject to further investigation to see if they exhibit physical and genetic interactions with SZY-20. Ultimately, we hope to identify new regulators of centrosome size and provide a detailed description of the molecular pathways involved.

1094B

Identification of serine/theonine kinases that phosphorylate CDC-25.1 during embryogenesis in *C. elegans*. **Christopher St-François**, Richard Roy. Department of Biology, Mcgill University, Montreal, Quebec, Canada.

In eukaryotic cells, CDC25 phosphatases play precise roles in positively regulating the cell cycle during embryonic growth and development. Proper control of CDC25 expression levels is pivotal for the correct formation of tissues and organs. In mammalian cells, CDC25A removes inhibitory phosphates on CDK2 and is a critical G1/S phase target that is regulated downstream of DNA damage signals. This is controlled through its timely degradation which is initiated by a highly conserved F box protein called β-TrCP/LIN-23, upon recognition of phosphorylated serine residues within the CDC25A (CDC-25.1 in *C. elegans*) phosphodegron motif. We have isolated a *cdc-25.1 gf* mutation that disrupts this domain, presumably affecting its appropriate recognition, allowing it to resist degradation by β-TrCP/LIN-23. As a result, this critical cell cycle regulator perdures and causes a supernumerary round of cell divisions, although exclusively in the intestine. Since phosphorylation of the CDC-25.1 phosphodegron likely precedes this event, we are trying to identify the kinase(s) responsible for this/these critical modifications. We have made use of bioinformatics to identify candidate *C. elegans* serine/threonine kinases, the activities of which, we have eliminated using RNA interference. By screening for intestinal hyperplasia in a *elt-2::GFP* background, we hope to identify all the kinases that are involved in triggering this event. Because the *cdc-25.1 gf* mutant causes hyperplasia exclusively in the gut, all candidate kinases that cause a supernumerary round of intestinal cell division will be subjected to a secondary screen in transgenic worms expressing GFP in an alternative lineage (MS), where the *cdc-25.1 gf* mutant has no obvious effect on proliferation. This screen will allow us to narrow down candidates by eliminating kinases involved in other developmental processes (*i.e.* cell fate specification). We are currently expressing our candidates in HEK293T cells and performing *in vitro* kinase assays on synthetic peptides representing the *C. elegans* phosphodegron motif, to test their ability to directly phosphorylate this region.

1095C

High throughput positional cloning of *C.elegans* mutant loci and immobilizing worms with microfluidic nanotechnology. **M.H. Price**[1], D.W. Turnbull[1], V. Davis Haug[1], D.R. Hamill[2], M.L. Drummond[1], L.A. Carter[1], E.A. Johnson[1], S.R. Lockery[1], B.A. Bowerman[1]. 1) Univ Oregon, Eugene, OR; 2) Ohio Wesleyan Univ, Delaware, OH.

To positionally clone mutant loci in essential *C.elegans* genes more rapidly and to examine developmental processes in live worms without anesthetics, we are developing new methods that we hope will be of general interest to the *C.elegans* research community. To revive chemical mutagenesis and classical genetics to study early *C.elegans* embryogenesis, we are developing new high throughput genetic mapping and genome sequencing approaches. First, we are applying RAD mapping[1] to rapidly position ts mutations to roughly 1-3 MB intervals. Second, rather than individually sequencing candidate genes, we are using magnetic beads to pull down 1-3 MB intervals of mutant genomic DNA for subsequent Illumina sequencing. To isolate MB intervals of mutant genomic DNA, we first link amplified, wild-type genomic DNA from the same interval to beads. The wild-type genomic DNA intervals are obtained from contiguous sets of genomic fosmid clones[2]. Mutant genomic DNA is then hybridized to the beads for pulldown. Thus far, up to 40% of the sequence reads align to targeted interval, with an average coverage of over 150X possible. Using this approach, we determined that one mutation, *or627*ts, is an allele of the gene *bmk-1*. We are currently sequencing genomic intervals that cover five additional mutants: *or452*ts, *or600*ts, *or643*ts, *or660*ts, and *or683*ts, which have defects in either meiotic or mitotic spindle assembly and function.

To study meiotic spindle assembly using live cell imaging with GFP fusions and spinning disk confocal microscopy, one must immobilize adult worms and image meiotic spindle assembly *in utero*. To avoid the use of anesthetics, which in our experience decreases ovulation rates and/or fails to fully immobilize adult worms, we have taken advantage of microfluidic nanotechnology. We have developed a PDMS (Polydimethylsiloxane) device well suited for live cell microscopy of multiple immobilized adult hermaphrodites. These devices have multiple channels that each hold one worm. The channels are made to a specific height and step down in width from 90 to 50 microns to accommodate worms of various sizes, firmly immobilizing each worm and eliminating the need for anesthetic; worms can be recovered after imaging if required. We will present both the design of these devices and documentation of their efficacy. With minor modifications the use of such chambers could easily be extended to live cell imaging in larval stage worms. [1]Miller et al. 2007. Gen Res. [2]Perkins et al. 2005. International Worm Meeting.

1096A

Development of small molecule proteasome inhibitors using *Caenorhabditis elegans*. **Michela Fiaschi**[1], Natalie Stabenow[1], Erica Tabakin[2], David Hunt[2], Sudhir Nayak[1]. 1) Department of Biology The College of New Jersey 2000 Pennington Rd. Ewing, NJ 08628; 2) Department of Chemistry The College of New Jersey 2000 Pennington Rd. Ewing, NJ 08628.

The proteasome is involved in a variety of processes including regulation of the cell cycle, morphogenesis, differentiation, cell surface receptor modulation, DNA repair, and numerous others. Due to its critical role in the cell, inhibition of the proteasome function results in cell cycle arrest and/or apoptosis. This has generated considerable attention in the development of proteasome inhibitors as anti-cancer therapeutics. We have initiated development of a high throughput screen for small molecule proteasome inhibitors using the real-time analysis of *C. elegans* germ line development and early embryogenesis. The basic strategy for the development of the novel small molecule templates incorporates structural elements from naturally occurring proteasome inhibitors epoxomicin and NPI-0052. To screen for bioactive derivatives we are taking advantage of a functional GLD-1::GFP (GLD-1 fused to GFP) that allows for the real-time assessment of gross germ line morphology. As a secondary screen, bulk DAPI (4',6-diamidino-2-phenylindole) staining was used to identify disruptions in nuclear morphology. In wild-type worms, the GLD-1 protein has a tightly restricted expression pattern with low levels present in mitosis, high levels of accumulation during meiotic progression, and low levels during oogenesis. We reasoned that any compound that interrupts the cell cycle would result in a breakdown in germ line polarity and would be identifiable by a change in expression of the GLD-1::GFP transgene. The basic protocol developed in 6 well plates has been scaled to a 96-well format and has been used to identify multiple novel small molecules that result in a change in GLD-1::GFP expression and abnormal nuclear morphology without non-specific toxicity. Our future directions include confirming the effects of the novel proteasome inhibitors using direct approaches for the disruption in proteasome function and testing the feasibility of adapting the procedure to 384-well format. Our preliminary data suggests that *C. elegans* can be used as a sensitive real-time whole animal screening system in the identification and development of bioactive compounds. Further development and refinement of this assay system should allow for the high throughput screen of novel inhibitors and other chemotherapeutic agents.

1097B

The in vivo function of *C. elegans* 53BP1 homolog in DNA repair. **Sang-Jo Kang**, Hyeon-Sook Koo. Dept Biochemistry, Col Life Sci & Biotech, Yonsei Univ, Seoul, Korea.

The tumor suppressor protein 53BP1 is known to participate in DNA damage checkpoint and DNA repair. After ionizing-irradiation, the protein is phosphorylated by ATM and recruited to double-strand break sites in the downstream of MDC1, RNF8 and in the upstream of CHK2. Recently, 53BP1 has turned out to be recruited to the methylated histones. Although its function in DNA damage checkpoint and repair was confirmed, the mechanism how the protein affects these reaction pathways has not been clearly elucidated. This evolutionarily conserved protein shows a homology with HSR-9 in *Caenorhabditis elegans*. To examine the function of this protein, we used a *C. elegans* mutant deleted in HSR-9. The mutant showed hypersensitivity to gamma rays, as inferred from the reduced hatching rate of embryos. After the irradiation, recruitment of 53BP1 to nuclei of the germ cells, representing its activation, was observed by immunostaining. However, the mutant was ineffective in inducing G2 phase arrest in response to gamma rays. These results suggest that the 53BP1 homolog in *C. elegans* is involved not in DNA damage checkpoint but probably in DNA repair. We also observed that HSR-9 is activated in the downstream of ATM although it is not involved in DNA damage checkpoint. Moreover, we observed the effect of histone methylation on activation of the 53BP1 homolog by knocking down histone methylases. We are now investigating by genetic analysis in which of the repair pathways, homologous recombination and nonhomologous end-joining, the protein participates.

1098C

The cloning of three conditional fast-inactivating *icp-1* (INCENP) alleles. **Julie C. Canman**[1], Bruce Bowerman[2], Karen Oegema[1]. 1) Ludwig Inst Cancer Research University of California at San Diego La Jolla, CA; 2) Institute for Molecular Biology University of Oregon Eugene, OR.

Cell division is a dynamic process controlled by a number of proteins, among which some are multifunctional and are required at multiple steps. Loss-of-function of these master regulators, by classical techniques of reverse genetics, leads to a complex pleiotropic phenotype due to the combination of defects at these various steps. The role of these master regulators in a specific aspect of cell division is thus challenging to address. One such important master regulatory complex is the Chromosome Passenger Complex (CPC), which participates throughout meiosis, mitosis, and during cytokinesis. Indeed, disrupting function of the CPC by traditional forward or reverse genetics results in massive chromosome segregation defects as well as a failure to divide. To examine the role of the CPC during cytokinesis specifically, we have taken a forward genetics approach in *C. elegans* to isolate conditional alleles which phenocopy loss-of-function of the CPC showing both massive chromosome segregation and cytokinesis defects. Using this approach we found three mutant lines that are all completely impaired in chromosome segregation and cytokinesis. These mutants all failed to complement with each other indicating they are alleles of the same gene. Using traditional visible marker mapping, we mapped these mutants to the left arm of chromosome I, which contains the *C. elegans* INCENP homolog, ICP-1^{INCENP}, the core scaffolding protein of the CPC. We sequenced the *icp-1* locus in these mutants and found that all three mutants contain single mis-sense mutations in *icp-1* resulting in single amino acid changes. All three alleles are fast-inactivating, showing a fully penetrant loss-of-function phenotype within seconds of shifting to the restrictive temperature. Thus, they will be powerful tools for studying the role of the CPC in cytokinesis independent of chromosome segregation during meiosis and mitosis.

1099A

FACS-RNAi screening identifies new *mel-28* genetic interactors. **Anita G. Fernandez**[1,2], Emily Mis[2], Fabio Piano[2]. 1) Dept Biol, Fairfield University, Fairfield, CT; 2) Center for Genomics and Systems Biology and Department of Biology, New York University, New York, NY, USA.

MEL-28/ELYS is a large AT-hook protein required for nuclear envelope integrity and chromosome segregation in metazoans. As expected by its fundamental function, MEL-28 is ubiquitously expressed in all cells analyzed. However, mutations in the *mel-28* gene are maternal-effect, causing embryonic lethality in the progeny of homozygous mutant mothers that are otherwise wild-type. Therefore, the function of MEL-28 in most cells is predicted to be buffered by other molecules. To identify additional proteins working with MEL-28 we looked for synthetic phenotypes in *mel-28* homozygous animals using RNAi. The challenge in this type of screen, as with any modifier screen of *mel* mutants, is to collect large numbers of homozygous animals. To accomplish this in high throughput, we generated a strain with the *mel-28(t1684)* mutation balanced by a GFP-marked chromosome and used a fluorescence-activated cell sorter (FACS) to isolate non-fluorescent *mel-28* homozygous L1 larvae from the rest of the population. Using this approach we have collected, in one sitting, as many as 100,000 larvae, over 99% of which are *mel-28* homozygous. We subjected the *mel-28* larvae to RNAi in 96-well plates and recorded results by high-throughput digital imaging. We present here the results from screening essentially all the genes encoded on Chromosome I. Of the 2260 clones tested, we found 14 that are synthetic sterile with *mel-28*. Among these genes we found members of expected molecular complexes. For example, most the nucleoporins known to be on Chromosome I were uncovered. In addition, we find other classes of proteins, like histones, as genetic interactors of *mel-28* suggesting new molecular connections between MEL-28 and chromatin organization. Our results also show that FACS-RNAi screening is a powerful way to uncover tissue-specific roles for pleiotropic genes.

1100B

Uncovering the Biological Roles for the Histone 3 Lysine 9/36 Tri-demethylases from Worm and Human. Emily Forbes, Josh Black, Ryan Walsh, Michelle Longworth, Katrin Tschoep, Nick Dyson, **Johnathan Whetstine**. Massachusetts General Hospital Cancer Center and Department of Medicine, Harvard Medical School.

Cancer is one of the leading causes of mortality. The stability of the genome is a factor involved in warding off tumorigenesis. Genomic instability arises from both extrinsic factors like radiation and intrinsic factors such as aberrant cell signaling, gene mutations, or perturbations in the higher order structure of the genome. DNA, histones, and other chromosomal proteins create this higher ordered structure called chromatin. Chromatin contains histones that have a plethora of post translational modifications (PTMs). The combination and degree of PTMs that occur on the histones impact DNA-dependent processes such as transcription and DNA damage response. Histone methylation is one such mark that is tightly regulated so that cell fate and genomic stability are maintained. A number of chromatin modifying enzymes are responsible for keeping these methylation states balanced. There is a fundamental gap in our understanding about the physiological context under which these modifying proteins and their associated methylation states function(s). Our laboratory attempts to bridge this gap in our understanding by combining the power of *C. elegans* genetics and human cell culture. Our approach allows us to study molecular mechanisms, physiological outcomes, and gain insights into cancer and other DNA damage-related pathologies.

We recently identified the JMJD2 family of histone 3 lysine 9/36 tri-demethylases in both human and *C. elegans*. Depletion of the only functional homolog of the JMJD2 family of enzymes (JMJD-2) in *C. elegans* results in increased histone lysine 9 and 36 tri-methylation, DNA damage, and p53-dependent apoptosis in the adult germline. These phenotypes are observed with both RNAi and a loss of function allele. Our most recent analysis indicates a role for JMJD-2 in preventing DNA damage in both mitotic and meiotic nuclei within the adult germline. We have also observed cell cycle-related abnormalities in the germline that are influenced by p53-dependent pathways and alterations in chromatin structure. Additionally, we identified a human JMJD2 member with the same phenotype in cell culture models. Our data suggest that the *C. elegans* germline and human cell culture phenotypes are a function of alterations in chromatin structure, which impacts the spatial and temporal control of chromatin-templated processes, and in turn, cell cycle and DNA damage-induced apoptosis. Our observations in worms and human cells provide insights into molecular mechanisms that may be contributing to cancer pathophysiology.

1101C

Activation of heat-shock–inducible genes: interaction with nuclear envelope components. **Peter Meister**, Sabine Rohner, Benjamin D. Towbin, Susan M. Gasser. Functional Implications of Nuclear Organisation, FMI-Novartis Research Foundation, Basel, Switzerland.

Nuclear organisation has been implicated in gene expression in unicellular eukaryotes and some mammalian lineages. We have examined changes in nuclear organization during the differentiation of the multicellular organism C. elegans. Using fluorescence in situ hybridisation (FISH) in embryos and GFP-lacI/lacO systems, we showed that developmentally regulated promoters direct subnuclear positioning of genes and gene arrays. The conclusion of these studies is that silent genome segments and arrays are located at the nuclear periphery while active promoters are retained centrally. Surprisingly, heat-shock (HS) promoters behave in a different manner : an array containing a transcriptional fusion of *hsp*-16.2 fused to GFP is located at the nuclear periphery before activation and expands upon transcriptional activation but stays at the nuclear rim. This prompted us to ask whether HS promoters where anchored at the nuclear envelope. Using FISH, we show that the genomic *hsp*-16.2 is also preferentially located at the nuclear periphery and that enrichment for peripheral localisation is further increased after heat shock. Using microparticle bombardment, we generated chromosomally integrated low-copy transgenes that contain arrays of LacO sites and the *hsp*-16.2 promoter driving mCherry, as well as control transgenes containing only lacO sites. These were then observed in vivo by expression of a nucleus-targeted GFP-lacI fusion. We observed that the promoter alone is able to direct the transgene toward the nuclear periphery, while HS treatment of the embryos leads to an increase of the proportion of transgenes located at the nuclear rim. We are examining various components of the nuclear envelope as anchors for the HS promoter. These investigations will be presented.

1102A

Mechanism of X-chromosome dosage compensation: probing X-chromosome higher-order structure in *C. elegans* . **Agnès Michel**, Cathy Pickle, Barbara Meyer. HHMI/ UC Berkeley, Berkeley, CA 94720.

Males and females differ in their number of X chromosomes, yet both require a similar level of X-chromosome products. Dosage compensation is an essential, universal, chromosome-wide regulatory process that equalizes the somatic expression of X-linked genes between males and females/hermaphrodites.

A dosage compensation complex (DCC) is directed to both X chromosomes of *C. elegans* hermaphrodites to reduce gene expression by half. DCC binds to discrete, dispersed cis-acting elements on X. Of the many sites bound by DCC, few can serve as DCC recruitment sites (recruitment element on X, *rex*). We have shown that the recruiting ability is at least partially conferred by a consensus motif (MEX) that is shared among *rex* sites. Most sites (dependent on X, *dox*) fail to recruit the DCC autonomously, therefore must be bound through a different mechanism. The similarity of DCC to condensin, a conserved protein complex essential for chromosome compaction, resolution, and segregation, suggests that DCC loading along X is associated with changes in chromosome structure. Long distances separate *rex* from *dox* sites, implying that long-range interactions are important for DCC distribution.

We have recovered animals that carry homozygous deletions of *rex* sites. These strains will be used to test the hypothesis that DCC binding to *rex* sites is necessary for DCC binding to *dox* sites via a mechanism of long-range interaction. Chromatin immunoprecipitation experiments using DCC-specific antibodies will allow us to determine whether *dox* sites become unoccupied in the *rex* deletion strains. In parallel, Chromosome Conformation Capture will be used to determine whether the *rex* and *dox* sites contained in two well-characterized 190kb regions interact at long distances. Finally, we will establish the network of interactions of all the DCC binding sites present in a large representative portion of the X chromosome, using the method of Chromosome Conformation Capture Carbon Copy.

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1103B

Regulation of the synptonemal complex assembly during meiosis in *C. elegans* . Ceyda Bilgir[1], Joshua Skodack[1], Carolyn Dombecki[1], Anne Villeneuve[2], **Kentaro Nabeshima**[1]. 1) Dept Cell & Dev. Bio, Univ Michigan, Ann Arbor, MI; 2) Dept Dev. Bio, Stanford University, Stanford, CA.

During sexual reproduction, haploid gametes are generated by a specialized cell division program (meiosis) in which homologous chromosomes pair and their association is stabilized by a protein structure, the synaptonemal complex (SC). SC formation between homologous chromosomes is essential for reciprocal recombination that is required for faithful segregation of homologous chromosomes. In order to identify the genes responsible for proper homologous chromosome association during *C. elegans* meiosis, we have been conducting a genome-wide screen using: 1) a system for visualizing homologous pairing in live animals, and 2) a feeding RNAi library to knock-down gene function. This screen has allowed us to identify genes that were not previously known to function in pairing and/or synapsis. Here we report novel aspects of SC assembly discovered through the analyses of some of the newly identified genes. First, we found that *pgl-1*, a gene encoding a protein associated with P granules, was transiently required for the initiation of SC assembly after the shift to a high temperature. We found that the initiation of SC assembly was heat sensitive even in the wild type, which was further sensitized in *pgl-1* mutant, suggesting that PGL-1 is regulating a factor(s) for the initiation of SC assembly. Second, we found that *gld-2* and *gld-3*, components of a cytoplasmic poly(A) polymerase that is known to function in a mitosis-meiosis switch, were required for prompt SC assembly. In these mutants, SC formation was significantly delayed, and non-pairing center (PC) ends of chromosomes rarely showed homologous association. Interestingly, PCs showed stable homologous association during meiosis in these mutants, indicating SC was assembled at least at pairing centers. This observation suggests that initiation and progression of SC assembly are separable and *gld-2* and *gld-3* are involved in the latter process. Third, we found that *mrg-1*, a chromo-domain protein required for germ line development and X chromosome silencing in the germline, was required for proper synapsis establishment. The *mrg-1* mutants showed partial synapsis and pairing defect at the non-PC ends (but not at the PC ends) of chromosomes. SC assembly initiated and progressed with the apparently normal timing and kinetics. This finding suggests that there is a mechanism to establish and/or maintain SC that is dedicated to non-PC region of chromosomes, possibly through chromatin modification. We are currently investigating how this mechanism is operating using cytological and genetic analyses.

1104C

Dissecting the functions of the conserved Nup107-160 nuclear pore subcomplex. **Eduardo Rodenas**, Peter Askjaer. Universidad Pablo de Olavide-CSIC, Seville, Spain.

The nuclear envelope (NE) is the physical barrier between the nucleus and the cytoplasm. In addition, the NE plays important roles in regulation of gene expression, nuclear stability and anchoring of the chromatin. Within the NE we find macrostructures called nuclear pore complexes (NPC), that are evolutionary conserved assemblies that allow the traffic of proteins and RNA between the nucleus and the cytoplasm. These NPCs are composed of multiple copies of around 30 different proteins called nucleoporins. These nucleoporins interact with each other to form in some cases subcomplexes, such as the NPC subcomplex Nup107-160, consisting of 9 different nucleoporins. The Nup107-160 subcomplex plays important roles in nucleocytoplasmic transport, chromatin organization and kinetochore function. One of the main interests in our lab is to understand the mechanisms underlying the function of the Nup107-160 subcomplex through the analysis of its individual members. Among metazoa, Caenorhabditis elegans is an attractive model system to dissect genetically the functions of the individual Nup107-160 subcomplex members. Mutant alleles have been isolated for several of the Nup107-160 subcomplex genes1. We have initiated our studies with the analysis of deletion alleles of Nup107/npp-5 (ok1966, tm3039) and Nup133/npp-15 (ok1954). Using a broad variety of reporter strains we are investigating how these mutations affect to kinetochore functions, DNA segregation, nucleocytoplasmic transport and cellular response to stress during all stages of development. 1 http://celeganskoconsortium.omrf.org/ and http://www.shigen.nig.ac.jp/.

1105A

HIM-8 interacts with SUN-1. **Philip M. Meneely**, Lauren Burch, Scott Thompson, Maral Daou. Dept Biol, Haverford Col, Haverford, PA.

During homolog recognition and pairing in meiosis, pairing centers at or near one end of each meiotic chromosome are localized to the nuclear envelope. SUN-1 is an evolutionarily conserved protein located in the inner nuclear membrane that appears to be directly involved in mediating telomere attachment in mice and yeast; in worms, a direct interaction between pairing center proteins and SUN-1 at the nuclear envelope has not yet been proved (1, 2). In *sun-1* null mutants, none of the meiotic chromosomes are paired but the pairing centers are localized to the nuclear envelope (2). HIM-8 is a C2H2 zinc finger protein that binds specifically to the pairing center on the X chromosome and is necessary for the pairing of the X chromosome and for synapsis (3). When a zinc finger is mutated in *him-8*, such as in *e1489* or the *tm611* deletion, the protein does not bind to the X pairing center, the X chromosomes do not pair, but the chromosomes are still located at the nuclear envelope. Thus, pairing and nuclear envelope attachment may be separable activities for the pairing center proteins. The autosomes are paired normally in *him-8* mutants. The three ZIM proteins, which are highly related to HIM-8 in sequence, mediate pairing of the autosomes (4). We find that SUN-1 and HIM-8 physically associate with each other in a yeast two-hybrid assay. Preliminary evidence suggests a similar physical interaction occurs between SUN-1 and at least one of the ZIM proteins. This suggests a model whereby meiotic chromosomes are tethered to the nuclear envelope for pairing via a direct interaction between SUN-1 in the inner membrane and HIM-8 or one of the ZIM proteins on each specific chromosome. 1.Penkner et al. 2007. Develop. Cell 12: 873-885 2.Fridkin, et al., 2009. Cell Mol. Life Sci. 66: (in press) 3.Phillips et al. 2005 Cell 123: 1051-1063 4.Phillips and Dernburg 2006 Develop. Cell 11: 817-829.

1106B

ZHP-3 is regulated by multiple pathways during meiotic prophase. **Catherine L. Randall**, Needhi Bhalla. Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA.

The faithful segregation of chromosomes is tightly regulated to prevent aneuploidy. This is particularly true during meiosis: Defects in meiotic chromosome segregation can produce aneuploid embryos that are typically inviable but can also result in developmental disorders, such as Down syndrome. To ensure proper chromosome segregation during meiosis, homologous chromosomes pair, synapse, and undergo crossover recombination, resulting in their physical linkage. The homolog pair is then dramatically restructured around the crossover to generate a bivalent functional for meiotic chromosome segregation. Recent work has demonstrated that ZHP-3 has two distinct roles during meiotic prophase. First, it is required to promote crossover formation during early pachytene. Additionally, ZHP-3 is required for proper remodeling of the chromosomes to form a functional bivalent. In line with its multiple functions, ZHP-3 undergoes a dramatic relocalization during prophase. It is first localized along the synaptonemal complex (SC) in pachytene. Prior to SC disassembly, ZHP-3 localization becomes restricted to one part of the chromosome. Finally, in late pachytene and diplotene, ZHP-3 is restricted to a single focus on each pair of homologs which marks the site of the crossover. It remains unclear how ZHP-3 is regulated to complete its distinct functions and what controls its relocalization during late prophase. Several independent experiments are being performed to shed light on these mechanisms. First, a non-complementation screen is being performed to isolate *zhp-3* alleles with point mutations that specifically disrupt the late prophase role of ZHP-3. We expect that sequencing of these alleles will reveal residues important for the regulation of ZHP-3 in late prophase and provide insight into how ZHP-3 function is modulated to accomplish its two roles. In addition, we have found that ZHP-3 is regulated by the MAP Kinase (MAPK) pathway. The MAPK signaling cascade is required for a switch in the mode of double strand break repair, coincident with the restriction in ZHP-3 localization. Further, the asymmetric distribution of ZHP-3 is lost in *mpk-1(ga111ts)* strain at the restrictive temperature. We are testing for a genetic interaction between *zhp-3* and *mpk-1* by evaluating the phenotype of double mutants. Another intriguing feature of ZHP-3 is the presence of a conserved RING finger motif (RFM), a hallmark of ubiquitin E3 ligases. Experiments are underway to determine if ZHP-3 modifies meiotic proteins by the addition of ubiquitin. Together, these experiments will further our understanding of how ZHP-3 is regulated and how it acts to promote accurate chromosome segregation during meiotic cell division.

