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C. elegans Genetics

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Full Abstracts

Plenary and Parallel | Plenary Session 1

Program number is in **bold** above the title. Presenting author is noted in **bold**.

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Control of protein homeostasis from a distance: the expanding world of stress-kines. **Andrew Dillin**. Salk Institute, La Jolla, CA.

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Caenorhabditis Genetics Center. **Aric L. Daul**, Theresa Stiernagle, Julie Knott, Ann E. Rougvie. CGC, Univ of 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 and will continue its duties of acquiring, maintaining and distributing worm stocks. The CGC now has over 13,000 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 some strains from the modENCODE project and strains that express various fluorescent protein 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; the CGC no longer accepts orders submitted by fax. 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, and now accepts credit payments for invoices directly through the CGC website. Payments must be made by check or credit card; electronic bank transfer may not be used for making payment. We provide quarterly reports to the NIH with statistics that reflect our services to the worm community. We 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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WormBook News. **Jane E. Mendel**[1], Todd Harris[1,2], Qinghua Wang[1], Paul Sternberg[1,3], Martin Chalfie[4]. 1) Division of Biology, California Institute of Technology, Pasadena, CA; 2) Ontario Institute for Cancer Research, Toronto, Ontario; 3) Howard Hughes Medical Institute, Pasadena, CA; 4) Department of Biological Sciences, Columbia University, New York, NY.

WormBook (http://www.wormbook.org/) is a comprehensive, open-access collection of original peer-reviewed chapters covering the biology of C. elegans and other nematodes. We have recently initiated a major series of chapter revisions so that WormBook remains comprehensive and current. Additionally, we have commissioned several chapters on nematodes other than C. elegans. These will provide content to support the sequence data contained in WormBase, and will include chapters on individual species and taxonomic groups, as well as transverse chapters devoted to morphological features and physiological functions. In the past two years, WormBook has successfully relaunched the Worm Breeder's Gazette as an on-line informal newsletter. To date, we have published 70 articles by over 200 authors from 16 countries. The Worm Breeder's Gazette has become a widely accessible forum for the distribution of current information about all aspects of nematode research including preliminary results, sequence data, and new methods.

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Introducing: The Next Generation of WormBase. **Todd W. Harris**[1], The WormBase Consortium[1,2,3,4]. 1) Ontario Institute For Cancer Research, Toronto, Ontario, Canada; 2) California Institute of Technology, Pasadena, CA, USA; 3) Washington University, St. Louis, MO, USA; 4) Wellcome Trust Sanger Institute, Hinxton, UK.

Since its introduction in 2001, WormBase has served as the central data repository for the *C. elegans* research community. Our goals have been threefold: 1) place data described in the published literature into a rich intellectual context through a process of deliberate manual curation; 2) correct and annotate gene models, sequences, and large scale datasets thereby providing a high confidence foundation for downstream studies; and 3) develop novel methods to standardize, store, query, and visualize these data. In pursuing these goals, WormBase now contains a vast array of data curated to a very high standard, ranging from molecular level descriptions of gene function to systems biology and back, all contained in a unified user interface.

In the past year we've added a number of substantial datasets to WormBase. modENCODE data -- already in use by WormBase curators to improve gene models -- is now available on the Genome Browser. The addition of new species including plant and animal parasites continues the transition of WormBase into a true clade-level resource. Ongoing literature curation has greatly expanded the breadth and depth of described genes, functions, phenotypes and available reagents.

Now, we're excited to introduce the next generation of the WormBase website for public testing. Designed from the ground up to be fast, stable, and easy to extend, the new site introduces novel ways to browse, search, share, and compare data. For example, the user interface is highly customizable with sections that can be hidden and rearranged to give a custom view of the data of greatest interest. Entries that are consulted frequently can be bookmarked and tracked for changes. New social features let users assist in the curatorial process by leaving public or private comments for curators and other users.

Two entirely new programming interfaces increase options for data mining. An extensive API for the Perl programming language and a RESTful interface, both backed by a dedicated server, also provide interested users with the tools to build "mashups": websites that draw from and complement the data and functionality available at WormBase.

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The Million Mutation Project - A *C. elegans* Mutation Resource. **Donald G. Moerman**[1], Pnina Strasbourger[2], Owen Thompson[2], Iasha Chaudhry[1], Mark Edgley[1], Brent Ewing[2], Lisa Fernando[1], Stephane Flibotte[1], Amber High[2], LaDeana Hillier[2], Joanne Lau[1], Norris Lee[1], Angela Miller[1], Greta Raymant[1], Jay Shendure[2], Jon Taylor[1], Emily H. Turner[2], Robert Waterston[2]. 1) Departments of Zoology and Michael Smith Laboratory, University of British Columbia, Vancouver, BC, Canada; 2) Department of Genome Sciences, University of Washington, Seattle, WA, U.S.A.

We are developing a library of 2,000 mutagenized strains and sequencing each to a depth of 15x as a community resource. From previous estimates this should yield a library of half to one million SNP's with 5-10 non-synonymous changes per average gene across the collection. After testing various mutagens we are using EMS, ENU and a cocktail of EMS plus ENU. In the F1 we select for *unc-22* mutations to ensure that the genome has been mutagenized. In the F2 we select for non-*unc-22* animals and then these animals are self-crossed for 10 generations to ensure that the final isolate is homozygous across all regions of the genome. We will supplement these strains with natural isolates to recover additional mutations. For sequencing we load size-selected, barcoded samples on either a GAII, or Hi-Seq sequencing machine (Illumina) and do paired-end reads. Data analysis employs BWA, SamTools and custom filters. Sequence data will be deposited in WormBase and the individual strains will be available from the Caenorhabditis Genetics Center for detailed study. Eventually we hope to distribute the 2,000 strains as a single kit, allowing parallel experimentation on a wide spectrum of mutant genes.

To date we have constructed and tested libraries for more than 1,000 strains and completed analysis of 451 strains. The analysis has yielded 142,566 SNP's including 32,835 non-synonymous changes in 13,685 genes. These comprise 1,404 nonsense mutations in 1,310 genes, 804 splicing mutation in 777 genes, 30,607 missense mutations in 13,269 genes and 20 readthrough mutations in 20 genes. Of 2,200 genes with either a nonsense, or a splicing alteration, over 1,000 have no previously reported mutations. Based on read numbers, the rDNA repeat copy number is surprisingly variable, with some strains having fewer than 60 copies and a few having more than 200. By the meeting in Los Angeles we will report on the analysis of the first 1,000 strains.

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Towards a mutation in every gene: A report from the front lines. **Mark Edgley**[1], Robert Barstead[2], Shohei Mitani[3], Don Moerman[4], and the Barstead, Mitani and Moerman Labs. 1) Life Sciences Centre, University of British Columbia, Vancouver, BC, Canada; 2) Oklahoma Medical Research Foundation, Oklahoma City, OK, USA; 3) Tokyo Women's Medical University School of Medicine, Tokyo, Japan; 4) Deparment of Zoology, University of British Columbia, Vancouver, BC, Canada.

Currently the combined efforts of the Barstead, Mitani and Moerman labs have identified over 7,000 deletions in 6,013 genes. About ¼ of these mutants are inviable as homozygotes. Most of the mutations identified to date have been isolated using the PCR deletion detection protocol but we are also using Comparative Genome Hybridization (CGH) and massively parallel short read sequencing technologies to identify alterations. While the facilities are largely request driven, we have made a concerted effort to identify mutations in a number of large critical genes families, including transcription factors (TF's) and kinases. To date we have identified mutations in close to 900 of the 935 TF's and 381 of the 416 annotated kinases.

The efforts of our three facilities when combined with efforts from the community and other more recent results from Deep Sequencing bring the total numbers of genes with either a deletion, a stop codon or a splicing defect to 8,000 genes.

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A recombinant Mos1 transposon can carry large DNA fragments. **Christian Frøkjær-Jensen**[1,4], MW Davis[1], M Sarov[2], J Taylor[3], ZC Stevenson[1], D Moerman[3], EM Jorgensen[1]. 1) Biology, HHMI, U. of Utah, Salt Lake City, UT; 2) Max Planck Institute, Dresden, Germany; 3) Dept of Zoology, U.ø of British Columbia, Canada; 4) Dept. Biomedical Science, U. of Copenhagen, Denmark.

P element-mediated transgenesis has been an important tool in Drosophila for the past 30 years. For example, P-elements have been used extensively to insert transgenes, to generate loss of function mutants and to probe the genome for regulatory regions. We have developed a minimal recombinant Mos1[A] transposon (miniMos) that behaves much like a P-element. Exogenous DNA placed inside a recombinant transposon is inserted into the *C. elegans* genome at high frequencies by simple injection (60% insertion freq.) or by heat-shock. In characterized lines, 46% inserted in intergenic regions, 43% in introns and 11% in exons (n = 54). The method is easy, very robust and works well with different mutant selection markers (*unc-119* and *unc-18*) as well as antibiotic markers (neomycin[B] and puromycin[C]). At lower frequencies (2-14%), miniMos can carry large pieces of DNA derived from fosmids (45kb). We have verified by comparative genome hybridization (CGH) that fully intact fosmids are inserted and all 18 independent *air-2::gfp* fosmid insertions express visible GFP. This technique makes the generation of single-copy transgenes rapid and easy. In addition, miniMos transgenes can be used for gene-traps and for genome-wide chromatin studies.

Gene trapping. The miniMos element can be used to simultaneously disrupt genes and to determine their expression pattern. We have constructed a miniMos gene-trap carrying a promoterless mCherry with an SL2 trans-splice leader sequence. In a pilot screen, we have isolated 20 lines that express mCherry in specific patterns. Each line contains a transposon inserted into a transcription unit (either exon, intron or UTR).

Chromatin and gene regulation. The miniMos element can be used to probe the genome for permissive and silenced regions. Germline expressed genes are highly enriched in introns that contain phased DNA[D] and are virtually absent from the X chromosome[E]. We can test the effects of genomic environment and phasing by inserting germline expressed transgenes. For example, a phased *Ppie-1::GFP::H2B* construct results in stable germline fluorescence in 67% (12 of 18) of inserts whereas similar, but non-phased *Pgld-1* and *Pmex-5* constructs result in much lower frequencies of fluorescence.

(A) Bessereau et al., (2001) (B) Giordano-Santini et al., (2010) (C) Semple et al., (2010) (D) Fire et al., (2006) (E) Bender et al., (2006)

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LIN-42/PERIOD Controls Cyclical and Developmental Progression of C. elegans Molting. **Gabriela C. Monsalve**[1], Cheryl Van Buskirk[2], Paul W. Sternberg[2], Alison R. Frand[1]. 1) Biological Chemistry, University of California, Los Angeles, Los Angeles, CA; 2) Howard Hughes Medical Institute, California Institute of Technology, Pasadena, CA.

The timing mechanisms that coordinate cyclical and successive events in development are not well understood. The molting cycle of *C. elegans* involves both the renovation of larval exoskeletons and idiosyncratic behaviors including quiescence. These rhythmic processes are coordinated with progressive development of the epidermis, which is programmed by the heterochronic gene regulatory network. Although the molts occur at regular intervals of 8 hours, the anticipated pacemaker has not been identified. Here we show that inactivation of the period-like, heterochronic gene *lin-42d* causes arrhythmic molting uncoupled from epidermal blast cell dynamics. Conversely, forced expression of the *lin-42d* isoform triggers precocious behavioral quiescence and molting in underdeveloped larvae. These results identify LIN-42D as a key component of an endogenous timer that controls rhythmic behaviors and cell biological events critical for molting. Our findings further suggest that cyclical and stage-specific actions of LIN-42/PER coordinate periodic molts with continuous development.

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Developmental Regulation of miRNA Activity. **Dana M King**, Christopher Hammell. Cold Spring Harbor Laboratories, Cold Spring Harbor, NY.

While our understanding of the genetic circuitry regulating spatial patterning has illuminated strategies shared by most animals, how temporal gene expression is coordinated remains obscure. In C. elegans, conserved classes of heterochronic genes control the invariant post-embryonic cell lineages. Characterization of mutants that alter stage-specific developmental programs has revealed a common paradigm in which the expression of critical timing genes such as transcription factors, RNA binding proteins and other regulatory components that function in early stages of development are repressed by the activity of specific microRNAs (miRNAs). While much is known about the transcriptional activation and biochemical maturation of miRNAs, our understanding of how these processes are coordinated at the molecular and cellular levels to create sharp transitions in gene expression programs is limited. To identify common molecular components that modulate the temporal activity of miRNAs, we screened for extragenic suppressors of mutations effecting essential miRNA processing components or specific miRNAs required for normal developmental timing. While these mutations result in distinct post-embryonic cell lineage defects that occur at different times during development, each screen resulted in the identification of a unique complementation group capable of suppressing all of these diverse lineage phenotypes. This class of suppressors is represented by multiple alleles of lin-42, encoding the C. elegans homologue of the human and Drosophila circadian regulatory component Period. In a manner similar to the expression of its circadian homologues, LIN-42 levels cycle in abundance with peak expression occurring between larval stages and sharply declining when individual miRNAs function to repress their targets. Molecular analysis of lin-42 mutants suggests that LIN-42 functions to antagonize the accumulation of mature heterochronic and non-heterochronic miRNAs by dampening their expression at a metabolic step at or upstream of pre-miRNA processing. Importantly, the precocious heterochronic phenotypes associated with lin-42 mutations arise as a consequence of an inappropriate over-accumulation of the heterochronic miRNAs during the inter-molt periods of larval development. These results suggest that miRNA maturation or activity can be directly coupled to the regulatory machinery controlling developmental timing.

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Hormonal signal amplification mediates environmental conditions during development and controls an irreversible commitment to adulthood. **O. Schaedel**[1], B. Gerisch[2], A. Antebi[2], P. Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, Caltech, Pasadena, CA; 2) Max Planck-Institute for Biology of Aging, Koeln, Germany.

Many animals can choose between different developmental fates to maximize fitness. Despite the complexity of environmental cues and life history, different developmental fates or polyphenisms, are executed in a robust fashion. The mechanisms that guarantee robust execution of a development choice in such environments remain unknown. The nematode *Caenorhabditis elegans* serves as a powerful model to examine this phenomenon because it has an advanced toolkit for cellular and genetic manipulations, and can adopt one of two developmental fates depending on environmental conditions. Nematodes grown in favorable conditions (sufficient food, low population density) develop into adults, whereas nematodes grown in unfavorable conditions (insufficient food, high population density) arrest development as a stress-resistant diapause form called dauer. The steroid hormone dafachronic acid (DA), product of DAF-9/cytochrome P450, directs development to adulthood by regulating the transcriptional activity of the nuclear hormone receptor DAF-12. The known role of DA suggests that it may be the molecular mediator of environmental condition effects on the developmental fate decision, although the mechanism is yet unknown. We hypothesize that information from the environment is integrated and reduced to a single cell non-autonomous environmental integrator, thereby explaining the tight binary nature of the developmental fate decision. We demonstrate that commitment to reproductive adult development occurs during the second larval stage when DA levels produced in the neuroendocrine XXX cells, exceed a threshold. As a result, DAF-12 dependent expression of *daf-9* in the epidermis is amplified and propagated from anterior to posterior, dispersing high amounts of DA throughout the body. The dispersion of DA drives adult programs in the gonad and epidermis. Furthermore, we demonstrate that the XXX cells are not necessary for maintaining the adult fate after the signal amplification has started. This indicates that the epidermal amplification also confers the irreversibility of the decision by uncoupling the execution of the decision from the environmental integrator. We propose that this relay serves as a robust fate-locking mechanism to enforce an organism wide binary decision, despite noisy and complex environmental cues.

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NSBP-1, a novel sterol binding protein, mediates the insulin/IGF-1 signaling pathway in response to cholesterol in *Caenorhabditis elegans*. **Mi Cheong Cheong**, Hyoung-Joo Lee, Keun Na, Hyoe-Jin Joo, Young-Ki Paik. Department of Biochemistry and Integrated Omics for Biomedical Science (WCU Program), College of Life Science and Biotechnology and Yonsei Proteome Research Center, Yonsei University, Seoul, 120-749, Korea.

Here we investigated how cholesterol influences the Insulin/IGF-1 signaling (IIS) pathway in *Caenorhabditis elegans*. Cholesterol affects many IIS functions, including dauer formation rate, oxidative stress resistance, fat storage, and longevity. Moreover, DAF-16 transcriptional activity in IIS is also modulated by cholesterol. These responses are mediated by a novel protein called nematode sterol binding protein (NSBP)-1, which is a homolog of human nucleosome assembly protein 1 encoded by D2096.8. NSBP-1 is expressed at sites of cholesterol accumulation and translocates to the nucleus independent of DAF-16 in response to IIS. Site-directed mutagenesis revealed possible NSBP-1 phosphorylation at Thr203. Co-immunoprecipitation studies demonstrated that NSBP-1 associates with DAF-16 and that this interaction can be affected by cholesterol concentration. Experiments using RNAi against NSBP-1 and DAF-16 demonstrated that these proteins work together to regulate the effects of cholesterol on the IIS pathway, as well as cholesterol homeostasis, in *C. elegans*. Our results may be applicable to screening of novel therapeutic targets for ameliorating insulin-resistant diabetes which can be caused by hyperactivation of FOXO in mammals.

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Germline tumors and loss of intestinal integrity in aging *C. elegans*. **Matthew D. McGee**[1], Darren Weber[1], Nicholas Day[1], Cathy Vitelli[1], Danielle Crippen[1], Laura A. Herndon[2], David H. Hall[2], Simon Melov[1]. 1) Buck Institute for Age Research, Novato, CA; 2) Center for *C. elegans* Anatomy, Albert Einstein College of Medicine, Bronx, NY.

The roundworm *C. elegans* is widely used as an aging model, with hundreds of genes identified that modulate aging. The development and bodyplan of the 959 cells comprising the adult have been well described and established for more than 25 years. However, morphological changes with age in this optically transparent animal are less well understood, with only a handful of studies investigating the pathobiology of aging. We have combined histological staining of serial-sectioned tissues, confocal microscopy, and transmission electron microscopy to characterize age-related morphological changes of multiple wild-type and mutant individuals at different ages. Using these three techniques, we have identified several novel pathologies with age in *C. elegans*, particularly in the intestine and germline. We describe an age-related loss of intestinal nuclei and degradation of the intestinal lumen. The intestinal nuclei gradually degrade with age, most likely due to autophagy. The intestinal lumen also appears to become irregular with age, with microvilli degraded or completely absent in some cells. We also describe an age-related onset of tumors that develop in the germline that may be analogous to age-related cancers in other species. The unfertilized germline cells endoreduplicate, forming large tumorous growths in the uterus and proximal gonad arm that distend tissue architecture to occupy most of the diameter of the worm. These large acellular tumors contain various proteins and DNA. Many nuclei are visible, but there appears to be a progressive breakdown of nuclear morphology. To our knowledge, this is the first description of age-related tumors in wild-type *C. elegans*.

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Modulators of small RNA pathways determine immunity against a natural virus of *C. elegans*. **Eric A. Miska**[1], Alyson Ashe[1], J. Piffaretti[2], G. Wu[3], I. Nuez[2], T. Bélicard[2], Y. Jiang[3], G. Zhao[3], C.J. Franz[3], Leonard D. Goldstein[1], M. Sanroman[2], D. Wang[3], M.A. Félix[2]. 1) Gurdon Institute, University of Cambridge, Cambridge, United Kingdom; 2) Institut Jacques Monod, CNRS-University of Paris-Diderot, Paris, France; 3) Departments of Molecular Microbiology and Pathology & Immunology, Washington University in St. Louis School of Medicine, St. Louis, Missouri, USA.