1107C

CRA-1 reveals a PC-independent and crossover-dependent pathway for assembly of SC proteins on unsynapsed axes. **Sarit Smolikov**, Sophie Zaaijer, Monica Colaiácovo. Dept of Genetics, Harvard Medical School, Boston, MA.

The proper assembly of the synaptonemal complex (SC) is essential for the formation of crossover events during meiosis. The SC consists of lateral element (LE) proteins that associate along the chromosomal axes, which in turn are joined by the central region (CR) proteins, resulting in chromosome synapsis. In mice, plants and yeast, SC formation is DSB-dependent. In contrast, SC formation is DSB-independent in both worms and flies. The evolutionarily conserved CRA-1 protein plays a key role in the assembly of the CR and in the stabilization of homologous pairing. Analysis of *cra-1* mutants revealed that DSB formation and repair play an important role in the polymerization of CR components along the unsynapsed chromosomal axes. *cra-1* double mutants with genes in the recombination pathway, exhibit severely impaired loading of CR proteins onto unsynapsed chromosomes. However, the mechanism of CRA-1 function remains unknown. We have undertaken several approaches to investigate this novel mechanism of recombination-dependent polymerization of SC components activated in *cra-1* mutants. To examine the progression of recombination in *cra-1* mutants we assessed the localization of ZHP-3, a marker for crossover sites. Although most chromosomes fail to undergo crossover recombination in *cra-1* mutants, ZHP-3 foci were observed on all chromosomes. These results suggest that crossover recombination is impaired after crossovers have been designated, but before they are implemented to form chiasmata. To test whether the recombination-dependent polymerization of SC components activated in *cra-1* mutants initiates in coordination with the DSB repair machinery, we examined the frequency of co-localization between RAD-51 (a strand-exchange protein) and SYP-1 (a CR protein) upon entrance to meiosis. Frequent co-localization of SYP-1 and RAD-51 was not observed in *cra-1* mutants, indicating that the assembly of SYP-1 may not initiate at all DSB repair sites, but instead, may be restricted to crossover designated sites. If recombination-dependent CR polymerization is coordinated with the DSB repair machinery, it may not be pairing center (PC)-dependent. In wild type, the chromosome localization of CR components requires PC proteins such as ZIM-2, which promotes pairing of chromosome V. However, SYP-1 is still able to localize to chromosome V in *cra-1;zim-2* mutants. This indicates that in the absence of *cra-1*, polymerization of CR components along unsynapsed chromosome axes is mostly PC-independent. These data, in combination with currently ongoing studies, will lead to a better understanding of the interplay between crossover formation and CR assembly in C. elegans meiosis.

1108A

Assembly and dynamics of meiosis-specific chromosome structures. **Weibin Zhang**, M. Zastrow, N. Miley, S. Mlynarczyk-Evans, G. Chen, A. Villeneuve. Stanford University, Stanford, CA, USA.

During meiosis, chromosomes assemble specialized structures that promote: 1) establishment and maintenance of homolog pairing, 2) crossing over between homologs and 3) segregation of homologs at the meiosis I division. We are taking several approaches to investigate the dynamic nature of these structures and how their assembly is regulated and coordinated with homolog pairing. During meiotic prophase, axial elements are assembled along the lengths of chromosomes and axes of aligned homologs are connected by the synaptonemal complex (SC). Axial elements are built on a foundation of the cohesin complex, made up of SMC-1, SMC-3, REC-8 and SCC-3. Whereas previous analysis (Chan et al. 2003) had suggested that SMC subunits can load independently of non-SMC subunits, we find that SMC-1 does not localize to chromosomes in *scc-3(ku263)* mutants, implying that SMC loading does require SCC-3. Instead SMC-1 is present in aggregates that also contain SC components SYP-1 and HIM-3. These aggregates are lost in *syp-1 scc-3* double mutants but chromosomal localization of SMC-1 is not restored, implying that SCC-3 plays a positive role in chromosomal loading of cohesin and does not function solely to inhibit premature associations of SC components and cohesin subunits. Our results indicate that SCC-3 is involved in the proper chromosomal assembly of cohesin and SC.

Prior to SC assembly, homolog pairing is accompanied by nuclear reorganization of chromosomes into a clustered configuration. Such nuclear reorganization is absent in pairing-defective *hal-2* mutants. In addition to defective pairing, *hal-2* mutants load SYP-1 incorrectly on unpaired homologs. Pairing at the pairing centers is substantially restored in *hal-2; syp-2* double mutants, implying that incorrect loading of SYP proteins is partially responsible for inhibiting homolog pairing. *hal-2; syp-2* mutants lack the extended region of clustered chromosome configuration seen in *syp* mutants, however, implying that HAL-2 has additional roles in promoting normal chromosome organization beyond inhibiting association of SYP proteins with unpaired homologs. Thus, we have identified HAL-2 as a novel component of the meiotic machinery involved in coordination of early meiotic events.

While EM images give an impression of the SC as a static scaffold-like structure, recent findings suggest that the SC is more dynamic than previously thought. We will conduct FRAP analysis to investigate SC protein dynamics, using strains expressing GFP- and mCherry-tagged SC components that correctly localize to chromosomes.

1109B

Size- and tissue-dependent endocytic transport of oral-administered nanoparticles. **Shin Sik Choi**, Joel Rothman. NRI, University of California Santa Barbara, Santa Barbara, CA.

Recent attention has been focused on nanomaterials owing to their broad potential for biological and medical applications. Nanoparticles fabricated from metals or polymers have been used to enhance the quality of biomedical images by increasing contrast of specific targets. Moreover, nanomaterials have been combined with medicines in an effort to prolong their in vivo half-lives. Most research into medical applications of nanoparticles has involved injecting them into an animal. We have found that polystyrene nanoparticles can undergo in vivo dispersion in *C. elegans* through oral administration. We found that orally administered nano-size polystyrene beads pass through the pharynx and are taken up into the intestine. When we fed animals with 50, 100, 200, and 500 nm polystyrene beads, only 50 and 100 nm particles were mobilized beyond the gut and were found to accumulate in the gonad and epidermis. Interestingly, nanoparticles were not detected in muscles or neurons. At a certain concentration, we found that nanoparticles induced toxic effects accompanied by pathological events, including defects in egg laying and disruption of the intestine. To explore the requirements for nanoparticle transport, we tested uptake of 50 and 100 nm particles in worms defective for genes involved in endocytosis and found that movement from the intestine to other sites was blocked in such mutants. Thus, inter-organ transport of nanoparticles from the intestine to the gonad and other tissues appears to involve endocytosis. These findings suggest that the upper limit in this size of nanoparticles that can undergo endocytic transport from the gut to other organs may be less than 200 nm.

1110C

A novel factor involved in ciliary specialization. **Anique Olivier-Mason**[1], David Doroquez[1], Ali Sarkeshik[2], John Yates[2], Piali Sengupta[1]. 1) Dept Molecular Cellular Biol, Brandeis Univ, Waltham, MA; 2) The Scripps Research Institute, La Jolla, CA.

Primary cilia are highly conserved sensory organelles present on almost all mammalian cell types. Cilia contain a central microtubular axoneme, are surrounded by a membrane, and house receptors and other signaling molecules for correct environmental sensation. Cilia are formed via intraflagellar transport (IFT), which traffics axoneme precursors and membrane proteins via anterograde kinesin and retrograde dynein motors. A subset of cilia, like the connecting cilium in photoreceptor cells and the kinocilium in the inner ear, have specialized functions and exhibit diverse morphologies. Although IFT is required for the formation of these specialized cilia, the mechanisms by which ciliary diversity is generated are unclear.

C. elegans has ~60 ciliated neurons, a subset of which exhibit highly specialized ciliary morphologies. Preliminary data from our lab suggest that ciliary diversity can arise from the differential deployment of IFT proteins and motors in different cilia types, as well as by environmental experience. In order to identify additional molecules that may play roles in ciliary morphological diversification, we immunoprecipitated GFP-tagged functional motor and IFT proteins from mixed stage animals, and identified associated ciliary proteins via mass spectrometry (also see abstract by Doroquez et al). In further mutant and expression analyses of candidate ciliary proteins, we identified a predicted motor protein gene, mutations in which disrupt the structures of only specific cilia, suggesting that this gene may play a role in generating ciliary structural diversity. Additional analyses of this motor protein, as well as analyses of other ciliary proteins will be presented.

1111A

P granules extend the nuclear pore environment in the C. elegans germ line. **Dustin Updike**, Susan Strome. Molecular Cell & Developmental Biology, University of California Santa Cruz, Santa Cruz, CA.

Germ granules are large, non-membrane-bound, ribonucleoprotein (RNP) organelles found in the germline cytoplasm of most, if not all, animals[1]. Like germ granules across species, P granules in C. elegans are found at the nuclear periphery[2], and are closely associated with nuclear pores in the germ line[3]. The C. elegans VASA homologs, GLH-1, GLH-2, and GLH-4, which are constitutively associated with P granules, resemble nuclear pore (NUP) proteins in being rich in FG (PheGly) repeats[4]. We hypothesized that the association between P granules and nuclear pores is facilitated by hydrophobic interactions between the FG repeats of the GLHs and NUPs. Consistent with this, P granules are dispersed when hydrophobic interactions are disrupted by aliphatic alcohols. In nuclear pores, hydrophobic interactions between FG repeat domains create a size exclusion barrier. We have shown that P granules impose a similar size exclusion barrier. The integral connection between P granules and nuclear pores is supported by results from a genome-wide RNAi screen for components required for proper P granule assembly and localization, in which we identified several nuclear pore associated factors. We are currently using a sensitive assay to measure interactions between FG repeat domains from GLH-1, GLH-2, GLH-4 and nuclear pore components identified in our screen. We propose that P granules extend the nuclear pore environment and provide a unique cytoplamic domain for post-transcriptional regulation in the germ line. 1.Eddy, E.M., Germ plasm and the differentiation of the germ cell line. Int Rev Cytol, 1975. 43: p. 229-80. 2.Strome, S. and W.B. Wood, Immunofluorescence visualization of germ-line-specific cytoplasmic granules in embryos, larvae, and adults of Caenorhabditis elegans. Proc Natl Acad Sci U S A, 1982. 79(5): p. 1558-62. 3.Pitt, J.N., J.A. Schisa, and J.R. Priess, P granules in the germ cells of Caenorhabditis elegans adults are associated with clusters of nuclear pores and contain RNA. Dev Biol, 2000. 219(2): p. 315-33. 4.Gruidl, M.E., et al., Multiple potential germ-line helicases are components of the germ-line-specific P granules of Caenorhabditis elegans. Proc Natl Acad Sci U S A, 1996. 93(24): p. 13837-42.

1112B

Drug-induced mitochondrial toxicity in *C. elegans*. **Richard de Boer**, Stanley Brul, Hans van der Spek. Molecular Biology and Microbial Food Safety, Swammerdam Institute for Life Sciences, Amsterdam, Netherlands.

Introduction: In recent years it has become increasingly clear that mitochondrial dysfunction can be a direct consequence of treatment with therapeutic drugs. The best studied example comprise the antiviral drugs used to treat HIV-1 infection, which have been shown to inhibit the mitochondrial (mt) DNA polymerase and deplete cells of mtDNA. Many studies have indicated mitochondrial toxicity as a cause of these side effects but the exact mechanism remains unknown. It has been shown that antiviral drugs can affect mtDNA replication, mitochondrial assembly and function, ROS production, and induce apoptotic and necrotic processes with severe clinical symptoms. However, most results have been obtained from patient- or cell culture studies. This poses limitations on the experiments that can be performed. Progress in this field is highly dependent on the development of good model systems. This project aims to address fundamental questions concerning the effects of drug-related toxicity on mitochondria, using *C. elegans* as a model system. **Methods:** To quantify mitochondrial DNA, Real Time PCR (ABS 7300 Real-Time PCR System) is used. Mitochondrial morphology is studied in a *C. elegans* strain with mitochondrially localized GFP using Fluorescent Microscopy. Quinone pool was studied by HPLC analysis. O_2 consumption rates were measured using a Clark-type electrode. Rates were normalized to protein content. **Results:** Several assays have been set up to study the effect(s) of anti-retroviral drugs on different aspects of mitochondrial function in *C. elegans*. Using Real-Time PCR we have been able to show a (concentration-dependent) decline in mtDNA copies when worms were cultured in the presence of anti-retroviral drugs. Using fluorescence microscopy we have been able to show that exposure to antiretroviral drugs can result in disruption of the mitochondrial network. Respiratory chain function has been studied by quinone reduction:oxidation levels and by oxygen consumption analysis. Preliminary data indicate that treatment with several drugs results in a more reduced quinone pool and to a decreased O_2 consumption. Interestingly, the observed effects are not necessarily coupled. **Conclusions:** Anti-retroviral drug treatment results in mitochondrial dysfunction in *C. elegans*, as indicated by mtDNA depletion, aberrant mitochondrial morphology, decreased lifespan, a shift in ubiquinone redox state and reduced oxygen consumption. Several of the effects are similar to those observed in patients on anti-retroviral therapy, indicating that *C. elegans* is a suitable model organism to study drug induced mitochondrial dysfunction.

1113C

C. elegans Nephrocystin-2 functionally interacts with the other nephrocystins to regulate cilia shape and position. **Andrew Jauregui**[1], Simon Warburton-Pitt[1], Chunmei Li[2], Michel Leroux[2], Maureen Barr[1]. 1) Rutgers University, Piscataway, NJ; 2) Simon Fraser University, Canada.

Nephronophthisis is a rare autosomal recessive nephropathy causing progressive renal failure in children and adolescents and is caused by mutation in one of nine different genes (NPHP1-NPHP9), which account for approximately 30% of all cases. Many NPHP genes are evolutionarily conserved, and the NPHP gene products localize to cilia in diverse organisms, however their role in cilia is largely unclear. We previously showed that *C. elegans* NPHP-1 and NPHP-4 regulate ciliary length and shape, and proposed that the nephrocystins are components of a transition zone (TZ) complex that regulates ciliary protein import and export. Our goal is develop *C. elegans* as a model organism to study the role the nephrocystins play in cilia and to define their molecular and genetic interactions.

We have begun to characterize the *C. elegans Inversin* homolog NPHP-2, to define its role in cilia, and to examine its interactions with the other nephrocystins. Nephronophthisis type 2 is caused by mutation in a gene called *Inversin*. In humans, *Inversin*/NPHP2 is associated with the infantile form of nephronophthisis causing situs inversus, enlarged kidneys, cyst formation and renal failure by the age of five. The *Inversin* protein localizes to cilia and is involved in the switch between canonical and non-canonical Wnt signaling, thus implicating the cilium in the regulation of Wnt signaling.

In *C. elegans, nphp-2* is expressed in the ciliated sensory nervous system, and encodes at least two splice forms with overlapping, yet distinct, localization patterns. *nphp-2* mutants have TZ placement and orientation defects that are not due to perturbation of the intraflagellar transport machinery required to build all cilia and flagella. While TZ localization of NPHP-1 or NPHP-4 was not altered in *nphp-2* mutants, a dramatic increase in NPHP-2::GFP fluorescence was seen in *nphp-4* mutants. *nphp-2* mRNA levels are not increased, suggesting increased stability or decreased degradation of NPHP-2 in *nphp-4* mutants. We are currently exploring the basis of this observation and hypothesize that NPHP-4 may be involved in the switch between the canonical and non-canonical Wnt pathways. Finally, using a yeast-two hybrid approach to study interactions between the nephrocystins, we have identified a nucleoporin that physically interacts with NPHP-4, NPHP-2, and OSM-6 and localizes to the ciliary base. We are currently exploring the function of this candidate and testing the ciliary TZ complex hypothesis.

1114A

The NPHP SynDyf genes act redundantly to regulate *C. elegans* ciliogenesis. **Simon R.F. Warburton-Pitt**, Andrew R. Jauregui, Maureen M. Barr. Rutgers University, Dept. of Genetics, Piscataway, NJ 08854.

In humans, cilia defects can lead to developmental abnormalities and disease. Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease and the most common genetic cause of end stage renal disease in children and young adults. NPHP is caused by mutation in one of at least nine different genes (*NPHP1-NPHP9*) account for less than 50% of all cases, hinting at the existence of other disease causing loci. NPHP extrarenal manifestations may include retinal degeneration, *situs inversus*, and neurological disorders, phenotypes that are also observed in Meckel (MKS), Senior-Løken (SLS), and Joubert (JBTS) Syndromes. In some instances, NPHP, MKS, SLS, and JBTS share loci.

C. elegans has proven to be a robust system for examining the roles of human ciliary disease gene homologs, many of which have conserved function and localization. *C. elegans* genome encodes *nphp-1*, *nphp-2*, *nphp-4*, *nphp-8*, and *nphp-9* homologs. We previously showed that the nephrocystins NPHP-1 and NPHP-4 act at the ciliary transition zone (TZ) to modulate ciliary length and shape. Using several cellular, behavioral and molecular assays, we have begun to unravel the role of *nphp-2*. While *nphp-2(gk653)* animals exhibit normal osmotic avoidance, foraging, and mating behaviors, *nphp-2* mutants have subtle phasmid dye filling (Dyf) defects. Utilizing fluorescent intraflagellar transport reporters, we observe that *nphp-2* mutants have defects in cilia length, and abnormal TZ placement. These errant morphological features suggest that *nphp-2* is involved in cilia position, construction, or maintenance.

Although *nphp*-2 single mutants exhibit a very subtle phasmid Dyf phenotype, *nphp-1; nphp-2* and *nphp-2 nphp-4* double mutants exhibited a severe amphid and phasmid Dyf phenotype. This synthetic Dyf (SynDyf) phenotype indicates that two pathways lead to normal cilia development, one involving *nphp-1* and *nphp-4*, the other involving *nphp-2*. To identify genes that act in the *nphp-2* and *nphp-1*/*nphp-4* pathways, we will use a candidate gene approach coupled with forward genetic screens for SynDyf mutants. The identification of new *nphp* genes or modifiers will provide valuable insight to the functions of the nephrocystins, cilia biology, and human ciliary diseases.

1115B

Vesicle trafficking in defense against pore forming toxins. **Ferdinand C O Los**, Cheng-Yuan Kao, Raffi V Aroian. Dept Cell & Dev Biol, UC San Diego, La Jolla, CA.

Pore forming toxins (PFTs) form the largest class of proteinaceous bacterial virulence factors. Many well known pathogenic bacteria, such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, rely greatly on their PFTs for host infection. Since many pathogenic bacteria use these virulence factors, learning how the immune system neutralizes them may have important implications for antibiotic-independent therapies that target them. The *Bacillus thuringiensis* (Bt) proteins Cry5B and Cry21A are PFTs that target *Caenorhabditis elegans*. Our lab is using *C. elegans* and these proteins to study, in an intact organism, the intrinsic cellular pathways and responses that defend against PFTs. Here, I will describe our work using various fluorescent markers for the intestinal apical plasma membrane and vesicle trafficking pathways. The effect of PFTs on *C. elegans* intestinal cells is very striking; we find dramatic changes in how membrane markers behave in response to the insertion of a PFT at the plasma membrane. These data suggest cells may be altering their endosomal recycling and lysosomal trafficking pathways to defend against membrane pores. In addition, initial studies of mutations or knock-downs of various vesicle trafficking components suggest loss of these genes may lead to hypersensitivity to PFTs, although these results are complicated by the pleiotropic effects of knocking down essential pathways. We conclude from these data, that, in line with recent results in mammalian cell systems, *C. elegans* employs endocytosis and vesicle trafficking pathways to try to neutralize pore forming toxins.

1116C

The GRP-1 Arf GEF and the CNT-2 Arf GAP proteins define two Arf cycles that regulate asymmetric neuroblast divisions in *C. elegans*. **K. Talavera**[1], S. Cordes[1,2], A. Singhvi[1,3], G. Garriga[1]. 1) University of California, Berkeley, Berkeley, CA; 2) Genentech, San Francisco; 3) Rockefeller Unviersity, New York, NY.

During asymmetric cell division (ACD) a mother cell divides forming two daughter cells that adopt different fates to generate cellular diversity. We have been studying genes required for the asymmetric division of the Q.p neuroblast, which divides to produce a cell that dies and the A/PVM-SDQ precursor. Loss of GRP-1 or CNT-2 causes Q.p to divide symmetrically to generate two precursors. This transformation results in an extra A/PVM and an extra SDQ. GRP-1 is the *C. elegans* ortholog of the cytohesin family of ARF guanine nucleotide exchange factors (GEFs) for Arf6. CNT-2 is a member of the AGAP family of Arf GAP proteins that stimulate the GTPase activity of Arf1. ADP ribosylation factors (Arfs) are a family of small GTPases that regulate vesicular trafficking. We have shown that the GRP-1 GEF activity and CNT-2 GAP activity is essential for their roles in asymmetric cell division.

We find that ARF-1 (class I Arf) and ARF-6 (class III Arf) are also involved in the Q.p division. Loss of either *arf* alone does not cause an obvious Q.p division defect, but *arf-1; arf-6* double mutants produce extra A/PVMs about 30% of the time. The simplest model would be that GRP-1 and CNT-2 define a single Arf cycle for the two Arfs that act redundantly, but genetic interactions suggest that the situation is more complex. Loss of ARF-1, but not ARF-6, enhances the Q.p defect of *grp-1* mutants, consistent with GRP-1 acting as a GEF for ARF-6. Loss of ARF-1, but not ARF-6, suppresses the Q.p defect of *cnt-2* mutants. We rationalize this result by proposing that the *cnt-2* mutant phenotype is caused by its cognate Arfs being trapped in an activated GTP bound state resulting in the mutant phenotype. Eliminating an Arf is predicted to decrease the amount of activated Arf activity and suppress the defect. Consistent with this interpretation, loss of ARF-1 but not ARF-6 enhances the Q.p division defect caused by a CNT-2 mutant protein with unregulated GAP activity. These results suggest that GRP-1 and ARF-6 define one cycle and that CNT-2 and ARF-1 define another. They also predict that genetic approaches can identify additional molecules that are part of the two cycles.

1117A

G protein signaling and the GMAP210 homologue SQL-1 modulate Intraflagellar Transport in *C. elegans*. **Joost Broekhuis**, Suzanne Rademakers, Martijn Dekkers, Jan Burghoorn, Gert Jansen. Cell Biology, ErasmusMC, Rotterdam, Zuid-Holland, Netherlands.

The cilia of *C. elegans'* amphid channel neurons can be divided into a middle and distal segment. Anterograde intraflagellar transport (IFT) in these cilia is mediated by two kinesin-2 complexes, kinesin II and OSM-3. In the middle segment OSM-3 and kinesin II move together at a speed of 0.7 μm/s, and in the distal segments OSM-3 moves alone at 1.2 μm/s. In the absence of *osm-3* kinesin II moves alone at 0.5 μm/s. The architecture of *C. elegans'* cilia suggests that cilia length and function can be dynamically regulated. To investigate whether sensory signals can modulate cilia or IFT we examined the cilia of *gpa-3* mutant animals. GPA3 is a sensory Gα protein that is expressed in all amphid neurons and involved in various sensory processes. Loss of *gpa-3 (lf)* does not affect cilia morphology, while a dominant active (*gpa-3QL*) mutation results in shortened cilia. In addition, we examined animals exposed to dauer pheromone, since previous studies have shown that dauer formation changes in the morphology of some cilia. Furthermore, mutations of gpa-3 affect dauer formation. We found that in both *gpa-3 (lf)* and *gpa-3QL* mutants, as well as in animals exposed to dauer pheromone, kinesin II and OSM-3 are at least partially uncoupled, while structural IFT particle proteins move at speeds intermediate to the two kinesins. This suggests that the cilia of *gpa-3* mutant animals contain two, possibly three, types of IFT particles: particles transported by OSM-3 or kinesin II alone, and perhaps a small subset transported by both kinesins. We propose a model in which GPA-3 regulated docking of either kinesin II, OSM-3 or both, determines entry of IFT particles into the cilia subdomains. This mechanism would allow plasticity of cilia structure and function. We performed a genetic screen for suppressors of *gpa-3QL* and identified *sql-1*, which encodes the homologue of the mammalian Golgi protein GMAP-210. *sql-1(lf)* suppresses the effect of *gpa-3QL* on cilia length, but does not seem to affect cilia morphology by itself. Speed measurements showed that in the middle segment of *sql-1(lf)* animals OSM-3 moves faster (0,85 μm/s) and kinesin II moves slower (0,6 μm/s), suggesting that the two kinesins are at least partially uncoupled. However, both complex A and B proteins move at the same speed as OSM-3. This suggests that in *sql-1(lf)* animals IFT is predominantly mediated by OSM-3 kinesin.

1118B

res-1 cooperates with *tat-1* to regulate PS asymmetry and membrane trafficking in *C. elegans*. **Baohui Chen**, Yue Jiang, Jiacong Yan, Sheng Zeng, Xin Li, Lina Yu, Xiaochen Wang. National Institute of Biological Sciences, Beijing, 102206, China.