From wild isolates of *C. elegans* and *C. briggsae* with unusual morphological phenotypes in intestinal cells, we identified two novel RNA viruses distantly related to known nodaviruses, one infecting specifically *C. elegans* (Orsay virus), the other *C. briggsae* (Santeuil virus). Infected animals continuously maintained viral infection for 6 mo (~50 generations), demonstrating that natural cycles of horizontal virus transmission were faithfully recapitulated in laboratory culture. In addition to infecting the natural *C. elegans* isolate (JU1580), Orsay virus readily infected laboratory *C. elegans* mutants defective in RNAi and yielded higher levels of viral RNA and infection symptoms as compared to infection of the corresponding wild-type N2 strain. These results demonstrated a clear role for RNAi in the defense against this virus. Furthermore, different wild *C. elegans* isolates displayed differential susceptibility to infection by Orsay virus, thereby affording genetic approaches to defining antiviral loci. This discovery establishes a bona fide viral infection system to explore the natural ecology of nematodes, host-pathogen co-evolution, the evolution of small RNA responses, and innate antiviral mechanisms. More recently we have been able to demonstrate that the difference in viral immunity between the laboratory reference N2 and the wild isolate JU1580 is due, at least in part, to a defect in the antiviral small RNA response. Sequencing of small RNA populations in N2, *rde-1* mutants and JU1580 after infection with the Orsay virus suggests that this defect is downstream of the generation of Dicer-dependent primary siRNAs, but upstream of the generation of antiviral secondary siRNAs. We have used a candidate screen and QTL mapping to identify genes required for an effective antiviral small RNA response. In addition, we have analysed mRNA expression profiles of N2, *rde-1* mutants and JU1580 before and after infection with the Orsay virus. We find that viral infection results in the upregulation of a specific set of innate immunity genes. Finally, we find that small RNA pathways not only raise an antiviral response, but also modulate the expression of innate immunity genes.

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C. elegans Germline Defense Against Retroelements. **Shannon M. Dennis**[1,2], Ujwal Sheth[2], Jessica L. Feldman[2], Jeff N. Molk[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.

The *C. elegans* genome contains an unusually small number of retroviral and retrotransposon sequences compared to other animal genomes, suggesting nematodes have efficient defenses against such elements. Because retroelements that infect non-dividing cells must bind to and transit nuclear pores, and most of the nuclear pores in nematode germ cells are covered by P granules, we are interested in how nematode retroelements confront/circumvent P granules. We used electron microscopy to search for wild nematode strains with virus-like particles (VLPs) in germ cells and identified several strains that express germ cell VLPs, including temperature-dependent expression in the laboratory N2 strain. Using RNAi we show that the N2 VLPs are products of the Cer1 retrotransposon, and that Cer1 contributes to some cell deaths that normally occur in the N2 gonad. Microtubule organizing centers (MTOCs) contain the minus ends of microtubules and are found near the nucleus in many types of animal cells; some retroelement capsids traffic to the nucleus via association with minus-end directed motors such as dynein. Using antibodies and GFP reporters, we find that nearly all Cer1 capsids are associated with germ cell microtubules. Cer1 capsid localization is dynein-dependent, suggesting that capsids may traffic on microtubules via dynein. In contrast, we found that *C. japonica* VLPs show no association with microtubules, and instead form grape-like clusters directly on P granules. The Cer1 capsids in N2 cluster near, but not on, pachytene nuclei, where P granules cover most nuclear pores. However, capsids are abundant on post-pachytene nuclei that enlarge and acquire P-granule free nuclear pores. Integration events in these latter nuclei might be limited by their extensive chromatin compaction. Most capsids that enter oocytes appear to be degraded, and thus prevented from entering mitotic germ cell precursors of the embryo. We propose that association with microtubules might allow capsids to probe pachytene nuclei for free NPCs, while maintaining anchorage against cytoplasmic flow through the gonad core.

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The conserved microRNA miR-80 negatively regulates *C. elegans* longevity through inhibition of the dietary restriction pathway. **Mehul Vora**, Mitalie Shah, Silvana Ostafi, Monica Driscoll. Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ.

Dietary restriction—limitation of caloric intake without deleterious reduction in essential nutrients—extends lifespan and reduces the incidence of age-associated disease across species. Identification of regulators of the DR state that might bypass the calorie limitation requirement but nonetheless engage metabolism to promote healthy aging are of considerable interest. We previously identified a unique fluorimetric signature for the *C. elegans* DR state (Excitation maximum peak shift for naturally fluorescent age pigments and lipofuscin[1]) that is not found in other long-lived mutant strains. We exploited this discovery to screen through the available miRNA deletion mutants to identify *mir* mutants that might persist in a constitutive DR-like state.

We identified a single *mir* mutant, affecting the conserved *mir-80* gene, which robustly exhibits the fluorimetric DR signature and lives longer than wild type. *mir-80* mutants also exhibit reduced fecundity and appear pale and thin. Like DR-animals, *mir-80(nDf53)* is hypersensitive to the DR mimetic drug metformin, which is the drug response expected for animals that are already in DR (pushed over the edge into starvation)[2]. Interestingly, a transcriptional GFP reporter for *mir-80* is responsive to food (upregulated in the presence of food and down-regulated in absence of food) and a key molecular reporter of DR, the transcription factor skn-1, is activated by *mir-80* deletion. We will present preliminary data on several genes that are needed for *mir-80(nDf53)*-regulated DR benefits.

In conclusion, we will provide evidence that miR-80 negatively regulates genes that are involved in DR metabolism. We report the first instance of a metazoan microRNA that affects longevity through dietary restriction.

1.Gerstbrein, B., et al., In vivo spectrofluorimetry reveals endogenous biomarkers that report healthspan and dietary restriction in *Caenorhabditis elegans*. Aging Cell, 2005. 4(3): p. 127-137. 2.Onken, B. and M. Driscoll, Metformin induces a dietary restriction-like state and the oxidative stress response to extend *C. elegans* Healthspan via AMPK, LKB1, and SKN-1. PLoS ONE, 2010. 5(1): p. e8758.

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Proteomic Analysis of Age-dependent Changes in Protein Solubility Identifies Genes that Modulate Lifespan. **Pedro R. Rodrigues**, Gregg Czerwieniec, Theodore Peters, Uday S. Evani, Mark Lucanic, Silvestre Alavez, Emily A. Gaman, Sean D. Mooney, Bradford W. Gibson, Robert E. Hughes, Gordon J. Lithgow. Buck Inst, Novato, CA.

While it is generally recognized that misfolding of specific proteins can cause late-onset disease states, the contribution of protein aggregation to the normal aging process is less well understood. To address this issue, a mass spectrometry-based proteomic analysis was performed to identify proteins that adopt sodium dodecyl sulfate (SDS)-insoluble conformations during aging. SDS Insoluble proteins purified from young and aged *C. elegans* were chemically labelled by isobaric tagging for relative and absolute quantitation (iTRAQ) and identified by liquid chromatography and mass spectrometry. Two hundred and three proteins were identified as being significantly enriched in an SDS-insoluble fraction in aged nematodes and were largely absent from a similar protein fraction in young nematodes. The SDS-insoluble fraction in aged animals contains a diverse range of proteins including a large number of ribosomal proteins. Transgenic nematodes expressing three proteins identified in the insoluble fraction, DAF-21, RPS-0 and EFT-3 fused to Green Fluorescent Protein (GFP) showed the formation of visible aggregates by fluorescence microscopy. In the case of RPS-0 and EFT-3, these aggregates appeared immobile as measured by Fluorescence Recovery after Photobleaching (FRAP). Expression of genes encoding insoluble proteins observed in aged nematodes was knocked-down in using RNAi and effects on lifespan were measured. Forty of 100 genes tested were shown to extend lifespan after RNAi. These data indicate that genes encoding proteins that become insoluble with age are modifiers of lifespan. These data also demonstrate that proteomic approaches can be used to identify genes that modify lifespan. Finally, these observations indicate that aggregation of a diverse range of proteins may be a general feature of aging.

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Asymmetric Arginine Dimethylation Determines Lifespan in *C. elegans* by Regulating Forkhead Transcription Factor DAF-16. **Yuta Takahashi**[1,3], Hiroaki Daitoku[1], Keiko Hirota[1], Hiroko Tamiya[1], Atsuko Yokoyama[1], Kayo Yasuda[2], Naoaki Ishii[2], Akiyoshi Fukamizu[1]. 1) Life Science Center of TARA, University of Tsukuba, Japan; 2) Department of Molecular Life Science, Tokai University School of Medicine, Japan; 3) Research Fellow of the Japan Society for the Promotion of Science.

Arginine methylation is a widespread posttranslational modification of proteins catalyzed by a family of protein arginine methyltransferases (PRMTs). It is well established that PRMTs are implicated in various cellular processes, but their physiological roles remain unclear. In this study, we show that *prmt-1*, the major asymmetric arginine methyltransferase, is a positive regulator of longevity in *C. elegans*. Loss of *prmt-1* shortens worm lifespan, while the restored expression of *prmt-1* fully rescues the lifespan in an enzymatic activity-dependent manner. Moreover, this regulation is dependent on DAF-16/FoxO transcription factor, which is negatively regulated by AKT-mediated phosphorylation downstream of the DAF-2/insulin signaling. Biochemical analyses indicate that PRMT-1 methylates DAF-16 at arginine residues within AKT consensus motif (RXRXXS/T), thereby blocking its phosphorylation by AKT. Disruption of PRMT-1 induces phosphorylation of DAF-16 with a concomitant reduction in the expression of longevity-related genes. Thus, we provide a mechanism by which asymmetric arginine dimethylation acts as an anti-aging modification in *C. elegans*.

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Reproduction and Longevity are Positively Correlated in *C. elegans*. **Christopher L. Pickett**, Kerry Kornfeld. Department of Developmental Biology, Washington University in Saint Louis, Saint Louis, MO.

The relationships between reproduction and aging are important for understanding mechanisms of aging and evaluating evolutionary theories of aging. To investigate the effects of progeny production on reproductive and somatic aging, we conducted longitudinal studies of *C. elegans* hermaphrodites. For mated animals, progeny production was positively correlated with reproductive and somatic aging, demonstrating that individuals that generated more progeny also reproduced and lived longer than those that generated fewer progeny. These results indicate that progeny production does not accelerate somatic aging. The longitudinal data demonstrated that cumulative progeny production through day 5 is an early-stage biomarker of aging that is a robust predictor of longevity. Furthermore, early progeny production in mated animals was positively correlated with late progeny production indicating that early progeny production does not accelerate reproductive aging. To independently evaluate the relationships between progeny production and aging, we compared self-fertile hermaphrodites that generated relatively few progeny with mated hermaphrodites that generated many progeny. Somatic aging was not significantly different between these groups, confirming that progeny production does not accelerate somatic aging. The disposable soma and antagonistic pleiotropy theories of the evolution of aging suggest the existence of trade-offs between reproduction and longevity. The results reported here address key predictions of these theories and indicate that individual *C. elegans* hermaphrodites do not display a trade-off between progeny production and longevity. These studies rigorously define relationships between progeny production, reproductive aging, and somatic aging, define new biomarkers of aging, and contribute to the experimental evaluation of prominent evolutionary theories.

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In situ study of oxidative stress using a new optogenetic tool, KillerRed. **Donald Fu[1]**, Ippei Kotera[2], Po-An Su[3], Hiroshi Suzuki[1,2]. 1) Dept Physiology, Fac Med; 2) Tanz Centre for Research in Neurodegenerative Diseases, University of Toronto, Canada; 3) Div Engineering Sci, Fac App Sci & Engineering.

Parkinson's Disease (PD), characterized by the progressive degeneration of dopaminergic (DA) neurons, leads to locomotive deficits. Genetic studies suggests a role for oxidative stress in impairing activity and viability of DA neurons. Conventional methods induced stress in *C. elegans* by administering mitochondrial respiratory chain inhibitors (such as rotenone and paraquat) to the growth medium. However, these drugs diffuse and affect all cells in animals, and the effects have mostly been scored by the animals' death. It is difficult to assess how drugs affect only DA neurons and locomotive deficits. To overcome these limitations, we adopt a new optogenetic tool, KillerRed: a red fluorescent protein engineered to generate oxygen radicals on photoactivation. KR can be genetically targeted to cells by cell-specific promoters, and to specific subcellular compartments by site-localization motifs. We can also control irradiation time and intensity to manipulate timing and duration of stress. Studies have shown its potency in inducing cell death in mammalian cultured cells and Zebrafish. To test this tool in *C. elegans*, we investigated its effects on the mechanosensory system by expressing KR solely in touch neurons using *mec-4p* and scoring morphology and behavior (forward escape mediated by PLM neurons). We established a method to confirm activation: after photoactivation, KR fluorescence diminished to <1% of its initial levels, with a decay half-life of ~5.43s. Irradiation of PLM neurons for 10mins lead to profound changes in cell shape or their disappearance within 2h, without affecting neighboring KR(-) cells. Behavioral analysis revealed 73% reduction in touch sensitivity. We are now applying this tool to study oxidative stress in DA neurons; we targeted KR to the mitochondria using *dat-1p* and cytochrome-c oxidase motif. To quantitatively examine locomotion dependence on DA neurons, we employ a computer behavior analysis system: Our new worm tracker allows comparison of stressed worms to wild-type, to DA-deficient *cat-2* mutants, and directly address DA-activity suppression via optogenetic tools (*dat-1p::NpHR*). We are also expressing KR in other subcellular compartments and crossing them with mutants of PD genes (such as *pdr-1, djr-1.1/2, pink-1*) to examine how they protect against stress in DA neurons. Thus, we propose that KR is a useful tool to study oxidative stress and for *in vivo* ablation of neurons in *C. elegans*.

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A heritable effect of environmental stress on embryonic mRNA profiles. **Daniel H. Schott[1]**, Itai Yanai[2], Craig Hunter[1]. 1) Molec & Cellular Biol, Harvard Univ, Cambridge, MA; 2) Biology, Technion, Haifa, Israel.

For centuries, biologists have sometimes wondered whether animals and plants can inherit acquired traits, particularly responses to the environment. Epidemiological studies have provided a tantalizing hint that environmentally-triggered multigenerational changes impact human health, but such changes are difficult to study in humans. Despite recent evidence for inheritance of reversible changes in both plants and animals, its generality, as well as the role it plays in responses to ecologically relevant stimuli, remain open questions. Because of the ability to precisely control growth conditions, we chose *C. elegans* as a model animal to study these questions. We used microarrays to identify transcripts that remain at stress-altered levels after one generation return to non-stressful culture. Specifically, we compared mRNA levels in early embryos collected from mothers kept at 20°C, with embryos whose mothers were also reared at 20°C but whose grandparents had been reared at the mildly stressful temperature of 25°C. We found that changes in transcript abundance for multiple genes persist for one generation after the stress is removed, providing evidence for transgenerational memory. At least one gene followed by quantitative PCR shows effects that persist for two or three full generations. Additionally, we have evidence that Infection with *M. nematophilum* also has heritable effects. Within minutes individuals show transcriptional responses to new environments, while over many generations, populations show adaptive genetic change by natural selection. Adaptation to environmental conditions varying or recurring over an intermediate timescale is still poorly understood. Studying this in *C. elegans* will give a unique perspective to a field that is still largely unexplored territory.

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Neurite Sprouting and Synapse Deterioration are Features of the Aging C. elegans Nervous System. **Marton Toth[1]**, I Melentijevic[1], L Shah[1], A Bhatia[1], K Lu[1], A Talwar[1], H Naji[1], C Ibanez-Ventoso[1], P Ghose[2], A Jevince[3], L Herndon[3], G Bhanot[1], C Rongo[2], D Hall[3], Monica Driscoll[1]. 1) Department of Molecular Biology & Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ., NJ; 2) Department of Genetics, Rutgers, The State University of New Jersey, Piscataway, NJ; 3) Center for C. elegans Anatomy, Albert Einstein College of Medicine, Bronx, NY.

C. elegans is a powerful model for analysis of the conserved mechanisms that modulate healthy aging, from organismal to molecular levels. As animals age, tissues deteriorate at different rates. Neuronal death and/or detectable loss of processes, however, are not readily apparent in the aging *C. elegans* nervous system. Because dendrite restructuring and loss of synaptic integrity are hypothesized to contribute to human brain decline and dysfunction, we combined whole neuron fluorescence microscopy and electron microscopy to screen for nervous system changes in aging *C. elegans* at high resolution. We report two major aspects of morphological change in the aging *C. elegans* nervous system: 1) accumulation of novel outgrowths from specific neurons; and 2) decline in synaptic integrity. Novel outgrowth phenotypes, including branching from the main dendrite or new growth from somata, appear at a high frequency in some aging neurons, but not all. Lowered DAF-2 InsR signaling confers robust maintenance of touch neuron structural integrity into old age. Both DAF-16/FOXO and heat shock factor transcription factor HSF-1 exert protective functions in maintaining neuronal architecture. EM evaluation in synapse-rich regions revealed a decline in synaptic presynaptic vesicle numbers and a dimunition of presynaptic density size. Interestingly, animals that maintain locomotory prowess into old age exhibit less synaptic decline than same age decrepit animals, suggesting synaptic integrity correlates with locomotory healthspan. Our data reveal similarities between the aging *C. elegans* nervous system and mammalian brain, suggesting conserved neuronal responses to age. Dissection of neuronal aging mechanisms in *C. elegans* may thus influence development of anti-brain aging therapies.

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TOR signaling and rapamycin modulate aging by regulating SKN-1- and DAF-16-driven transcription. Stacey Robida-Stubbs[1], Kira Glover-Cutter[1], Prashant Raghavan[1], Masaki Mizunuma[1,3], Theresa Operana[1], **TK Blackwell[1,2]**. 1) Joslin Diabetes Ctr, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston MA; 3) Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University, Higashi-Hiroshima, Japan.

The TOR (target of rapamycin) kinase is central to growth regulation, and influences aging in diverse species. In the context of the TORC1 complex, TOR promotes protein synthesis in response to nutrient and growth signals. Less is understood about the related TOR-containing complex TORC2. TORC1 signaling has been implicated in the effects of dietary restriction on longevity, and inhibition of TOR through genetics or treatment with rapamycin, a clinically used TORC1 inhibitor, extends lifespan from yeast to mice. It is a central challenge in the aging and growth signaling fields to understand how TOR influences longevity and resistance to stresses.

Previous C. elegans studies showed that reduction of TOR kinase activity extends lifespan independently of DAF-16/FOXO, suggesting that TOR influences aging through different mechanisms from the insulin/IGF-1 signaling (IIS) and germline stem cell (GSC) pathways. We have determined that specific inhibition of TORC1 increases longevity and stress resistance by acting through both the SKN-1/Nrf and DAF-16 transcription factors. Adulthood knockdown of individual TORC1 complex genes increases longevity dependent upon both *skn-1* and *daf-16*. This longevity increase does not involve the GSC pathway, which we show also inhibits SKN-1 as well as DAF-16. In contrast to specific TORC1 inhibition, rapamycin treatment confers *skn-1*-dependent, *daf-16*-independent longevity. This appears to involve inhibition of both TORC1 and TORC2, because we find that TORC2 inhibition increases lifespan independently of *daf-16*. The *daf-16*-independence of TORC2-associated longevity can explain the previously observed *daf-16*-independence of the effects of TOR kinase inhibition on aging. Importantly, both SKN-1 and DAF-16 upregulate transcription of protective downstream target genes in response to either TORC1 inhibition or rapamycin treatment. We conclude that the TOR, IIS, and GSC pathways each influence aging by acting through distinct mechanisms to inhibit gene activation by SKN-1 and DAF-16. The data have important implications for understanding effects of TOR-based therapies, and relationships between nutrient availability, growth regulation, and aging.