The asymmetrical distribution of phosphatidylserine (PS) across plasma membrane is disrupted in apoptotic cells, which leads to the exposure of PS on the cell surface that serves as an "eat-me" signal for their clearance. How PS asymmetry is maintained and regulated during apoptosis and in other cellular processes is poorly understood. From a genetic screen for regulators of engulfment signal, we isolated several alleles of *tat-1* (transbilayer amphipath transporters) and one allele of *res-1* (regulator of engulfment signals). *tat-1* encodes a P-type ATPase that is suggested to promote the inward movement of aminophospholipids such as PS and PE, which restricts them to the inner leaflet of cell membrane. Consistent with previous findings (1), we found that in the *tat-1* mutants, the surface-exposed PS is detected not only on apoptotic cells, but also on living cells. Identical phenotypes were also observed in the *res-1*(*qx36*) mutants, indicating that *res-1* is also involved in maintaining PS asymmetry on plasma membrane. Interestingly, we found that both *tat-1* and *res-1* mutants display severe defects in membrane trafficking including intestinal vacuolation and endocytosis defects in both intestine and coelomocytes. TAT-1 is localized to plasma membrane and intracellular vesicles, which is consistent with its function in maintaining PS asymmetry across cell membrane and in endocytosis (1, 2). Importantly, the membranous and vesicular localization of TAT-1 is totally disrupted in the *res-1*(*qx36*) mutants. On the other hand, RES-1::GFP showed similar localization pattern to that of TAT-1, which is abolished in the *tat-1*(*qx30*) mutants. Therefore, RES-1 and TAT-1 likely cooperates with each other to regulate PS asymmetry and membrane trafficking in *C. elegans*. We will report our characterizations of RES-1 and TAT-1 in the meeting. 1.Monica Darland-Ransom, Xiaochen Wang, Chun-Ling Sun, James Mapes, Keiko Gengyo-Ando, Shohei Mitani, Ding Xue, 2008. Role of *C. elegans* TAT-1 protein in maintaining plasma membrane phosphatidylserine asymmetry. Science 320: 528-531 2.Anne-Francoise Ruaud, Lars Nilssoon, Fabrice Richard, Morten Krog Larsen, Jen-Louis Bessereau and Simon Tuck, 2009. The *C. elegans* P4-ATPase TAT-1 regulates lysosome biogenesis and endocytosis. Traffic 10(1): 88-100.

1119C

TBC-2 is a New Regulator of Rab GTPase-mediated Endocytic Trafficking. **Laetitia Chotard**, Marc-Andre Sylvain, Christian Rocheleau. Dept Medicine, McGill Univ, Montreal, PQ, Canada.

Rab GTPases are key regulators of vesicular trafficking and act as molecular switches, alternating between an active GTP-bound state and an inactive GDP-bound state. Rab5 and Rab7 regulate endocytic trafficking from the plasma membrane to the lysosome. While Rab5 is required for endocytosis and trafficking of early endosomes, Rab7 is required for trafficking of cargo from the early endosome to the late endosome and the subsequent fusion with the lysosome. Different views had been proposed to explain how cargo transport occurs from early to late endosomal compartments. The current model is the Rab5 to Rab7 conversion based a "cut-out switch" in which active Rab5 recruits the HOPS complex, a GTPase Exchange Factor (GEF) for Rab7, and thus might facilitate the recruitment of Rab7 on endosomes. Then, Rab7 may inactivate Rab5 through a negative feedback loop. However, the molecular mechanisms that drive the dynamic of Rab conversion are not fully understood. TBC-2 is a putative Rab GTPase Activating Protein (RabGAP), and thus a potential negative regulator of Rab GTPase activity. We found that *tbc-2(tm2241)* mutants accumulate enormous endosomes in the intestine with refractile material. While few of these are RAB-5-positive early endosomes, the majority are RAB-7 and LMP-1 positive late endosomes/lysosomes. Reducing the activity of *rab-7* can completely suppress the large endosome phenotype, and while expression of *tbc-2(+)* in the intestine can rescue the phenotype, a predicted catalytically inactive version *tbc-2(R687K)* cannot, consistent with TBC-2 negatively regulating RAB-7 activity. This is further supported by the strong colocalization seen between TBC-2 and RAB-7 on late endosomes and the accumulation of autophagic proteins within the large late endosomes. Surprisingly, we found that expression of constitutively active RAB-5 in the intestine can phenocopy the *tbc-2* mutant, suggesting that RAB-5 could be the target of TBC-2 activity. To identify the Rab substrate of TBC-2, we are using binding assays and in vitro GAP assays in collaboration with David Lambright's laboratory at the University of Massachusetts Medical School. Our data suggests that activated RAB-5 can drive activation of RAB-7, consistent with the Rab conversion model, and suggests that TBC-2 regulates this process.

1120A

Autophagy can rescue cellular but not developmental defects of endosomal maturation mutants. **Abderazak Djeddi**, Xavier Michelet, Adriana Alberti, Renaud Legouis. CGM, CNRS, GIF-SUR-YVETTE, France.

Endocytosis and autophagy are two major processes required for lysosome-dependant degradative pathway in eukaryotic cells. Endocytosis plays a key role in degrading and recycling extracellular and plasma membrane proteins and downregulating receptor-mediated signalling. Autophagy is responsible for non-selective intracellular components degradation allowing cell survival under starvation conditions and plays an essential role during C. elegans development. During autophagy, double-membrane vesicles named autophagosomes sequester cytoplasm and fuse with lysosomes. Our aim is to understand the interaction between autophagy and endocytosis during C. elegans development. We have previously demonstrated that the inactivation of Class E vps genes required for endosome maturation leads to various developmental defects ranging from embryonic lethality to no obvious phenotype. Using an RNAi approach against 9 vps-E genes we monitored the localisation of an endosomal and an autophagosomal marker respectively VPS-27::GFP and GFP::LGG-1. RNAi inactivation of lvps-E genes leads to an accumulation of enlarged endosomal structures and subsequently an increase of the autophagic process. Noticeably, the apparition of enlarged endosomes in vps-E mutants was not directly correlated with the stage of lethality which, in contrast was always concomitant with the increase of autophagy. We hypothesized that GFP::LGG-1 subcellular localisation in vps-E mutants could result either from autophagosomes-lysosome defective fusion or from an indirect response to maintain homeostasis. In higher eukaryotes, autophagosomes are able to fuse with late endosomes, so we first asked whether autophagosomes and endosomes share common compartments. We have performed immuno-colocalisation experiments of the autophagic marker GFP::LGG-1 with markers of early or late endosomes. No vesicle was positive for both markers neither in wild-type animals nor in vps-E mutants suggesting that there is no common compartment between autophagosomes and endosomes in C. elegans. Thus, the autophagosomal accumulation is not due to a blockage of phagosome-to-lysosome fusion. Next we used a genetic approach to analyse whether autophagy is responsible for lethality in vps-E mutants. Using RNAi , we induced or reduced the level of autophagy in several vps-E mutant backgrounds. We observed that an induction of autophagy may correct vps-E cellular defects while a reduction hastens cellular degradation. However an increase of autophagy could only delay but not suppress the lethal phenotype. According to these results we propose that in vps-E mutants autophagy is a secondary response in an attempt to preserve the homeostasis of the cell from endocytosis defects.

1121B

The cation diffusion facilitator gene cdf-2 mediates zinc metabolism in Caenorhabditis elegans. Diana Davis[1], Hyun Roh[1], Krupa Deshmukh[1], Janelle Bruinsma[1], Daniel Schneider[1], James Guthrie[2], J. David Robertson[2], **Kerry Kornfeld[1]**. 1) Department of Developmental Biology, Washington University School of Medicine, Saint Louis, MO; 2) Research Reactor Center and Department of Chemistry, University of Missouri, Columbia, MO.

Zinc is essential for many cellular processes. To use C. elegans to study zinc metabolism, we developed culture conditions allowing full control of dietary zinc and methods to measure zinc content of animals. Dietary zinc dramatically effected growth and zinc content; wild-type worms survived from 7 μM to 1.3 mM, and zinc content varied 27 fold. We investigated cdf-2, which encodes a predicted zinc transporter in the cation diffusion facilitator family. cdf-2 mRNA levels were increased by high dietary zinc, suggesting cdf-2 promotes zinc homeostasis. CDF-2 protein was expressed in intestinal cells and localized to cytosolic vesicles. A cdf-2 loss-of-function mutant displayed impaired growth and reduced zinc content, indicating that CDF-2 stores zinc by transport into the lumen of vesicles. The relationships between three cdf genes, cdf-1, cdf-2, and sur-7, were analyzed in double and triple mutant animals. A cdf-1 mutant displayed increased zinc content, whereas a cdf-1 cdf-2 double mutant had intermediate zinc content, suggesting cdf-1 and cdf-2 have antagonistic functions. These studies advance C. elegans as a model of zinc metabolism and identify cdf-2 as a new gene that has a critical role in zinc storage.

1122C

LGG-1 and LGG-2 play redundant but unequal roles in autophagy and longevity in C. elegans. Adriana Alberti, Abderazak Djeddi, Xavier Michelet, **Renaud Legouis**. CGM, CNRS, Gif-sur-Yvette, France.

Autophagy is a general term for the degradation of cytoplasmic components which is essential for survival, differentiation, development and homeostasis. Autophagy involves the formation of double membrane vesicles containing a portion of the cytoplasm, named the autophagosomes, which finally fuse with the lysosomes. During this process two ubiquitin-like proteins Atg8 and Atg12 are associated to the membrane of the autophagosomes. In this report we have investigated the precise function and localization of the C. elegans homologs of Atg8. C. elegans genome contains two Atg8 homologues named LGG-1 and LGG-2. Phylogenetic and functional analyses revealed that LGG-1 is the paralogue of the yeast protein Atg8p and is closely related to human GABARAP (respectively 53% and 83% identity) whereas LGG-2 is homolog to HsMAPLC-3A (59% identity) which has been shown to be an autophagosomal marker in mammals. Melendez and colleagues have previously demonstrated that during dauer larva formation, a process in which autophagy is induced, GFP::LGG-1 changes its localization in the lateral hypodermal seam cells from diffuse in the cytoplasm to a punctuate pattern. Using GFP fusion proteins, we show that LGG-2 and LGG-1 present overlapping expression patterns and localizations during development. Both proteins present a dual localization with a diffuse cytoplasmic pattern and the presence of discrete punctuated structures. We then analyzed the modifications of their localization in several biological conditions during which autophagy is induced, namely the dauer formation, senescence and starvation. Upon induction of autophagy, the localization of both proteins is modified but the dynamic of accumulation of puncta is different. The increase in LGG-1 expression is rapid and transient whereas LGG-2 accumulates over time. Using RNAi feeding approach to deplete LGG-2 and LGG-1 we demonstrated that LGG-2 is also essential for dauer induction and longevity of the worm. Moreover, the depletion of both LGG-2 and LGG-1 produces a synergistic defect on development and life span. Our data indicate that the proteins have partially redundant functions but could also have specific functions.

1123A

Identification of proteins that regulate intraflagellar transport. **W.Y. Leong**, S. Rademakers, G. Jansen. Cell Biology, Erasmus MC, Rotterdam, South Holland, Netherlands.

The cilium is an important microtubule (MT)-based apparatus that performs diverse roles in motility, sensory perception and signaling. Building, maintaining and functioning of cilia require IFT (Intraflagellar Transport). IFT mediates transport from the base of the cilia to the tip (anterograde), using kinesin-2 motor complexes and back to the base (retrograde), using dynein complexes. Recent studies suggest that IFT regulates cilia length and signaling. We study the regulation of IFT in *C. elegans*. *C. elegans* has 60 ciliated neurons including eight pairs of amphid channel neurons exposed to the environment. The cilia of these amphid neurons can be further divided into a middle segment with nine doublet microtubules and a distal segment with nine singlet microtubules. In the middle segments, the heterotrimeric kinesin II (encoded by *klp-11*, *klp-20*, and *kap-1*) and homodimeric kinesin OSM-3 mediate anterograde transport whereas only OSM-3 mediates transport in the distal segments. We recently identified the *dyf-5* gene, a conserved MAP kinase, which is involved in the regulation of IFT in *C. elegans*. *Dyf-5* loss-of-function (*lf*) mutants were found to have elongated cilia, while animals over-expressing *dyf-5* have shorter cilia. In animals that lack *dyf-5*, IFT particles are only transported by the kinesin II and not OSM-3, although OSM-3 is still present and moves along the microtubular axoneme, but at a reduced speed. In addition, kinesin II is no longer restricted to the middle segments but also enters the distal segment. In order to understand the role of DYF-5 in the regulation of IFT, we will use a biochemical and a genetic approach. The biochemical approach involves the use of in vivo biotinylation of DYF-5, pull-down of biotinylated complexes and mass spectrometry to find proteins which function in the same complex as DYF-5. C- and N-terminally tagged constructs of the wild type *dyf-5* as well as their kinase dead equivalents have been made. Currently, we are establishing transgenic strains that carry these constructs in combination with a birA biotin ligase expression construct. In the genetic approach we are performing suppressor screens to identify mutants that suppress the dye-filling defect of *dyf-5* loss- or gain-of-function mutations.

1124B

Exploring the molecular basis of glycosphingolipid function. Esther Marza[1], **Giovanni M. Lesa[1,2]**. 1) MRC Laboratory for Molecular Cell Biology, UCL, London, United Kingdom; 2) Department of Cell and Developmental Biology, UCL, London, United Kingdom.

Glycosphingolipids (GSLs) are complex glycolipids in the lipid bilayer. Their ubiquitous distribution and complexity suggest that they have important functions, but what these are in vivo is still poorly understood. GSLs consist of a ceramide lipid moiety (a fatty acid chain linked to a sphingoid base) embedded in the cell membrane, which is attached to a wide variety of oligosaccharide structures that extend from the non-cytosolic leaflet of the lipid bilayer.

The precursor of all *C. elegans* glycosphingolipids, glucosylceramide, is synthesized in the lumenal leaflet of the Golgi membrane by ceramide glucosyltransferase (CGT), which catalyzes the addition of glucose to ceramide.

The *C. elegans* genome encodes for three CGTs, *cgt-1*, *cgt-2* and *cgt-3* and we have shown that animals lacking these enzymes do not synthesize GSLs, arrest growth at the first larval stage (L1) and display defects in a small subset of cells in their digestive tract. These defects impair larval feeding resulting in starvation-induced growth arrest. We have also found that restoring CGT function in these digestive tract cells is sufficient to rescue the phenotype associated with loss of CGT function, suggesting that GSLs are only essential in those cells.

To start clarifying the molecular basis of GSL function, we carried out a pilot Ethyl Methane Sulfonate-based genetic screen designed to identify mutations that result in growth arrest/death only in the absence of *cgt-1* activity. For this, we used balanced *cgt-1* mutants animals expressing *cgt-1(+)* DNA and performed an F2 clonal screen. We mutagenized approximately 2,000 gametes and found two candidate mutations, provisionally named 54C6 and S143M. Both mutations map on chromosome I and don't complement each other, suggesting that they are mutations in different genes. In addition, while 54C6 is synthetically lethal with both *cgt-1* and *cgt-3*, S143M is synthetically lethal only with *cgt-1*.

We used SNIP SNP mapping to place 54C6 close to and on the right of *unc-29*. We are now testing candidate genes by RNAi for their ability to phenocopy the mutant.

Finding out the molecular nature of 54C6 and S143M will shed light on the molecular basis of GSL function.

1125C

EXC-5 Maintains Subcellular Organelle Distribution. **Brendan C. Mattingly**, Elinor M. Brown, Matthew Buechner. Molecular Biosciences, Univ Kansas, Lawrence, KS.

The *C. elegans* excretory canal cell is a long "H"-shaped tubular structure that serves as the osmoregulatory organ for the animal. Null mutations in *exc-5* cause the canals to swell into large fluid-filled cysts, sometimes expanding to as large as the entire body diameter of the worm. These cysts form as a result of a mechanical failure of the actin-based cytoskeleton lining the canal apical surface. Overexpression of *exc-5* leaves the apical surface intact, but causes defects in the formation of the basal surface of the canal. This results in a convoluted tubule phenotype, similar to that of laminin alpha chain mosaics.

EXC-5 represents the sole nematode homologue of a family of guanine nucleotide exchange factors called FGD, that is conserved throughout animal evolution. Humans carrying defective copies of either FGD1 or FGD4 develop the genetic disorders FacioGenital Dysplasia (Aarskog Syndrome) or Charcot-Marie-Tooth Disease type 4H, respectively. In the latter disease, the single-celled tubular glia of the peripheral nervous system are no longer able to maintain their apically-secreted myelin layer.

In order to determine the subcellular processes altered by loss or overexpression of *exc-5*, we created lines of animals expressing subcellular markers (the generous gift of Barth Grant) within the canals. mCherry-labeled proteins include EEA-1 (early endosomes), CHC-1 (clathrin), CDC-42, RAB-5 (endocytic vesicles) EEA-1 (early endosomes), RAB-7 (late endosomes), RAB-11 and RME-1 (recycling endosomes), GRIP (Golgi), and GLO-1 (lysosomes). These lines show punctate location and organization of many of these compartments along the length of the canals.

In addition, we have crossed these labeled strains to an *exc-5* (*rh232*) null mutant, and to a line overexpressing *exc-5*. We have examined the overlapping expression of these markers with EXC-5 to determine the subcellular location of this protein. In addition, the effects of mild overexpression of EXC-5::GFP on canal morphology are enhanced when combined with expression of some of the markers. We conclude that EXC-5 activity on the actin cytoskeleton regulates passage of material through recycling endosomes to regulate replacement of material necessary for maintaining the apical tubular surface.

1126A

EM analysis of the adaptor complex AP-1 loss of function phenotype reveals polarity and endocytosis defects. Massi Shafaq-zadah[1], Yannick Schwab[2], Michel Labouesse[2], **Gregoire Michaux[1]**. 1) IGDR, Rennes, France; 2) IGBMC, Strasbourg, France.

We are interested in the function of the AP-1 complex, a clathrin adaptor which is implicated in membrane traffic. AP-1 loss of function induces a developmental arrest at the 3-fold stage (see also Shafaq-zadah & Michaux abstract). In order to study this phenotype, we decided to use electron microscopy (EM). WT adults were RNAi treated by feeding for three hours and the embryos were fixed 10 hours later by high pressure freezing, followed by freeze substitution. We examined hypodermal and intestinal cells for polarity and trafficking defects. In the hypodermis, all intracellular organelles looked normal, including ER, Golgi and various classes of endosomes and lysosomes. Although the cuticle was sometimes irregular in thickness alae could be identified even in 3-fold arrested embryos, indicating no major problem in cuticle synthesis and secretion, as also shown by a normal morphology and number of light multivesicular bodies (MVB) containing exosomes (Liégeois et al, JCB, 2006). Furthermore it demonstrates that the arrest in elongation does not affect the rest of the developmental process. However apical junctions were abnormal, too long and mislocalised along the lateral membrane instead of being subapical; additionally seam cells were sometimes bulging. We are now planning to perform immuno-EM to analyse the precise localisation of various junction markers. In the intestine, several phenotypes were observed. First, there was an accumulation of MVBs and lysosomes between the apical pole and the nucleus. We also observed that small and dense vesicular structures of about 100nm in diameter and restricted to the apical cortex were absent in AP-1(RNAi) embryos. Based on the disappearance of RAB-11::GFP positive apical endosomes in these embryos, as seen by confocal microscopy, we propose that these vesicular structures easily identified by EM could be apical recycling endosomes. Immuno-EM will allow us to test that hypothesis. We also observed defects at the apical pole with microvilli disorganised and/or abnormally long. Finally, apical junctions displayed abnormalities in length, although not misplaced as in the hypodermis. A surprising observation was that the intestinal lumen was full of cellular debris, as well as the space between the embryo and the eggshell. We interpret that as leaking of cells or of parts of cells which then float in the egg cavity and can be ingested; basically the embryo is feeding on itself. This is likely due to the loss of adherence between hypodermal cells, leading to cellular debris detaching from the hypodermal cells. Indeed these cells often have irregular shapes compared to control embryos; contrary to this intestinal cells do not look affected in a similar way.

1127B

Does UNC-89 mediate excitation-contraction coupling in *C. elegans*? Patrick Spooner, **Kenneth Norman**. Center for Cell Biology & Cancer Research, Albany Medical College, Albany, NY.

Ca2+ is a ubiquitous signaling molecule that is either directly or indirectly involved in most if not all cell physiological functions. Previously, we have found that the Rho GTPase family guanine nucleotide exchange factor, VAV-1 is a crucial component involved in mediating rhythmic activities by regulating Ca2+ oscillations. To understand the role VAV-1 plays in Ca2+ regulation, we have generated transgenic strains that overexpress constitutively active VAV-1. These animals display abnormal rhythmic activities, including altered defecation cycle, ovulation and uncoordinated locomotion. To gain insight into the mechanism(s) underlying VAV-1's role in regulating Ca2+, we have conducted a suppressor screen to identify mutations that can suppress the Unc phenotype associated with expression of constitutively active VAV-1 (see A. Fry's poster). From the suppressors isolated, two suppressors are alleles of *unc-89*. *unc-89* encodes several isoforms that are part of the titin immunoglobulin (Ig) superfamily, which includes several large Ig rich isoforms (>750 kDa) and two small isoforms (~160 kDa) (Benian et al., 1996; Small et al., 2004). UNC-89 and its mammalian homolog, obscurin, have been implicated in myofilament assembly and maintenance. To understand how *unc-89* mutants can suppress constitutively active VAV-1, we are addressing the following questions: 1) Does the disorganization of the myofilaments in *unc-89* mutations lead to disrupted actomyosin contraction, 2) Is the sarco-endoplasmic reticulum (SR) disorganized in *unc-89* mutants, 3) Do *unc-89* mutations affect Ca2+ signaling events required for excitation-contraction coupling? Thus far, we have found that the alleles of *unc-89* that suppress constitutively active VAV-1 are slightly slow moving but show normal sinusoidal locomotion. This argues that contraction is not disrupted but may be reduced since these animals are slow moving. Additionally, we have found that in the late embryo and early larvae the myofilaments are normally arranged in *unc-89* mutants; however, in late larval/adult stages the myofilaments are disorganized. This suggests that UNC-89 is important for growth and/or maintenance of the myofilaments. Additionally, we have found that the SR as well as UNC-68/Ryanodine receptors are also disorganized in *unc-89* adults, which could lead to abnormal Ca2+ influx from the SR. Thus far, our data suggests that UNC-89 is important for myofilament/SR organization in adults and that loss of UNC-89 activity leads to reduced muscle activity either by reducing the amount of Ca2+ inducing myofilament contraction or altering myofilament sensitivity to Ca2+. Benian et al., 1996 JCB 132: 835-848. Small et al., 2004 J Mol Biol 342: 91-108.

1128C

The characterization and molecular identification of *glo-2*, a gene necessary for gut granule biogenesis. **Becca Salesky**, Allison Weis, Elizabeth Kwan, Beverley Rabbitts, Greg Hermann. Department of Biology, Lewis & Clark College, Porltand, OR.

Caenorhabditis elegans intestinal cells are characterized by the presence of gut granules, lysosome-related organelles that contain autofluorescent and birefringent material. Gut granule formation requires the activity of AP-3 subunits, the HOPS complex, and GLO-1/Rab38, genes whose homologues function in trafficking to lysosomes and lysosome-related organelles. Mutations in these genes result in the loss and/or mislocalization of birefringent material into the embryonic intestinal lumen (the *glo* phenotype). Here we present our phenotypic and molecular analysis of an uncloned gene required for gut granule biogenesis, *glo-2*. *glo-2* was defined by a single mutant allele, *zu455*, which has a maternal effect gut granule biogenesis defect. *glo-2* mutants contain significantly reduced numbers of acidic, fat containing, and terminal endocytic gut granules in their intestinal cells. Embryonic intestinal cells lack multiple markers associated with gut granules. Our genetic studies implicate *glo-2* function in gut granule biogenesis pathways distinct from those currently implicated in gut granule formation. We localized *glo-2* to a 0.4 map-unit region of chromosome I. We found that a *tm* deletion allele in this region phenocopied and did not complement *glo-2(zu455)*. A fosmid clone spanning the region containing the tm deletion rescued the glo phenotypes of both the *tm* and *zu* alleles of *glo-2*. We are currently performing experiments to identify the gene affected in *glo-2(-)*.

1129A

Multiple types of lysosomal compartments co-exist within *C. elegans* embryonic intestinal cells. **Emily Scavarda**, Sage Coe Smith, Brian King, Greg Hermann. Department of Biology, Lewis and Clark College, Portland, OR.

We are examining whether *C. elegans* embryonic intestinal cells contain multiple types of lysosomes. Ours and others results support the idea that three lysosomal compartments, gut granules, yolk platelets, and LMP-1::GFP containing organelles, are present within the *C. elegans* intestinal primordium. To address their lysosomal character we have examined these organelles for: (1) acidification, (2) the presence of subunits of the V-ATPase, (3) terminal endocytic activity, (4) the presence of a transporter predicted to mediate export from lysosomes, and (5) the presence of putative degradative proteases and lipases predicted to function within lysosomes. Our results indicate that gut granules, while being acidified and containing subunits of the V-ATPase, do not appear to be a major site of degradation. Instead LMP-1::GFP labeled compartments, which are not acidified and do not contain subunits of the V-ATPase, contain a number of proteins predicted to function in degradative lysosomal processes and a transporter predicted to function in the export of degraded products into the cytoplasm and therefore appear to function as degradative sites within embryonic intestinal cells. In our studies, yolk platelets do not appear to have characteristics of lysosomal compartments. Our work points to gut granules and LMP-1::GFP containing compartments as lysosomes within the embryonic intestine and provide a genetic system to investigate how multiple lysosomal compartments are formed in the same cell.

1130B

The adaptor complex AP-1 is required for maintenance of epithelial polarity and several endocytic routes. **Massi Shafaq-zadah**, Gregoire Michaux. IGDR, Rennes, France.