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Death Made Visible: The Necrosis Pathway Generates A Burst Of Blue Fluorescence Marking Organismal Death In *C. elegans*. **Cassandra Coburn[1]**, Keith Nehkre[2], Filip Matthijssens[3], Grahame Fischer[1], Filipe Cabreiro[1], Caroline Araiz[1], Bart Braeckman[3], David Gems[1]. 1) G.E.E., University College London, London, United Kingdom; 2) Departments of Pharmacology and Physiology, and Medicine, University of Rochester Medical Center, Rochester, NY, USA; 3) Department of Biology, Ghent University, Ghent, Belgium.

Under UV light, blue fluorescence is visible in the intestinal cells of *C. elegans*. Mean levels of this fluorescence increase in aging worm cohorts(1). It has been suggested that the fluorescent substance is lipofuscin, a complex aggregate of oxidised proteins and lipids. Lipofuscin occurs in aging mammalian cells and has similar spectral properties(2). Blue fluorescence is thus often used as a biomarker of aging in *C. elegans*. When we monitored individual worms (rather than cohorts) as they aged, we saw no increase in fluorescence. Instead, as the worms died, we saw a rapid and striking increase in fluorescence (~5 fold, p = 2.8e-13; time lapse photography). This fluorescence was propagated in a wave along each worm as it died. A similar increase was also seen in young adult worms when we killed them. Hyperoxia caused increased protein oxidation but did not affect levels of blue fluorescence. Altogether, our findings strongly imply that the blue fluorescent material is not lipofuscin or any kind of biomarker of aging, but rather an indicator of death. We then asked how such death fluorescence is generated. In the necrotic calpain-cathepsin protease pathway of mammals(3) and nematodes(4), lysosomal lysis causes cytosolic acidosis, which leads to cell death via peptidase activation. We employed necrosis pathway mutants to block the pathway, and tested effects on death fluorescence. Knockdown of intracellular calcium release (*crt-1*, *unc-68*), or cysteine (*tra-3*) or aspartyl (*cad-1*) proteases, or inhibition of lysosomal acidification (*vha-12*) all significantly decreased death fluorescence levels. Moreover, cytosolic acidosis immediately precedes death fluorescence. This suggests that death fluorescence is a terminal product of the necrotic cell death pathway. The fluorescent material, which is non-proteinaceous, can be purified using HPLC. Mass spectrometry identifies a single molecular species, which we are now analyzing. Our work implies that the blue fluorescent substance in the worm intestine is not lipofuscin but, instead, a terminal product of necrotic death. Moreover, it suggests that intestinal necrosis, and its propagation along the worm, plays a major role in *C. elegans* organismal death. (1) Gerstbrein, Aging Cell, 2005 (2) Klass, Mech Ageing Dev, 1977 (3) Yamashima, Cell Calcium, 2004 (4) Xu, Neuron, 2001.

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The germline influences *C. elegans'* longevity through Wnt signaling. **Monika Suchanek**, Cynthia Kenyon. Dept of Biochemistry & Biophysics, University of California San Francisco.

Germ cell ablation extends the life span of *C. elegans* by up to 60%. This effect is not due to sterility, as the removal of the entire reproductive system does not prolong life span. Similarly, the reproductive tissues of flies and mice control their lifespan. These findings are very exciting, because they suggest evolutionary conservation of the pathway linking reproductive status of the organism to its longevity.

In *C. elegans*, the life-prolonging effect of germline ablation depends on the nuclear translocation of the DAF-16/FOXO transcription factor in intestinal cells, illustrating the existence of long-range communication between gonad and other organs. In intestinal nuclei, DAF-16 activates the transcription of multiple life-span extending genes.

To understand how the intestine senses the absence of the germline, I screened a signaling component of the *C. elegans* genome for RNAi clones clones that interfere with germline signaling in *C. elegans*, utilizing both the subcellular localization of DAF-16::GFP in the intestine, as well as the intensity of fluorescence of a DAF-16 downstream target gene (SOD-3::GFP) in the germline-defective *glp-1* worms. Screening of 1304 RNAi clones led to identification of 115 (8.8%) potential signal candidates. Among the genes that robustly affected DAF-16 localization and SOD-3 expression were multiple components of a Wnt-signaling pathway, suggesting the intriguing possibility that a Wnt signal communicates the status of the reproductive system to the rest of the organism.

To identify the Wnt/β-catenin that functions to signal to the intestine in response to germline loss, I tested effects of all *C. elegans* Wnts and β-catenins on DAF-16 localization, SOD-3 expression, and lifespan. RNAi against *wrm-1* (β-catenin) and *mom-2* (Wnt) specifically affected the lifespan of animals lacking germ cells (and not that of other long-lived mutants or wild-type worms), therefore genetically behaving as expected for the signal to intestine. Both *wrm-1* and *mom-2* are components of a non-canonical Wnt signaling pathway that during *C. elegans'* development mediates communication between two early blastomeres (P_2 and EMS) at the 4-cell stage. Interestingly, the blastomere EMS is the precursor of the intestine and P_2 becomes the germline, raising an intriguing possibility that the same pathway that is used during development is later on during the adulthood reused to signal the status of the reproductive system to the intestine.

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Defining longevity-specific targets of AMPK. **William Mair**[1,2,3], Ianessa Morantte[1,2,3], Ana Rodrigues[4], Gerard Manning[4], Marc Montminy[3], Reuben Shaw[1,2,3], Andrew Dillin[1,2,3]. 1) Molecular and Cellular Biology Labratory, The Salk Institute for Biological Studies, La Jolla, CA; 2) Howard Hughes Medical Institute, The Salk Institute, La Jolla, CA; 3) The Glenn Foundation for Medical Research, The Salk Institute, La Jolla, CA; 4) Razavi Newman Center for Bioinformatics, The Salk Institute, La Jolla, CA.

Dietary restriction increases lifespan in organisms from yeast to primates and reduces severity of a range of age-related pathologies. Targeting the molecular framework underpinning this phenomenon may therefore provide novel therapeutics that confer resistance to multiple diseases via a single intervention. AMP-activated protein kinase is a pro-longevity kinase activated when energy is low, which lies upstream of a variety of nutrient sensing pathways known to mediate organismal lifespan. We have shown that a critical longevity target of AMPK in *C. elegans* is the CREB-regulated transcriptional coactivator CRTC-1. CRTCs are a family of cofactors involved in diverse physiological processes including energy homeostasis, cancer and endoplasmic reticulum stress. CRTC-1 is a direct AMPK target, and interacts with the CREB homologue-1 (CRH-1) transcription factor *in vivo*. The pro-longevity effects of activating AMPK decrease CRTC-1 and CRH-1 activity and induce transcriptional responses similar to those of *crh-1* null worms. Downregulation of *crtc-1* increases lifespan in a *crh-1*-dependent manner and directly reducing *crh-1* expression increases longevity, substantiating a role for CRTCs and CREB in aging. This finding allows us to uncouple causal longevity-specific processes regulated by AMPK from casually associated downstream targets. We demonstrate the specific role of CRTC-1 in the longevity function of AMPK, separable from the effect of AMPK on growth and reproduction. Further, we show that AMPK activates a family of genes involved in the unfolded protein response (UPR^{ER}) and that these targets modulate the effect of AMPK on lifespan. Together these data illustrate a molecular pathway activated under dietary restriction to induce transcriptional changes that up-regulate protein homeostasis and longevity.

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Tissue specific 14-3-3 activities in *Caenorhabditis elegans* lifespan regulation. Z. Yang, S. Yu, Z. Yu, D. Zhao, **Y. Wang**. School of Life Sciences, Xiamen University, Xiamen, Fujian, China.

The 14-3-3 proteins are a family of ubiquitiously expressed and highly conserved proteins. They play key roles in the regulation of central physiological processes through interactions with multiple proteins. Our previous studies demonstrated that two *C. elegans* 14-3-3 proteins, PAR-5 and FTT-2 can interact with DAF-16 and SIR-2.1 to regulate lifespan and overexpression of PAR-5 and FTT-2 in *C. elegans* extended longevity significantly. To further explore the role of 14-3-3 proteins in the regulation of DAF-16 and SIR-2.1 for the longevity, we investigated in which tissue(s) PAR-5 and FTT-2 activity may be required for the lifespan regualtion of *C. elegans*. By using tissue specific promoters to drive PAR-5::GFP and FTT-2::GFP expression, we could specifically express PAR-5::GFP and FTT-2::GFP in different tissues of the transgenic worms. Since *par-5* is widely expressed in different tissues of *C. elegans*, we used *unc-119* promoter, *ges-1* and *spl-1* promoters, *lin-26* promoter and *unc-54* promoter to drive PAR-5::GFP expression in the nervous system, the intestine, hypodermis and the body-wall muscle, respectively. For the tissue specific expression of FTT-2::GFP, we used *unc-119*, *ges-1* and *spl-1*, *lin-26*, *unc-54* and *myo-2* promoters to specifically express FTT-2::GFP in the nervous system, the intestine, hypodermis, the body-wall and pharyngeal muscles, respectively. Then we examined the lifespan of these transgenic lines. Compared with the control, the transgenic line overexpressing PAR-5::GFP in the nervous system and hypodermis, where both DAF-16 and SIR-2.1 are expressed, extended mean lifespan significantly. The transgenic lines overexpressing PAR-5::GFP in other tissues had no obvious influence on the lifespan. The transgenic lines overexpressing FTT-2::GFP in the intestine and pharygenal muscle extended the lifespan significantly. Taken together, our results suggest that PAR-5 and FTT-2 may regulate lifespan through interaction with DAF-16 and SIR-2.1 in different tissues. Key words: *C. elegans*, 14-3-3 protein, tissue specificity, lifespan.

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NDG-4 determines Aging and Stress Resistance. Jeanette Brejning, Lone Schoeler, Tine Moeller, Helle Jakobsen, **Anders Olsen**. Department of Molecular Biology , Aarhus Universtiy, Aarhus, Denmark.

The gene *ndg-4* encodes a transmembrane protein with largely unknown function. *ndg-4* belongs to a small group of genes causing nose contraction resistance to the anti-depressive drug fluoxetine (Nrf phenotype). Some Nrf mutants, including *ndg-4*, lay pale eggs, presumably due to yolk and lipid deficiency [1]. We have isolated *ndg-4* in a whole genome RNAi screen for resistance to stalled replication forks induced by treatment with hydroxy urea (HU). Interestingly, we find that two different alleles of *ndg-4* both confer large increases in lifespan (more than 50 percent) and stress resistance in *C. elegans*. Both the stress resistance and the longevity of *ndg-4* mutants are, at least in part, independent of the insulin signaling pathway as mutation of *ndg-4* can increase both stress resistance and lifespan of *daf-16* null mutants. Furthermore, we observe a large synergistic effect on lifespan when *ndg-4* mutants are treated with *daf-2* RNAi which doubles the already long lifespan. *ndg-4* appears to function in the same stress resistance and longevity pathway as the checkpoint protein *chk-1* [2], since inactivation of *chk-1* does not further increase stress resistance and lifespan of *ndg-4* mutants. Nrf mutants with the pale egg phenotype are resistant to sterility caused by the dietary lipid DGLA, suggesting a failure to distributing lipophilic substances in these mutants [3]. We find that two other Nrf mutants that display the pale egg phenotype, *nrf-5* encoding a putative lipid binding protein and *nrf-6* encoding an *ndg-4* homolog, are also long-lived and stress resistant. In contrast, other Nrf mutants not conferring the pale egg phenotype have normal lifespan. Therefore, it is tempting to speculate that the increase in stress resistance and longevity in *ndg-4*, *nrf-5* and *nrf-6* mutants may be connected to lipid signaling. 1.Choy, R.K., J.M. Kemner, and J.H. Thomas. Genetics, 2006. 172(2): p. 885-92. 2.Olsen, A., M.C. Vantipalli, and G.J. Lithgow. Science, 2006. 312(5778): p. 1381-5. 3.Watts, J.L. and J. Browse. Dev Biol, 2006. 292(2): p. 381-92.

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Parallel evolution of domesticated Caenorhabditis species targets pheromone receptors. **Patrick T. McGrath**[1], Yifan Xu[1], Jennifer Garrison[1], Michael Ailion[2], Rebecca Butcher[3], Cornelia Bargmann[1]. 1) Rockefeller University, New York, NY; 2) University of Utah, Salt Lake City, UT; 3) University of Florida, Gainesville, FL.

Evolution can follow predictable genetic trajectories, indicating that discrete environmental shifts can select for reproducible genetic changes. Conspecific individuals are an important feature of an animal's environment, and a potential source of selective pressures. We show here that adaptation of two Caenorhabditis species to growth at high density, a feature common to domestic environments, occurs by reproducible genetic changes that affect specific pheromone receptor genes. Chemical communication through pheromones that accumulate during high density growth causes young nematode larvae to enter the long-lived but non-reproductive dauer stage. Two strains of Caenorhabditis elegans grown at high density have independently acquired multigenic resistance to pheromone-induced dauer formation. Resistance to one pheromone, C3, results from the disruption of two adjacent chemosensory receptor genes; independent deletions in these two genes were fixed in both strains. Rescue, localization, misexpression, and calcium imaging experiments suggest that these genes encode redundant G protein-coupled receptors for C3. Similar multigenic resistance to dauer formation has also arisen in high-density cultures of a different nematode species, Caenorhabditis briggsae, resulting in part from deletion of a paralogous to these chemoreceptor genes. These results demonstrate rapid remodeling of the chemoreceptor repertoire as an adaptation to specific environments, and indicate that parallel changes to a common genetic substrate can affect life history traits across species.

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Behavioral adaptation of soil nematodes facilitates escape from predacious fungi. Sean Maguire, Christopher M. Clark, Jennifer K. Pirri, **Mark J. Alkema**. Dept Neurobiology, Univ Massachusetts Med, Worcester, MA.

Animal nervous systems need to respond to challenges in their natural environment. This is epitomized by predator-prey interactions where survival depends on the animal's ability to escape. However, we have few insights into how natural selection has shaped the molecular and neural mechanisms of behavior. We investigated predator-prey interactions between C. elegans and predacious fungi that catch and devour nematodes using constricting rings as trapping devices. Gentle touch to the anterior half of the body of C. elegans induces an escape response in which the animal reverses and suppresses exploratory head movements. We found that the constricting rings of Drechslerella doedycoides catch early larval stages with a diameter similar to the trap opening. There is a delay between the ring entry and ring closure, which allows the animal to withdraw from the trap before getting caught. Tyramine-signaling mutants that fail to suppress head movements in response to touch are caught more efficiently than the wild type in constricting fungal rings. Competition experiments show that the suppression of head movements in response to touch is an ecologically relevant behavior that allows the C. elegans to smoothly retract from a fungal noose and evade capture. Behavioral analysis of other soil nematodes suggests that selective pressures imposed by predacious fungi have shaped the evolution of escape behavior of free-living soil nematodes.

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A new family of nematode signaling molecules promotes social aggregation behavior in *C. elegans*. **Jagan Srinivasan**[1], Stephan von Reuss[2], Neelanjan Bose[2], Parag Mahanti[2], Margaret Ho[1], Oran O'Doherty[2], Paul Sternberg[1], Arthur Edison[3], Frank Schroeder[2]. 1) HHMI and Division of Biology, California Institute of Technology, Pasadena, CA 91125; 2) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853; 3) Department of Biochemistry and Molecular Biology, University of Florida, PO Box 100245, Gainesville, Florida 32610-0245.

The free-living nematode *C. elegans* has been used extensively as a model system for social behaviors such as foraging, population density sensing, mating, and aggregation. Recently a family of small molecule signals, the ascarosides have been shown to control both population density sensing and mating behavior [1]. We hypothesized that *C. elegans* aggregation behavior is also mediated by small-molecule signals as no intraspecific signals promoting attraction or aggregation of wild-type hermaphrodites have been identified. Using a comparative metabolomics approach [2], we have isolated a novel group of ascarosides that incorporates an indole moiety in the ascaroside structure. Behavioral assays demonstrated that these indole ascarosides serve as potent intraspecific attraction and aggregation signals for hermaphrodites, in contrast to ascarosides lacking the indole group, which are repulsive to hermaphrodites[3]. Hermaphrodite attraction to indole ascarosides is dependent on the ASK-amphid sensory neurons. Previous studies have shown that the ring interneuron RMG integrates attraction and aggregation signals from ASK and other sensory neurons[3]. However, we found that attraction to indole ascarosides does not require the RMG interneurons. The role of the RMG interneuron in mediating aggregation and attraction is thought to depend on the neuropeptide-Y-like receptor NPR-1, because solitary and social *C. elegans* strains are distinguished by different *npr-1* variants. We show that indole ascarosides promote attraction and aggregation in both solitary and social *C. elegans* strains, independently of npr-1 locus. The identification of indole ascarosides as aggregation signals reveals a highly developed chemical language for social communication in *C. elegans*. 1. Srinivasan, J. et al. (2008). A blend of small molecules regulates both mating and development in *Caenorhabditis elegans*. Nature 454, 1115. 2. Pungaliya, C., et al. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 106, 7708. 3. Macosko, E.Z et al (2009). A hub-and-spoke circuit drives pheromone attraction and social behavior in *C. elegans*. Nature 458, 1171.

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Two insulin-like peptides antagonistically regulate olfactory learning in C. elegans. **Zhunan Chen**[1], Astrid Cornils[2], Wolfgang Maier[2], Joy Alcedo[2], Yun Zhang[1]. 1) Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA; 2) Friedrich Miescher Institute for Biomedical Research, Maulbeerstrasse 66, CH-4058 Basel, Switzerland.

The insulin/insulin-like growth factor signaling (IIS) is a conserved pathway that regulates key events of animal physiology across taxa, ranging from invertebrates to vertebrates. Aside from its well-characterized role in metabolism and development, it has been implicated in behavioral plasticity. However, the mechanism underlying the function of IIS in learning remains largely unknown. Here we address this important neurobiology question using behavioral assays that quantify C. elegans olfactory learning. We find that two insulin-like peptides (ILPs), INS-6 and INS-7, antagonistically regulate the learning process. ins-6 mutants are defective in learning, which is surprisingly rescued by mutations in ins-7. In cell-specific rescue experiments, wild-type INS-6 rescues the learning defect of ins-6 single mutants when it is expressed in the ASI sensory neurons, and wild-type INS-7 restores the learning defect of the ins-6; ins-7 double mutants when it is expressed in the URX neurons. To explore further the mechanism behind the epistatic interaction between ins-6 and ins-7, we have characterized the expression pattern of ins-7 in the nervous system, using an integrated GFP transcriptional reporter for ins-7. We have measured the expression level of ins-7 in wild type and ins-6 mutant animals. Intriguingly, we have observed an increase in ins-7 expression in the ins-6 mutant background specifically in the URX neurons, but not in other neurons that express ins-7. In addition, we find that overexpression of INS-7 in URX produce a learning defect in wild-type animals. Together our data suggest that INS-6 from ASI promotes learning, whereas INS-7 acts in URX to inhibit it; and that INS-6 negatively regulates the transcription of ins-7 in URX to achieve the antagonistic effects on learning regulation. This work not only characterizes the complex nature of ILP signaling in behavioral plasticity, but also reveals a novel IIS pattern that hierarchically engages multiple ILPs in a selective set of cells. This multi-step ILP-to-ILP signaling pattern employs both molecular diversity and cellular identity to achieve the sophisticated coordination of different ILP functions.

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The function of TGF-β signaling in olfactory learning of *C. elegans*. **Xiaodong Zhang**[1], Yun Zhang[2]. 1) Department of Molecular and Cellular Biology, Center for Brain Science, Harvard University, Cambridge, MA; 2) Department of Evolutionary and Organismic Biology, Center for Brain Science, Harvard University, Cambridge, MA.

Recent studies have started to reveal that many molecules essential for early development also play important roles in adult neuronal plasticity. Here we investigate the function of the transforming growth factor-β (TGF-β) signaling pathway in animal learning. TGF-β signal transduction is critical in diverse physiological processes such as embryonic development, tissue homeostasis and immune responses. Although a couple of studies have implicated TGF-β in neural plasticity, the function of TGF-β in learning was never directly tested and the underlying cellular and physiological mechanism is essentially unknown.

Utilizing a behavioral assay that measures olfactory learning in adult animals, we have found that the Sma/Mab TGF-β signaling pathway is required for aversive olfactory learning of *C. elegans*. Mutations in the genes of this pathway, including the *dbl-1* ligand, *sma-6* and *daf-4* receptors, and *sma-3* Smad, abolish the learning ability of adult animals. We further explored the cellular mechanisms of this pathway in learning through expression analysis, cell/tissue-specific rescues, calcium imaging and behavioral characterization. We identified a set of interneurons that are critical for olfactory sensorimotor response as the essential release site of DBL-1 ligand in regulating learning. The neuronal activity of the interneurons is repressed by training, accompanied by an increase in the expression and secretion of DBL-1 from the interneurons. We also found that downstream Sma/Mab signaling components act in hypodermis and/or sensory neurons to promote learning. In addition, we present evidence that the function of TGF-β signaling in aversive learning of adult animals can be separated from its roles in body size determination and innate immunity based on different signaling strength as well as temporal and spatial requirements. Our results support a model in which training induced changes in neuronal activity lead to alteration of the Sma/Mab TGF-β signaling, which in turn modulates the olfactory response of adult animals.

Because TGF-β superfamily is conserved throughout metazoans, we expect our study to shed light on the function of TGF-β signaling in adult behavioral plasticity in other organisms as well.

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Probing the potassiome: K+ channel function in sleep behavior. **Cheryl L. Van Buskirk**[1], Julie Cho[1], Paul W. Sternberg[1,2]. 1) Division of Biology, Caltech, Pasadena, CA; 2) Howard Hughes Medical Institute.

Prior to each molt *C. elegans* enters lethargus, a period of cuticle remodeling accompanied by sleep-like behavior that is characterized by an inhibition of feeding, locomotion and sensory responses. This behavioral quiescence is regulated in part by EGF signaling and can be induced at any time, including adulthood, through forced expression of LIN-3/EGF. We have found that EGF-induced sleep behavior is dependent on EGFR signal transduction within a single neuron, ALA. It remains unknown, however, what signals are released by ALA and how these lead to dampened activity within the nervous system.

Potassium channel activity is known to modulate neuron excitability by tuning membrane resting potential, and short-sleeping mutants in *Drosophila* have been found to carry mutations in the Kv1 voltage-gated potassium channel *Shaker*. To gain insight into the regulation of neuronal excitability during *C. elegans* sleep, we have performed a functional analysis of the 'potassiome' with a set of ~80 RNAi clones comprising potassium channel genes and known regulators. Using a *lin-15b; eri-1; hs:LIN-3C* strain, we screened for suppression of the EGF-inducible adult sleep phenotype. While RNAi against *C. elegans* Kv1 *(shk-1)* had no detectable effect, we identified the UNC-103/ERG Kv11 potassium channel as a contributor to locomotory quiescence. We then analyzed the role of UNC-103 during L4 lethargus using RNAi, mutant analysis, and ERG-blocking compounds.

Human ERG has been studied extensively for its role in cardiac muscle contraction, and *unc-103* has been characterized for its role in male spicule protractor muscle function. More recently, ERG has been implicated in sleep regulation in a small-molecule screen for modulators of zebrafish behavior. We will present the results of our RNAi screen along with mutant and pharmacological analyses of UNC-103/ERG function in *C. elegans* lethargus.

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Long term calcium imaging of AFD thermosensory neuron revealed behavioral strategy for exploratory movements during thermotaxis. **Yuki Tsukada**[1,2], Tomoyasu Shimowada[1], Noriyuki Ohnishi[1,2], Atsushi Kuhara[3], Ikue Mori[1,2]. 1) Graduate Sch Sci, Nagoya Univ, Nagoya, Japan; 2) CREST, Japan; 3) Dep of Bio, Konan Univ, Hyogo, Japan.

Despite the identification of neurons, their connectivity, and molecules related to the specific mechanisms of neural circuits, little is known about the mechanism of information processing in *Caenorhabditis elegans*. An essential obstacle is the lack of methods that can monitor time course of information flow during information processing tasks. To address this issue, we focus on thermotaxis behavior of *C. elegans* because the information flow is relatively simple: the essential neural circuit related to thermotaxis is thought to consist of only several neurons. We quantitatively monitored information input (temperature) and output (behavior) by using thermography and our live imaging/tracking system that pictures freely moving single animals; the time-lapse images of a single animal were recorded up to several hours at about 30 frames/sec video rate with migratory trajectory. We simultaneously monitored activity of AFD thermosensory neuron and behavior during thermotaxis by combining the tracking system with the calcium imaging system with genetically encoded calcium indicator, Cameleon YC 3.60. Thus we quantified exact temperature, AFD activity, and behavioral state of freely moving animal during thermotaxis. Wild-type N2 animals on an agar plate with thermal gradient showed thermotaxis behavior depending on their cultivated temperature or food availability. Long-term monitoring of AFD activity showed spike-like increments of calcium concentration despite the continuous, slow temperature change of about 0.1°C/min. By comparing AFD activity and the behavioral states, we found that the peak of AFD activity corresponded to the timing of turn, although the AFD activity and turning behavior was not one-to-one coupling and one activity peak seemed to correspond to a few turns. This relationship changed when the animals were conditioned with off-food plate, by experiencing the animal off-food for two hours before the thermotaxis assay. Off-food conditioned animals tended to show one AFD activity peak for one turn or AFD activity peaks without any turn. According to these results, we suggest that AFD thermosensory neurons recognize thermal environment as discrete system even in shallow continuous thermal environment and that the animals change behavioral strategy during thermotaxis by adjusting relationship between AFD activity input and turning output. These hypotheses brush up biased random walk model of exploratory behavior of *C. elegans* and shed light on a specific type of information processing systems which utilize random signal.

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Recognition of familiar food stimulates feeding via endocrine serotonin signaling in Caenorhabditis elegans. **B. Song**, L. Avery. Dept Molecular Biol, Univ Texas SW Medical Ctr, Dallas, TX.

Familiarity discrimination allows animals to respond appropriately to previously encountered stimuli by altering behavior. It is poorly understood by which mechanisms the recognition memory alters behavior. In this study, we show that the nematode C. elegans is capable of familiarity discrimination. Worms form a memory of particular bacteria after experience and display behavioral plasticity, increasing the feeding response when they subsequently recognize the familiar bacteria. Using this behavior, we identified a neural pathway that is activated by recognition of familiar bacteria and delineated the mechanism by which the pathway increases the feeding response. Recognition of familiar food increases serotonin release from the pair of ADF chemosensory neurons. The released serotonin acts humorally and directly activates SER-7, a type 7 serotonin receptor, in MC motor neurons in the feeding organ whose activation increases the feeding response. Our study provides insight into the mechanism by which familiarity discrimination alters behavior.

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Sexual identity of a single sensory neuron pair controls *C. elegans* behavioral choice. **Renee M. Miller[1,2]**, Kyung-Hwa Lee[3], Deborah A. Ryan[1], Douglas S. Portman[1,2]. 1) Ctr Neural Dev & Disease, Univ Rochester, Rochester, NY; 2) Dept of Biomedical Genetics, Univ Rochester, Rochester, NY; 3) Lewis-Sigler Institute for Integrative Genomics and Dept of Mol Biol, Princeton University, Princeton, NJ.

Sex differences in morphology and behavior exist in all animals to ensure fitness through optimal prioritization of basic needs such as food and drives such as mate seeking. It is known that *C. elegans* males readily leave food to satisfy a mate searching drive, while hermaphrodites remain on or near a food source indefinitely. In addition, *C. elegans* males and hermaphrodites display characteristic sex-specific chemotaxis behaviors to a variety of volatile attractive cues. Here we show that sexual dimorphism in the expression of the olfactory receptor ODR-10 in the AWA neurons is a key contributor to the observed behavioral differences. While hermaphrodites express ODR-10, which detects the food-related chemical diacetyl, males lack appreciable ODR-10. Several lines of evidence indicate that food-leaving behavior is enhanced when ODR-10 levels are low, while staying on food is associated with high ODR-10 levels. We find that *odr-10* mutant hermaphrodites leave food significantly more than wild type hermaphrodites. Like all known sex differences in *C. elegans*, ODR-10 expression is under control of the sex determination pathway. We therefore also asked whether re-programming the sexual identity of AWA is sufficient to evoke sex-specific behavior characteristic of the opposite sex. We have demonstrated that genetic sex-reversal of the AWA neurons is sufficient to sex-reverse ODR-10 expression, diacetyl chemotaxis, and exploratory behavior in males and hermaphrodites. Therefore the sexual identity of a single neuron pair, AWA, through the modulation of a sensory receptor ODR-10, controls the olfactory response to diacetyl and the decision to remain on food. Since starvation is known to delay food-leaving behavior in males, we asked whether the absence of food regulates ODR-10 levels. Indeed, we found that ODR-10 is robustly expressed in starved males. Over a period of several days without food, ODR-10 expression increases in both sexes. Thus, environmental stimuli such as food act in parallel with sex to set and modify ODR-10 levels in AWA to allow for the appropriate food-related chemotaxis and exploration behaviors in males and hermaphrodites.

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Integrin Signaling Modulates Touch Sensitivity In Dauer Touch Receptor Neurons. **Xiaoyin Chen**, Martin Chalfie. Department of Biological Sciences, Columbia University, New York, NY 10027 USA.

Modulation of sensory perceptions enables animals to sense a wide range of signals and to adapt to different biological conditions. In *C. elegans*, sensing gentle touch requires the touch receptor neurons (TRNs). We find that dauer formations subtly modulate gentle touch sensitivity in *C. elegans*. In fact, dauers respond well to gentle touch at the head or tail. Unlike starved L3 larvae, which move backward when plates are tapped, dauer larvae move forward or backward at equal probabilities. This modulation requires both integrin and insulin signaling. Previous RNAi experiments demonstrated that reduction of gene activity for the integrins and focal adhesion proteins results in reduced touch sensitivity. We used mosaic analysis to examine the phenotype of complete loss of gene activity from focal adhesion genes, including *pat-2* (α-integrin), *pat-3* (β-integrin), *pat-6* (actopaxin), *unc-97* (Pinch) and *unc-112* (Mig-2) in the TRNs. Because mosaics of null mutations in these genes reduced, but did not eliminate, touch sensitivity in the head, the integrins and focal adhesion proteins probably modulate touch sensitivity. Null mutations in these genes had little effect on touch sensitivity in the tail. Furthermore, the modulation acts upstream of calcium wave propagation because ALMs lacking these genes respond normally to activation by channelrhodopsin. Thus, integrins and focal adhesion proteins could directly modify the activity of the MEC-4 transduction channel, perhaps by changing the force response. Using SID-1-dependent feeding RNAi, we tested several genes whose products have been shown to modulate integrin-related signaling to determine which are needed for touch sensitivity. Reduction of genes associated with four major signaling pathways affected touch sensitivity: the Rho GTPases, actin cytoskeleton, Ras/MAPK pathway, and the insulin-like pathway. Animals mutated in *daf-2* (insulin receptor) or *age-1* (PI3 kinase) responded less to touch in the head. The reduction of touch sensitivity was partially rescued by overexpression of the focal adhesion proteins UNC-112 and PAT-6 in the TRNs, suggesting that the insulin-like pathway modulates touch sensitivity through the focal adhesion proteins. Furthermore, overexpressing UNC-112 and PAT-6 restored the tap response of the dauers to that of starved L3 larvae. We conclude that the insulin signaling pathway working through integrin-signaling complexes alters the balance between anterior and posterior touch sensitivities in dauers. The modulation in the balance of touch sensitivity may allow dauers to conserve energy by moving less when stimulated by non-localized vibration, while still being able to escape from direct contact with foreign objects.

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Two novel DEG/ENaC channel subunits expressed in glia play an essential role in *C. elegans* touch sensitivity. **Lu Han[1]**, Ying Wang[1], Yun Lu[2], Shai Shaham[2], Laura Bianchi[1]. 1) Dept Physiology & Biophysics, Univ Miami, Miami, FL; 2) Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

DEG/ENaC ion channels play critical roles in sensory perception, including touch sensation, pain sensation, thermosensation and proprioception. To date, DEG/ENaC channels have been shown to be expressed in sensory neurons. Surprisingly, we recently discovered that DEG/ENaC channels are also present in *C. elegans* glia associated with sensory neurons and play a major role in sensory perception. DEG/ENaCs are expressed in mammalian glia, including astrocytes and Schwann cells. However, virtually nothing is known about the role that they play in these cell types. Here, we show that novel DEG/ENaC subunits *delm-1* (degenerin-like channel mechanosensory linked-1) and *delm-2* (degenerin-like channel mechanosensory linked-2) are expressed in OLQ and IL1 socket glia. Knock-out of *delm-1* and *delm-2* caused insensitivity to touch to the nose and defects in foraging, consistent with dysfunction of OLQ and IL1 sensory neurons. Electron microscopic analysis revealed that in *delm-1* knock-out, OLQ and IL1 sensory neurons and associated glia are normal, supporting that DELM-1 is needed for the function of OLQ and IL1 neurons rather than for development or structural integrity. Cell specific rescue experiments support the finding that DELM-1 and DELM-2 are required cell-autonomously in socket glia to maintain nose-touch sensitivity. To gain insight into the mechanism underlying DELM-1 and DELM-2 regulation of OLQ and IL1 sensory neurons function, *in vivo* Ca^{2+} imaging experiments and electrophysiological analysis of the channels reconstituted in the *Xenopus* oocytes expression system are being conducted. Our analysis reveals that DELM-1 is a constitutively open channel, permeable to Na^{+} and Li^{+} and blocked by amiloride. Whether DELM-1 and DELM-2 are part of the same channel complex and whether DELM-1 and DELM-2 are sensitive to mechanical forces are also being investigated.

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DEG-1 and not OSM-9 is the major mechanoeletrical transduction channel in ASH. **Shana Geffeney**[1], Juan Cueva[1], Dominique Glauser[1], Joseph Doll[2], Tim Lee[1], Misty Montoya[1], Snetu Karania[1], Arman Garakani[3], Beth Pruitt[2], Miriam Goodman[1]. 1) Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA; 2) Mechanical Engineering, Stanford University, Stanford, CA; 3) Reify Corporation, Saratoga, CA.

Mechanoreceptor neurons, including nociceptors, detect, encode and transmit mechanical stimuli as electrical signals. Understanding how mechanoreceptors detect force is critical for understanding how these neurons function, however the molecules responsible for mechanotransduction remain obscure except in two *C. elegans* neurons, PLM and CEP. Our goal was to identify the channels required to detect force in a *C. elegans* nociceptor ASH. In this study, we combined *in vivo* whole-cell patch-clamp recording and genetic dissection to deconstruct mechanoreceptor currents (MRCs) in ASH neurons. We found that forces 100-fold larger than those needed to activate a gentle touch receptor neuron, PLM, are required to activate MRCs in ASH. These results demonstrate that, like other nociceptors, ASH has a high threshold for cell activation. MRCs in ASH activate within milliseconds of stimulus application, like MRCs in touch receptor and CEP neurons. These results suggest that the channels carrying these currents are directly activated by force. MRCs in ASH are both Na^+-dependent and inhibited by amiloride, properties of DEG/ENaC channels. Indeed in this study, we identify DEG-1, a DEG/ENaC channel, as the major mechano-electrical transduction (MeT) channel in ASH. These findings demonstrate that MRCs in both nociceptors and touch receptors rely on DEG/ENaC channels. But, DEG-1 is not the only mechanotransduction channel in ASH: loss of *deg-1* reduced MRC by 80% and revealed a second minor current, which is not formed by another DEG/ENaC channel. Thus, ASH nociceptors rely on two genetically-distinct, MeT channels. Surprisingly the minor current is also independent of two TRPV channel genes, *osm-9* and *ocr-2*, that are co-expressed in ASH. Though null mutations in *osm-9* and *ocr-2* inhibit ASH-dependent behavioral responses to noxious stimuli, loss of OSM-9 and OCR-2 channels has no effect on mechanoreceptor currents or potentials in ASH. We propose that TRPV channels are not essential for detecting force, but contribute to encoding and transmitting information. Because mammalian and insect nociceptors as well as other C. elegans nociceptor neurons also co-express DEG/ENaCs and TRPVs, the cellular functions elaborated here for these ion channels may be conserved.

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Contribution of cyclic nucleotide gated (CNG) channel subunits to behavioral plasticity in *C. elegans*. **Damien M. O'Halloran**[1,3], Xiao-Dong Zhang1[1], Chantal Brueggemann[1], Svetlana Altshuler-Keylin1[1], Yawei Yu[1], Tsung-Yu Chen[1,2], Noelle D. L'Etoile[1,3]. 1) Neuroscience, UC Davis, Davis, CA; 2) Department of Neurology, U.C. Davis, Davis, CA; 3) Department of Psychiatry and Behavioral Sciences, U.C. Davis, Davis, CA.

The *Caenorhabditis elegans* AWC neurons are responsible for sensation of a range of attractive volatile odors (Bargmann et al., 1993). Prolonged odor exposure in the absence of food leads to reversible decreases (adaptation) in the animal's attraction to that odor (Colbert and Bargmann, 1995; L'Etoile et al., 2002; Nuttley et al., 2002; Kaye et al., 2009; O'Halloran et al., 2009). It has been shown previously that the odor specificity of adaptation is determined by the feeding status of the animal (Colbert and Bargmann, 1997). That is, if a well-fed worm is exposed to benzaldehyde for a sustained period, it will adapt to both benzaldehyde and isoamyl alcohol (both sensed by AWC), this process is termed cross adaptation. In contrast, an unfed (starved) worm will adapt to benzaldehyde and its response to isoamyl alcohol will remain intact. The TAX-4 and TAX-2 cyclic nucleotide-gated (CNG) channel subunits are required for AWC-mediated olfactory responses (Coburn and Bargmann, 1996; Komatsu et al., 1999). TAX-4 is an α subunit that can form homomeric channels while TAX-2 is a β subunit that requires TAX-4 to form a functional channel (Coburn and Bargmann, 1996; Komatsu et al., 1999). C. elegans encodes two additional predicted α subunits, CNG-1 and CNG-3 (Cho et al., 2004a; 2004b; Coburn thesis, 1996). Here we report that CNG-1 is required in AWC to promote short-term (30-mins) cross adaptation between benzaldehyde and isoamyl alcohol. The ability of food to induce this cross adaptation is also dependent on the ASI sensory neurons. We also demonstrate that CNG-3 is required in AWC for adaptation to short-term (30-mins) exposures of odor. By using FRET, Bio-molecular Fluorescent Complementation assays, and genetically encoded calcium imaging we find that TAX-2::TAX-4::CNG-3 channels may promote short-term adaptation in AWC. Our data suggests the TAX-2::TAX-4::CNG-3 channel adopts a 3α:1β stoichiometry. By examining the electrophysiology of the CNG subunits in AWC, we also demonstrate that fast closing dynamics appear critical for proper short-term adaptation responses. Taken together our findings provide more understanding into the mechanisms and circuitry of how CNG channels shape olfactory plasticity.