The adaptor complex AP-1 is implicated in several post-Golgi trafficking routes, including TGN–endosomes and targeting to the basolateral membrane in mammalian epithelial cells. In *C. elegans* complete loss of function of AP-1 by RNAi leads to embryonic arrest at the 3-fold stage. We have decided to analyse this phenotype to understand the cause of lethality and better characterise the function of AP-1 during embryogenesis. We will show that AP-1 is required for maintenance of epithelial polarity, receptor mediated endocytosis, late endosome maturation and recycling. Because arrest during elongation can be triggered by defects in epithelial polarity or endocytosis, we have analysed these two processes. Although the establishment of polarity is normal as assessed with various fluorescent markers, electron microscopy (EM) studies have revealed a default in apical junction maintenance in hypodermal epithelial cells, with longer junctions migrating toward the basal pole along the lateral membrane instead of being strictly subapical. FRAP experiments and immuno-EM localisation of junction markers will be used to analyse this phenotype in more details. We also characterised endocytic routes and show that early endosomes are not affected. However, late endosomes and lysosomes accumulate in intestinal epithelial cells, as shown both with fluorescent markers and by EM. Performing RNAi after embryonic development also allows us to study AP-1 function during larval development. We examined yolk uptake by oocytes and showed that AP-1(RNAi) adults display an Rme (Receptor Mediated Endocytosis) phenotype presumably through a defect in RME-2–the yolk receptor–trafficking, a hypothesis we are now testing. Finally, we examined recycling endosomes and found that RAB-10 basolateral endosomes are unaffected, while RAB-11 apical endosomes disappear from the apical membrane in intestinal cells. Taken together, these results suggest new functions for AP-1 in maturation of late endosomes and recycling, roles not detected previously. We also found that AP-1 depletion does not affect secretion of various markers including ss::GFP, UNC-52, cuticle and yolk. To analyse the functions of the different AP-1 subunits, we specifically targeted each of them in turn. All these phenotypes were observed when the sigma (*aps-1*) or gamma (*apg-1*) subunits were targeted. We were also able to recapitulate most of these observations in the mu1-II (*apm-1*) knock-down, albeit at a later stage. On the contrary, mu1-I (*unc-101*) mutants did not display any of these phenotypes. This confirms a general requirement for AP-1-APM-1 and suggests a more specific role for AP-1-UNC-101 in neurons.

1131C

Calcineurin regulates differential dynamin-dependent endocytic processes in *C. elegans*. **Hyun-Ok Song**[1], Jungsoo Lee[2], Yon Ju Ji[3], Jeong Hoon Cho[4], Byung-Jae Park[5], Joohong Ahnn[6]. 1) Cell Dynamics Research Center, Department of Life Science, GIST, Gwangju 500-712, Republic of Korea; 2) Ernest Gallo Clinic and Research Center, Department of Neurology, Programs in Neuroscience and Biomedical Science, University of California, San Francisco, Emeryville, California 94608, USA; 3) Laboratory of Cell and Developmental Signaling, National Cancer Institute-Frederick, Frederick, Maryland 21702, USA; 4) Division of Biology Education, College of Education, Chosun University, Gwangju 501-759, Republic of Korea; 5) Department of Life Science, Hallym University, Chunchon, Gangwondo 200-702, Republic of Korea; 6) Laboratory of Developmental Genetics, Department of Life Science, College of Natural Sciences, Hanyang University, Seoul 133-791, Republic of Korea.

C. elegans has been an ideal model organism to reveal in vivo mechanisms of clathrin-mediated endocytosis. One major clathrin-mediated endocytic process in *C. elegans* utilizes 6 macrophage-like scavenger cells called coelomocytes to endocytose foreign molecules in the body cavity. Recently, several genes are identified to be probably involved in coelomocyte endocytosis using *C. elegans in vivo* monitoring assay system. However, the exact mechanism is still unknown. Here, we report a possible function of calcineurin, which is evolutionally, conserved a Ca^{2+}/calmodulin-dependent Ser/Thr protein phosphatase, in coelomocyte endocytosis. We observed that calcineurin mutants were defective for coelomocyte endocytosis and function upstream of the nicotinic acetylcholine receptor CUP-4 to regulate the receptor. Calcineurin also interacts directly with the *C. elegans* dynamin homolog DYN-1, a large GTPase protein that we show here also regulates CUP-4. Although calcineurin does not appear to be involved in receptor-mediated endocytosis in the oocytes, we observed that both calcineurin and dynamin are also necessary for synaptic vesicle recycling in neurons. We speculate that calcineurin may regulate multiple endocytic processes in the nematode by dephosphorylating DYN-1.

1132A

Comparisons of the subcellular localization and mutant phenotypes of intracellular ABC transporters HAF-4 and HAF-9 with LMP-1 in *C. elegans* intestinal cells. **Takahiro Tanji**[1], Hirohisa Shiraishi[1], Yasuo Yoshida[2], Koujiro Tohyama[2], Keiko Gengyo-Ando[3], Shohei Mitani[3], Masatomo Maeda[4], Ayako Ohashi-Kobayashi[1]. 1) Department of Immunobiology, School of Pharmacy, Iwate Medical University, Yahaba, Iwate, Japan; 2) The Center for Electron Microscopy and Bio-Imaging Research, Iwate Medical University, Morioka, Iwate, Japan; 3) Department of Physiology, School of Medicine, Tokyo Women's Medical University, Tokyo, Japan; 4) Department of Molecular Biology, School of Pharmacy, Iwate Medical University, Yahaba, Iwate, Japan.

HAF-4 and HAF-9 are half-type ABC transporters highly homologous to human lysosomal transporter TAPL (TAP-like; ABCB9). Although TAPL putatively conveys peptides from cytosol to lysosome in various tissues, the physiological role remains to be elucidated. Previously we reported that HAF-4::GFP localizes on the membrane of intestinal granules, and that the deletion mutants for *haf-4* and *haf-9* show the granular defect (15th International *C. elegans* Meeting). We carried forward the characterization of those granules.

Both HAF-4::GFP and HAF-9::GFP localized on the membrane of non-acidic but LAMP (lysosome-associated membrane protein) homolog LMP-1-positive intestinal granules. On another front, they localized on terminally maturated late lysosomes enlarged in the *ppk-3* mutant, but not on the RAB-5-positive early endosomes enlarged in the *rab-10* mutant as LMP-1 does.

As for the characterization of granules lost in the *haf-4* and *haf-9* deletion mutants, the mislocalization of LMP-1 in those mutants suggests that the lost granules are LMP-1- and HAF-4/9-positive ones. On the other hand, the transmission electron microscopic analysis revealed that the *haf-4* and *haf-9* mutants showed different granular phenotype from the *lmp-1* deletion mutant, although they show the indistinguishable phenotype to the *lmp-1* mutant at the optical microscopic level.

These results suggest that HAF-4/9 and LMP-1 play similar but distinct roles in the biogenesis of some populations of intestinal lysosome-related organelles.

1133B

The identification of *glo-1(-)* suppressors. Brian King, **Travis Walton**, Emily Scavarda, Greg Hermann. Department of Biology, Lewis & Clark College, Portland, OR.

Lysosome-related organelles (LROs) comprise a class of cell-type specific compartments with specialized functions. In mammals, defects in their formation result in Hermansky-Pudlak syndrome. The genes controlling the biogenesis of LROs are conserved in metazoa and include subunits of the AP-3 and HOPS complexes and Rab32/38. GLO-1, a homologue of Rab32/38, is localized to and is required for the formation of *C. elegans* gut granules, LROs containing fat and birefringent material found only within intestinal cells. In an effort to better understand the activity of GLO-1, we have carried out a screen for mutations the suppress gut granule biogenesis defects associated with the loss of *glo-1* function. To date, we have molecularly characterized 16 mutant alleles of *glo-1*: 6 are missense alleles altering amino acid residues conserved in all Rab GTPase, 3 are amber nonsense alleles, 2 are opal nonsense alleles, 2 are alleles predicted to alter splicing, and 2 are alleles that delete the entire *glo-1* gene. All of the alleles result in the complete loss of birefringent material from embryonic intestinal cells and its mislocalization into the embryonic intestinal lumen. In order to identify both bypass and allele specific suppressors we screened for suppressors of *glo-1(kx8)*, an allele that changes Asp67 to Asn. In other small GTPases, this residue has been shown to be essential for their function. We carried out a screen of F2 embryos within approximately 100,000 mutagenized *glo-1(kx8)* F1 adults for the presence of birefringent compartments within their intestinal cells. From the screen we identified 8 *glo-1* suppressors (*kx101-kx108*) that fall into 2 distinct classes. Six of the alleles are recessive and weakly restore birefringent compartments in *glo-1(kx8)* embryos. However, other markers of gut granules do not appear to be similarly restored, suggesting that these suppressors alter the trafficking or formation of the birefringent material. Two suppressors, *kx105* and *kx106*, dominantly and with high penetrance, suppress the loss and mislocalization of birefringent material. Suppressed embryos contain organelles that exhibit all of the hallmarks of normal gut granules. *kx105* suppresses the loss of gut granules in multiple *glo-1* alleles, including those containing very early premature stop codons, indicating that it bypasses the function of *glo-1* in gut granule biogenesis. We are currently investigating whether the dominant suppressors can similarly suppress the defects in gut granule biogenesis exhibited by AP-3 and HOPS mutants. The dominant suppressors are not present on the X chromosome where *glo-1* is located, and we are carrying out assays to identify the gene(s) altered by these alleles.

1134C

Exploring the link between cell polarity and endocytosis. **Ann M. Wehman**[1], Barth D. Grant[2], Jeremy F. Nance[1]. 1) Skirball Inst, New York Univ, New York, NY; 2) Rutgers Univ, Piscataway, NJ.

Contact between cells can induce inner-outer (apical-basal) polarities that are vital for many processes, including cell movement and epithelial function. The PAR proteins are known regulators of polarity and inner-outer PAR polarity is important for the timely ingression of the endodermal precursors in *C. elegans* (Nance *et al.*, 2003). At the 4 cell stage, the anterior PAR proteins PAR-3, PAR-6, and PKC-3 are initially localized symmetrically around the plasma membrane. Within one cell cycle, they are relocalized away from cell contacts and enriched on the outer surface of cells. How these proteins are moved away from cell contacts is not known.

Previous work has placed the anterior PAR proteins in control of endocytosis (Balklava *et al.*, 2007). In an RNAi screen for regulators of cell contact-mediated PAR polarity, we identified several genes implicated in endocytosis and membrane trafficking. Polarized trafficking is a promising mechanism for transporting the PAR proteins away from cell contacts.

One interesting protein identified in the screen is TAT-5, a putative aminophospholipid translocase. RNAi of *tat-5* results in multiple endocytic defects similar to anterior par mutants, such as in recycling endosomes and clathrin-mediated endocytosis. In addition, *tat-5* RNAi embryos have a novel defect in the endocytosis of midbodies after scission. We are currently analyzing mutants affecting these processes to determine whether PAR proteins are mislocalized after the 4 cell stage. Establishing the cell biological mechanism for relocalization of the PAR proteins may inform the mechanism behind other cell contact-induced polarization events, such as polarization during compaction of the mammalian embryo and apicobasal polarization of epithelial cells.

1135A

The colocalization of FoF1 ATP synthase with polycystin-1 on cilia is evolutionarily conserved from worm to mammals. **Qing Wei**, Jinghua Hu. Mayo Clinic, Rochester , MN.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic diseases. Mutations in two proteins, polycystin-1 (PC1) and polycystin-2 (PC2), are responsible for nearly all ADPKD cases. The major functional site for PC1 and PC2 is suggested to be on primary cilia, however, how PC1 and PC2 function on cilia remains elusive. Recently, C. elegans has been used as a success model to study human ADPKD. The C. elegans LOV-1 and PKD-2 proteins are homologues of human PC1 and PC2, respectively. LOV-1 and PKD-2 colocalize and function in the cilia of male-specific sensory neurons. Previously, we have shown that mitochondrial FoF1 ATP synthase beta subunit unexpectedly associates with evolutionarily conserved LOV-1 PLAT domain and locates to cilia. Our evidences support the hypothesis that the whole ATP synthase locates to cilia and is involved in polycystin signaling in C. elegans. Most interestingly, we demonstrate that the ATP synthase colocalize with polycystin-1 on mammalian kidney cilia. Pharmacological assay suggests that the ciliary ATP synthase may also play a pivotal role in ciliogenesis. Our data indicate that the ectopic ciliary ATP synthase plays a conserved role in cilia function and/or ciliogenesis. Using both worm and mammal models, we will explore the molecular mechanisms underlying the ciliary targeting of the ATP synthase and its involvement in human ADPKD disease.

1136B

Distinct roles for *C. elegans* clathrin adaptin complexes AP1 and AP2 in mediating protein trafficking to cilia. Oktay Kaplan, Katarzyna Kida, **Oliver Blacque**. University College Dublin, Dublin, Ireland.

Clathrin in association with four adaptor complexes (AP1-4) facilitates membrane trafficking and protein sorting between various cellular compartments. Despite increasing evidence implicating these systems in ciliary processes, the role of metazoan clathrin and AP complexes in cilium formation and function remains poorly understood. Here we addressed this question in *C. elegans* sensory neurons. First, we found that clathrin heavy chain (CHC-1), AP1 (UNC-101, APS-1) and AP2 (DPY-23, APA-2) subunits, but not AP3 subunits, are required for normal cilia integrity, morphology and ultrastructure. Subcellular localisation analysis using rescuing GFP reporters revealed that AP1 and AP2 subunits display distinct non-ciliary localisations, with UNC-101 and APS-1 predominantly within the perinuclear region, whereas DPY-23 is found more diffusely throughout the cell, including a region near the ciliary base. Loss of CHC-1, AP1 and AP2 subunit function results in overlapping yet distinct defects in ciliary protein trafficking. In *chc-1* and *unc-101* mutants, ODR-10 (transmembrane protein) is trapped at the plasma membrane and fails to undergo dendritic translocation, whereas in *dpy-23* mutants, ODR-10 translocates normally along dendrites but frequently accumulates within the distal dendrite region. Consistent with differential effects of AP1 and AP2 subunits on membrane trafficking to cilia, the assembly of enlarged AWB ciliary membrane fans in *grk-2* mutants requires *unc-101*, but not *dpy-23*. Similarly, loss of CHC-1 and AP1/2 function differentially affects intraflagellar transport, with IFT grossly normal in *unc-101* mutants, partially slowed in *dpy-23* mutants and absent altogether in *chc-1(RNAi)* animals. Finally, loss of *chc-1* and *dpy-23* function, but not *unc-101*, results in the distal dendrite accumulation of IFT proteins. Together, these data demonstrate roles for metazoan CHC, AP1 and AP2, but not AP3, in cilium formation. In addition, these findings suggest that AP1 and AP2 operate at distinct ciliary protein sorting compartments, mediating differential effects on membrane trafficking to cilia and intraflagellar transport.

1137C

Analysis of Oxysterol-binding protein (OSBP)-related genes in *C. elegans*. **Hiroyuki Kobuna**[1,2,3], Takao Inoue[2,3], Keiko Gengyo-Ando[1,3], Shohei Mitani[1,3], Hiroyuki Arai[2,3]. 1) Dept. Physiol., TWMU, Tokyo, Japan; 2) Grad. Sch. Parm. Sci., Univ. of Tokyo, Tokyo, Japan; 3) Crest, JST.

Oxysterol-binding protein (OSBP) was originally identified as a cytosolic protein that bound to an oxygenated cholesterol. OSBP is translocated from the cytosol to the Golgi in response to cellular sterol content, and recently it has been shown that OSBP facilitates the transfer of cholesterol between membranes in vitro, suggesting that OSBP mediates non-vesicular cholesterol transport to the Golgi. A family of OSBP-related proteins (ORP family) has been identified in a variety of species ranging from yeast to mammals. The ORP family members are also implied to be involved in cholesterol transport; however, the physiological functions in vivo remain to be elucidated.

In this study, we generated the deletion mutants of all four ORP genes in *C. elegans* (*obr-1-4*). All single, double and triple *obr* mutants were viable and showed no apparent abnormalities. However, *obr-1;obr-2;obr-3;obr-4* quadruple mutants lacking all *obr* genes exhibited embryonic lethality (~11%) and slow growth during larval development (~18%). Of the *obr* quadruple mutant embryos that did not hatch, the majority (94%) developed to the late embryonic stage (2- to 4-fold stage), indicating that there is no strict requirement for OBR proteins during early embryogenesis. Hatched *obr* quadruple mutants were able to develop to adults and produce subsequent progeny; however, they had a reduced brood size (60% of wild-type) and showed defects in gonadal cell migrations with incomplete penetrance (~10%). These data suggest that OBR proteins (OBR-1~4) act redundantly on embryonic development and larval growth in *C. elegans*. To gain insight into the molecular mechanisms of embryonic lethality in *obr* quadruple mutants, we conducted a synthetic lethal RNAi screen to identify genes which functionally compensate for the *obr* molecules. We tested 2434 RNAi clones in the Ahringer library and identified 28 genes that showed specific synthetic lethality in the *obr* quadruple mutant background, but not in the wild-type background. These enhancer genes include the genes encoding vesicular transport-related proteins, signaling proteins, and nuclear proteins. At the meeting, we will present detailed analysis of genetic interactions between *obr* and the enhancer genes.

1138A

Stability of the synaptic vesicle kinesin motor, UNC-104, depends on specific binding to cargo. **Jitendra Kumar**[1], Bikash Chowdhary[1], Raghu Metpally[1], Sowdhamini Ramanathan[1], Dieter Klopfenstein[2], Sandhya Koushika[1]. 1) NCBS-TIFR, Bangalore; 2) Center for Molecular Physiology of the Brain, Georg August University Gottingen, Germany.

It is important to understand the fate of motors unbound to cargo since such motors are likely to occur naturally at the synapse and at other locations in the cell wherever cargo is loaded or released. To address this question we studied the essential and conserved synaptic vesicle motor UNC-104/KIF1A that had lost its ability to bind cargo, and explored the consequences on the motor itself. The *C. elegans* motor UNC-104 transports synaptic vesicles by binding to $PI(4,5)P_2$ through its PH-domain. The *unc-104(e1265)* allele carries a point mutation in the $PI(4,5)P_2$ binding pocket. This mutation nearly abolishes specific binding to $PI(4,5)P_2$ *in vitro* while still binding other lipids, and is insensitive to $PI(4,5)P_2$ levels *in vivo* assayed by indirect measurements of synaptic transmission. Moreover, *unc-104(e1265)* animals show highly reduced levels of UNC-104 occurring at least partly through the ubiquitin pathway. We isolated two intragenic suppressors, *unc-104(e1265tb107)* and *unc-104(e1265tb120)*, within the PH-domain. Each suppressor partially restored *in vitro* $PI(4,5)P_2$ binding specificity, showed *in vivo* sensitivity to $PI(4,5)P_2$ levels (assayed by indirect measurements of synaptic transmission) and correspondingly showed partial restoration of UNC-104 protein levels. Blocking the ubiquitin-mediated degradation pathway in the two intragenic suppressors also improved indirect measures of synaptic transmission. An additional mutation in the PH-domain reversing the suppressor effect of *unc-104(e1265tb120)* again lacked *in vitro* binding to $PI(4,5)P_2$ and had reduced levels of UNC-104 *in vivo*. Preliminary data suggest that in an *uba-1* mutant UNC-104 motor levels increase at synapses. Our results suggest that it is the lack of cargo binding that causes the motors to be targeted for degradation inside neurons. This provides the first evidence for a long-proposed mechanism that the degradation of the anterograde motor at the synapse is a key regulatory step in setting up directionality of axonal transport. It further suggests that the balance of free motors in cells could be regulated by degradation.

1139B

Identification of a gene required for localizing the KLP-6 motor and its cargo PKD-2. **Julie Maguire**, Maureen Barr. Rutgers University, Department of Neuroscience, Department of Genetics, Piscataway, NJ 08854.

In the male *C. elegans* nervous system, the LOV-1-PKD-2 transient receptor potential polycystin (TRPP) receptor-channel complex localizes to ciliated endings of sensory dendrites. In humans, the polycystins localize to cilia of renal epithelia cells, and mutations in the TRPP genes PKD1 and PKD2 cause autosomal dominant polycystic kidney disease. In male worms, GFP-tagged PKD-2 localizes to cell bodies and cilia of CEM, RnB and HOB neurons, and can be visualized as moving puncta in dendrites. When PKD-2 and LOV-1 are absent, males exhibit response and location of vulva (Lov) defects. The kinesin-3 KLP-6 is required for normal localization of PKD-2 as well as male response and vulva location behavior.

We previously screened for mutants exhibiting mislocalization of PKD-2::GFP[1]. In the ciliary localization (Cil) defective mutant *my16,* we observe an abnormal accumulation of PKD-2::GFP in the ciliary base and cilium proper of CEM neurons, though the dendrites had normal localization of PKD-2::GFP[1]. *my16* males are response and Lov defective, presumably due to the mislocalization of PKD-2. We are interested in identifying the gene mutated in *my16* animals and understanding its function in ciliary receptor trafficking and male mating behaviors.

In wild-type males, KLP-6::GFP is expressed in both the CEM and IL2 neurons and localizes throughout the neuron. In contrast, *my16* mutant males display abnormal localization of KLP-6::GFP in the form of both globular structures and small puncta located laterally along the ciliary ending of the CEMs. One possibility is that KLP-6 is trapped on its cargo as indicated by the puncta. In hermaphrodites, which contain IL2 and lack CEM neurons, KLP-6 localization appears wild type. We conclude that the *my16* allele affects KLP-6::GFP localization only in CEMs, and are currently determining whether the *my16* mutation specifically affects male sensory neurons.

Motor-based transport is important for precise cargo delivery, and we are interested in determining how the *my16* mutation affects the KLP-6 motor and its cargo PKD-2. To test the interdependence between *pkd-2*, *klp-6*, and *my16*, we will examine KLP-6::GFP localization in a *pkd-2; my16* double mutant background, and PKD-2::GFP localization in a *klp-6; my16* double mutant background. We will also study the effects of *my16* on male ciliated neuronal development and function, including examination of genetic interactions between *lov-1*, *pkd-2*, *klp-6*, and *my16* on male mating behaviors.

1. Bae YK, Lyman-Gingerich J, Barr MM, Knobel KM. 2008. Identification of genes involved in the ciliary trafficking of *C. elegans* PKD-2. Dev Dyn.

1140C

The Arf-like GTPase ARL-8 is required for lysosome biogenesis in *C. elegans*. **I. Nakae[1]**, T. Fujino[1], T. Kobayashi[1], Y. Kikko[1], S. Harada[1], M. Fukuyama[1], K. Gengyo-Ando[2,3], S. Mitani[2,3], K. Kontani[1], T. Katada[1]. 1) Dept of Physiol Chem, Grad Sch of Pharmaceut Sci, The Univ of Tokyo, Japan; 2) Dept of Physiol, Tokyo Women's Med Univ, Sch of Med, Japan; 3) CREST, JST.

Lysosomes are the major digestive compartments with many hydrolytic enzymes and acidic lumenal pH. For efficient degradation of macromolecules, lysosomes repeatedly undergo dynamic fusion events, which include both homotypic fusion and heterotypic fusion with late endosomes, phagosomes and autophagosomes; yet cells are able to maintain an approximately constant number and size of lysosomes. Clearly, lysosomal fusion/scission events must be tightly regulated for appropriate lysosomal biogenesis and function; however, the molecular mechanism of their regulation is poorly understood.

Arl8 is the first small GTPase that has been shown to be localized primarily to lysosomes in mammalian cells. We conducted the first genetic analysis of an *arl-8*-deletion mutant using *C. elegans*, and found that ARL-8 is an essential GTPase for lysosome biogenesis in the macrophage-like coelomocytes. *arl-8* mutants display the unique combination of lysosomal phenotype that are unprecedented in previous studies. First, loss-of-function of ARL-8 leads to supernumerary lysosomes that are smaller than wild type. Second, the defects of these aberrant lysosomes are not limited to their morphology: Distributions of V-ATPase subunits among lysosomes are disorganized, and most lysosomes are defective for their luminal acidification in *arl-8* mutant coelomocytes. The localization of lysosomal aspartic protease ASP-1 is also compromised in *arl-8* mutants: While ASP-1 is distributed among virtually all lysosomes in wild-type, it localizes to a small population of lysosomes in *arl-8* mutants. *arl-8* mutant coelomocytes can endocytose macromolecules; however, the endocytosed macromolecules accumulate in a lysosomal fraction with relatively low levels of ASP-1. These results imply that loss of *arl-8* leads to the generation of numerous small lysosomes, where lysosomal enzymes and endocytosed macromolecules accumulate in distinct lysosome subpopulations. Furthermore, we found that *arl-8* acts upstream to *cup-5*, which is the orthologue of human mucolipin-1 implicated in mucolipidosis type IV. Previous studies have shown that *cup-5* mutant coelomocytes display enlarged lysosome-like vacuoles probably due to defects in the maturation/scission process of lysosomes. Interestingly, loss of *arl-8* strongly suppresses formation of enlarged lysosome-like vacuoles in *cup-5* mutants. Collectively, our findings suggest that ARL-8 acts as an essential node that tightly links appropriate maintenance of number and size of lysosomes with their intrinsic functions.

1141A

tra-1 is a suppressor of the *tbc-2* large endosome phenotype. **Christian E. Rocheleau**, Anna Chavlovski, Krittika Bhende. Dept Med, McGill Univ, Montreal, PQ, Canada.

The *tbc-2* gene encodes for a putative Rab GTPase activating protein. Mutations in *tbc-2* result in the accumulation of large late endosomes in the intestine, as well as trafficking phenotypes in oocytes and coelomocytes (see abstract by Chotard et al.). To identify genes required for the *tbc-2(-)* intestinal phenotype we performed a pilot genetic screen for suppressors of the large late endosome phenotype. One of the suppressor mutations, *vh8*, is male in character. The *vh8* animals lack a hermaphrodite vulva and have a male tail displaying varying degrees of development. The somatic gonad appears to be male, however it develops both sperm and oocyte-like cells. Loss of function mutations in the *tra* genes *(tra-1, -2, and -3)*, "Transform" XX hermaphrodites into phenotypic males, and hypomorphic *tra-1* XX pseudo-males can develop oocytes similar to *vh8*. To determine if *vh8* could be an allele of one of the *tra* genes, we tested if *vh8* is balanced by the translocations *hT2 I; III* and *nT1 IV;V,* or the chromosomal inversion *mIn1 II*, which balance *tra-1, tra-3* and *tra-2* respectively. We found that *vh8* is balanced by *hT2*, but not *nT1* or *mIn1*, suggesting that *vh8* might be an allele of *tra-1*. Consistent with this idea we found that *tra-1(e1099)* could also suppress the *tbc-2(tm2241)* intestinal phenotype. Finally, we found that *vh8* fails to complement *tra-1(e1099)* for the Tra male tail phenotype and thus *vh8* is likely a new allele of *tra-1*. TRA-1 is a GLI-type transcription factor, suggesting that it acts indirectly to regulate the *tbc-2(-)* endocytic phenotype in the intestine. In hermaphrodites TRA-1 functions to suppress male traits and thus promotes hermaphrodite specific development. Therefore, a simple explanation would be that the *tbc-2(-)* phenotype is hermaphrodite specific. However we find that *tbc-2(-)* males also display the large intestinal endosome phenotype indicating that the phenotype is not sex specific and suggesting that *tra-1* might function independently of sex determination to affect trafficking in the intestine. It has previously been shown that *tra-1* is involved in the development of the somatic gonad in both males and hermaphrodites and therefore could regulate other aspects of animal development/physiology in both sexes. We are keen to determine if mutations in other *tra* genes can also suppress the *tbc-2(-)* intestinal phenotype, or if TRA-1 is functioning independently of the sex determination pathway to affect endocytic trafficking in the intestine.

1142B

A Screen for Endocytic Regulators of EGFR Signaling. **Olga Skorobogata**, Christian Rocheleau. Department of Medicine, McGill University, Montreal, Quebec, Canada.

Epidermal Growth Factor Receptor (EGFR)/Ras/Mitogen Activated Protein Kinase (MAPK) signaling is involved in regulation of cell proliferation, migration and apoptosis. Thus it is not surprising that mutations resulting in increased Ras signaling may lead to cancer. There is an interplay between the signaling and trafficking of the internalized receptor, namely upon the endocytosis of the stimulated EGFR into early endosomes where it can continue to signal to downstream target proteins. The small GTPase Rab7 mediates EGFR trafficking through late endosomes to lysosomes where the receptor is degraded. It has been shown that blocking Rab7 activity prevents EGFR degradation, however the impact on signaling is still unknown.

In C.elegans LET-23 EGFR signaling is required for vulval cell fate specification. Our genetic data indicates that RAB-7 activity antagonizes LET-23 EGFR signaling during vulval induction, consistent with it having a role in LET-23 EGFR degradation. This suggests that the non-degraded LET-23 EGFR remains competent to signal and that Rab7 could possibly function as a tumor suppressor in mammals. The strongest effect of *rab-7(ok511)* is observed on the LIN-2 CASK/ LIN-7 Veli/ LIN-10 Mint11 complex required for basolateral localization of LET-23 EGFR in the Vulval Precursor Cells (VPCs). Mutations in *lin-2*, *lin-7*, *lin-10* or *let-23(sy1)* result in a strong Vulvaless (Vul) phenotype due to the mislocalization of LET-23 to the apical membrane domain of the VPCs. *rab-7(ok511)* can strongly suppress Vul phenotype of these mutants. We are currently performing Whole Mount Immunostaining to determine whether the suppression is due to restored localization of LET-23 to basolateral membrane of VPCs. The *rab-7(ok511)* mutant is maternal effect embryonic lethal and would not have been identified in previous genetic screens for suppressors of *lin-2*, *lin-7*, *lin-10* or *let-23(sy1)*. To identify additional genes that might regulate late endocytic trafficking of the LET-23 EGFR, we conducted a pilot forward genetic screen for suppressors of the *lin-2(e1309)* Vul phenotype using a strategy that would recover maternal effect lethal mutants. We identified seven candidate suppressor mutants. Our preliminary analysis of these suppressors identifies additional phenotypes that are suggestive of vesicular trafficking defects and other phenotypes that distinguish these mutants from previously identified negative regulators of EGFR/Ras/MAPK signaling. We are currently mapping two mutants to chromosomal regions using balancers and snip-SNP techniques. Thus far one mutant, *vh4*, appears to map to the right arm of LGI. We are testing candidate genes by RNAi for suppression of the *lin-2* Vul phenotype.

1143C

Genetic and dietary regulation of lipid droplet size in *C. elegans*. Shaobing Zhang, Andrew Box, Johan le Men, Jingyi Yu, Ningyi Xu, **Ho Yi Mak**. Stowers Institute for Medical Research, Kansas City, MO.

Lipid droplets are subcellular organelles in which neutral lipids including triglycerides (TAG) is stored. The number and size of lipid droplets reflect the metabolic status and dietary intake of an animal. In order to understand how the size of lipid droplets is regulated, we isolated genetic mutants that accumulate enlarged intracellular lipid droplets. Cloning of four mutants from our collection revealed the involvement of a peroxisomal fatty acid beta-oxidization pathway, including DHS-28 and DAF-22, in the regulation of enlarged lipid droplet formation. We found that *dhs-28* and *daf-22* mutants have elevated TAG level and their lipid droplet size is diet-dependent. We are currently trying to identify specific lipid species in different bacterial strains that may modulate the intracellular lipid droplet size in *C. elegans*.

1144A

Loss of mitochondrial fusion causes acidification in worms and mammalian cells. **David W. Johnson**, Keith Nehrke. Dept Biochemistry, Univ Rochester, Rochester, NY.

Mitochondrial structural dynamics occur through the opposing processes of membrane fission and fusion. Although the physiologic role(s) of changes in morphology are incompletely understood, mitochondrial synthesis, transport and perhaps function are all thought to be regulated by morphology. In fact, genetic mutations that reduce the function of protein components of the fission or fusion machinery have been associated with mammalian disease, and a complete loss of these proteins causes embryonic lethality in mice. However, loss-of-function mutations in genes coding for fission and fusion orthologs is tolerated in worms, making *C. elegans* one of the only models for studying the physiologic effects of mitochondrial morphology. We have found that fragmentation of the mitochondrial network through mutation of either *eat-3* or *fzo-1*, required for inner and outer membrane fusion, respectively, causes cellular acidification in all of the tissues tested. These genes are epistatic to one another, and mutations in the profission gene *drp-1* alleviate acidosis in an *eat-3(ad426)* mutant with reduced function (rf). Neither the rf nor complete deletion of drp-1 on its own has significant effects on intracellular pH, suggesting that the changes in morphology that cause acidification are specific to fragmentation. We further show that mutations in the electron transport chain cause lactic acidosis and also reduce the ATP content of the worms, but in contrast morphologic acidosis is not caused by lactate accumulation, although the ATP content is reduced in *eat-3* mutants. We are currently testing if the extent of acidification correlates with energy demand in worms. We have extended these observations to include cultured mammalian cells. Clone 9 cells were transfected with either a construct overexpressing the profission Fis1, or with dsRNA targeting Opa1, the mammalian ortholog of EAT-3. These treatments resulted in mitochondrial fragmentations and caused cellular acidification, suggesting an evolutionarily-conserved link between morphology and pH homeostasis. We have also found that quiescence induced by serum starvation causes acidification on its own, likely resulting from the loss of Na+/H+ exchange activity; however, fragmentation of the mitochondria in quiescent cells does not cause further acidification. This suggests that either fragmentation can regulate Na+/H+ exchange activity or that, as we predict occurs in worms, energy demand correlates with acidification. Finally, we present a model where fragmentation-induced acidification plays a physiologic role under high energy demand, in addition to contributing to the patho-physiology of morphology diseases.

1145B

The complex roles of the JNK signaling scaffold molecule, UNC-16, in regulating mitochondrial distributions in neurons. **Guruprasada Reddy Sure**, Swathi DeviReddy, Swetha Mohan, Eva Romero, Anjali Awasthi, Sandhya Koushika. Dept Neurobiology, NCBS-TIFR, Bangalore, Karnataka, India.

To study the regulation of mitochondrial transport, a transgenic strain, *jsIs609* was built in which GFP is targeted to the mitochondrial matrix in all six mechanosensory neurons. Co-localization with mitotracker labeling in mechanosensory neurons in cultures suggest that *jsIs609* labels nearly all mitochondria and at least partially co-localize with a synaptic vesicle marker at mechanosensory neuron synapses. Mitochondria number increases with the age/length of the animal/axon. In axons most of the mitochondria are stationary while moving mitochondria exhibit saltatory movements. Mitochondrial flux varies with developmental stages however size and density of mitochondria remain the same through out development. Mutants in *unc-116* and *km-11* (both together form the Kinesin-I motor) decrease the number of mitochondria in axons while a mutant in dhc-1 does not. Genes such as Milton that link the Kinesin-I motor to mitochondria are absent from the *C. elegans* genome, suggesting that other proteins may play roles in regulating mitochondrial axonal transport. To understand the role of known interactors of the Kinesin-I complex in mitochondrial transport, genetic analyses with mutants in these components was carried out. Kinesin-I forms a complex with regulatory proteins such as UNC-76, UNC-14, UNC-51, UNC-69 and adaptor proteins like JIP-3/UNC-16 to facilitate vesicle transport. *unc-16* mutants show increased number of axonal mitochondria unlike that observed in Kinesin-I motor complex mutants. *unc-116 unc-16* and *unc-16; klc-2* double mutants show improved anterograde transport of mitochondria compared to *unc-116* and *klc-2* animals alone. Further, in *unc-16* mutants the population of smaller mitochondria in axons increases. Since certain UNC-16 effects on mitochondria are independent on the Kinesin-I motor, we hypothesize that UNC-16 may act in the following ways: (1) recruiting or activating an alternate anterograde mitochondrial motor (2) regulating the size and numbers of axonal mitochondria through fission (3) by decreasing the recruitment of dynein thereby reducing retrograde transport. These possibilities are currently being tested.

1146C

Mitochondrial fusion and autophagy aid in removal of bulky mitochondrial DNA adducts. **Amanda M Smith**, Maxwell Leung, Autumn Bernal, Tracey Crocker, Joel Meyer. Nicholas School of the Environment and Earth Sciences, Integrated Toxicology Program, Duke University, Durham, NC.

Mitochondrial DNA (mtDNA) integrity is critical for human health; however, it is unclear how bulky mtDNA adducts formed after exposure to environmentally important genotoxins such as ultraviolet radiation and PAHs are handled. mtDNA may be particularly susceptible to these genotoxins due to the absence of nucleotide excision repair, the primary repair mechanism for bulky DNA adducts in nuclear DNA. We investigated the removal of bulky mtDNA adducts via mitochondrial fusion and autophagy in Caenorhabditis elegans. Larval fusion and autophagy mutant C. elegans were exposed to serial UVC doses over 48 hours. This exposure protocol allows for accumulation of mtDNA damage without lasting damage to nuclear DNA and results in measurable larval growth arrest. Strains carrying mutations in the autophagy gene atgr-18 and fusion genes fzo-1 and eat-3 exhibited exacerbated larval growth arrest with little to no growth recovery after 72 hours. We concluded that these proteins are required for normal recovery from mtDNA damage-induced larval growth arrest. In order to test directly the contribution of autophagy and fusion proteins to removal of UVC-induced DNA damage, we performed RNAi knockdown of autophagy and fusion genes in UVC treated adult glp-1 C. elegans. Knockdown of autophagy and fusion genes inhibited removal of UVC-induced DNA damage, as measured by a quantitative PCR-based assay. These data suggest that autophagy and fusion processes are involved in the removal of bulky DNA damage in mitochondria. Mitochondrial dysfunction as a result of bulky mtDNA damage may trigger mitochondrial remodeling. Preliminary results indicate the relative ATP levels are reduced in UVC treated animals. Therefore, we hypothesize that UVC-induced mtDNA damage is removed via fusion-mediated mitochondrial remodeling and subsequent autophagy, possibly triggered by mitochondrial dysfunction. This research was supported by funding from the National Institutes of Environmental Health Sciences, 1 P30 ES-011961-01A1.

1147A

Ciliopathy-associated proteins maintain the structural and functional integrity of the ciliary gate. Chunmei Li[1], Nathan Bialas[1], Peter Inglis[1], Katarzyna Kida[1], Oliver Blacque[2], **Michel Leroux[1]**. 1) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada [leroux@sfu.ca]; 2) School of Biomolecular and Biomedical Science, The Conway Institute, University College Dublin, Belfied, Ireland.

Cilia are membrane-ensheathed organelles with motile and/or sensory functions that are important for a wide array of physiological and developmental processes. Many of the proteins destined for localisation to cilia are trafficked on post-Golgi vesicles that must fuse at the base. The proteins may then be captured by a non-vesicular intraflagellar transport (IFT) machinery that docks at transitional fibers and is mobilised within the organelle by virtue of kinesin motor(s). The transitional fibers, along with a neighbouring region (transition zone) possessing Y-shaped crosslinks, join the microtubule axoneme to the membrane and as such form a 'ciliary gate' that restricts vesicle entry and perhaps also acts as a molecular gatekeeper akin to the nuclear pore complex. However, the components that form or maintain such a ciliary gate are unknown. Candidates include approximately 12 different proteins, all of which are implicated in ciliopathies collectively characterised by retinal degeneration, kidney disease and other ailments; these proteins localise at or near the transition zone and perform essentially unknown roles related to cilia function. Here, we demonstrate using *C. elegans* that a functional interaction between a novel C2 domain-containing protein (MKS-6), recently implicated in the ciliopathy Meckel syndrome, and the Nephrocystin protein NPH-4, is required for the structural integrity of this ciliary gate. The loss of these two proteins causes a disruption in the ultrastructure of the transition zone region, characterised by an expanded membrane, absence of Y-links and the accumulation of membranous-type material. Our findings expand the interaction network of proteins present within the ciliary transition zone region, and point to the importance of ciliopathy-associated proteins in maintaining a functional ciliary gate.

1148B

mboa-7 is required for selective incorporation of polyunsaturated fatty acids into phosphatidylinositol in *C. elegans*. **Hyeon-Cheol Lee**[1], Takao Inoue[1,3], Keiko Gengyo-Ando[2,3], Shohei Mitani[2,3], Hiroyuki Arai[1,3]. 1) Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan; 2) Department of Physiology, Tokyo Women's Medical University School of Medicine, Tokyo, Japan; 3) Crest, JST, Japan.

Phosphatidylinositol (PI) is a component of membrane phospholipids functioning both as a signaling molecule and as a compartment-specific localization signal in form of polyphosphoinositides. Arachidonic acid (AA) is the predominant fatty acid in the *sn*-2 position of PI in mammals. LysoPI acyltransferase (LPIAT) is thought to catalyze formation of AA-containing PI; however, the gene that encodes this enzyme has not yet been identified. In this study, we established a screening system to identify genes required for utilization of exogenous polyunsaturated fatty acids in *C. elegans*, in which eicosapentaenoic acid (EPA) instead of AA is dominant in PI. We found that a previously uncharacterized gene, which we named *mboa-7*, is required for incorporation of PUFAs into PI. Incorporation of exogenous EPA into PI of the living worms and the *in vitro* incorporation of EPA into lysoPI by the microsomal fraction were greatly reduced in *mboa-7* mutants while the incorporation of EPA into other phospholipids was unaffected in both systems. siRNA of human *mboa-7* homologue (*MBOAT7*) in HeLa cells also reduced AA incorporation into PI at cellular level and *in vitro*. Furthermore, the membrane fractions of transgenic worms expressing recombinant MBOA-7 and MBOAT7 exhibited remarkably increased LPIAT activity but not other lysophospholipid acyltransferase activity. Finally, *mboa-7* mutants had greatly reduced EPA content in PI but not in other phospholipids. These data demonstrated for the first time that *C. elegans mboa-7* and its mammalian homologue *MBOAT7* are LPIAT functioning as a determinant of PI molecular species.

1149C

mboa-6 is required for incorporation of polyunsaturated fatty acids into phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine in *C. elegans*. **S. Matsuda**[1], T. Inoue[1,3], K. Gengyo-Ando[2,3], S. Mitani[2,3], H. Arai[1,3]. 1) Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan; 2) Department of Physiology, Tokyo Women's Medical University School of Medicine, Japan; 3) Crest, JST, Japan.

Glycerophospholipids, one of the major components of biological membranes, are comprised of various molecular species with different fatty acyl moieties. Previous studies have established that fatty acids of cellular phospholipids are distributed asymmetrically. In general, saturated fatty acids are esterified at the *sn*-1 position while polyunsaturated fatty acids (PUFAs) are esterified at the *sn*-2 position. It is widely believed that PUFAs are incorporated into glycerophospholipids after their de novo synthesis by remodeling of fatty acyl chains of newly synthesized phospholipid species. Over 40 years ago, it has been proposed that phospholipids of biological membranes are metabolically active and participate in a series of deacylation-reacylation reactions, which may lead to accumulation of PUFAs at the *sn*-2 position of the glycerol backbone; however, the genes that encode these enzyme have not yet been identified. Very recently, using *C. elegans* RNAi screen, we have identified lysophosphatidylinositol (LPI) acyltransferase (*mboa-7*/*LPIAT*) which specifically transfers PUFAs into the *sn*-2 position of LPI. *mboa-7*/*LPIAT* belongs to the membrane-bound *O*-acyltransferase (MBOAT) family and is widely conserved in variety of species ranging from *C. elegans* to mammals. In this study, we found *mboa-6*, another member of the MBOAT family in *C. elegans*, as an acyltransferase which catalyzes incorporation of PUFAs into lysophosphatidylcholine (LPC), lysophosphatidylserine (LPS) and lysophosphatidylethanolamine (LPE). Knockdown of *mboa-6* reduced incorporation of exogenous PUFAs into phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) in *C. elegans*. In *in vitro* assays, overexpression of *mboa-6* and its human orthologue resulted in great increases in LPC, LPS and LPE acyltransferase activities but not in LPI or lysophosphatidic acid (LPA) acyltransferase activities. These results indicate that *mboa-6* is a lysophospholipid acyltransferase (LPLAT) acting preferentially on LPC, LPS and LPE. Knockdown of *mboa-6* caused several developmental defects such as early larval arrest, slow growth, and a dumpy morphology during postembryonic development, suggesting that PUFA-containing PC, PS, or PE are required for normal larval growth and morphology. Identification of *mboa-7*/*LPIAT* and *mboa-6*/*LPLAT* will reveal the physiological significance of fatty acid molecular species in membrane phospholipids.

1150A

Three dimensional structure of the Ward body. **Kristin A. Politi**[1], KD Derr[2], William J. Rice[2], Kevin Fisher[1], Chris Crocker[1], David H. Hall[1]. 1) Neuroscience, Albert Einstein Col Medicine, Bronx, NY; 2) New York Structural Biology Center, 89 Convent Ave, New York, NY.

The plasma membrane of the nematode hypodermis features a unique set of stacked membrane invaginations that we presume to be associated with cuticle deposition, although there is still no experimental evidence to prove it. These structures were first noted by TEM in C. elegans by Sam Ward in the early 1970s, and briefly mentioned in WormBook I by John White (1). Here we present electron microscopic evidence for their organization in the wild type adult, combining thin sections, freeze fracture and electron tomography to view their organization in groupings along the body wall. The so-called "Ward body" resembles a Golgi body that has been rotated on end so that each succeeding membrane leaflet can fuse directly to the plasma membrane. Ward bodies are often clustered nearby to one another, but the orientation of each Ward body seems to be independent of its neighbors, and seemingly has no fixed angle with respect to the body axis. In some cases their leaflets lie virtually parallel to the plasma membrane. Similar membrane infoldings have also been seen in the excretory duct's luminal membrane, but are not known to occur in seam cells or other epithelia in the nematode. We hypothesize that large extended proteins, such as collagens, may co-assemble within the protected environment of a Ward body leaflet prior to deposition in the cuticle. Supported by NIH RR 12596 to DHH. 1. W. Wood (Ed), The nematode C. elegans. Chapter 4, "The Anatomy", Fig 2a, 1988.

1151B

Role of *bec-1* in autophagy and endocytosis in *C. elegans*. **A. Ruck**[1,2], J. Attonito[2], A. Meléndez[1,2]. 1) Biology, The Graduate Center, City University of New York, New York, NY; 2) Biology, Queens College, Flushing, NY.

We have previously shown that *bec-1* (the *C. elegans* ortholog of Atg6/Vps30/Beclin 1) has functions in various fundamental biological processes, including survival, longevity, dauer and reproductive development. In addition *bec-1* and other autophagy genes have also been shown to be required for the lifespan extension of dietary-restricted animals. In yeast, the ATG6p functions in two separate complexes: one with autophagy function and one with vacuolar protein sorting (Vps) function. Interestingly, the *C. elegans* BEC-1 and human Beclin 1 proteins complement the yeast autophagy function whereas they do not complement the VPS function of yeast Atg6/Vps30p, suggesting that the mechanisms of Beclin 1/BEC-1 function in tumor suppression and in longevity involves a class III PI3K Vps34-dependent autophagy, but not vacuolar protein sorting. However, screens for genes that mediate the increased life span of *C. elegans* insulin-like mutants have identified a high number of endocytosis genes. Thus, it is important to determine whether *bec-1* has functions in endocytosis and if its many roles are due to autophagy, endocytosis, or both. To this end, we have crossed *bec-1* mutant animals with a panel of fluorescent markers previously developed by the Grant and Fares labs that label different endocytic compartments. GFP::RAB-5 labels early endosomes that appear as small punctate structures near the basolateral and apical plasma membrane in intestinal cells, while GFP::RAB-7 labels similar early endosomes near the plasma mmbrane, and abundant larger ring-like structures thought to be late endosomes. In addition, we have also examined markers for intestinal recycling endosomes, including GFP::RAB-10, GFP::RAB-11 and GFP::RME-1, in *bec-1* deficient animals. *bec-1* homozygous animals that segregate from a heterozygous parent die as young adults and display an accumulation of large aberrant vacuoles. Our data suggests that these mutant vacuoles are of late endosomal nature. Furthermore, an early endosomal marker localizes to distinct domains within the aberrant vacuoles and displays an asymmetric localization suggesting that once the early endosome fuses, it no longer displays localization of the marker. Lastly, vesicle trafficking between the endoplasmic recycling complex and the plasma membrane is also affected, as recycling endosome markers show weak association with the mutant vacuoles in *bec-1* homozygous animals. We will provide evidence that BEC-1 has a role in endocytic trafficking in *C. elegans*. We are currently determining the cellular focus of action for *bec-1* and conducting colocalization studies between endocytosis and autophagy markers to understand the functional relationship between the two processes.

1152C

Genetic analysis of the complete heparan sulfate modification network. **Robert Townley**, Hannes Buelow. Albert Einstein College of Medicine, New York, NY.

Heparan sulfates (HS) are unbranched polysaccharides and a defining component of the extracellular matrix in metazoans. HS functions to regulate cellular communication through HS motifs that are composed of blocks of modified saccharide subunits. These motifs are generated by a set of conserved enzymes that catalyze specific sulfations and epimerizations. The molecular and cellular mechanisms that govern the synthesis of HS motifs in vivo are only poorly understood. In order to gain insight into this important process, we have undertaken an integrated genetic and biochemical analysis of the complete set of known modifying enzymes. We have measured the disaccharide composition of heparan sulfate purified from worms with defined deletions or point mutations in one or more Hs modifying enzymes. From these measurements we have developed a comprehensive model of heparan sulfate synthesis that contains novel quantitative and qualitative features. We find that the overall sulfate content of the heparan polymer is initially determined by the amount of sulfate-donor (PAPS) that is available in the lumen of the Golgi. PAPS is transported into the Golgi by a selective transporter (pst-1). The phenotype of pst-1 mutants can account for the puzzling observation made previously that removal of 2-O or 6-O sulfation alone has little effect on overall sulfation. Additionally, our data define the order in which the modifications occur. Most published models and discussions place 2-O sulfation before 6-O, however, our data indicate 6-O and 2-O sulfation can occur concomitantly and/or 6-O can precede 2-O sulfation. Finally, measurements of disaccharide composition in the 6-O endosulfatase (sul-1) mutant that selectively removes individual sulfations, quantify the extent to which the sulfation motifs initially established are dynamic and reversible. Taken together our results establish for the first time for any organism the genetic ground state of the complete heparan modification network.

1153A

B9 domain proteins are required for the regulation of ciliary membrane composition. **Corey L Williams**, Marlene E Winkelbauer, Bradley K Yoder. Cell Biology, University of Alabama at Birmingham, Birmingham, AL.

Cilia are microtubule-based and membrane-bound organelles that serve diverse functions in various organisms and tissues. The ciliary membrane is a tightly regulated region that is enriched with many proteins not found throughout the rest of the cell membrane. Likewise, many proteins found in the cell membrane are excluded from the cilium. The mechanisms by which the cell membrane and ciliary membrane are differentially regulated are unknown. It is proposed that proteins at the base of the cilium participate in cell/ciliary membrane protein sorting, but their molecular identity has not been uncovered. Here, we demonstrate that the B9 domain proteins, TZA-1(Y38F2AL.2) and TZA-2(K03E6.4), function in such a capacity. MKS-3(F35D2.4), a transmembrane protein implicated in the human developmental disorder Meckel-Gruber Syndrome (MKS), normally localizes specifically to the ciliary base in *C. elegans*. In the absence of TZA-1 or TZA-2, MKS-3 freely enters the cilium, suggesting a requirement of these proteins in restricting ciliary access. Additionally, the transmembrane protein TRAM-1a (C24F3.1a), which normally concentrates directly adjacent to the ciliary base at the dendritic tip, is allowed ciliary access in the absence of TZA-1 or TZA-2. These data provide important insight into a mechanism by which ciliary membrane composition is regulated.