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Multiple Wnts redundantly orient polarities of epithelial stem cells in *C. elegans*. Yuko Yamamoto[2,3], Hisako Takeshita[2], **Hitoshi Sawa[1,2,3]**. 1) Multicellular Organization, National Institute of Genetics, MIshima, Shizuoka, Japan; 2) Center for Developmental Biology, Kobe, Japan; 3) Department of Biology, Kobe University, Kobe, Japan.

During development, cells are often polarized in a coordinated manner to harmonize tissue patterning and morphogenesis. However how cell polarities are synchronized by extrinsic signals are not understood. In *C. elegans*, most mitotic cells are polarized along the anterior-posterior axis and divide asymmetrically. Although this process is known to be regulated by a Wnt signaling pathway called Wnt/β-catenin asymmetry pathway, how Wnt genes control cell polarities are poorly understood. We analyzed how Wnts and their receptors control polarities of seam cells that are epithelial stem cells in *C. elegans*. We found that multiple Wnt genes redundantly regulate orientation of the cell polarities. In compound Wnt mutants including animals with mutations in all the five Wnt genes, most seam cells were still polarized but orientation of polarities was randomized. In contrast, in animals in which functions of three Wnt receptors *(lin-17, mom-5 and cam-1*) were disrupted, stem cells were not polarized undergoing symmetric divisions, suggesting that Wnt receptors are essential for generation of polarities and can function even in the absence of Wnts. All seam cells except V5 can be properly polarized by one of the multiple Wnt genes that are expressed either anterior or posterior to seam cells, suggesting that each seam cell can be properly oriented irrespective of positions of and distances from Wnt sources. Ectopic expression of posteriorly expressed Wnts in an anterior region and vice versa rescued polarity defects in the compound Wnt mutants. Our results suggest the presence of novel mechanisms that orient cell polarities by Wnt genes.

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EGL-27/MTA1 interacts with SEM-4/SALL and other pluripotency factors to potentiate the Y-to-PDA cell reprogramming event. **A. Ahier**, K. Kagias, N. Fischer, S. Jarriault. IGBMC-Institut de Genetique et de Biologie Moleculaire, Illkirch, Alsace, France.

The cell plasticity describes the ability for a differentiated cell to be reprogrammed and adopt another identity. When the final identity is also differentiated, this phenomenon is called transdifferentiation (TD). We recently characterized such an event in *Caenorhabditis elegans*, in which an epithelial rectal cell (Y) switches to a neuronal cell (PDA) during the L2-L3 larval stages transition (Jarriault et al, 2008). This phenomenon occurs as a multistep process in which the Y cell, and not its neighbours, first acquires the competence to change its identity, initiates the TD process and transits through discrete non-pluripotent steps before adopting the PDA identity (Richard et al, 2011). Here, we have focused on the TD initiation and addressed the role of factors involved in chromatin remodeling. Indeed, many evidences point to a role of chromatin factors in the maintenance of cell identity memory and gene expression regulation. Moreover chromatin structure appears to be widely remodeled during reprogramming events. Through a targeted RNAi screen for factors impacting on chromatin modifications, we identified *egl-27*, an homologue of the human MTA1, a NuRD (Nucleosome Remodeling and Deacetylase complex) component, as a key player in this process. In absence of *egl-27/mta1* activity, the Y cell is unable to change its identity and no TD is initiated, suggestive of a lack of competence. Interestingly, mta-1 is necessary for the maintenance of ES cells pluripotency in mammals. Investigation of potential interactors for *egl-27/mta1*, using RNAi and/or corresponding mutant analysis, revealed the implication of another conserved nuclear factor previously described in the initiation of Y TD: *sem-4/sall*. This is an exciting partner, as SEM-4/SALL is important for ES cells pluripotency and enhances IPS reprogramming. Furthermore, conventional biochemical approaches allowed us to show that EGL-27 physically interacts with SEM-4/SALL and other conserved pluripotency factors. Thus, EGL-27/MTA1 and SEM-4/SALL appear to be components of a conserved cell plasticity complex which is necessary to allow the Y cell to be reprogrammed. Our data highlight a new function for these ES cell factors for the first time in a natural TD event, in vivo, and suggest that common mechanisms may be at play between different cell plasticity phenomena. The determination of the molecular mechanisms allowing a cell to be plastic has significant implications on our ability to better and safely reprogram cells for regenerative medicine purposes.

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A biased competition model for distinct tube fates in the C.elegans excretory (renal) system. **Ishmail Abdus-Saboor**, Vincent Mancuso, Kelly Howell, Katherine Palozola, Meera Sundaram. Gen, Univ Pennsylvania, Philadelphia, PA.

Signaling through Receptor Tyrosine Kinases and Ras is critical for tube formation and branching morphogenesis in many systems, but how signaling controls these processes is poorly understood. Here we describe sequential roles for Notch and Epidermal Growth Factor (EGF)-Ras-ERK signaling in the development of epithelial tube cells in the C. elegans excretory (renal) organ. This simple organ consists of three tandemly connected unicellular tubes, the excretory canal cell, duct and G1 pore. *lin-12* and *glp-1/Notch* are required to generate the canal cell, which is a major source of lin-3/EGF expression and is required for duct and G1 pore tubulogenesis. Asymmetric positioning of the canal cell appears to bias which of two competing precursors will, in a *let-60/Ras*-dependent manner, adhere to the canal cell, adopt the duct fate, and undergo auto-fusion to form a permanent seamless tube. The remaining precursor adopts the default G1 pore fate and forms a transient seamed tube that later withdraws from the organ. Ras signaling is required over an extended time period to promote sequential steps in duct development, and acts primarily through transcriptional effectors including LIN-1/Ets and EOR-1. These results reveal multiple roles for Ras signaling in tubulogenesis as well as similarities to Ras-mediated control of branching morphogenesis in more complex organs, including the mammalian kidney. The relative simplicity of the excretory system makes it an attractive model for addressing basic questions about how cells polarize, organize into tubes and (in some cases) decide to withdraw from a tube and take on new identities.

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The F-box protein SEL-10 inhibits signaling pathways critical for vulval development in *C. elegans*. **Claire de la Cova**, Iva Greenwald. HHMI/Dept Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

Multiple mechanisms ensure the correct spatial and temporal activity of LET-23/EGFR and LIN-12/Notch signaling during vulval fate patterning. We report here that SEL-10/Fbw7, an F-box and WD repeat containing protein that promotes ubiquitination and proteasome-mediated degradation of phosphorylated substrates, regulates the activity of the two main signaling pathways that specify vulval fate pattern.

Previous work showed that *sel-10* inhibits *lin-12* activity during *C. elegans* vulval fate patterning and that LIN-12/Notch proteins are conserved substrates of SEL-10/Fbw7. We now find that *sel-10* also negatively regulates the activity of the Ras pathway through *lin-45*, a *C. elegans* B-Raf and key component of the EGFR signal transduction pathway. Loss of *sel-10* suppresses defects resulting from partial loss of *lin-45* activity. The hypomorphic alleles *lin-45(n2506)* and *lin-45(n2018)* greatly reduce *lin-45* activity; most mutant larvae die during L1 with a rod-like morphology. This rod-like lethality is efficiently suppressed by *sel-10(ar41)*. In addition, loss of *sel-10* enhances vulval defects caused by a hyperactive *lin-45*. The transgene *lin-45(ED)* carries mutations that render LIN-45 constitutively active. Tissue-specific expression of LIN-45(ED) in vulva precursor cells (VPCs) results in a Multivulva (Muv) phenotype with large ventral protrusions characteristic of excessive Ras/Raf/MAPK signaling. The Muv phenotype of animals carrying *lin-45(ED)* is enhanced by *sel-10(ar41)*.

We have also analyzed the sequences mediating SEL-10 regulation of LIN-12 and LIN-45. Previous work showed that SEL-10 binds the C-terminal PEST sequence of intracellular LIN-12. We have found that both LIN-12 and LIN-45 proteins contain peptide sequences that match reported Fbw7 binding sites in human proteins. Although VPC-specific expression of LIN-12(intra) does not produce vulval defects, we found that mutation of the predicted SEL-10 binding site, which lies within the PEST sequence of LIN-12(intra), results in a Muv phenotype characteristic of hyperactive *lin-12(d)* alleles. In LIN-45, the predicted SEL-10 binding site lies within a mostly unconserved region; however, the corresponding residues in mammalian Raf proteins are highly phosphorylated. We found that mutation of the predicted SEL-10 binding site within LIN-45(ED) enhances the severity and penetrance of the resulting Muv phenotype. Given the effects on signaling in this important paradigm, we are now investigating the developmental regulation and role of *sel-10*.

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Sumoylation of *C. elegans* nuclear receptor NHR-25 promotes proper organogenesis. **Jordan D. Ward**[1], Nagagireesh Bojanala[2], Teresita Bernal[1], Marek Jindra[2], Kaveh Ashrafi[1], Keith R. Yamamoto[1], Masako Asahina[2]. 1) UCSF, Mission Bay Campus, San Francisco, CA; 2) Biology Centre ASCR and Univ. South Bohemia, Budweis, Czech Republic.

Tissue-specific regulatory programs governed by nuclear receptors (NRs) are a critical component of metazoan development and homeostasis with aberrations in these programs leading to pathophysiology. We are using the simplicity and powerful genetics of *C. elegans* to deconvolute how regulatory inputs differentially converge on NRs in specific cell and tissue-types. The single *C. elegans* NR5A family receptor, NHR-25, coordinates diverse tissue-specific developmental events such as molting, seam cell differentiation, fat metabolism, cell fate decisions and vulva formation. To uncover novel co-regulators of NHR-25 activity we performed a genome-wide yeast two-hybrid (Y2H) screen that uncovered the *C. elegans* SUMO1 homolog (SMO-1) as an NHR-25 interacting protein. SUMO Y2H interactions can reflect both covalent sumoylation and non-covalent interaction; to distinguish between these possibilities we performed mutational analysis. Both deletion of the SMO-1 C-terminal di-glycine repeat and mutations in the SMO-1 beta sheet, which abrogate covalent sumoylation and non-covalent binding, respectively, prevented NHR-25 binding in Y2H assays. *in vitro* biochemical assays confirmed the sumoylation of NHR-25. We have identified three lysines in NHR-25 responsible for the interaction, as well as non-covalent Sumo interacting motifs (SIM) located in the ligand binding domain of NHR-25. These data argue that NHR-25 initially binds SMO-1 non-covalently in order to promote its sumoylation, similar to thymine DNA glycosylase sumoylation. The *C. elegans* vulva is a paradigm of organogenesis since the progression from 22 epithelial precursor cells to the seven toroidal disks in the completed organ involve a combination of reproducible cell divisions, migrations, remodeling of adherens junctions, cell fusions and muscle attachments. Deletion of the *smo-1* gene causes severe protruding vulva (Pvl) and weak multivulva (Muv) phenotypes. When NHR-25 activity was reduced in a *smo-1* mutant, Muv induction in P3.p, P4.p and P8.p was enhanced. Interestingly, daughters of P(5-7).p vulval precursor cells (VPCs) were also affected and could not complete the full program of vulval cell divisions, thereby suppressing the smo-1 Pvl phenotype. Our work suggests that SMO-1 and NHR-25 function together during VPC cell division in a cell context-dependent manner. These data highlight how the sumoylation of nuclear receptors fine-tunes target gene regulation and thus ensures proper organ development. Supported by GACR 204/09/H058, 204/07/0948, NPVII 2B06129, TFF postdoctoral fellowship 700046, NIH grant CA020535.

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Hidden and apparent effects of a non-synonymous polymorphism in *C. elegans*. **Fabien Duveau**, Marie-Anne Félix. Inst. J. Monod - CNRS/ Université Paris 7, France.

Some biological systems give rise to highly reproducible phenotypes despite variation in environmental conditions. In such robust systems, phenotypic variation may occur within the developing system yet be buffered at the level of its output. This buffering allows the accumulation of "cryptic" mutations affecting the system without changing its end product. Cryptic genetic variation affecting the activity of the vulval signaling network was previously uncovered among *C. elegans* wild isolates (Milloz et al., 2008). One approach was to sensitize the system by introducing into different wild backgrounds a mutation that affects vulva cell fate induction. For example, the *let-23(sy1)/egfr* allele causes stronger vulva induction defects in the N2 background than in the AB1 background. To identify the underlying molecular variation, we used a quantitative genetic approach. We built 60 recombinant inbred lines between strains carrying *let-23(sy1)* in the N2 and AB1 backgrounds. Quantitative trait locus analyses detected one major-effect locus on chromosome I. Fine-mapping followed by functional analyses indicated that the causative polymorphism was a non-synonymous SNP in *nath-10*, the homolog of the human N-acetyltransferase 10 gene whose function is largely unknown in *C. elegans*. The identified polymorphism modifies an otherwise conserved amino acid in the putative acetyltransferase domain. The N2 allele of *nath-10* behaves as a hypomorph. By genotyping other wild isolates and LSJ1, a lab relative of N2, we found that the *nath-10(N2)* allele likely appeared during the early lab culture of N2. In addition to its cryptic role in the vulva system, the *nath-10* polymorphism also affects in a non-cryptic manner two life history traits with potentially opposite effects on fitness: the *nath-10(N2)* allele confers a ≈10% larger brood size and an older age at maturity than the AB1 allele. Both effects can be explained through a modulation in the timing of the sperm/oocyte switch in hermaphrodites. Indeed, *nath-10(N2)* animals produce more sperm and present a delay in oogenesis onset. These results show that cryptic genetic variation does not necessarily accumulate neutrally during the evolution of robust systems because of pleiotropic effects. Together with previous studies (McGrath et al., 2009; Weber et al., 2010), our results also indicate that the laboratory environment, which differs in several aspects from natural habitats, has driven fast phenotypic evolution of the N2 lab strain. We are currently performing competition assays to determine whether the N2 allele of *nath-10* confers a greater adaptive value in lab conditions.

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Changes in the Wnt and Netrin pathways that may give rise to new gonad morphologies. **D. Rudel**[1], H. Tian[2], B. Wyatt[1], R.J. Sommer[2]. 1) Department of Biology, East Carolina University, Greenville, NC; 2) Department of Evolutionary Biology, Max-Planck Institute for Dev. Biol., Tuebingen, Germany.

How new morphologies are generated has been a subject of speculation for centuries by such likes as Charles Darwin and Jean-Baptiste Lamarck. Changes in the shape of organs are an essential component to the development of new body plans, new species, and the capacity to take advantage of new ecological niches. However molecular investigations into the advent of novelties are in their infancy. Here, we show that within Diplogastridae, the hermaphrodite gonad has a novel path of gonad arm extension and makes a unique ventral migration in comparison to many other representative nematodes including *C. elegans*. This novel ventral migration can be prevented in *Pristionchus pacificus* by laser ablation or genetic ablation of the vulva. Ablation of all three vulval precursor cells (VPCs) results in all gonad arms failing to migrate ventrally. Wnt signaling plays a putative direct role in instructing the distal tip cells (DTCs) to migrate from the dorsal to the ventral side of the animal as the extending arms come back to the anterior-posterior center of the animal. In *P. pacificus* hermaphrodite animals where the Wnt pathway members *bar-1*, *mom-2* and *cwn-2* are mutated, gonad arms fail to extend ventrally. *Ppa*-BAR-1 is expressed in the nuclei of DTCs consistent with a signaling role for BAR-1 in these cells. *Ppa*-MOM-2 is expressed in the developing vulva at the appropriate stage suggesting it is part of a complex Wnt signal from the vulva to the DTCs. Taken together it is likely that cooption of Wnt signaling members has allowed for the advent of new organ morphology. We hypothesize that the Wnt signal is permissive and acts through the Netrin cell guidance system in *P. pacificus*; this hypothesis is based upon the observation that members of this pathway are necessary for all post-embryonic dorsal-ventral migrations in *C. elegans*. We are currently investigating the expression of Netrin receptors in the DTCs of *P. pacificus* hermaphrodites. Intriguingly analysis of the *P. pacificus* genome and the available expressed sequence tags (ESTs), suggest the composition of the Netrin pathway and its molecular functional organization operate differently in *P. pacificus* than *C. elegans*. This system provides in roads towards investigating the acquisition of new signaling functions and altered molecular networks and their roles in generating new organ shapes and body plans.

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Conserved Eukaryotic Fusogens can Fuse Viral Envelopes to Cells. **Ori Avinoam**[1], Karen Fridman[1], Clari Valansi[1], Inbal Abutbul[2], Tzviya Zeev-Ben-Mordehai[3], Ulrike Maurer[4], Amir Sapir[1], Dganit Danino[2], Kay Grünewald[3,4], Judith White[5], Benjamin Podbilewicz[1]. 1) Department of Biology, Technion - Israel Institute of Technology, Haifa 32000, Israel; 2) Department of Biotechnology and Food Engineering, Technion - Israel Institute of Technology, Haifa 32000, Israel; 3) Oxford Particle Imaging Centre, Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, UK; 4) Department of Molecular Structural Biology, Max-Planck Institute of Biochemistry, D-82152 Martinsried, Germany; 5) Department of Cell Biology, University of Virginia, Charlottesville, VA 22908, USA.

Cell-cell fusion is a fundamental process that occurs during development of most eukaryotes. In *C. elegans* cell-cell fusion is necessary for fertilization and organogenesis of the hypodermis, pharynx, vulva, uterus, glands, and male tail. Moreover, cell fusion has been implicated in myogenesis, bone formation, placentation, neuronal function, stem-cell reprogramming and carcinogenesis. Despite the relative abundance of cell fusion events during development, the molecular mechanism of cell-fusion is unknown. The first family of proteins that mediate membrane fusion (i.e fusogens) was identified in *C. elegans*. Its first members, AFF-1 and EFF-1 (CeFFs) are essential for cell fusion and their expression is sufficient to fuse cells that normally do not fuse both in vivo and in cell-culture. Furthermore, CeFFs are required on both membranes in order for them to fuse. We have shown that FFs are functionally conserved within and beyond the nematode phylum and that divergent members from the nematode *T. spiralis* and the chordate *B. floridae* can mediate fusion of mammalian cells. We use CeFFs as prototypic fusogens to understand the principles of cell-cell fusion machineries. In order to study the molecular mechanism of FF proteins we generated pseudotyped Vesicular Stomatitis viruses that express CeFFs on the viral membrane, in place of the endogenous fusogenic glycoprotein. We found that CeFFs are able to rescue the infectious activity of the virus and mediate viral infection via virus-cell fusion. In addition, the viral mode of fusion and infection became dependent on the expression of FFs on the virus as well as on the target cell membrane. We investigated the structure of AFF-1 at nano-resolution using electron microscopy and tomography and found that it formed distinct supercomplexes resembling pentameric and hexameric flowers on pseudoviruses. Thus, the evolution, structure and function of FFs begin to unravel as they converge with glycoproteins of enveloped viruses as minimal fusogenic machineries capable of fusing membranes independently of other cellular co-factors.