1154B
New and Improved: WormAtlas 2.0 and WormImage. **Laura A. Herndon**, Zeynep F. Altun, Chris Crocker, Tylon Stephney, David H. Hall. Center for *C. elegans* Anatomy, AECOM, Bronx, NY.

We announce the release of WormAtlas2.0. This new version of WormAtlas includes all previous information, but features a new look and many new additions. The main page adopts a tighter format by using drop down menus and more image based icons for intuitive navigation throughout the site. The Handbook is reorganized and provides a table of contents in a static frame for each section to allow for easy maneuvering to subsections and figures. The Hermaphrodite Handbook has updated content including an entirely new section covering the nervous system. Each section features new and updated figures and each figure now links to a separate page with the figure and legend and also to a high resolution version showing even greater detail. The Male Handbook also has been converted to the new layout and we are planning to introduce new content in the near future. Similarly, the Individual Neuron pages are being updated to a new format and will contain new figures, including a major expansion of the Male-specific neurons and their wiring patterns (see Emmons et al., this meeting). Slidable Worm is also gaining new pages and we have initiatives underway to streamline and accelerate this process. It is our hope that WormAtlas2.0 will be simpler to navigate and that beginners will find it more accessible. We encourage feedback from members of the *C. elegans* community on ways to improve the new version of WormAtlas.

The WormImage website houses thousands of unpublished electron micrographs and related data, and has been expanding steadily. It now presents more data from mutant animals, particularly for genes affecting the nervous system. We continue to rely heavily on MRC datasets, but we are also adding more from the Riddle and Hall lab files, among others. We encourage more laboratories to share their best archival TEM and SEM images so that this resource can continue to grow and serve the *C. elegans* community. We are very grateful to many people who have already contributed ideas, advice and experimental results that are featured on these websites. This work is supported by NIHRR 12596 to DHH.

1155C
At the intersection of bugs and worms: development of a joint microbiology-eukaryotic genetics undergraduate lab course. **Michael Y. Chao**, Paul Orwin. Dept Biol, California State Univ, San Bernardino, CA.

A major challenge for teaching science labs at the undergraduate level is balancing the need for “canned” lab exercises that demonstrate important biological principles to students with a spontaneous lab experience that more accurately conveys the excitement and realities of scientific discovery. Building on our own research interests and on a recent surge in publications that explore interspecies interactions between *C. elegans* and bacteria, we decided to explore a joint microbiology-*C. elegans* genetics undergraduate upper-division lab-based course for Spring quarter 2009. Due to their rapid life cycles, ease of handling, and inexpensive cost, both microorganisms and worms have major advantages over other organisms in an undergraduate teaching lab setting, particularly in large public universities on the 10 week quarter system. The question we wish to address is: can undergraduate students (most of whom have never had a microbiology and/or genetics course), given the most basic laboratory skill set and being limited within the framework of microbe-nematode interspecies interactions, formulate testable hypotheses and design and implement experiments to directly test them? (with some guidance, of course!) Assessment vehicles will be developed to compare effectiveness in student learning relative to “traditional” microbiology/genetics courses that are scheduled for the 2010-2011 academic year.

This study is funded by a Course Development Grant awarded to M.Y.C. and P.O. by the Teaching Resource Center at California State University San Bernardino.

1156A
A discovery-based molecular techniques course aimed to develop reagents for rab gene function studies. **Maria E. Gallegos**. Dept Biol, Cal State Univ, East Bay, Hayward, CA.

I have developed a discovery-based lab course at California State University, East Bay (CSUEB) entitled Advanced Molecular Techniques. This lab course has three aims: 1) to promote the National Science Education Standards mandate to replace “cookbook” lab courses with inquiry-based learning; 2) to teach a variety of molecular biology techniques applicable in both academia and industry settings and 3) to develop molecular tools useful to the C. elegans community for gene function studies. During the quarter, students learn a variety of standard molecular techniques in addition to QuikChange Site-Directed Mutagenesis (Stratagene) and Gateway Recombinational Cloning (Invitrogen). To prepare for and execute each step of the project, students must also master software tools such as NCBI BLAST (NLM), CLUSTAL W (EMBL-EBI), Primer Generator (Lawler and Turchin), A Plasmid Editor (Wayne Davis) and Image J (NIH), all of which are available free online. Students use these software tools and molecular techniques to modify Orfeome entry clones and create expression vectors containing dominant negative and constitutive active forms of the rab monomeric GTPases (entry clones kindly provided by Dr. Kang Shen). To date we have focused on the rab family of GTPases for several reasons: The rab family is manageable in size with 22 members. The average coding sequence is short, about the length of a traditional sequencing run. Dominant negative and constitutive active forms are well characterized. And finally, rab GTPases play important and diverse roles in cell and developmental biology. Each pair of students is assigned a single gene. One will create the dominant-negative while the other will create the constitutive active form. Thus each student owns a unique project with unique solutions while still having access to backup reagents (when necessary) and advice from peers. To provide each student a unique project, I needed to reduce costs. The QuikChange Site-Directed Mutagenesis kit by Stratagene is the most expensive component of this course thus each year we have also experimented with its protocol. Importantly, our results indicate that you do not need to use PAGE purified mutagenic primer pairs (as recommended), there is no reduction in mutagenesis efficiency if half the reaction volume is used and you can still get a sufficient number of transformants with half the volume of competent cells. Our progress will be reported. Any tools that have been created are freely available to the worm community.

1157B

Understanding linkage and recombination using *C. elegans* RNAi mutants. **Julie Hall**[1], Theresa Zucchero[2]. 1) Laboratory of Molecular Toxicology, National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC; 2) Department of Biology, Methodist University, Fayetteville, NC.

Although students often grasp the concepts of genetic inheritance and DNA structure easily, they sometimes struggle with understanding linkage and recombination frequencies. We set out to design a guided investigative project for undergraduate genetic students to help them better understand the concepts of linkage and recombination frequencies and how these concepts are used in research to genetically map an unknown gene. The goal of this laboratory exercise is to help students understand how a genetic model organism is used in the study of genetics. More specifically, students will be able to determine linkage using basic genetic crosses with marker strains, apply concepts of mapping, and calculate recombination frequencies to map a gene. For this five week project, students work in pairs to map the location of an "unknown" mutant which is defective for a gene involved in RNA interference (RNAi). When mutated, these genes result in RNAi resistance, an easily testable phenotype. Using linkage analysis, students map their "unknown" mutant to one of three chromosomes. Once they have determined on which chromosome their mutant lies, students pick strains from a provided list of mutants with visible phenotypes to use in a three-factor cross. Using the data collected from their three-factor cross, students determine recombination frequencies and an approximate map position. Finally, with the help of the professor, students navigate Wormbase.org to determine the identity of their "unknown" gene. The data the students collected did lead them to map locations that allowed them to correctly predict the identity of their "unknown" gene. Background information was introduced in the form of review articles and primary literature. Worksheets requiring the students to fill in the predicted results were provided to help them better understand the crosses being performed. Additionally, at the end of the project, students were asked to submit a journal-style paper based on their results. Although students were guided through the project, they were required to think critically about their results and the next step of the experiment. This project allowed students to apply knowledge they had learned in the genetics course to an experiment that mimicked a procedure often performed in research.

1158C

Ultraviolet light mutagenesis for integration of extrachromosomal arrays in Caenorhabditis elegans. Jenna D. Hill, Kristy N. Chiles, Candice C. Lightbourne, **Peter Barrett**. Department of Biology, Xavier University of Louisiana, New Orleans, LA.

Caenorhabditis elegans is an ideal organism for use in a Genetics Laboratory course, due to its relatively short generation time, ease of culture, and tractable genetics. The purpose of our experiments was to generate integrated transgenic lines of this organism using ultraviolet light (UV) mutagenesis. We did this both for the intrinsic goal of having these lines integrated, as well as for the pedagogical purpose of determining whether a mutagenesis experiment could be incorporated as a module into the Genetics Laboratory course at our institution. In the worm, transgenic DNA arrays are normally carried extrachromosomally, but can be integrated into a chromosome by any of a number of mutagens, including UV irradiation. We chose UV because of its common availability and ease of use, and also its relative safety as a mutagen in a classroom setting. Chromosomally integrated transgenic arrays can be easily identified by their conferring 100% transmission. Although UV mutagenesis has been used previously for this purpose, the results have not always been consistent and reproducible, perhaps due to differences in the wavelength, dosage, and experimental setup and evaluation protocols used. We will present data suggesting that: 1) existing protocols for integration with UV can be improved; 2) UV can work reproducibly and consistently as a mutagen and at higher efficiency than has been previously reported; and 3) the mutagenesis protocol can be easily adapted to become a part of the Genetics Laboratory curriculum at any primarily undergraduate institution.

1159A

Killing two birds with one stone: Getting your undergraduate class to map your mutants. **Lucinda Carnell**. Dept Biological Sciences, Central Washington, Ellensburg, WA.

Science education is moving towards more authentic and inquiry-based approaches to enhance learning. Laboratory courses that provide genuine research experiences are one strategy for generating excitement and enthusiasm. I have used this approach in a one-quarter upper-level majors Molecular Biotechniques course by performing a *C. elegans* mapping project. During the first third of the class, the students are introduced to and perform the techniques necessary to map the molecular lesion of a mutant to a chromosome. These include isolation of genomic DNA, PCR, and restriction digestion of restriction fragment length polymorphisms (RFLPs) also called snip-SNPs. In addition, the students learn about the broader significance of single nucleotide polymorphisms (SNPs) in phenotypic variation of organisms and disease by identifying examples through literature searches of primary research papers. In the last three weeks of the course, the students apply theses techniques to map a mutant previously isolated in a genetic screen. Each student was given an F2 recombinant from a cross between the mutant (in the N2 background) and the polymorphic mapping strain CB4856, with the goal of collectively mapping the mutation to a chromosome. The students isolated genomic DNA, cloned snip-SNP fragments for each of the six chromosomes, and digested the snip-SNPs using a single restriction enzyme, DraI (Davis et al. 2005). On the last day of the class, the students compiled their results together as a class (the total number of students was 22). The class data was analyzed and suggested that the mutation is on Chromosome V. As an independent research project, two students are going to continue to more finely map the lesion on Chromosome V. Students' comments indicate that they enjoyed the challenge of such a project, especially when their experiments lead to novel research findings. The novelty of the project can be maintained by using a new mutant each year.

Davis et at. 2005 BMC Genomics 2005, 6:118doi:10.1186/1471-2164-6-118.

1160B

Open Science in Practice: C. elegans Researchers and Their Use of Open Access Resources. **Lily Farris**[1], Don Moerman[2], Ed Levy[3], Emily Marden[3]. 1) Zoology/Centre for Applied Ethics, UBC, Vancouver, BC, Canada; 2) Zoology, UBC, Vancouver, BC, Canada; 3) Centre for Applied Ethics, UBC, Vancouver, BC, Canada.

As integrated GE3LS (genomics, ethics, environment, economics, law and society) researchers with the C. elegans Gene Knockout Consortium we are exploring how publicly available data is accessed and used and how this process informs scientific advancement. Our survey asked researchers about their scientific resource use, sharing of information, and handling of information with proprietary potential. The results from this survey provide additional understanding of the relationship between the researcher's use and exchange of information and their scientific innovation. Methods We invited researchers currently using C. elegans as a model organism who were 19 years of age or older to complete an online survey (www.wormsurvey.com) to provide information about their current use and exchange of research materials. Compiling a list of C. elegans researchers from the list of registered researchers in WormBase, we contacted a random sample of respondents five times in order to unsure higher response rates. The survey was fielded in October, 2008. We also requested that researchers circulate the survey to all members of their research team including graduate students, technicians, and research assistants who conduct any C. elegans related research. Preliminary Results Of the 349 respondents who conducted research with C. elegans, the majority supported the work of C. elegans Gene Consortium and supported the statement that their research would function differently if this resource was not available. Most respondents used WormBase on a daily or weekly basis and few respondents held patents. Overall, C. elegans researchers use and access a variety of resources with unrestricted access using these resources to produce both basic science and commercial innovation.

1161C

Biological Consequences of Poverty: A New Undergraduate Course on Poverty and Health. **James L. Lissemore**. Biology Dept., John Carroll University, University Heights, OH.

Most improvements in human health and life expectancy in developed countries over the last century can be attributed to public health efforts like immunization, sanitation, and water treatment. However, many of these public health advances have not been fully implemented in developing countries where HIV/AIDS, tuberculosis, malaria and other diseases are found at levels much higher than in developed countries. In addition, the poor in wealthy countries, e.g. the United States, face increased risks for a host of health problems, such as HIV/AIDS, obesity, and diabetes. Differences in health based on economic or other circumstances are known as health disparities, health inequities, or health inequalities. Beyond extending standard public health approaches to poor communities, research is needed to develop new treatments and preventive approaches for a variety of diseases and conditions. To this end, the scientific community has been paying increased attention to the links between poverty and health. For example, The Council of Science Editors recently produced a Global Theme Issue on Poverty and Human Development, in which major science journals, e.g. the Nature journals, Science, PNAS, Cell, and the PLoS journals, simultaneously published papers related to this topic in October 2007. The stated goal of this project was "to stimulate interest and research in poverty and human development and disseminate the results of this research as widely as possible." Engaging scientists and health professionals in developing creative approaches to reducing health disparities is clearly required. Additionally, efforts to raise awareness of the connections between poverty and health among the next generation of scientists and health professionals are essential. Therefore, I developed a new course for undergraduate biology majors, Biological Consequences of Poverty, that addresses the links between poverty and health. Some of the goals of the course are to provide students with: 1) a basic understanding of global and domestic poverty, health disparities, public health, and epidemiology; 2) basic knowledge and understanding of global and US health disparities with respect to HIV/AIDS, obesity/diabetes, tuberculosis, and malaria; and 3) an understanding of evolutionary factors in disease. Results from student surveys that address changes in student learning and attitudes and a detailed description of the course will be presented.

1162A

C. elegans as a Platform for Teaching Introductory Genetics and Experimental Biology. **Alice M. Rushforth**, Dennis H. Kim. Dept of Biology, MIT, Cambridge, MA.

Introduction to Experimental Biology and Communication (Course 7.02) is an introductory laboratory course offered by the Department of Biology at MIT. The course enrolls 100 undergraduates and provides an opportunity to learn experimental techniques in molecular biology, biochemistry, and genetics. The course integrates basic principles of these subjects with experimental design and data analysis to prepare students for subsequent research experiences. Course 7.02 is required of all Biology majors and is also taken by nearly all pre-medical students. 7.02 is a one semester course that is divided into two approximately 6 week-long modules, the first module emphasizes basic concepts and methods of molecular biology and biochemistry; students perform site-directed mutagenesis, express and purify the mutant protein and then apply two assays to assess mutant protein function. In the second module, students use molecular genetic methods to explore genetics, development, anatomy, and behavior in *C. elegans*. Students start by learning about forward genetics by examining mutants for phenotypes and learning about the gene that has been disrupted. They go on to investigate genetic relationships between genes through epistasis analysis. These experiments also allow students to learn about organogenesis through characterizing vulval development mutants and to gain an appreciation for complex regulatory pathways by examining the role of the *daf-2* signaling pathway in both the dauer developmental decision and in aging. Students also perform classic two-factor mapping to determine the linkage between two genes and then use single nucleotide polymorphism (SNP) mapping to identify the chromosomal region to which these genes map in the genome. Students explore reverse genetics methods by investigating a specific gene at WormBase and performing RNA interference (RNAi) on the gene of interest. Finally, to investigate aspects of *C. elegans* behavior students perform two experiments; one in which they measure pumping rates in wild-type and mutant strains, and the other in which they investigate chemotaxis behavior. Through these experiments students are introduced to error analysis and perform basic statistical tests on their data. Experimental protocols, the laboratory manual, and educational assessment and support materials are available to non-profit teaching institutions upon request. Acknowledgement: Curriculum development, instructional equipment, and teaching resources were supported in part by an undergraduate institutional research grant from the Howard Hughes Medical Institute, and by MIT Alumni Class Funds for undergraduate curriculum development.

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The following index is composed of keywords selected by presenting authors.
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Allison Lynn Abbott
Marquette Univ
allison.abbott@marquette.edu

Linu S Abraham
Rosalind Franklin University
linu.abraham@rosalindfranklin.edu

Daniel Ackerman
Inst Healthy Ageing
daniel.ackerman@ucl.ac.uk

Aude S Ada-Nguema
Univ Utah
adanguema@biology.utah.edu

Cristina Aguirre-Chen
Albert Einstein College of Med
caguirre@aecom.yu.edu

Joohong Ahnn
Hanyang Univ
joohong@hanyang.ac.kr

Michael Ailion
Univ Utah
ailion@biology.utah.edu

Segen Aklilu
Agnes Scott College
saklilu@agnesscott.edu

Silvestre Alavez
Buck Inst
salavez@buckinstitute.org

Amelia Alessi
Life Sciences Institute
aalessi@umich.edu

Ashley Elizabeth Alker
Central Michigan University
alker1ae@cmich.edu

Taylor Allen
Oberlin Col
taylor.allen@oberlin.edu

Scott Alper
National Jewish Health
alpers@njhealth.org

Christopher Alvaro
Muhlenberg College
ca235106@gws1.muhlenberg.edu

Daphne Anastasiades
Columbia Univ
dca2106@columbia.edu

Erik C Andersen
Princeton University
erik.andersen@gmail.com

Giana Angelo
FHCRC
gangelo@fhcrc.org

Javier Apfeld
Harvard Med Sch
javier_apfeld@hms.harvard.edu

H Efsun Arda
Univ Massachusetts Med Sch
efsun.arda@umassmed.edu

Swathi Arur
sarur@genetics.wustl.edu

Peter E.A Ash
Mayo Clinic Florida
ash.peter@mayo.edu

Matthew Attreed
AECOM
mattreed@aecom.yu.edu

Jason Avery
University of Tulsa
jason-avery@sbcglobal.net

Felipe Avila
University of Texas at Arlington
avilafelipe@uta.edu

Srinivas Ayyadevara
Univ Arkansas Medical Sci
srini54@hotmail.com

Nasrin Babadi
Univ Ottawa
nhabi102@uottawa.ca

Charles F Baer
Univ Florida
cbaer@ufl.edu

Adam K Bahrami
Harvard Univ
bahrami@fas.harvard.edu

Christopher C Baldi
UMDNJ
baldicc@umdnj.edu

Sophie Marie Bamps
Leeds Univ
fbssb@leeds.ac.uk

Zhirong Bao
Sloan-Kettering Institute
baoz@mskcc.org

Jeff W. Barclay
University of Liverpool
barclayj@liverpool.ac.uk

Kathleen Barnhart
Union Biometrica
kbarnhart@unionbio.com

Peter Barrett
Xavier University of Louisiana
pbarrett@xula.edu

Enkelejda Bashllari
Columbia University
eb2277@gmail.com

Ryan Baugh
Duke University
ryan.baugh@duke.edu

Daphne Bazopoulou
University of Michigan
dafbaz@umich.edu

Maria L Begasse
Max Planck Institute
begasse@mpi-cbg.de

Jean-Michel Bellanger
CRBM-CNRS
jean-michel.bellanger@crbm.cnrs.fr

Claire Y Benard
Columbia Univ
cb2213@columbia.edu

Ingrid Benirschke
Cold Spring Harbor Lab. Press
cshpress@cshl.edu

Laura A Berkowitz
Univ Alabama
laberkowitz@bama.ua.edu

Yelena B Bernadskaya
UMDNJ/RWJMS
patelfb@umdnj.edu

Stefano Berri
University of Leeds
s.berri@leeds.ac.uk

Axel Bethke
Baylor Col Med
abethke@bcm.tmc.edu

Matthew H Beverly
Brandeis Univ
bev@brandeis.edu

Puneet Bharill
UAMS
pbharill@uams.edu

Raja Bhattacharya
Albert Einstein Col Med
rajabhattacharya8@gmail.com

Laura Bianchi
Univ Miami
lbianchi@med.miami.edu

Allison Billi
University of Michigan
acbilli@umich.edu

Bilge Birsoy
Univ California, Santa Barbara
birsoy@lifesci.ucsb.edu

Joshua C Black
MGH
blackjc@gmail.com

Oliver E Blacque
University College Dublin
oliver.blacque@ucd.ie

Dan Blick
University of Washington
blick@u.washington.edu

Tom Blumenthal
Univ Colorado
tom.blumenthal@colorado.edu

Max E Boeck
Univ Washington
maxboeck@u.washington.edu

Justin S Bois
MPI-CBG
bois@mpi-cbg.de

Olaf Bossinger
RWTH Aachen University
bossinger@mac.com

Konstantinos Boulias
MIT
boulias@mit.edu

Charles E Brabin
University of Oxford
charles.brabin@magd.ox.ac.uk

Christian Braendle
CNRS, Univ Nice
braendle@unice.fr

Katjusa Brejc
UC Berkeley/HHMI
kbrejc@berkeley.edu

John Lennon Brenner
Marquette Univ
john..brenner@marquette.edu

Sarah Elizabeth Brisbin
Queen's Univ
sarah.brisbin@queensu.ca

Thomas Matthew Brodigan
LMB/NIDDK/NIH
thomasbr@intra.niddk.nih.gov

Alison Brooks
Fred Hutchinson Cancer Res Ctr
anbrooks@u.washington.edu

Jacqueline M Brooks
Harvard Univ
jbrooks@mcb.harvard.edu

Yelena V Budovskaya
Stanford Univ
yelenab@stanford.edu

Hannes E Buelow
Albert Einstein/Yeshiva Univ
hbuelow@aecom.yu.edu

Julien Burger
Institut Jacques Monod, CNRS
burger.julien@ijm.jussieu.fr

Kirk Benjamin Burkhart
Univ Wisconsin
kburkhart@wisc.edu

Rebecca A Butcher
Harvard Medical Sch
rebecca_butcher@hms.harvard.edu

Alex Butschi
Univ Zurich
alex.butschi@molbio.uzh.ch

Filipe Cabreiro
Univ College London
f.cabreiro@ucl.ac.uk

Guy A Caldwell
The University of Alabama
gcaldwel@bama.ua.edu

Peter R Canavello
Tulane University
pcanavel@tulane.edu

Vincent Cannataro
SUNY College at Geneseo
vlc2@geneseo.edu

Yossi Capua
NYU
josef.capua@med.nyu.edu

Juan J Carmona
Harvard Med School
carmona@fas.harvard.edu

Ines Carrera
ic2255@columbia.edu

Michael Cary
UCSF
michael.cary@uscf.edu

Raymond Caylor
raycay3@ku.edu

Julian Ceron
FUNDACIO IDIBELL
jceron@idibell.cat

QueeLim Ch'ng
King's College London
queelim@kcl.ac.uk

Martin Chalfie
Columbia Univ
mc21@columbia.edu

Jason P Chan
Univ So California
jasonpch@usc.edu

Howard Chang
MIT
hcchang@mit.edu

Lucy Fang-I Chao
UMass Medical School
Lucy.Chao@umassmed.edu

Jamie O Chapman
Univ Kansas
chapman@ku.edu

Daniel Chase
UMass
danchase@biochem.umass.edu

Daniel Marques Chaves
Univ Massachusetts Med Sch
daniel.chaves@umassmed.edu

Di Chen
Buck Inst
dchen@buckinstitute.org

Grace L Chen
Stanford University
glin1@stanford.edu

Ling Chen
Univ California, Santa Barbara
l_chen@lifesci.ucsb.edu

Lu Chen
Albert Einstein Col Medicine
luchen@aecom.yu.edu

Pan Chen
The University of Alabama
pchen3@bamamail.ua.edu

Wen Chen
California Inst Technology
wchen@cco.caltech.edu

Xiaoyin chen
columbia University
xc2123@columbia.edu

Yushu Chen
Columbia University
yc2332@columbia.edu

Poh Hui Chia
Stanford
pchia@stanford.edu

Wei-Chung Chiang
University of Michigan
weichun@umich.edu

Daisuke Chihara
NYU
Daisuke.Chihara@nyumc.org

Ian D Chin-Sang
Queen's Univ
chinsang@queensu.ca

Andrew Chisholm
Univ California, San Diego
chisholm@ucsd.edu

Julie Cho
Calif Institute of Technology
juliec@caltech.edu

Myung-gyu Choi
Seoul National Univ
mgm9@naver.com

Shin Sik Choi
Univ California, Santa Barbara
choi@lifesci.ucsb.edu

Trushal V Chokshi
Univ Michigan
tchokshi@umich.edu

King-Lau Chow
Hong Kong Univ Sci & Technol
bokchow@ust.hk

Nikos Chronis
Univ Michigan
chronis@umich.edu

Jeffrey Shih Chieh Chu
SFU
jeff.sc.chu@gmail.com

Hediye N Cinar
Food & Drug Admin
hediye.cinar@fda.hhs.gov

Thais G Cintra
San Francisco State University
thais_gc@hotmail.com

Scott G Clark
University of Nevada, Reno
sgclark@unr.edu

Brian Coblitz
Columbia Univ
bcoblitz@columbia.edu

Max Cohen
University of Colorado
max.cohen@colorado.edu

Monica P Colaiacovo
Harvard Medical Sch
colaiaco@receptor.med.harvard.edu

Sara L Collier
Washington University of STL
CollierS@Wustl.edu

Annie L Conery
Massachusetts General Hospital
aconery@molbio.mgh.harvard.edu

Vince Contreras
East Carolina Univ
vc0307@ecu.edu

Astrid Cornils
Friedrich Miescher Inst
astrid.cornils@fmi.ch

Pauline Cottee
Univ Alabama at Birmingham
pcottee@uab.edu

Hannah Craig
Univ Leeds
h.craig@leeds.ac.uk

Emily Crane
Univ California, Berkeley
emily@crane.net

James F Cregg
Univ California, San Diego
jcregg@ucsd.edu

Helen Marie Crook
Newcastle Univ
h.m.crook@ncl.ac.uk

Melanie Croydon-Sugarman
University of Toronto
m.croydon.sugarman@utoronto.ca

Mingxue Cui
Univ Colorado
cuim@colorado.edu

Philip R Cumbo
McMaster University
cumbop@mcmaster.ca

Asher D Cutter
Univ Toronto
asher.cutter@utoronto.ca

Nooreen Suzanne Dabbish
University of Pennsylvania
nooreen@mail.med.upenn.edu

Caroline Lund Dahlberg
Tufts University
lina.l.dahlberg@gmail.com

Krishnakali Dasgupta
Univ. of Southern California
kdasgupt@usc.edu

Keith G Davies
Univ Oxford
keith.davies@bioch.ox.ac.uk

Amanda Day
University of Michigan
dayama@umich.edu

Sasha De Henau
Ghent University
Sasha.DeHenau@UGent.be

Claire de la Cova
Columbia University
ccd24@columbia.edu

Alexandre de Lencastre
Yale University
alexandre.delencastre@yale.edu

Vronique de Vaux
University of Fribourg
veronique.devaux@unifr.ch

Sarah E DeGenova
College New Jersey
degenov2@tcnj.edu

John DeModena
California Inst Technology
demoj@caltech.edu

Shannon M Dennis
FHCRC
smm04@u.washington.edu

Tracey DePellegrin Connelly
Carnegie Mellon Univ
td2p@andrew.cmu.edu

Micah Depper
Lewis & Clark College
mdepper@lclark.edu

Abby F Dernburg
Univ California, Berkeley
afdernburg@lbl.gov

Krupa Deshmukh
Washinton university St.Louis
kdeshmukh@wustl.edu

Christoph Dieterich
Max Delbruck Ctr Molec Med
chrisd@tuebingen.mpg.de

James C Dillon
Univ Southampton
jcd@soton.ac.uk

Ivan D Dimitrov
Washington University in St. Lou
dimitrovi@wustl.edu

Abderazak Djeddi
CNRS
abderazak.djeddi@cgm.cnrs-gif.fr

Jung H Doh
University at Albany
jd885462@albany.edu

Maria Doitsidou
Columbia Univ
md2398@columbia.edu

Meng-Qiu Dong
NIBS, Beijing
dongmengqiu@nibs.ac.cn

Ryan Doonan
University College London
r.doonan@ucl.ac.uk

Alana V Doty
Univ Maryland College Park
adoty@umd.edu

Kevin McCoy Drace
Mercer University
drace_km@mercer.edu

Thomas F Duchaine
McGill University
thomas.duchaine@mcgill.ca

Kathleen Dumas
LSI, University of Michigan
kjdumas@umich.edu

Denis Dupuy
IECB
d.dupuy@iecb.u-bordeaux.fr

Meenakshi Dwivedi
Hanyang University
meena.dwivedi@gmail.com

Ralf Eberhard
University of Zurich
ralf.eberhard@molbio.uzh.ch

Theresa Leigh Brix Edelman
brixx001@umn.edu

Arthur S. Edison
UNIVERSITY OF FLORIDA
art@mbi.ufl.edu

Tyson Edwards
Yale University
tyson.edwards@yale.edu

Toshihiko Eki
Toyohashi Univ Tech
eki@eco.tut.ac.jp

Ronald E Ellis
UMDNJ-SOM
ron.ellis@umdnj.edu

Taishi Emmei
Nagoya Univ
emmei.taishi@b.mbox.nagoya-u.ac.jp

Maria A Ermolaeva
University of Cologne
ermolaem@uni-koeln.de

Kathleen Anne Estes
UC San Diego
esteskt@gmail.com

Marina Ezcurra
MRC-LMB
marina@mrc-lmb.cam.ac.uk

Kei C. Fan
HKUST
timmysfxc@gmail.com

Chris Fang-Yen
Harvard University
cfangyen@gmail.com

Brian M Farley
Univ Massachusetts Med Sch
brian.farley@umassmed.edu

Michael Fazzio
Union Biometrica
mfazzio@unionbio.com

Jessica L Feldman
Fred Hutchinson Cancer Res Ctr
jlfeldma@fhcrc.org

Marie-Anne Felix
CNRS - Inst Jacques Monod
felix@ijm.jussieu.fr

Dingxia Feng
Iowa State University
fengdxia@iastate.edu

Denise M Ferkey
University at Buffalo, SUNY
dmferkey@bio.buffalo.edu

Michela Fiaschi
College New Jersey
fiaschi2@tcnj.edu

Sylvia E J Fischer
Massachusetts General Hosp
fischer@molbio.mgh.harvard.edu

David Fitch
NYU- Silver 1009
david.fitch@nyu.edu

Stephane Flibotte
BC Cancer Research Centre
sflibotte@bcgsc.ca

Emily Forbes
MGH/ HMS
emma244@gmail.com

Eric P Foss
Central Washington Univ.
fosse@cwu.edu

Juan Jose Fraire-Zamora
Univ California
jfrai001@student.ucr.edu

Melissa Y Frederic
Simon Fraser University
mfa34@sfu.ca

Lilach Friedlander
Technion-Israel Inst Tech
lilachf@tx.technion.ac.il

Christian Frokjaer-Jensen
Univ Utah
christianfj@gmail.com

Ya Fu
Albert Einstein Col Medicine
yfu@aecom.yu.edu

Masashi Fujita
RIKEN ASI
m-fujita@riken.jp

Masamitsu Fukuyama
Univ Tokyo
mfukuyam@mol.f.u-tokyo.ac.jp

Tomoyuki Furuta
Nagoya Unisversity
tomoyuki.furuta@c.mbox.nagoya-u.ac.jp

Johnie J Gallagher
Univ Kansas
meercat515@hotmail.com

Christopher Michael Gallo
Johns Hopkins Univ
cgallo2@jhmi.edu

Francis Amrit Raj Gandhi
University of Birmingham
frg681@bham.ac.uk

Shangbang Gao
Samuel Lunenfeld Research Inst.
gao@mshri.on.ca

Susana M Garcia
Massachusetts Gen Hosp
garcia@molbio.mgh.harvard.edu

Jacob Matthew Garrigues
UC Santa Cruz
garrigues@biology.ucsc.edu

Jeb Gaudet
Univ Calgary
gaudetj@ucalgary.ca

Laura Gaydos
UC Santa Cruz
ljgaydos@biology.ucsc.edu

Sara Gelino
Burnham Institiute Med Res
sgelino@burnham.org

Birgit Gerisch
MPI Biol Ageing
gerisch@molgen.mpg.de

Vikas Ghai
Univ Calgary
vghai@ucalgary.ca

Piya Ghose
Rutgers Univ
piyag@waksman.rutgers.edu

Anindya Ghosh Roy
UCSD, Room2429, Bonner Hall
aghoshroy@ucsd.edu

Matthew S Gill
Buck Institute
mgill@buckinstitute.org

Chris R Gissendanner
Univ Louisiana, Monroe
gissendanner@ulm.edu

Aiza Cathe A Go
San Francisco State University
aizacathe@gmail.com

Andy Golden
NIDDK/NIH
andyg@mail.nih.gov

Miriam B Goodman
Stanford Univ
mbgoodman@stanford.edu

Vidya Gopinath
Haverford College
pmeneely@haverford.edu

Lakshmi Gorrepati
UMBC
lakshmi2@umbc.edu

Monica Gotta
Geneva University
monica.gotta@unige.ch

Elena Govorunova
Albert Einstein College of Medic
egovorun@aecom.yu.edu

Jo M Goy
Univ North Texas
jmgoy@harding.edu

Liane R Greaver
CSU San Bernardino
greaverl@csusb.edu

David Greenstein
Univ Minnesota
green959@umn.edu

Stephan W Grill
MPI-CBG/MPI-PKS
grill@mpi-cbg.de

Kelly A Grussendorf
University of Kansas
grusseke@ku.edu

Shouhong Guang
Univ Wisconsin, Madison
sguang@wisc.edu

Kristin C Gunsalus
New York Univ
kcg1@nyu.edu

Yiqing Guo
UMDNJ
guoyi@umdnj.edu

Mark Gurling
UC-Berkeley
mgurling@berkeley.edu

Stephan Gysi
Univ Zurich
stephan.gysi@molbio.unizh.ch

Leonard A Haas
Rutgers, The State University NJ
lenhaas@eden.rutgers.edu

Gizelle Hacopian
Cal Poly Pomona
ghacopian1@gmail.com

Jeong-Hoon Hahm
Yonsei Univ
hahmjh@proteomix.org

David H Hall
Albert Einstein Col Med
hall@aecom.yu.edu

Julie Hall
NIEHS
hallj2@niehs.nih.gov

Elissa A Hallem
California Inst Technology
ehallem@caltech.edu

Danielle R Hamill
Ohio Wesleyan Univ
drhamill@owu.edu

Marc Hammarlund
Yale University
marc.hammarlund@yale.edu

Michael Han
Wellcome Trust Sanger Inst
mh6@sanger.ac.uk

Sungmin Han
Univ Alabama, Birmingham
han@uab.edu

Xue Han
Univ of Alberta
xue.han@ualberta.ca

David D Hansen
Univ Calgary
dhansen@ucalgary.ca

Mariah Hanson
Central Michigan University
hanso1ml@cmich.edu

Vera Marie Hapiak
Univ Toledo
hapiakv@yahoo.com

Sharon Harel
UQAM
sharon.harel@gmail.com

Adam John Harrington
Univ Alabama
ajharrington@bama.ua.edu

Gareth P Harris
University of Toledo
gazalad2@hotmail.com

Martin Harterink
Hubrecht Institute
m.harterink@niob.knaw.nl

Tamar Hashimshony
Technion
thashimi@tx.technion.ac.il

Gal Haspel
NIH
haspelg@ninds.nih.gov

Nancy Caroline Hawkins
Simon Fraser Univ
nhawkins@sfu.ca

Bin He
Baylor Col Medicine
bh147985@bcm.tmc.edu

Bree Heestand
Baylor College Med
bheestand@bcm.tmc.edu

Cassandra Heighington
cassington2@hotmail.com

Andrew Bennett Hellman
Stanford Univ
abhellman@hotmail.com

Johan Henriksson
Karolinska Inst
johan.henriksson@ki.se

Greg J Hermann
Lewis & Clark College
hermann@lclark.edu

Ramon Antonio Herrera
New York University
rantonio.herrera@gmail.com

Karanda Jean Hildebrand
Univ Kansas
karanda@ku.edu

Andrea Hinas
Harvard Univ
hinas@mcb.harvard.edu

Shizuka Hino
Ritsumeikan Univ
cb005045@is.ritsumei.ac.jp

Takashi Hirose
MIT
thirose@MIT.EDU

Oliver Hobert
Columbia Univ
or38@columbia.edu

Jonathan Hodgkin
Univ Oxford
jonathan.hodgkin@bioch.ox.ac.uk

Ray Hong
California State Univ
ray.hong@csun.edu

Pamela E Hoppe
Western Michigan Univ
pamela.hoppe@wmich.edu

Kelly L Howell
Univ Pennsylvania
kehowell@mail.med.upenn.edu

Uladzislau Hryshkevich
Technion Israel Inst Tech
hryshkev@tx.technion.ac.il

Jinghua Hu
Mayo Clinic
jhu@pharmacy.wisc.edu

Shuang Hu
University of Toledo
shuang.hu@utoledo.edu

zhitao Hu
Massachusetts General Hosp
huz@molbio.mgh.harvard.edu

Elvis Huarcaya Najarro
Univ Kansas
bioelvis@ku.edu

Thomas J Hubert
UCSD
thubert@ucsd.edu

Martin L Hudson
Univ Kansas
mlhudson@ku.edu

Agnes K. Y. Hui
HKUST
hkyaa@ust.hk

Piper R Hunt
National Inst on Aging
huntpr@mail.nih.gov

Jerrod Wayne Hunter
OMRF
oumagicman@yahoo.de

Steven J Husson
K.U.Leuven/Goethe-Univ Frankfurt
steven.husson@bio.kuleuven.be

Ara Banya Hwang
POSTECH
ara.hwang@postech.ac.kr

Mouna Ibourk
Northeastern University
ibourk645@yahoo.com

Yuichi Iino
Univ Tokyo
iino@biochem.s.u-tokyo.ac.jp

Akitoshi Inoue
Kyushu Univ
sl20054@s.kyushu-u.ac.jp

Takao Inoue
National University of Singapore
bchti@nus.edu.sg

Richard Elwyn Isaac
Univ Leeds
r.e.isaac@leeds.ac.uk

Takeshi Ishihara
Fac Sci, Kyushu Univ
takeiscb@mbox.nc.kyushu-u.ac.jp

Hiroaki Iwasa
Rutgers Univ
iwasa@biology.rutgers.edu

Ryo Iwata
Univ Tokyo
ss077133@mail.ecc.u-tokyo.ac.jp

Belinda M Jackson
Univ Maryland, Baltimore Cnty
bjacks1@umbc.edu

Tracy James
Virginia Tech University
tjames84@vt.edu

Sophie Jarriault
IGBMC
sophie@igbmc.u-strasbg.fr

Britta Jedamzik
MPI-CBG
jedamzik@mpi-cbg.de

Louise Jensen
Aarhus Univ
ltj@mb.au.dk

dahye Jeong
Postech
sona0126@gmail.com

Pan-Young Jeong
UCLA, Santa Barbara
pyjeong@lifesci.ucsb.edu

Xinnong Jiang
Huazhong Univ of Sci & Tech
jiangxn88@mail.hust.edu.cn

Tomasz J Jodlowski
Montefiore Medical Ctr
tomaszjod@gmail.com

David W Johnson
Univ Rochester
david_johnson@urmc.rochester.edu

Nicholas Michael Johnson
Hubrecht Institute
n.johnson@niob.knaw.nl

Tamako Jones
Loma Linda Univ
tjones@dominion.llumc.edu

Antony M Jose
Harvard Univ
amjose@mcb.harvard.edu

Maelle Jospin
Univ de Lyon 1, UMR CNRS 5123
jospin@univ-lyon1.fr

Paola Jurado
Nagoya Univ
paola.nagoya.univ@gmail.com

Eriko Kage-Nakadai
Tokyo Women's Medical University
nakadaie@research.twmu.ac.jp

John M Kalb
Canisius College
kalbj@canisius.edu

Andrea K Kalis
Univ Minnesota
horn0135@umn.edu

Shaunak Kamat
Rutgers Univ
kamata@eden.rutgers.edu

Chanhee Kang
UT Southwestern Medical Center
chanhee.kang@utsouthwestern.edu

Sang-Jo Kang
Yonsei Univ
ksjojo602@hanmail.net

Cheng-Yuan Kao
Univ California, San Diego
c2kao@ucsd.edu

Pankaj Kapahi
Buck Inst
pkapahi@buckinstitute.org

Takeshi Karashima
Tokyo Univ
karashima-tky@umin.ac.jp

Jonathan E Karpel
Claremont Colleges
jkarpel@jsd.claremont.edu

Luv Kashyap
Univ Pittsburgh
vfa2@pitt.edu

Mihoko Kato
California Inst Technology
mkato@caltech.edu

Amanda L Kauffman
Princeton University
alpeters@princeton.edu

Kayo Kawano
Tottori University
heart_beat135@yahoo.co.jp

Tsuyoshi Kawano
Tottori Univ
kawano@muses.tottori-u.ac.jp

Trupti Kawli
Stanford Univ
trupti@stanford.edu

Lisa Kennedy
Columbia University
lmk2146@columbia.edu

Roongpetch Keowkase
Univ Maryland Baltimore
rkeow001@umaryland.edu

Rossio K Kersey
NIDDK/NIH
kerseyr@niddk.nih.gov

Sana Khan
Queens College
skhan119@qc.cuny.edu

Vishal Khivansara
Life Sci Inst
kvishal@umich.edu

Michael Tommy Kiedrowski
Hofstra University
mkiedr1@pride.hofstra.edu

Chun-A Kim
Seoul National university
kimchuna@hotmail.com

Grace S Kim
Albert Einstein Col Med
gsykim@mit.edu

Hongkyun Kim
Rosalind Franklin University
hongkyun.kim@rosalindfranklin.edu

Jeongmin Kim
Hanyang University
innovatorjm@naver.com

Kyuhyung Kim
Brandeis University
khkim@brandeis.edu

Seongseop Kim
University of Minnesota
kimx1285@umn.edu

Sunhong Kim
Univ Colorado
sunhong@colorado.edu

Tsubasa Kimata
Nagoya University
kimata.tsubasa@a.mbox.nagoya-u.ac.jp

Kotaro Kimura
Osaka Univ
kokimura@bio.sci.osaka-u.ac.jp

Jason M Kinchen
Univ of Virginia
jmk8q@virginia.edu

Jason A Kintzele
Western Michigan University
jason.a.kintzele@wmich.edu

Edward T Kipreos
Univ Georgia
ekipreos@cb.uga.edu

Keisuke Kiriyama
Kyushu Univ
3sl09037s@sls.kyushu-u.ac.jp

Ranjana Kishore
California Inst Technology
ranjana@its.caltech.edu

Gunnar Kleeman
Princeton University
kleeman@princeton.edu

Anna Kleshayeva
Univ Louisiana at Monroe
kleshaa@tribe.ulm.edu

Phil Knezevich
San Jose State University
Vonkonigsberg@yahoo.com

Daniela Knoefler
University of Michigan
knoefler@umich.edu

Hiroyuki Kobuna
TWMU, Tokyo, Japan
kobuna@research.twmu.ac.jp

Paulina M Kolasinska-Zwierz
Univ Cambridge
pmk35@cam.ac.uk

Irina Koloteuva
IGBMC - CERBM
ert@igbmc.fr

Richard W Komuniecki
University of Toledo
rkomuni@uoft02.utoledo.edu

Kenji Kontani
University of Tokyo
kontani@mol.f.u-tokyo.ac.jp

Janet Koon
York Univ
jk_2002@yorku.ca

Hendrik C Korswagen
Hubrecht Laboratory
rkors@niob.knaw.nl

Jerome Peter Korzelius
Utrecht Univ
jkorzelius@gmail.com

Zdenek Kostrouch
Charles Univ
zdenek.kostrouch@lf1.cuni.cz

Marta Kostrouchova
Charles Univ
marta.kostrouchova@lf1.cuni.cz

Nikos Kourtis
FORTH
kourtis@imbb.forth.gr

Ismar Kovacevic
Northeastern Univ
ismar.k@neu.edu

Hiroshi Koyama
National institute of Genetics
hkoyama@lab.nig.ac.jp

Predrag Krajacic
University of Pennsylvania
predrag@mail.med.upenn.edu

Elsa Kress
University of Geneva
elsa.kress@unige.ch

Eva Krpelanova
Erasmus MC
e.krpelanova@erasmusmc.nl

Terry J Kubiseski
York University
tkubises@yorku.ca

Atsushi Kuhara
Nagoya Univ
atsushi_kuhara@cc.nagoya-u.ac.jp

Ashish Kumar
Univ California, Santa Barbara
kumar@lifesci.ucsb.edu

Hirofumi Kunitomo
University of Tokyo
kunitomo@biochem.s.u-tokyo.ac.jp

Vinod Kurumathurmadam Mony
Kansas State Univ
vinodkm@ksu.edu

Koji Kyoda
RIKEN ASI
kkyoda@riken.jp

Robert Labiento
rlabie1@pride.hofstra.edu

Mary Lee Ladage
mary.ladage@gmail.com

Bernard Lakowski
Pasteur Inst
lakowski@pasteur.fr

Julie D Lamarche
CR-CHUM
julie.demers-lamarche@umontreal.ca

Craig W Lamunyon
California State Polytech Univ
cwlamunyon@csupomona.edu

Karen Lange
University of Alberta
klange@ualberta.ca

Nhien Lao
University of Calgary
laom@tcd.ie

Renee A Larson
Canisius College
larsonr@canisius.edu

Laura Lscarez
IFC - UNAM
lascarez@ifc.unam.mx

Patrick Laurent
MRC-LMB
patrick@mrc-lmb.cam.ac.uk

Robert M Layne
University of Toledo
robert.layne@utoledo.edu

Michelle G LeBlanc
NYU
michelle.g.leblanc@gmail.com

Anna Y-W Lee
McGill Univ
anna@mcb.mcgill.ca

Hanee Lee
IMBG
danji122@snu.ac.kr

Insuk Lee
Yonsei Univ
insuklee@yonsei.ac.kr

Jihyun Lee
Seoul National University
jlee00@snu.ac.kr

KyungHwa Lee
Univ Rochester
kyunghwa.lee@gmail.com

Myeongwoo Lee
Baylor Univ
myeongwoo_lee@baylor.edu

Raymond Lee
California Inst Technology
raymond@caltech.edu

Siu S Lee
Cornell Univ
ssl29@cornell.edu

Teresa W Lee
UC Berkeley
teresalee@berkeley.edu

Renaud Legouis
CNRS
renaud.legouis@cgm.cnrs-gif.fr

Haiyan Lei
NIH/NIDDK
leihaiyan@niddk.nih.gov

Bennie Lemmens
Hubrecht Institute
b.lemmens@hubrecht.com

Luciana Leopold
UNC- Chapel Hill
lleopold@med.unc.edu

Giovanni M Lesa
UCL
giovanni.lesa@ucl.ac.uk

Maxwell C.K. Leung
Duke University
maxwell.leung@duke.edu

Steven Levitte
Lewis & Clark College
slevitte@lclark.edu

Chris Li
City Col New York
cli@sci.ccny.cuny.edu

Ji Li
Columbia Univ
jl2493@columbia.edu

Weiqing Li
Univ Washington
weiqing@u.washington.edu

Xiangqian Li
Divergence, Inc.
li@divergence.com

Yujie Li
Mayo Clinic
li.yujie@mayo.edu

Diana E Libuda
Stanford Univ
dlibuda@stanford.edu

Conny H C Lin
Univ British Columbia
conny@interchange.ubc.ca

Robyn Lints
Texas A & M Univ
rlints@mail.bio.tamu.edu

Gang Liu
Waksman Inst
evaeva8099@gmail.com

Jianfeng Liu
Huazhong Univ of Sci & Tech
jfliu@mail.hust.edu.cn

Jun Liu
Cornell Univ
jl53@cornell.edu

Oliver Liu
Stanford University
oliu@stanford.edu

Qinwen Liu
University of Maryland
qinwen@umd.edu

Wan-Ju Liu
UMBC
wanju1@umbc.edu

Yishi Liu
Texas A&M Univ
yliu@mail.bio.tamu.edu

Szecheng Lo
Chang Gung Univ
losj@mail.cgu.edu.tw

Cody J Locke
Univ California San Francisco
cody.locke@ucsf.edu

Curtis M Loer
Univ San Diego
cloer@sandiego.edu

Anne C. Logie
National Institute on Aging
logieac@nia.nih.gov

Ferdinand C O Los
UC San Diego
flos@ucsd.edu

Yu Lu
McGill Univ
yu.lu4@mail.mcgill.ca

Jim Lund
Univ Kentucky
jiml@uky.edu

Shijing Luo
Princeton Univ
sluo@princeton.edu

Allison Lynch
University of Wisconsin-Madison
amlynch2@wisc.edu

Eliska Machalova
Inst Inherited Metab Disorders
eliska.machalova@lf1.cuni.cz

Lisa Maduzia
New England Biolabs
maduzia@neb.com

Tetsuya Maeshiro
Univ Tsukuba
maeshiro@slis.tsukuba.ac.jp

Julie Maguire
Rutgers, The State Univ NJ
julimagu@eden.rutgers.edu

Andre FS Maia
University Medical Center Utrech
A.F.Maia@umcutrecht.nl

Eleanor M Maine
Syracuse Univ
emmaine@syr.edu

Will Mair
Salk Institute
mair@salk.edu

Vincent P Mancuso
Univ Pennsylvania
vincent.mancuso@gmail.com

Kumaran Mani
Univ Wyoming
kmani2@uwyo.edu

Arun Prasad Manoharan
Life Sciences Institute
arunpras@umich.edu

Xianrong Mao
Washington University
maox@morpheus.wustl.edu

Geraldine Maro
Stanford Univ
gmaro@stanford.edu

Ashley Anne Martin
University of Illinois Chicago
amarti7@uic.edu

Ichiro Maruyama
Okinawa Inst of Sci & Technol
ichi@oist.jp

Shinji Matsuda
University of Tokyo
shinji@mol.f.u-tokyo.ac.jp

Brendan C Mattingly
Univ Kansas
bjayhawk@ku.edu

Mirjam Mayer
MPI-CBG
mayer@mpi-cbg.de

Mark McCormick
Univ California, San Francisco
mark.mccormick@ucsf.edu

Tamara Jean McEwen
University of Missouri
tjmx9c@mizzou.edu

Debbie McLaggan
Univ Aberdeen
d.mclaggan@abdn.ac.uk

Ellen Meelkop
K.U.Leuven
ellen.meelkop@bio.kuleuven.be

Alicia Melendez
Queens Col - CUNY
alicia.melendez@qc.cuny.edu

Justine Melo
Massachusetts General Hospital
jmelo@molbio.mgh.harvard.edu

Jane E Mendel
California Inst Technology
mendelj@caltech.edu

Philip M Meneely
Haverford Col
pmeneely@haverford.edu

Jorge Merlet
Institut Jacques Monod, CNRS
merlet.jorge@ijm.jussieu.fr

Barbara J Meyer
Univ California, Berkeley
bjmeyer@berkeley.edu

Stephany G Meyers
Catholic Univ of America
meyers@cua.edu

Gregoire Michaux
IGDR
gmichaux@univ-rennes1.fr

William J Michels
(unaffiliated)
biotica@sbcglobal.net

Pavol Mikolas
Charles Univ
pavol.mikolas@lfl.cuni.cz

Dana L Miller
FHCRC
dlmiller@fhcrc.org

Kenneth G Miller
Oklahoma Med Res Fndn
millerk@omrf.org

Michael A Miller
Univ Alabama at Birmingham
mamiller@uab.edu

Renee M Miller
Univ Rochester
renee_miller@urmc.rochester.edu

Paul Minor
Caltech
minor@caltech.edu

Jennifer Anne Miskowski
Univ Wisconsin, La Crosse
miskowsk.jenn@uwlax.edu

Kota Mizumoto
Stanford University
mizumoto@stanford.edu

Ariz Mohd
IIT Kanpur
ariz@iitk.ac.in

Juan David Moncaleano
National Tsing Hua University
jdmoncaleanop@gmail.com

Gabriela C Monsalve
Univ California, Los Angeles
gmonsalve@ucla.edu

Taiowa Montgomery
Massachusetts General Hospital
montgomery@molbio.mgh.harvard.edu