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A positive feedback loop involving translational repression and protein degradation maintains totipotency of germline blastomeres. Rueyling Lin[1], Tugba Guven-Ozkan[1,2], Scott Robertson[1], **Rueyling Lin**[1]. 1) Department of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX; 2) Department of Neuroscience, Scripps Research Institute, Jupiter, FL.

Specification of primordial germ cells requires global repression of transcription. In *C. elegans*, primordial germ cells are generated through four rounds of asymmetric divisions, starting from the zygote P0, each producing a transcriptionally repressed germline blastomere (P1-P4). Repression in P2-P4 requires PIE-1, which is provided maternally in oocytes and segregated to all germline blastomeres. The germline blastomere-specific localization of PIE-1 depends on multiple mechanisms, one relies upon regulated degradation of PIE-1 in somatic cells. Degradation of PIE-1 is initiated by a cul-2 containing E3 ligase. We have shown that the temporal and spatial regulation of this E3 ligase activity is controlled by translation of the substrate-binding subunit, ZIF-1. Using a reporter containing the *zif-1* 3' UTR, we showed that *zif-1* is translated in somatic cells, where PIE-1 is degraded, but not in oocytes or germline blastomeres, where PIE-1 is present. We have shown previously that OMA-1 and OMA-2 are responsible for the translational repression of *zif-1* in oocytes. We investigated how temporal and spatial expression patterns of ZIF-1 are regulated in embryos where OMA proteins are no longer present. We show that the somatic cell-specific translation pattern of ZIF-1 results from both positive regulation in soma and repression in germline blastomeres. Deleting a small region of the *zif-1* 3' UTR resulted in the loss of expression of the *zif-1* translational reporter in soma and derepression in germline blastomeres. We showed that this region of *zif-1* 3' UTR is bound by four RNA-binding proteins, SPN-4, POS-1, MEX-5, and MEX-6, which together restrict the translation of *zif-1* only to somatic cells. Repression of *zif-1* in germline blastomeres requires SPN-4 and POS-1. MEX-5 and MEX-6, which are expressed only in somatic cells, promote the translation of *zif-1* in somatic cells. POS-1 is also a substrate for the ZIF-1-containing E3 ligase and POS-1 degradation in soma is critical for its germline blastomere-specific localization. We show that translation of *zif-1* in the somatic cell most recently separated from its sister germline blastomere results from a positive feedback loop. In these cells, translation of *zif-1*, promoted by MEX-5, degrades POS-1. As POS-1 level decreases, more ZIF-1 is generated, effectively creating the germline blastomere-specific localization pattern of many ZIF-1 target proteins, safeguarding germline totipotency.

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Antagonizing germline fate in somatic cells: opposing roles of the MES and synMuv B chromatin regulators. **Lisa N. Petrella**[1], Wenchao Wang[2,3], Caroline A. Spike[2,4], Andreas Rechtsteiner[1], Susan Strome[1,2]. 1) MCD Biol, Univ California, Santa Cruz, Santa Cruz, CA; 2) Department of Biology, Indiana Univ, Bloomington, IN; 3) Dana-Farber Cancer Institute, Boston, MA; 4) Department of Genetics, Cell Bio and Development, Univ of Minnesota, MN.

The newly fertilized embryo inherits a chromatin state with germline characteristics. As development proceeds, those cells destined to generate somatic tissues must be reprogrammed to abolish this germline chromatin state. Previous studies demonstrated that a subset of synMuv B mutants ectopically express germline-specific P-granule proteins in their somatic cells, suggesting a failure to properly orchestrate a soma/germline fate decision (Unhavaithaya et al. 2002 Cell 111:991; Wang et al. 2005 Nature 436:593). Surprisingly, this fate confusion does not affect viability at low to ambient temperatures. We have shown that, when grown at high temperature, a majority of synMuv B mutants irreversibly arrest at the L1 stage (Petrella et al. 2011 Development 138:1069). High temperature arrest (HTA) is preceded by the expression of germline genes in somatic cells starting in late embryogenesis. This leads to somatic expression of P-granule proteins, components of the synaptonemal complex, and other meiosis proteins. Somatic expression of germline genes is enhanced at elevated temperature, leading to developmentally compromised somatic cells and arrest of newly hatched larvae. HTA is suppressed by loss of global regulators of germline chromatin, including the MES proteins, revealing that arrest is caused by somatic cells possessing a germline-like chromatin state. We propose that synMuv B mutants fail to erase or antagonize an inherited germline chromatin state in somatic cells during embryonic and early larval development. Consistent with this, the somatic cells of synMuv B mutants display elevated levels of chromatin marks that are normally enriched in the primordial germ cells. We are currently exploring how chromatin states are affected in synMuv B mutants and by elevated temperature, using nuclear spot assays.

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CDK-1 and Wnt regulate cortical release of WRM-1/ β-catenin to control cell-division orientation in early C. elegans embryos. S. Kim[1], **T. Ishidate**[1,2], R. Sharma[1,2], M. Soto[3], M. Shirayama[1], C. Mello[1,2]. 1) Program in Molec Med, Univ Massachusetts Med.School, Worcester, MA; 2) Howard Hughes Medical Institute; 3) Univ. of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School.

Asymmetric cell division plays a fundamental role in both development and the homeostasis of tissues. In many cases the proper regulation of asymmetric cell division requires coordination between the cell-cycle machinery and the signals that establish tissue or organismal polarity. However, with few exceptions, the molecular intersections between cell cycle and polarity signaling pathways have yet to be defined. During endoderm induction in early *C. elegans* embryos the Wnt- and Src-signaling pathways function in parallel to induce both the cleavage orientation and fate of the endoderm precursor, called EMS. The β-catenin-related protein WRM-1 accumulates asymmetrically in the nuclei of EMS descendants and is required for endoderm specification, but has not been linked to control of EMS division orientation. Here we show that WRM-1 localizes to the cortex of all early blastomeres and, in a Wnt-responsive manner, is released during mitosis from the cortex proximal to the signaling cell P2. We show that cortical release of WRM-1 depends on CDK-1, which phosphorylates WRM-1 directly. Mutant WRM-1 protein, lacking the CDK-1 sites, is retained at the cortex and, in embryos with impaired Src-signaling, interferes with the rotation of the EMS spindle onto the polarized axis of the cell. These findings identify WRM-1 as a direct developmental target of CDK-1 and as a molecular link that integrates a Wnt-polarity signal with the control of the cell-division axis.

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The microRNA *mir-235* is essential for the insulin/IGF pathway-dependent quiescence in blast cells during L1 diapause. **Hidefumi Kasuga**, Masamitsu Fukuyama, Hiroaki Kajiho, Kenji Kontani, Toshiaki Katada. Department of Physiological Sciences, Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan.

Multipotent stem and blast cells reversibly arrest cell division and differentiation in response to harsh environments to maintain homeostasis in animals. When *C. elegans* L1 larvae are hatched in the absence of food, both germline stem and somatic blast cells are prevented from initiating their postembryonic developmental events and sustained at developmentally quiescent state (L1 diapause). Nutrients reactivate these undifferentiated cells via the insulin/IGF pathway to release them from the developmental dormancy (1-3). Recent studies have begun to show that some of miRNAs are involved in regulation of stem cell fate. Thus, we speculated that there may be a microRNA that acts during L1 diapause to keep quiescence in stem and blast cells under the control of the insulin/IGF pathway, by antagonizing genes that promote progression of L1 development. In order to identify such a miRNA, we screened for miRNA mutants that inappropriately initiate L1 development, defects caused by constitutive activation of the insulin/IGF pathway (1,2,4). We found that, among mutants of 111 miRNAs, only animals deleting *mir-235* showed striking defects in maintaining L1 diapause. *mir-235* mutants initiate L1 developmental processes in somatic blast cells such as P and M cells, and eventually undergo L1 molt even under starvation conditions. These defects were significantly restored by introduction of the genomic fragment containing *mir-235*. In contrast, restoration of the phenotypes was completely diminished when the *mir-235* genomic fragment was mutated in its seed sequence. These observations indicate that *mir-235* is required for maintaining L1 diapause. Northern blot analysis indicated that the amount of mature *mir-235* was upregulated during L1 diapause and downregulated in response to nutrients. Furthermore, this nutrient-dependent downregulation was abolished in *daf-2/insulin receptor* mutants. Altogether, these findings indicate that downregulation of *mir-235* by nutrients via the insulin/IGF pathway is a critical step for exit from L1 diapause. The role of *mir-235* in arresting L1 developmental programs is reminiscent of that of CKI-1, a cyclin-dependent kinase inhibitor, in arresting cell cycle during L1 diapause (5). Thus, identification of *mir-235* targets may reveal the core machinery for L1 developmental progression. 1: *Curr. Biol.* 16, 773. 2: *Curr. Biol.* 16, 780. 3; *Cell* 128, 577. 4: IWM 2005 Fukuyama et al. 5: *Development* 125, 3585. *Development* 126, 4861.

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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, MA 02115, USA.

Successful execution of the meiotic program depends on the timely establishment and removal of sister chromatid cohesion. 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 is therefore critically important to understand how the loading, maintenance and dissociation of cohesin complexes from DNA is regulated. LAB-1, a functional homolog of Shugoshin in *C. elegans*, is required to protect sister chromatids from the premature removal of REC-8, a meiosis-specific cohesin member, at metaphase I. Here we identify an earlier role for LAB-1 in promoting the establishment of sister chromatid cohesion during early prophase I. Utilizing fluorescence in situ hybridization (FISH) we find that *lab-1(RNAi)* reduces homologous pairing levels on both autosomes and the X chromosome compared to wild type. Moreover, nuclei with between 3 to 4 FISH signals were present from the pre-meiotic tip to the end of pachytene in *lab-1* depleted gonads, suggesting loss of sister chromatid cohesion. As a result, both the progression of meiotic recombination and synapsis are impaired, as evidenced by the elevated levels of a marker for DNA double-strand break repair (RAD-51 foci) and by the mixture of synapsed and unsynapsed chromosomes observed in pachytene, respectively. Further support for a role in the establishment of sister chromatid cohesion stems from the observation that both LAB-1 and REC-8 are required for the chromosomal association of SMC-3, a structural maintenance of chromosomes protein and cohesin member. Similar to *rec-8* mutants, depletion of *lab-1* results in partial loss of sister chromatid cohesion when combined with mutations in two of the *rec-8* paralogs, *coh-3* and *coh-4*, and further increases the number of separated chromatids when all three paralogs are mutated. LAB-1 has a putative degenerate Protein Phosphatase 1 (PP1) binding motif, and accordingly, during early meiotic steps, depletion of *lab-1* leads to reduction in the nuclear localization of the PP1 homolog GSP-2, and nuclear accumulation of the Aurora B Kinase homolog AIR-2. We propose that LAB-1 cooperates with the cohesin complex to link sister chromatids, and maintain cohesion by antagonizing AIR-2 via GSP-2 in early prophase, thereby facilitating the *C. elegans* meiotic program.

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AIR-2 guides condensin I to chromosomes and the spindle. K. Collette, J. Bembenek, N. Golenberg, **G. Csankovszki**. MCDB, Univ Michigan, Ann Arbor, MI.

Condensin complexes are essential for mitotic and meiotic chromosome segregation. *C. elegans*, like other metazoans, has two distinct mitotic/meiotic condensin complexes, condensins I and II. The two complexes occupy distinct chromosomal domains and perform non-redundant functions. Our laboratory focuses on determining how condensin I and condensin II are targeted to their sites of action and how they contribute to chromosome dynamics. Despite the differences in mitotic and meiotic chromosome behavior, we uncovered several conserved features. During both mitosis and meiosis, condensin II loads onto chromosomes in early prophase and condensin I at entry into prometaphase. During both mitosis and meiosis, the localization of condensin I closely parallels the localization of the chromosomal passenger kinase Aurora B/AIR-2. Consistently, AIR-2 affects the targeting of condensin I, but not condensin II, to chromosomes. Interestingly, AIR-2 appears to target condensin I not only to chromosomes, but also to the mitotic and meiotic spindle, as observed by both immunofluorescence and live cell imaging of the condensin I subunit CAPG-1. During mitosis, condensin I localizes to the spindle midzone, in addition to chromosomes, and to the midbody during abscission, late in cytokinesis. During anaphase of meiosis, condensin I associates with microtubules between separating chromosomes. Therefore, condensin shows a localization pattern similar to other chromosomal passenger complex proteins and we hypothesize that it may contribute to AIR-2's functions not only on the chromosome, but also on the spindle. AIR-2 coordinates many aspects of cell division, including kinetochore-microtubule attachments, chromosome orientation, anaphase spindle dynamics, and cytokinesis. Condensin I depletion leads to monopolar meiotic spindles and misaligned chromosomes, consistent with possible roles in regulating kinetochore-spindle attachments and spindle dynamics. We also observed multinucleate cells in condensin I depleted embryos, indicating a possible role in cytokinesis. Our results suggest that condensin I may contribute to multiple functions of the chromosomal passenger complex during cell division.

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Kleisin Subunit Identity Determines Cohesin Loading Mechanism, Distribution, and Function during *C. elegans* Meiosis. **Aaron F. Severson**, Barbara J. Meyer. HHMI and Dep't of Molecular and Cell Biology, University of California, Berkeley, CA USA.

Faithful transmission of the genome through sexual reproduction requires reduction of genome copy number during meiosis to produce haploid sperm and eggs. Three processes unique to meiosis are crucial for reducing ploidy. First, homologous chromosomes become linked through crossover recombination. Second, the two sister chromatids of each homolog attach to microtubules (MTs) from the same spindle pole (co-orient) in meiosis I and attach to MTs from opposite spindle poles (bi-orient) in meiosis II. Third, sister chromatid cohesion (SCC) is released in two steps to allow separation of homologs and then sisters during meiosis I and II. Work in yeast has shown that establishing the meiotic pattern of chromosome segregation requires that Scc1, the "kleisin" subunit of cohesin complexes that mediate mitotic SCC, is replaced by the meiosis-specific paralog Rec8. Subsequently, *rec8* mutations have been used to define meiotic cohesin functions in many organisms. We have shown that REC-8 is not the sole meiotic kleisin in *C. elegans*, and based on our work and other published data we propose that involvement of multiple kleisins is common in meiosis. *C. elegans* REC-8 and a pair of functionally redundant kleisins called COH-3 and COH-4 (COH-3/4 hereafter) perform specialized roles during meiosis. For example, although both REC-8 and COH-3/4 are required for meiotic SCC, only REC-8 cohesin can co-orient sister chromatids and mediate SCC that persists after anaphase I. Thus, cohesin complexes that differ in their kleisin subunit can perform distinct tasks in a single nucleus, and cohesin function is determined by kleisin identity. The kleisin also influences the mechanism by which cohesin loads onto chromosomes. The axial element protein HTP-3 is required for loading of REC-8 cohesin onto meiotic chromosomes, as has been shown previously for the timeless paralog TIM-1. Remarkably, neither HTP-3 nor TIM-1 is required for loading of COH-3/4 cohesin. Finally, REC-8 and COH-3/4 become asymmetrically distributed on meiotic chromosomes late in prophase of meiosis I, adopting an organization consistent with their distinct roles in meiotic chromosome segregation: COH-3/4 becomes enriched at regions where SCC is released at anaphase I and REC-8 becomes enriched where sister chromatids co-orient and SCC persists until anaphase II. Because REC-8 alone can co-orient sister chromatids and mediate cohesion that persists following anaphase I, we are currently testing whether establishing this reciprocal pattern of cohesin localization is important for the stepwise separation of homologs and sister chromatids.

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The RGA-3/4 RhoGAP and signalling by the mitotic asters restrict RhoA activity to cell equator during cytokinesis. **Esther Zanin**, Karen Oegema. Ludwig Institute for Cancer Research, UCSD, USA.

Cytokinesis is accomplished by constriction of a cortical contractile ring. To ensure that each cell receives a single genomic complement, contractile ring assembly is directed by signals from the anaphase spindle. The spindle is thought to direct ring assembly by patterning activation of the small GTPase RhoA to generate a narrow equatorial zone of activated RhoA, which in turn directs assembly of the contractile ring. During cytokinesis, the RhoGEF ECT-2 activates RhoA. Active RhoA is anchored in the plasma membrane with a lipid moiety and freely diffuses within the membrane. Theoretical studies have suggested that in addition to localized activation, generation of a narrow equatorial zone of active RhoA would also require rapid constitutive inactivation of RhoA. Rapid RhoA flux would prevent active RhoA from distributing over the membrane by diffusion (Bement et al., BioEssays 28:983-993, 2006). The importance of RhoA flux and the identity of the critical inactivating RhoGAP are important current questions. In C. elegans two highly similar RhoGAPs, RGA-3 and RGA-4, have been shown to act preferentially on RhoA to control cortical contractility during polarization of the embryo (Schonegg et al., PNAS 104:14976-14981, 2007; Schmutz et al., Development 134: 3495-3505, 2007). Here, we test whether RGA-3/4 also promote Rho flux during cytokinesis to constrain RhoA activation. rga-3/4 mutant embryos have a hypercontractile cortex and 25% of rga-3/4 embryos form no cleavage furrow. Since hypercontractility complicates analysis, we analyzed the contribution of RGA-3/4 to the patterning of the contractile ring protein anillin in embryos in which we suppressed contractility by inhibiting none-muscle myosin II (nmy-2). In both wild type and nmy-2(RNAi) embryos, anillin is cleared from the poles of the cell and localizes in a ~10µm wide equatorial zone in anaphase. In nmy-2(RNAi);rga-3/4 embryos anillin is cleared from the polar regions, but localizes to a zone that is two times broader than in controls. Our results suggest that the RGA-3/4 RhoGAP opposes the ECT-2 RhoGEF to promote RhoA flux, which is critical to confine the equatorial zone of active RhoA. However, our data also indicate that the centrosomal asters inhibit the accumulation of cortical contractility at the poles via a mechanism independent of RGA-3/4. Thus, the RGA-3/4 RhoGAP and the centrosomal asters make independent contributions to control the spatial activation of RhoA during cytokinesis.

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Condensin I and the spindle midzone prevent furrow regression induced by chromosome mis-segregation in *C. elegans* embryos. Koen J.C. Verbrugghe[1], Joshua N. Bembenek[2], Györgyi Csankovszki[2], **Raymond C. Chan[1]**. 1) Depts. of Human Genetics & Internal Medicine, University of Michigan, Ann Arbor, MI; 2) Dept. of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI.

During cell division, chromosomes invariably segregate before the cleavage furrow ingresses to partition the cell. Chromosomes that obstruct the furrow could block cytokinesis leading to altered cell ploidy, a defect common in cancer. Most cellular checkpoints minimize chromosome mis-segregation by delaying cell cycle progression until the chromosomes are replicated and bi-oriented to the spindle poles. However, this strategy may not be compatible with embryonic development, during which the timing of cell divisions must be closely coordinated. In *C. elegans* embryos, somatic cells with chromosome or spindle abnormalities can initiate cytokinesis (1-3). However, the cleavage furrow did not regress in embryos containing chromatin bridges induced by topoisomerase II *(top-2)* RNAi (4). To address whether this phenomenon of sustained furrow integrity is seen in other chromomsome mis-segregation events, we inactivated several genes essential for segregation. While most conditions that generate chromatin bridges do not trigger cleavage furrow regression, the loss of the condensin complexes does. Condensin I and II contain the Structural Maintenance of Chromosome 2 and 4 homologs, MIX-1 and SMC-4, and additional subunits unique to each complex. The disruption of a shared subunit of condensin I and II was sufficient to induce both chromosome mis-segregation and furrow regression. We found that disruption specific to each condensin complex could functionally separate these two defects. Loss of condensin II caused chromosome mis-segregation, while mutations in condensin I did not. Furrow regression is only seen when chromatin bridges occur in the condensin I mutants, suggesting that condensin I may be required to sustain furrow integrity. Condensin I is highly enriched on chromatin bridges. Surprisingly, condensin I normally localizes to the spindle midzone, and disruption of the spindle midzone via *spd-1* deficiency recapitulates this condensin I mutant phenotype. Together our data support the presence of a pathway, resembling the abscission checkpoint (5), which prevents furrow regression in the presence of chromatin bridges in *C. elegans* embryos.

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The SCF^{SEL-10} ubiquitin ligase complex is a key regulator of ZYG-1 levels. **Nina Peel**, Michael Dougherty, Kevin O'Connell. Lab Biochem & Gen, NIDDK, NIH, Bethesda, MD.

The correct segregation of DNA during cell division requires formation of a bipolar spindle, organized at each pole by a centrosome. Regulation of centrosome duplication such that each mitotic cell has exactly two centrosomes is therefore of central importance to cell division. Deregulation of centrosome duplication causes the appearance of supernumerary centrosomes, which are a hallmark of many cancer cells and can contribute to tumorigenesis. Ectopic expression of the kinase Plk4, which is required for centrosome duplication causes the formation of extra centrosomes, moreover aberrant Plk-4 expression levels are associated with cancer. Data from *Drosophila* and human cells suggests that Plk4 levels are regulated by the $SCF^{slimb/\beta Trcp}$ ubiquibin ligase and proteosomal degradation. In *C. elegans* it is unclear how levels of the functional homolog of Plk4, ZYG-1, are controlled. We show that levels of ZYG-1 are regulated by proteosomal degradation as inhibition of proteosome function leads to an increase in ZYG-1 protein levels. We further show that RNAi-mediated down-regulation of SCF components SKR-1/2 causes an increase in ZYG-1 levels, indicating that this complex is required to target ZYG-1 for degradation. We do not however find a role for the slimb/βTrcp homolog *lin-23* in ZYG-1 degradation. Surprisingly we find that the F-box protein SEL-10 is instead required to regulate ZYG-1 levels. Significantly, down-regulation of any of the SCF^{sel-10} components upregulates ZYG-1 activity sufficiently to restore centrosome duplication in a *zyg-1* hypomorphic mutant. Moreover, we find that components of the SCF complex show an overlapping pattern of localization with ZYG-1, consistent with their regulating ZYG-1 levels. Our results show that precise control of ZYG-1 levels is achieved through its proteosomal degradation directed by the SCF^{SEL-10} complex.

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Genomic and Epigenetic Regulation of Double Strand Breaks During Meiosis in C. elegans. **C.V. Kotwaliwale[1,2]**, A. Dose[1], A.F. Dernburg[1,2]. 1) Molecular and Cell Biology, University of California, Berkeley, CA; 2) Howard Hughes Medical Institute.

Sexual reproduction depends on meiosis, which enables the formation of haploid gametes from diploid cells. In order to segregate to different daughter cells, homologous chromosomes must first establish physical connections with each other via the process of homologous recombination. Meiotic recombination is not distributed randomly across the genome, but occurs in regions that are thought to be preferred sites for programmed DSB formation by the SPO-11 enzyme. In C. elegans, recombination is strongly biased to occur within distal regions (or "arms") of the chromosome. The central regions, which constitute roughly half of the chromosome length, are relatively "cold" for recombination events. It has been unclear whether this bias is a consequence of DSB distribution, or instead reflects a mechanism that biases the downstream repair outcome of DSBs. To address this, we generated a genome-wide map of meiotic DSBs by mapping the distribution of the sole C. elegans RecA-like recombinase, RAD-51, by ChIP-seq (chromatin immunoprecipitation/Illumina sequencing). RAD-51 is a good proxy for DSBs, since it binds to the resected ends and mediates the search for a homologous repair template. We have found that the RAD-51 binding pattern is strikingly similar, but not identical, to the known recombination distribution pattern in C. elegans. RAD-51 binding is highly enriched on chromosome arms. Previous work has shown that the arms of each chromosome differ in recombination rate. We find that these differences are mirrored by the distribution of DSBs. In addition, we find that DSB sites occur in regions of active histone marks such as H3K4 trimethylation and H3K27 acetylation. This is surprising because in general, silencing marks are more abundant on chromosome arms, while active marks are enriched on chromosome centers. This suggests that local gene expression activity may contribute to DSB formation. Consistent with this, we have found a correlation between recombination rate and gene expression. Previous work has shown that recombination rate strongly correlates with intron size. Strikingly, a large number of RAD-51 binding sites occur within introns. Although the DSB pattern is highly correlated with crossover frequency, there are interesting exceptions. In particular, the sub-telomeric regions are completely devoid of crossovers but are active DSB sites. This raises the possibility that additional mechanisms act downstream of DSBs in C. elegans to bias the repair outcome. Taken together, our data provide interesting insights into the role of recombination on the evolution of the C. elegans genome.

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Meiotic chromosome structural proteins influence centriole dynamics and spindle organization during spermatocyte meiosis. **Mara Schvarzstein**, Anne Villeneuve. School of Medicine, Stanford University, CA.

Successful embryonic development requires that sperm provide the zygote with both, a haploid complement of chromosomes and a single engaged pair of centrioles. Correct endowment of these components depend on events that occur during the spermatocyte meiotic divisions. We provide evidence that meiotic chromosome structural proteins and structural organization of the chromosomes themselves influence spindle organization during the spermatocyte meiotic divisions. First, we identified a previously unanticipated role for meiotic chromosome structural proteins in regulating centriole dynamics. We discovered that centriole behavior is altered in mutants lacking the meiosis-specific HORMA proteins HTP-1/2, previously implicated in prophase I and regulation of sister chromatid cohesion. Based on this observation, we analyzed centriole dynamics and spermatocyte spindle organization in the horma mutants (*htp-1, htp-1htp-2, htp-3, him-3*), a meiosis-specific cohesin mutant (*rec-8*), and in mutants defective in double strand break formation (*spo-11*) and synapsis (*syp-1 and syp-2*). This analysis reveals that the HORMA proteins have roles in preventing premature separation of centrioles during the spermatocyte meiotic divisions. Centriole dysfunction in *horma* mutants is not a secondary consequence of achiasmate chromosomes, as centriolar defects are absent or significantly less frequent in *spo-11, syp-1* or *syp-2* mutants. Further, similar centriolar defects were seen in the *rec-8* and *horma* mutants, suggesting that regulation of sister chromatid and centriole separation by HTP-1/2 could be mechanistically linked. Second, we find evidence that structural features of chromosomes established during meiotic prophase can have profound impact on spindle organization. Multipolar spindles were seen in a subset of spermatocytes in meiotic mutants where sister chromatids co-segregate together at meiosis I, but do not occur in mutants that undergo equational segregation at meiosis I. Conversely, mutant spermatocytes that enter meiosis II with separated sister chromatids have high frequencies of anucleate sperm, reflecting defective meiosis II division. Third, we find that premature centriole separation during spermatocyte meiosis can lead to multipolar mitotic spindles in zygotes, but it is not sufficient to trigger multipolar spindles during the spermatocyte meiotic divisions. However, separation of centrioles may exacerbate spermatocyte spindle assembly defects caused by chromosome structural features. Our data imply that an elaborate interplay between chromosomes, centrioles and microtubules that ensures that sperm will deliver the appropriate endowment of chromosomes and centrioles needed for correct zygote development.

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$CUL2^{LRR1}$ ubiquitin ligase mediates the degradation of CDK inhibitors to regulate the cell cycle in *C. elegans* germ cells and cell motility in human cells. **Natalia G. Starostina**, Edward T. Kipreos. University of Georgia, Athens, GA.

Cullin-RING ligase complexes (CRLs) constitute the most abundant class of ubiquitin ligases (E3s) and regulate a host of essential cellular processes including the cell cycle. CDK inhibitors (CKIs) are critical negative regulators of the cell cycle, with a primary role to bind and inhibit CDK/cyclin complexes in the nucleus. Our earlier work showed that CUL-2 promotes the G1-S phase transition in *C. elegans* germ cells by negatively regulating the level of CKI-1 [Nat Cell Biol 1999,1:486]. However, we did not know whether CKI-1 is directly targeted by a CUL-2 complex (CRL2) for degradation, and if so, what substrate recognition subunit (SRS) functions in the CRL2. In our current study we identified the leucine-rich repeat protein LRR-1 as a CUL-2 interactor and showed that it functions as the SRS for a CRL2 complex to degrade CKI-1 in germ cells to allow G1 phase progression. *lrr-1* deficient animals have fewer germ cells that are significantly enlarged, with 2C DNA content, similar to *cul-2* mutants. LRR-1 is co-localized with CKI-1 in germ cell nuclei. Similar to the downregulation of CUL-2, depletion of LRR-1 causes an accumulation of CKI-1 in germ cell nuclei; and the co-depletion of CKI-1 suppresses the G1 arrest observed in *lrr-1* deficient animals. In vitro assays demonstrate that LRR-1 directly interacts with CKI-1 and induces CKI-1 degradation. Notably, we found that $CRL2^{LRR-1}$ has a conserved function in human cells in targeting the CKI-1 homolog p21. Surprisingly, human $CRL2^{LRR1}$ does not regulate the cell cycle by degrading nuclear p21. Instead, human LRR1 is localized to the cytoplasm, and controls the levels of the cytoplasmic fraction of p21. Unlike nuclear-localized p21, which inhibits CDK-cyclin complexes to control the cell cycle, cytoplasmic p21 inhibits Rho kinase to control the actin cytoskeleton. siRNA knockdown of human LRR1 results in inactivation of the Rho pathway and the subsequent activation of the actin-depolymerizing protein cofilin that induces actin cytoskeletal rearrangements and cell motility. Therefore, human $CRL2^{LRR1}$ normally acts to degrade cytoplasmic p21 to maintain cells in a stationary, non-motile state. Importantly, the regulation of cell movement by LRR-1 does not appear to be conserved in *C. elegans*, as an analysis of the migration of distal tip cells, sex myoblasts, and touch receptor neurons in lrr-1 mutants did not reveal significant migration defects. This data provides an interesting case study in which an alteration in the subcellular localization of the LRR-1 protein during evolution changes the cellular function (the regulation of the cell cycle vs. control of cell movement), even though the substrate remains the same (Cip/Kip CKIs).

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Synaptonemal Complex mediates crossover interference during meiosis. **Diana E. Libuda**, Rayka Yokoo, Michiko Hayashi, Anne M. Villeneuve. Dept of Developmental Biology, Stanford University, Stanford, CA.

Crossover (CO) recombination events during meiosis are critical for the formation of the chiasmata that link homologous chromosomes together and ensure their proper segregation. Despite an excess of double strand DNA breaks (DSBs) that serve as initiating events for CO formation, most organisms make few COs, and formation of a CO tends to inhibit formation of other COs nearby on the same chromosome, a process known as CO interference. *C. elegans* exhibits particularly robust CO interference, with only a single CO forming between each pair of homologous chromosomes, making it an ideal model for investigating how COs form and how CO interference is mediated. Here we show that the synaptonemal complex (SC), a proteinaceous structure that forms between homologous chromosomes during meiosis, regulates CO formation both by directing localization of CO-promoting proteins and by mediating CO interference along the lengths of meiotic chromosomes. Our analysis used a GFP-tagged version of COSA-1, a conserved CO-promoting factor that we identified, as a marker of CO sites. Upon onset of the late pachytene stage of meiotic prophase, GFP::COSA-1 localizes to six bright foci per nucleus, corresponding to the single CO site on each homolog pair. These foci represent the sites of eventual concentration of other conserved CO-promoting factors (MSH-5 and ZHP-3) that initially exhibit broader distribution along chromosomes. Analysis of conditions where DSBs are either limiting or in excess demonstrated that COSA-1 foci represent a robust cytological readout of the CO interference process. We exploited this property to investigate involvement of the SC central region proteins (SYPs), in formation and regulation of COs. Our data demonstrate that SYPs both: 1) dictate where CO-promoting proteins will concentrate; and 2) function in inhibiting excess CO formation. In mutants where the SC forms on only a subset of chromosomes, CO-promoting proteins localize only to those chromosomes where SYPs are present. Further, CO-promoting proteins co-localize and become sequestered with SYPs in nuclear aggregates that form when SYPs are prevented from loading onto chromosomes. Most notably, despite the fact that the SYP proteins are required to form COs, we find that partial depletion of the SYP-1 protein actually results in an increase in COSA-1 foci, implying attenuation of CO interference. These and other data implicate the SC as a key player in the robust CO interference mechanism that normally limits the number of CO events to one per homolog pair during *C. elegans* meiosis.

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Activation of MOM-4 by SRC-1 and Wnt: A mechanistic link between spindle rotation and signaling pathways lowering nuclear POP-1 levels. **Sung Hee Ahn**, Scott Robertson, Rueyling Lin. Dept. of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX.

During *C. elegans* embryonic development, asymmetric divisions oriented along the anterior-posterior (A-P) axis play a crucial role in generating cell diversity. All posterior daughters from A-P divisions exhibit a reduced level of nuclear TCF/POP-1, compared to their anterior sister cells. Both POP-1 and its nuclear asymmetry are important for A-P asymmetric cell fates. Because cell divisions along the A-P axis rely on precise orientation of the spindle, there must be a mechanistic link between the pathways regulating spindle orientation with those regulating asymmetric cell fate specification. Two kinases in the MAPK pathway that regulates POP-1 levels have been identified: the MAP kinase (MAPK) LIT-1 and the MAP kinase kinase kinase (MAPKKK) MOM-4. Upstream regulators for the MAPK pathway are not known. The Wnt pathway crosstalks with the MAP kinase pathway, as mutations in the Wnt pathway result in variable defects in nuclear POP-1 levels. We have identified two molecular mechanisms by which this MAPK pathway is activated in embryos. First, we show that the SRC-1 tyrosine kinase phosphorylates MOM-4 on two specific tyrosine residues, and these tyrosine phosphorylations are both necessary and sufficient to activate MOM-4 kinase activity *in vivo*. Changing either tyrosine to aspartate or glutamate partially bypasses the requirement for SRC-1 *in vivo*. Second, we show that MOM-4 activity *in vivo* is negatively regulated by GSK-3-dependent phosphorylation of specific serine residues. Phosphomimicking residues at these serines inactivate MOM-4 activity whereas non-phosphorylatable changes activate. The Wnt signaling pathway activates MOM-4 activity by antagonizing this GSK-3 activity. We find that MOM-4 carrying non-phosphorylatable mutations at the GSK-3 sites can partially rescue the mutant phenotype that results from depletion of the Wnt ligand, MOM-2. These two mechanisms appear to be independent and function together to fully activate MOM-4. Embryos depleted of *mom-2* and *src-1* have a fully penetrant defect in MAPK-dependent POP-1 nuclear level throughout embryogenesis. In embryos, SRC-1 activity has been shown to be enhanced by a membrane protein, and together they regulate spindle orientation. We are currently investigating mechanisms by which asymmetric SRC-1 kinase activity is achieved. Our results demonstrate a mechanistic link between the pathways regulating spindle orientation with those regulating asymmetric cell fate specification, and provide a clue as to the mechanism by which a cell may track its position and polarity throughout embryogenesis.

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The mRNA splicing regulator SPK-1 is required for cell polarity in one-cell *C. elegans* embryos. **Martin Mikl**, Carrie R. Cowan. Research Institute of Molecular Pathology, Vienna, Austria.

Cell polarity is a prerequisite for asymmetric division, which gives rise to daughter cells with different developmental fates. In one-cell *C. elegans* embryos, cell polarity comprises distinct cortical domains of actomyosin contractility and PAR proteins. Polarity establishment is initiated by a centrosome-dependent cue. In an RNAi screen for polarity establishment defects we identified one gene, spk-1, that appeared to have normal centrosome assembly and only mildly reduced cortical activity but nonetheless failed to establish correct PAR protein localization. Consistent with defects in PAR polarity, *spk-1(RNAi)* embryos divided symmetrically to give rise to equivalent daughter cells.

SPK-1 is a kinase targeting the SR protein family of mRNA splicing factors and thereby potentially influences splice site selection. By analyzing splice form abundance of candidate polarity mediators in SPK-1 depleted worms, we identified a splicing change in the par-5 3'UTR. *par-5(RNAi)* embryos showed similar polarity establishment defects to *spk-1(RNAi)* embryos, and both *par-5(RNAi)* and *spk-1(RNAi)* embryos could be rescued by overexpressing PAR-2. Thus SPK-1 may facilitate polarity establishment by regulating PAR-5, which in turn controls PAR-2 availability for recruitment to the cortex.

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AP-1 controls an apical trafficking pathway required for PAR-6 localisation and epithelial polarity. Massi Shafaq-zadah, Lysiane Brocard, **Gregoire Michaux**. IGDR, Rennes, France.

The establishment and the maintenance of epithelial polarity are essential for animal growth and development and the PAR proteins play an essential role in that process. However little is known about the mechanisms required for their localisation in epithelial cells. We have uncovered an apical trafficking pathway dependent on clathrin/CHC-1, dynamin/DYN-1, RAB-5, RAB-11 and the clathrin adaptor complex AP-1 which are all required for the maintenance of PAR-6 apical localisation in intestinal epithelial cells. Because AP-1 was found to be implicated in basolateral targeting in mammalian epithelial cells we decided to focus on this new and essential function of AP-1 at the apical cortex during embryonic morphogenesis. We found that AP-1 depletion does not affect epithelial polarity establishment and the initial formation of the intestinal lumen. However it induces a complete loss of apico-basal polarity of PAR-6 localisation later during embryonic elongation; while other polarity determinants are unaffected PAR-6 becomes homogenously distributed at the apical and basolateral cortex. This loss of PAR-6 asymmetric localisation triggers de novo formation of ectopic intestinal lumens along the lateral membranes of adjacent cells as shown by light and electron microscopy. In addition we found that AP-1 sorts apical transmembrane cargos, is required for the maintenance of RAB-11 apical recycling endosomes and acts upstream of RAB-11. We also identified a function for RAB-11 itself in the maintenance of a single intestinal lumen. Finally we found that this AP-1 function is dependent on the mu1-II subunit APM-1 while the mu1-I subunit UNC-101 is not implicated. Altogether our results demonstrate an essential function for an AP-1 dependent apical trafficking pathway which is required for the maintenance of PAR-6 localisation and epithelial polarity.

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Breaking symmetry: polarization of the C. elegans embryo. **Geraldine Seydoux**. Johns Hopkins School of Medicine, Baltimore, MD.