Julia Moore
Sloan Kettering
moorej@mskcc.org

Natalie Moroz
Harvard University
natalie.moroz@gmail.com

Ali Mortazavi
Caltech
alim@caltech.edu

Fumio Motegi
Johns Hopkins University
fmotegi1@jhmi.edu

Fritz Mueller
Univ Fribourg
fritz.mueller@unifr.ch

Takashi Murayama
OIST
tmurayama@oist.jp

Michael J Muskus
Washington University
muskusm@wusm.wustl.edu

Priah Nadarajan
Univ Minnesota
nada0010@umn.edu

Akihisa Nakagawa
Univ Colorado
akihisa.nakagawa@colorado.edu

Shunji Nakano
MIT
shunji@mit.edu

Kenichi Nakazato
RIKEN
na@riken.jp

Johana Nakielna
Charles University
johana.nakielna@lf1.cuni.cz

Sri Devi Narasimhan
UMASS Medical School
sridevi.narasimhan@umassmed.edu

Sheila Nathan
National University of Malaysia
sheila@ukm.my

Scott J Neal
Brandeis Univ
sjneal@brandeis.edu

Ralda Nehme
Dartmouth Col
ralda@dartmouth.edu

Matthew Nelson
NYU
mdn233@nyu.edu

Stacey A Nerkowski
CSU San Bernardino
nerkowss@csusb.edu

Mario F Neto
Northwestern University
mjneto@fc.ul.pt

Brent Neumann
University of Queenland
b.neumann@uq.edu.au

Phuong Anh Thi Nguyen
SFU
tpn3@sfu.ca

Rachael A Nimmo
Yale University
rachael_nimmo@hotmail.com

Kiyoji Nishiwaki
Kwansei Gakuin Univ
nishiwaki@kwansei.ac.jp

Paola Nix
Univ Utah
pnix@biology.utah.edu

Scott L Noble
Univ Colorado Denver
scott.noble@uchsc.edu

Ken Norman
Albany Medical College
NormanK@mail.amc.edu

Amanda C Nottke
Harvard Medical Sch
anottke@fas.harvard.edu

Isabelle Nuez
CNRS, Inst J Monod
nuez@ijm.jussieu.fr

Lizbeth Nunez
Queens College of The CUNY
lizng20@gmail.com

Stefanie Nusser
University of Zurich
stefanie.nusser@zool.uzh.ch

Inish M O'Doherty
Cornell Univ
imo3@cornell.edu

Damien M O'Halloran
UC Davis
dmohalloran@ucdavis.edu

Michelle O'Meara
Columbia University
mmo2104@columbia.edu

Eyleen J O'Rourke
Massachusetts General Hosp
eorourke@molbio.mgh.harvard.edu

Shigekazu Oda
University of Tokyo
rustc.sgkz@gmail.com

Ken-ichi Ogura
Yokohama City Univ
kenogura@med.yokohama-cu.ac.jp

Johanna T Ohlmeyer
Columbia University
jo32@columbia.edu

Yasumi Ohshima
Sojo Univ
ohshima@life.sojo-u.ac.jp

Ayako Okazaki
Nagoya Univ
okazaki.ayako@b.mbox.nagoya-u.ac.jp

Monika Olahova
Newcastle Univ
m.olahova@ncl.ac.uk

Anders Olsen
Univ Aarhus
ano@mb.au.dk

Shuichi Onami
RIKEN ASI
sonami@riken.jp

Brian D Onken
Rutgers Univ
onken@biology.rutgers.edu

Laura Opperman
University of Wisconsin-Madison
ljopperman@wisc.edu

Rachel Leah Ostroff
Vanderbilt Univ
rachel.l.ostroff@vanderbilt.edu

Melissa Owraghi
Univ California, Riverside
mowra001@student.ucr.edu

Pamela A Padilla
Univ North Texas
ppadilla@unt.edu

John M Pagano
Univ Massachusetts Med Sch
john.pagano@umassmed.edu

Stephen C Pak
University of Pittsburgh
scp10@pitt.edu

Francesca Palladino
Ecole Normale Supriure de Lyon
francesca.palladino@ens-lyon.fr

Julia E Palter
Univ California, Santa Barbara
palter@lifesci.ucsb.edu

Saumya Pant
Rutgers Univ
spant@eden.rutgers.edu

Nicolas Paquin
MIT
npaquin@mit.edu

Sang-Kyu Park
Univ Colorado at Boulder
sangkyu@colorado.edu

Angela Parrish
Univ of CA, San Diego
arparrish@ucsd.edu

Frederick Alan Partridge
Univ Oxford
frederick.partridge@bioch.ox.ac.uk

Falshruti B Patel
UMDNJ/RWJMS
patelfb@umdnj.edu

Pranali P Pathare
Fred Hutchinson Research Ctr
ppranali@hotmail.com

Daniel Paz-Gomez
IFC-UNAM
dpaz@ifc.unam.mx

Nina Peel
NIH
peeln@niddk.nih.gov

Mark Watson Pellegrino
Institute of Zoology
mark.pellegrino@zool.uzh.ch

Maureen A Peters
Oberlin Col
maureen.peters@oberlin.edu

Emily L Petty
Univ Michigan
emilynnp@umich.edu

Carolyn M Phillips
Massachusetts Gen Hosp
phillips@molbio.mgh.harvard.edu

Chris Pickett
Washington University
cpickett@wustl.edu

Beverly Jean Piggott
Life Sciences Inst
bpiggott@umich.edu

Berangere Pinan-Lucarre
Rutgers Univ
pinan@biology.rutgers.edu

Lionel Pintard
Inst Jacques Monod, CNRS
pintard@ijm.jussieu.fr

John Plenefisch
Univ Toledo
jplenef@uoft02.utoledo.edu

Benjamin Podbilewicz
Technion-IIT
podbilew@tx.technion.ac.il

Kristin A Politi
Albert Einstein Col Med
kpoliti@fandm.edu

Daniel Avery Pollard
dpollard@gmail.com

Robert Harding Pollok
UTSouthwestern Medical Center
robert.pollok@utsouthwestern.edu

Aidan Porter
Harvard University
aporter@mcb.harvard.edu

Jess Porter Abate
Joslin Diabetes Ctr
jess.porter@joslin.harvard.edu

Jennifer R Powell
Massachusetts General Hosp
jpowell@molbio.mgh.harvard.edu

Vida Praitis
Grinnell Col
praitis@grinnell.edu

Stephan Preibisch
MPI-CBG
schneide@mpi-cbg.de

Meredith H Price
Univ Oregon
mhprice@molbio.uoregon.edu

Nathalie Pujol
INSERM CIML
pujol@ciml.univ-mrs.fr

Hiroshi Qadota
Emory Univ
hkadota@emory.edu

Yingchuan Qi
UC San Diego
billyqi@ucsd.edu

Yvonne Quan
McGill Univ
ton-yee.quan@mail.mcgill.ca

Hilary E Racher
University of Calgary
hleivo@ucalgary.ca

Mohammad M Rahman
University of Georgia
mrahman1@uga.edu

Edward J Ralston
Univ California, Berkley
eralston@uclink.berkeley.edu

Laura Ramsay
Simon Fraser Univ
lfr@sfu.ca

Catherine Randall
UC, Santa Cruz
randall@biology.ucsc.edu

Georgia Rapti
ENS-INSERM
rapti@biologie.ens.fr

Jeffrey P Rasmussen
FHCRC
rasmuss@u.washington.edu

Karine G.M Rebora
Inst Europeen Chimie et Biol
k.rebora@iecb.u-bordeaux.fr

April Rose Reedy
University of Claude Bernhard
april.reedy@gmail.com

Min Ren
Univ Chicago
mindyren@uchciago.edu

Claire E Richardson
MIT
clairer@mit.edu

Donald L Riddle
Univ British Columbia
driddle@msl.ubc.ca

Matthias Rieckher
Inst Molec Biol & Biotech
matthias@imbb.forth.gr

Gary Michael Riefler
UT MD Anderson Cancer Center
griefler@mdanderson.org

Niels Ringstad
MIT
ringstad@mit.edu

Valerie J P Robert
Inserm
vrobert@biologie.ens.fr

Christian E Rocheleau
McGill Univ
christian.rocheleau@mcgill.ca

Matthew Rockman
New York Univ
mrockman@nyu.edu

Ana P Rodrigues
Salk Institute
arodrigues@salk.edu

Casey Roehrig
Harvard Univ
croehrig@mcb.harvard.edu

Anthony S Rogers
Wellcome Trust Sanger Inst
ar2@sanger.ac.uk

Zoe Rogers
MIT
zoer@mit.edu

Christopher Rohde
MIT
crohde@mit.edu

Monica R Rohrschneider
New York Univ-Sch Med
mrohrsch@saturn.med.nyu.edu

Catalina Romero
Harvard Univeristy
cromero@fas.harvard.edu

Ashley J. Ross
University of Toronto
ashley.ross@utoronto.ca

Jason Rothman
CA State Polytechnic Univ.
jarothman@scupomona.edu

Veerle Rottiers
Massachusetts Gen Hosp
veerle@libertsweb.com

Charles Rubin
Albert Einstein Col Medicine
rubin@aecom.yu.edu

Sascha Elle Russel
Harvard Univ/Mass Gen Hosp
russel@amber.mgh.harvard.edu

Dror Sagi
Stanford Univ
drorsagi@stanford.edu

Mako Saito
Dartmouth Medical School
richard.m.saito@dartmouth.edu

Taro Sakamoto
Kitasato Univ
sakamotot@pharm.kitasato-u.ac.jp

Tassa K Saldi
University of Colorado, Boulder
Tassa.Saldi@colorado.edu

Jenifer Salhanha
Iowa State Univ
jenifers@iastate.edu

Chrysanthi Samara
Massachusetts Inst Tech
csamara@mit.edu

Buck S. Samuel
Mass General Hospital
bsamuel@molbio.mgh.harvard.edu

Andrew Vaughn Samuelson
Massachusetts General Hosp
samuelso@molbio.mgh.harvard.edu

Leticia Sanchez Alvarez
Univ Ottawa
leticiasa@yahoo.com

Matthew J Sanders
University College London
matthew.sanders05@gmail.com

Shigeki Sanehisa
OIST
ssanehis@oist.jp

Sharon Bree Sann
UCSD
ssann@ucsd.edu

Amir Sapir
California Inst Tech
amirsa@caltech.edu

Mihail S Sarov
MPI-CBG
sarov@mpi-cbg.de

Yutaro Sassa
Hiroshima Univ
m092390@hiroshima-u.ac.jp

Aya Sato
UC Berkeley
asato@lbl.edu

Hitoshi Sawa
RIKEN Center for Developmenta
sawa@cdb.riken.jp

Oren Schaedel
Caltech
orens@caltech.edu

Ursula Schaeffer
University of Freiburg
ursula.schaeffer@biologie.uni-freiburg.de

Meredith J Scheider
Univ Buffalo (SUNY)
mjs32@buffalo.edu

Gary Schindelman
California Inst Technology
garys@caltech.edu

Tobias Schmid
University of Zurich
tobias.schmid@zool.uzh.ch

Lone Vedel Scholer
Aarhus Univ
lvs@mb.au.dk

Sonia CV Schott
Ecole Normale Suprieure de Lyon
Sonia.Schott@ens-lyon.fr

Nathan E Schroeder
Rutgers, The State Univ NJ
schroeder@biology.rutgers.edu

Robbie D Schultz
Texas A&M University
rdszpf@tamu.edu

Jill M Schumacher
Univ Texas MD Anderson CA Ctr
jschumac@mdanderson.org

Erich M Schwarz
California Inst Technology
emsch@its.caltech.edu

Katerina Sebkova
First Fac Med, Charles Univ
katerina.sebkova@lf1.cuni.cz

Hannah S Seidel
Princeton Univ
hseidel@princeton.edu

Piali Sengupta
Brandeis Univ
sengupta@brandeis.edu

Scott Matthew Severance
University of Maryland
severanc@umd.edu

Geraldine Seydoux
Johns Hopkins Univ Sch Med
gseydoux@jhmi.edu

Muazzum Shah
University of Michigan
muazzum@umich.edu

William R Shaw
University of Cambridge
r.shaw@gurdon.cam.ac.uk

Gidi Shemer
Univ North Carolina
bishemer@email.unc.edu

Yu Shen
Harvard University
shenyupku@gmail.com

Ujwal Sheth
FHCRC
usheth@fhcrc.org

Tadasu Shin-i
National Inst Genetics
tshini@genes.nig.ac.jp

Yoichi Shinkai
Kyushu University
sc307058@s.kyushu-u.ac.jp

David Eli Shore
Harvard Medical Sch
david_shore@med.harvard.edu

Ludmila Shtessel
UNC Chapel Hill
luda@email.unc.edu

Derek S Sieburth
Univ Southern California
sieburth@usc.edu

Malan Silva
SUNY College at Geneseo
mss8@geneseo.edu

Alicia N Simmons
University of North Carolina at Chapel Hill
simmona@email.unc.edu

Jeffrey S Simske
Rammelkamp Ctr
jsimske@metrohealth.org

Gunasekaran Singaravelu
Waksman Institute of Microbiol
guna@waksman.rutgers.edu

Nirupama J Singh
Catholic University of America
50singh@cua.edu

Varsha Singh
Duke Univ
varsha.singh@duke.edu

Renuka Sivapatham
Aarhus Univeristy
res@mb.au.dk

Todd B Slaby
Univ California, Berkeley
slaby@berkeley.edu

Ann E Sluder
Scynexis
ann.sluder@scynexis.com

Amanda M Smith
Duke University
ams66@duke.edu

Harold E Smith
NIH/NIDDK
harold.smith.tarheel@gmail.com

Joseph R Smith
Univ Utah
jsmith@genetics.utah.edu

Ksenia Smurova
Technion
k.smurova@gmail.com

Shuhei So
Sojo Univ
shuhei_so0812@yahoo.co.jp

Gerard Somers
Massachusettus General Hospital
upsgerardsomers@yahoo.com

Erica M Sommermann
Univ California, Santa Barbara
sommerma@lifesci.ucsb.edu

Bo-mi Song
UTsouthwestern Medical Center
bomi.song@gmail.com

Mi Hye Song
NIDDK/NIH
mihyesong@mail.nih.gov

Martha C Soto
UMDNJ - RWJMS
sotomc@umdnj.edu

Andrew M Spence
Univ Toronto
andrew.spence@utoronto.ca

John Spieth
Washington Univ Sch Medicine
jspieth@watson.wustl.edu

Barbara Squiban
CIML
squiban@ciml.univ-mrs.fr

Jagan Srinivasan
Caltech
jsrini@caltech.edu

Christopher St-Francois
Mcgill
christopher.st-francois@mail.mcgill.ca

Stefan Staben
SFSU
staben@sfsu.edu

Gillian M Stanfield
Univ Utah
gillians@genetics.utah.edu

Daniel A Starr
Univ California, Davis
dastarr@ucdavis.edu

Andreas Steimel
Simon Fraser University
andreass@sfu.ca

Rachel Stewart
Oberlin College
rstweart@oberlin.edu

Randy F Stout
Univ Alabama at Birmingham
rstout@nrc.uab.edu

Susan Strome
U California, Santa Cruz
strome@biology.ucsc.edu

Monika Suchanek
Univ California, San Francisco
monika.suchanek@ucsf.edu

Asako Sugimoto
RIKEN CDB
sugimoto@cdb.riken.jp

Alyson L Sujkowski
The University of Toledo
alyson.sujkowski@utoledo.edu

Eisuke Sumiyoshi
National Inst Genetics
esumiyos@lab.nig.ac.jp

Meera V Sundaram
Univ Pennsylvania Sch Med
sundaram@mail.med.upenn.edu

Hyun Sung
Hanyang University
gyaooay@naver.com

Finger Susanne
Friedrich Miescher Institute
susanne.finger@fmi.ch

Motoshi Suzuki
motoshi-suzuki@bio.nagoya-u.ac.jp

Hiroaki Tabara
Kyoto Univ
htabara@hmro.med.kyoto-u.ac.jp

Shin Takagi
Nagoya Univ Sch Sci
i45116a@nucc.cc.nagoya-u.ac.jp

Karla M Talavera
Univ California, Berkeley
komarg@berkeley.edu

Ryusei Tanaka
Saga University
nema_god@yahoo.co.jp

Yoshinori Tanizawa
MRC Laboratory of Molecular Biol
tanizawa@mrc-lmb.cam.ac.uk

Elizabeth Tank
UCSF
elizabeth.tank@gmail.com

Maja Tarailo-Graovac
Simon Fraser Univ
mta57@sfu.ca

Arnaud Tauffenberger
Univ de Montreal
arnaud.tauffenberger@umontreal.ca

Eillen Tecle
Albert Einstein Col Med
etecle@aecom.yu.edu

Masahiro Terasawa
RIKEN CDB
mterasw@cdb.riken.jp

Maike Thamsen
University of Michigan
mthamsen@umich.edu

Kenneth W Thompson
Univ MD Baltimore County
kthomp1@umbc.edu

Chenxi Tian
Cornell Univ
ct267@cornell.edu

Tiffany Anne Timbers
University of British Columbia
ttimbers@interchange.ubc.ca

Heidi A Tissenbaum
Univ Massachusetts Med Sch
heidi.tissenbaum@umassmed.edu

Ulrike Topf
Friedrich Miescher Inst
ulrike.topf@fmi.ch

Marton L Toth
Semmelweis Univ
toth@biology.rutgers.edu

Robert Townley
Yeshiva University
rtownley@aecom.yu.edu

Hsin-Yue Tsai
University of Massachusetts Medical school
HSINYUE.TSAI@umassmed.edu

Michelle L Tucci
Univ Alabama
mtucci1@bama.ua.edu

Kwame Twumasi-Boateng
UC Berkeley
kwametb@gmail.com

Yonatan Tzur
Harvard Medical School
ytzur@genetics.med.harvard.edu

Oluwatoroti O Umuerri-Olusanya
Erasmus University MedicalCenter
o.olusanya@erasmusmc.nl

Dustin Lynn Updike
Univ California Santa Cruz
updike@biology.ucsc.edu

Sara Valentini
Univ College London
s.valentini@ucl.ac.uk

Kimberly Van Auken
California Inst Technology
vanauken@caltech.edu

Sander J L Van Den Heuvel
Utrecht Univ
s.j.l.vandenheuvel@uu.nl

Alexander M van der Linden
Brandeis Univ
slinden@brandeis.edu

Heather Alane Van Epps
Western Washington University
heather.vanepps@wwu.edu

Priscilla M Van Wynsberghe
UCSD
pvanwyns@ucsd.edu

Miri VanHoven
San Jose State University
miri.vanhoven@sjsu.edu

Maithili C Vantipalli
Buck Inst
mvantipalli@buckinstitute.org

Jessica J Vasale
University of Massachusetts
jessica.vasale@umassmed.edu

Olena K Vatamaniuk
Cornell Univ
okv2@cornell.edu

Victor Venegas
Baylor Col Medicine
vv139924@bcm.tmc.edu

Ismael A Vergara
Simon Fraser Univ
iav@sfu.ca

Adrian Verster
Univ Toronto
adrian.verster@utoronto.ca

Ryan D Viveiros
Univ British Columbia
viveiros@interchange.ubc.ca

Mehul Vora
Rutgers Univ
mehulv@dis.rutgers.edu

Roman Vozdek
Charles Univ
roman.vozdek@lf1.cuni.cz

Edward Vuong
University of Rochester
edward_vuong@urmc.rochester.edu

Tokumitsu Wakabayashi
Iwate Univ
wakat@iwate-u.ac.jp

Michael Walser
University of Zurich
michael.walser@zool.uzh.ch

Katherine M Walstrom
New Col Florida
kwalstrom@gmail.com

Christopher L C Wang
Univ Calgary
clcwwang@ucalgary.ca

Jennifer T Wang
Johns Hopkins Univ / HHMI
jwang110@jhmi.edu

John Wang
University of Lausanne
john.wang@unil.ch

Peng Wang
NIH
wangpeng@niddk.nih.gov

Wenqing Wang
Penn State University
wzw111@psu.edu

Xiaodong Wang
Caltech
xdwang@caltech.edu

Xin Wang
Univ Calgary
wangxin@ucalgary.ca

Yimin Wang
Institute for Bio3, uni-Freiburg
wangyiminsibs@yahoo.com.cn

Zhiping Wang
UCSD
z4wang@ucsd.edu

Nattha Wannissorn
Univ Toronto
nattha.wannissorn@utoronto.ca

Alex Ward
Univ Michigan
alexward@umich.edu

Rachael E Ward
MRC Lab Molec Cell Biol, UCL
rachael.ward@ucl.ac.uk

Kurt J Warnhoff
Truman State Univ
kjw879@truman.edu

Jennifer L Watts
Washington State Univ
jwatts@wsu.edu

Ann M Wehman
New York Univ
wehman@saturn.med.nyu.edu

Qing Wei
UMDNJ-SOM, GSBS
weiqi@umdnj.edu

Emily Wendland
McGill University
emily-marie.wendland@mail.mcgill.ca

Jennifer S Whangbo
Children's Hosp Boston
jennifer.whangbo@childrens.harvard.edu

Allyson J Whittaker
Caltech
awhitta@its.caltech.edu

Tobias Wiesenfahrt
RWTH Aachen University
tobias2278@gmx.de

Sarah Wignall
Stanford University
swignall@stanford.edu

Corey L Williams
Univ Alabama at Birmingham
coreyw@uab.edu

Gareth Williams
Wellcome Trust Sanger Inst
gw3@sanger.ac.uk

John H Willis
Univ Oregon
jhwillis@uoregon.edu

Jennifer Winn
jennifer.winn@utsouthwestern.edu

Martin Wojtyniak
Brandeis Univ
mwojtyni@brandeis.edu

Cathy A Wolkow
NIA, Biomedical Res Ctr
wolkowca@grc.nia.nih.gov

Garry Wong
Kuopio Univ
garry.wong@uku.fi

Ming-Ching Wong
Princeton Univ
mingwong@princeton.edu

Jordan Wood
University at Buffalo, SUNY
jfwood@buffalo.edu

Rachel T Wragg
Univ Toledo
rachelwragg@yahoo.co.uk

Edlyn Wu
McGill Univ
edlyn.wu@mail.mcgill.ca

Jui-ching Wu
San Francisco State Univ
jcwu@sfsu.edu

Xiaoyun Wu
Massachusetts General Hospital
wux@molbio.mgh.harvard.edu

David Wynne
Univ California, Berkeley
davewynne@berkeley.edu

Huajiang Xiong
UMDNJ/RWJMS
xiongh1@umdnj.edu

Ningyi Xu
Stowers Inst Med Res
nxu@stowers.org

Xiao Xu
Stanford Univ
xiaoxu@stanford.edu

Koji Yamada
Univ Tokyo
yamada@biochem.s.u-tokyo.ac.jp

Ayanori Yamakawa
Wakayama National Col
yamakawa@wakayama-nct.ac.jp

Tracy Mae Yamawaki
Univ California, San Francisco
tracy.yamawaki@ucsf.edu

Dong Yan
yandongdong@gmail.com

Itai Yanai
Technion - IIT
yanai@technion.ac.il

Chonglin Yang
Inst. of Gen. and Dev., CAS
clyang@genetics.ac.cn

Youfeng Yang
Univ Alabama at Birmingham
yangyoufeng@gmail.com

Judith L Yanowitz
Carnegie Inst Washington
yanowitz@ciwemb.edu

Kelvin Yen
UMass Med
kelvin@kyen.org

Jianghua Yin
Queens Col, CUNY
jianghua.yin@qc.cuny.edu

Bum Ho Yoo
Yonsei Univ
vdarios@naver.com

Kazushi Yoshida
University of Tokyo
kyoshida@biochem.s.u-tokyo.ac.jp

Matthew James Youngman
MIT
mjyoungman@yahoo.com

Weiqun Yu
University of Pennsylvania
weiqunyu@mail.med.upenn.edu

Duygu Yucel
University of Sydney
dyuc2466@usyd.edu.au

Lim Yun-Ki
Hanyang University
337jjang@naver.com

Tanya P Zand
Univ North Carolina
tzand@med.unc.edu

Alon Zaslaver
Caltech
alonzo@caltech.edu

Danna Zeiger
Brandeis University
danna@brandeis.edu

Monique C Zetka
McGill Univ
monique.zetka@mcgill.ca

Chi Zhang
Massachusetts General Hosp
czhang@molbio.mgh.harvard.edu

Feifan Zhang
Columbia University
fz2124@columbia.edu

Huimin Zhang
IGBMC - CERBM
zhangh3@igbmc.fr

Sihui Zhang
Virginia Polytechnic Inst
sihuiz07@vt.edu

Xiaodong Zhang
Harvard University
xzhang@fas.harvard.edu

Yuqian Zhang
York Univ
jyzhang@yorku.ca

Zhongying Zhao
Univ Washington
zhao@gs.washington.edu

Qun Zheng
Washington Univ
zheng@pcg.wustl.edu

Weiwei Zhong
Rice Univ
weiwei.zhong@rice.edu

Kang Zhou
Pennsylvania State University
kuz107@psu.edu

Shan Zhou
Univ Minnesota
zhoux095@umn.edu

Zheng Zhou
Baylor Col Med
zhengz@bcm.tmc.edu

Ilyass Zniber
Inst Europeen Chimie et Biol
i.zniber@iecb.u-bordeaux.fr

Olivier Zugasti
INSERM - CIML
zugasti@ciml.univ-mrs.f