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A kinase-independent role of Aurora A in the assembly of mitotic spindle microtubules. Mika Toya[1], Masahiro Terasawa[1], **Asako Sugimoto[1,2]**. 1) Laboratory for Developmental Genomics, RIKEN Center for Developmental Biology, Kobe, Japan; 2) Graduate School of Life Sciences, Tohoku University, Sendai, Japan.

Aurora A kinase is one of the key regulators of mitotic events including mitotic entry, centrosome maturation and spindle bipolarity. We previously found that *C. elegans* Aurora A (AIR-1) is responsible for the assembly of γ-tubulin-independent microtubules in early embryos (1); however, the mechanism by which AIR-1 contributes to microtubule assembly during mitosis has been unclear.

In other organisms, condensed chromatin induces spindle microtubule assembly by creating a local environment that is favorable for microtubules nucleation and stabilization. We found by live imaging and RNAi in *C. elegans* early embryos that AIR-1 has a crucial role in the assembly of chromatin-stimulated microtubules that is independent of the γ-tubulin complex. Surprisingly, the kinase activity of AIR-1 is dispensable for this process. Whereas the kinase-inactive form of AIR-1 was detected along the microtubules as well as on centrosomes, the kinase-active form of AIR-1—recognized with anti-phospho-AIR-1—was restricted to centrosomes. Furthermore, embryos expressing kinase-inactive forms of AIR-1 (in the absence of endogenous AIR-1) were able to assemble chromatin-stimulated microtubules, although they were defective in centrosome maturation.

TPXL-1 is a potent activator of the AIR-1 kinase activity *in vitro* (2), but we found that AIR-1 at centrosomes in *tpxl-1(RNAi)* embryos was kinase-active, suggesting that TPXL-1 is not the major AIR-1 kinase activator *in vivo*. Although AIR-1 does not localize along microtubules in *tpxl-1(RNAi)* embryos (2), short chromatin-stimulated microtubules were still formed by the contribution of AIR-1. Thus, we speculate that AIR-1 can mediate the formation of chromatin-stimulated microtubules without localizing along them, and that its localization along microtubules via TPXL-1 enhances its microtubule stabilizing effect.

Taken together, we propose that AIR-1 has a kinase-dependent role at centrosomes and a kinase-independent role for stabilizing spindle microtubules, and that coordination of these two roles is crucial for the assembly of mitotic spindles.

1. Motegi et al. Dev Cell 10, 509-20 (2006)
2. Ozlü et al. Dev Cell 9, 237-48 (2005).

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Cracking the Eggshell: Protective Barrier Assembly Following Fertilization of the *C. elegans* Embryo. **Sara K. Olson[1]**, Garrett Greenan[2], Ana Carvalho[1], Thomas Müller-Reichert[2], Karen Oegema[1]. 1) Ludwig Institute, UC San Diego, La Jolla, CA; 2) MPI-CBG, Dresden, Germany.

Metazoan oocytes have an extracellular coating that governs fertilization. Following fertilization, this covering is altered to prevent polyspermy and protect the developing embryo. In *C. elegans*, a vitelline layer covers oocytes prior to fertilization. Fertilization initiates conversion of the vitelline layer into a trilaminar eggshell consisting of an outer vitelline layer, a middle chitin-containing layer, and an inner layer proposed to serve as a permeability barrier. Here, we characterize CPG-1 and CPG-2, functionally redundant chondroitin proteoglycans that are the first described protein eggshell components. We show that CPG-1 and CPG-2 are delivered to the extracellular space after formation of the chitin layer by cortical granule exocytosis during meiosis I. Although they contain multiple chitin binding domains, CPG-1 and CPG-2 localize to the inner eggshell layer, whereas chitin is confined to the middle layer. We show that the inner eggshell layer is not the permeability barrier for small molecular weight solutes. Instead, this function resides in a previously undescribed layer that assembles between the eggshell and the plasma membrane following meiosis II. Disruption of the permeability barrier leads to solute permeability and osmotic stress. Disruption of the inner CPG-1/2 eggshell layer causes these phenotypes, as well as adhesion of the embryonic plasma membrane to the eggshell and cytokinesis failure. Interfering with chitin layer assembly results in the inner layer phenotypes, plus polyspermy and catastrophic eggshell rupture. We conclude that the eggshell layers and permeability barrier are laid down in a step-wise and cell cycle-dependent fashion, with later assembly events requiring successful completion of previous ones. To build on this work, we also conducted an RNAi screen to identify additional genes that regulate eggshell and permeability barrier assembly. Several screen hits rendered the eggshell permeable, with minimal deleterious effects on early embryonic development. We therefore developed a reliable method to permeabilize and immobilize embryos to allow temporally-controlled and acute drug/inhibitor treatment to study early embryonic processes with live imaging.

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Lysosome-Related Organelles in Intestinal Cells Function as a Zinc Storage Site in C. elegans. **Hyun C. Roh[1]**, Sara Collier[1], James Guthrie[2], J. David Robertson[2], Kerry Kornfeld[1]. 1) Dev Biol, Washington Univ in St Louis, St. Louis, MO; 2) Chemistry Department, University of Missouri, Columbia, MO.

Zinc is a trace element essential for a variety of biological processes and involved in human diseases. Animals require homeostatic mechanisms to store and mobilize zinc in response to dietary fluctuations, but these mechanisms are not well defined. Here we demonstrate that lysosome-related organelles called gut granules present in intestinal cells of C. elegans function as the major site of zinc storage. Labile zinc was detected in gut granules by a zinc-specific fluorescent dye, and gut-granule-loss (Glo) mutant animals that lack gut granules were defective in zinc storage. The cation diffusion facilitator protein CDF-2 plays a critical role in this process by transporting zinc into gut granules. Zinc storage in gut granules promotes detoxification and subsequent mobilization, since cdf-2 mutant and Glo animals were defective in both processes. In response to high dietary zinc, gut granules displayed structural changes characterized by a bilobed morphology with asymmetric distributions of molecular markers. Glo genes were required for this structural change of gut granules. These findings elucidate novel mechanisms of zinc storage, detoxification and mobilization in C. elegans and may be relevant to other animals.

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Cell Shape and Wnt Signaling redundantly Control the Division Axis of C. elegans Epithelial Stem Cells. **Marjolein Wildwater[1]**, Matilde Galli[1], Nicholas Sander[2], Geert de Vreede[1], Sander van den Heuvel[1]. 1) Developmental Biology, Utrecht University, Utrecht, Utrecht, Netherlands; 2) Department of Genetics, University of Minnesota, 321 Church St. SE, Minneapolis, MN 55455, USA.

Tissue-specific stem cells combine proliferative and asymmetric divisions to balance self-renewal with differentiation. Tight regulation of the orientation and plane of cell division is critical in this process. Here, we study the reproducible pattern of anterior-posterior oriented stem cell-like divisions in the *Caenorhabditis elegans* seam epithelium. In a genetic screen, we identified an *alg-1* Argonaute mutant with additional and abnormally oriented seam cell divisions. ALG-1 is the main subunit of the miRNA-Induced Silencing Complex (miRISC) and was previously shown to regulate the timing of postembryonic development. Time-lapse fluorescence microscopy of developing larvae revealed that reduced *alg-1* function successively interferes with cell adherence, orientation and timing of seam cell division, cell shape and Wnt signaling. We found that Wnt inactivation, through *mig-14* Wntless mutation, disrupts tissue polarity but not anterior-posterior division. However, combined Wnt inhibition and cell shape alteration resulted in disordered orientation of seam cell division, similar to the *alg-1* mutant. Thus, we identified additional *alg-1* regulated processes and uncovered a previously unknown function of Wnt ligand signaling in seam tissue polarity. Furthermore, we showed that Wnt signaling and geometric cues redundantly control the seam cell-division axis. In addition to the division axis, we observed that seam cells displace their mitotic spindle off center during asymmetric division, giving rise to a smaller anterior daughter cell (with a hyp7 fate) and a larger posterior daughter cell (with a seam cell fate). Interestingly, this spindle displacement is not observed during symmetric seam cell division. Initial observations revealed that the conserved coiled-coil protein LIN-5 (NuMA in mammals) regulates the orientation of the spindle within the seam cells. In addition, the non-muscle myosin motor NMY-2 localized posteriorly just prior to anterior movement of the spindle, suggesting that myosin II motors could be involved in the inhibition of spindle displacing forces. Currently we are investigating the mechanisms that control spindle displacement. Taken together, our findings establish a novel system to study the division plane in the context of an epithelial tissue.

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repo-1(or430ts): a C. elegans mutant with a reversed anterior-posterior body axis. **Reza Keikhaee**, Bruce Nash, John Yochem, Bruce Bowerman. Institute of Molecular Biology, University of Oregon, Eugene, Oregon, USA.

Over the course of one hour, following fertilization of an oocyte, the apolar one-cell C. elegans zygote is transformed into a highly polarized cell that divides asymmetrically to produce anterior and posterior daughters with different sizes, contents, cell cycle times and developmental potentials. While many of the molecules required for anterior-posterior (AP) cell polarity are conserved across animals, the mechanisms that establish it remain unclear. We have identified a dominant, temperature-sensitive, embryonic-lethal allele (or430ts) of the C. elegans ortholog of SF3a66 called or430ts. SF3a66 is a subunit of human SF3a (splicing factor 3a) that is involved in the processing of pre-mRNA, but recent studies have shown that SF3a66 may also act as a microtubule binding and bundling protein independent of its RNA splicing role, and the C. elegans ortholog is a synthetic multivulva (synMuv) protein. We found that at the restrictive temperature (26°C), embryos produced by or430ts/or430ts worms (hereafter called mutant zygotes or embryos) exhibit a remarkable reversal of AP cell polarity at the one-cell stage. Differential interference contrast microscopy (DIC) showed a decrease in cortical ruffling and pseudocleavage, and the sperm and egg pronuclei meet centrally in this mutant, instead of meeting toward the posterior pole as in wild type, suggesting a loss of AP polarity in repo-1(or430ts) mutant zygotes. Spinning disk confocal microscopy with GFP transgenic strains showed that the posterior polarity proteins PAR-2 and PIE-1 are localized to the anterior pole in one-cell stage mutant zygotes, confirming that polarity is reversed; hence the gene name repo-1, for reversed polarity. We also have observed microtubule defects in repo-1(or430ts) one-cell zygotes, including defective rotation of the centrosome/pronuclear complex, a lack of P0 mitotic spindle elongation, and chromosome segregation defects following the first embryonic mitosis. Since depletion of microtubules has been shown to delay AP polarity induction, we are interested in the possibility that the dominant or430ts mutation results in reversed polarity by affecting microtubule dynamics. Because arrested meiotic spindles also have been shown to cause a partial reversal of AP polarity, we currently are investigating meiotic spindle dynamics in repo-1(or430ts) mutant to determine if abnormalities in meiotic spindle assembly or dis-assembly might be responsible for the reversed polarity phenotype.

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The phospholipid flippase TAT-5 prevents the budding of extracellular vesicles during *C. elegans* embryogenesis. **Ann M. Wehman[1]**, Barth D. Grant[2], Jeremy Nance[1]. 1) Skirball Inst, New York Univ, New York, NY; 2) Dept of Mol Biol and Biochem, Rutgers Univ, Piscataway, NJ.

During cytokinesis and morphogenesis, embryos undergo dramatic changes in cell shape. While much is known about the role of the cytoskeleton in regulating these processes, we understand less about the role of the lipid bilayer in modulating cell shape. For example, the asymmetric partitioning of phosphatidylserine (PS) and phosphatidylethanolamine (PE) to one leaflet of the bilayer can affect membrane curvature and influence dynamic membrane events such as cytokinesis and endocytosis. We identified TAT-5, a P4 ATPase predicted to flip phospholipids to the cytoplasmic leaflet of the bilayer, in an RNAi screen for essential regulators of cell contact-induced polarity in *C. elegans*. Loss of TAT-5 also resulted in other plasma membrane defects including cell shape, cell-cell adhesion, cytokinesis, and morphogenesis.

GFP-tagged TAT-5 localized to the plasma membrane and TAT-5 prevented the externalization of PE, but not PS, on the surface of cells. In contrast, TAT-1 prevented the externalization of PS, but not PE. Since TAT-5 appears to act at the cell surface, we used electron tomography to examine the three-dimensional structure of the plasma membrane at high resolution. Strikingly, loss of TAT-5 caused the large-scale shedding of budding vesicles from the plasma membrane, which disrupted the structure of cell contacts and likely explains the defects in cell polarity and morphogenesis that we observed in *tat-5* embryos. The robust production of extracellular vesicles in *tat-5* embryos depended on the function of RAB-11, the recycling endosome-associated GTPase, as well as ESCRT complex proteins (HGRS-1, STAM-1, TSG-101, and VPS-28), which normally function in the formation of multivesicular bodies. Mammalian homologs of these proteins have been shown to regulate viral budding, suggesting mechanistic similarities between these topologically similar membrane budding events. Our findings define for the first time the essential role of a P4 ATPase in the regulation of PE asymmetry in a multicellular organism. Our results also suggest a novel mechanism whereby PE externalization influences dynamic remodeling of the plasma membrane during embryonic development.

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Replication-Coupled Nucleosome Assembly Is Required to Generate a Bilateral Asymmetry in the *C. elegans* Nervous System. **Shunji Nakano**[1], Bruce Stillman[2], Bob Horvitz[1]. 1) HHMI, MIT, Cambridge, MA; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Bilateral asymmetry in *C. elegans* can arise from left-right asymmetric cell lineages. The single left-right unpaired MI neuron descends from the right side of an otherwise left-right symmetric cell lineage that on the left generates the e3D epithelial cell.

We sought mutations that transform MI into an e3D-like cell or e3D into an MI-like cell, thereby generating symmetry in these cell lineages. We recovered 16 mutations in 7 genes that transform MI into an e3D-like cell. We previously showed that the establishment of the MI-e3D asymmetry requires asymmetric expression of a transcriptional cascade in which the *Otx* homeodomain protein CEH-36 is expressed in the MI grandmother cell but not in the e3D grandmother cell and that CEH-36 promotes asymmetric expression of two bHLH proteins, NGN-1 and HLH-2, in the MI mother cell but not in the e3D mother cell (Nakano et al., Development 137, 4017, 2010). Here we show that another isolate, *n5357*, is a gain-of-function allele of the gene *his-9*, which encodes a replication-dependent histone H3 protein. This *his-9(gf)* mutation acts cell-autonomously in the MI mother cell and affects a process downstream of or in parallel to the expression of CEH-36, NGN-1 and HLH-2.

Replication-dependent histone H3-H4 proteins are deposited onto nucleosomes by the CAF-1 complex during DNA replication. The human CAF-1 complex is composed of three subunits, p150, p60 and p48. Inactivation of *T06D10.2*, *Y71G12B.1* and *rba-1*, which encode proteins homologous to p150, p60 and p48, respectively, transformed MI into an e3D-like cell. That a gain-of-function mutation in a histone H3 gene phenocopied the loss of CAF-1 function suggests that the mutant H3 protein inhibits CAF-1-mediated nucleosome formation. We performed a nucleosome assembly reaction to monitor CAF-1 activity *in vitro* and observed that corresponding mutant histone H3 proteins indeed inhibited CAF-1-mediated nucleosome formation. Our results reveal that replication-coupled nucleosome assembly is required to generate the MI-e3D asymmetry. We suggest that during S phase of the MI mother cell CAF-1 assembles nucleosomes on which the NGN-1/HLH-2 complex recruits histone-modifying enzymes to generate a chromatin state necessary to specify MI and that CAF-1-mediated nucleosome formation and the asymmetric localization of the NGN-1/HLH-2 complex drive left-right asymmetric epigenetic regulation, leading to the establishment of the MI-e3D asymmetry.

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Ras effector switching from Raf to RalGEF promotes divergent fates in vulval patterning. Tanya Zand, Kimberly Monahan, Channing Der, **David Reiner**. Department of Pharmacology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC.

EGF secreted from the anchor cell induces multipotent vulval precursor cells (VPCs) to form an invariant 3°-3°-2°-1°-2°-3° pattern. EGF activation of the canonical Ras-Raf-MEK-ERK MAP Kinase cascade induces the most proximal VPC to become a presumptive 1° cell, which in turn secretes DSL ligands to induce Notch-dependent 2° fate in neighboring VPCs. EGF also contributes to 2° fate via an unknown mechanism. To ensure that 1° and 2° fates are mutually antagonistic, presumptive 2° cells express LIP-1/MAP kinase phosphatase to attenuate the Ras-Raf pro-1° signal, while presumptive 1° cells degrade pro-2° LIN-12/Notch. Using genetic epistasis analysis we showed that LET-60/Ras functions in parallel with and antagonistic to its Ras-Raf pro-1° function. In addition to Raf, Ras signals through RalGEF, a non-canonical Ras effector recently shown to be critical in several Ras-dependent cancers. The principal function of RalGEF is to activate the small GTPase Ral. As we found with LET-60/Ras, the *C. elegans* RalGEF and Ral orthologs RGL-1 and RAL-1 antagonize Ras-Raf pro-1° signaling, suggesting that the Ras-RalGEF-Ral signaling module is evolutionarily conserved and counteracts Ras-Raf pro-1° activity. Mutationally activated RAL-1 induced 2° fate in a weakly constitutive LIN-12/Notch background. Therefore, we used mutationally activated LET-60/Ras harboring a RalGEF-selective effector binding domain mutation (E37G) to similarly promote Notch-sensitized 2° fate, and this activity was RGL-1- and RAL-1-dependent. Likewise, endogenous mutant activated LET-60/Ras stimulated RGL-1- and RAL-1-dependent 2° fate when LIN-45/Raf was abrogated and LIN-12/Notch was sensitized. Therefore, Ras-RalGEF-Ral signaling is pro-2°, explaining its antagonism of Ras-Raf pro-1° signaling. During induction GFP expression driven by the *ral-1* promoter was restricted to presumptive 2° cells, suggesting that transcriptional exclusion of RAL-1 from the presumptive 1° cell provides a mechanism for LET-60/Ras effector switching from Raf to RalGEF-Ral. Thus, we have shown that Ras switches effectors as part of a developmental patterning program. Previous studies showed that strong EGF signaling induced 1° fate, while weaker EGF signaling induced 2° fate. We titrated the EGF (LIN-3) or EGF receptor (LET-23) signal to levels sufficient to promote 2° but not 1° fate, and found that pro-2° EGF signal was LET-60-, RGL-1- and RAL-1-dependent, implying that the pro-2° EGF signal is propagated through Ras activation of RalGEF-Ral signaling. We have therefore identified the long-missing mechanism by which EGF promotes 2° fate.

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Distinct and mutually inhibitory binding by two β-catenins, SYS-1 and WRM-1, coordinates TCF/POP-1 nuclear levels and activity. **X. Yang**[1], S. Huang[1], M. Lo[1], W. Xu[2], S. Robertson[1], R. Lin[1]. 1) Dept. of Molecular Biology, University of Texas Southwestern Medical Center, Dallas, TX; 2) Dept. of Biological Structure, University of Washington, Seattle, WA.

We have shown that specification of endoderm requires a high β-catenin/SYS-1 to TCF/POP-1 ratio in the nucleus of E blastomere. Similar to vertebrate β-catenin binding to the N-terminal ~50 amino acids of TCF, SYS-1 binds to the N-terminal domain of POP-1 and functions as a coactivator in the activation of endoderm genes. Elevation of SYS-1 levels in E is dependent entirely on the Wnt signaling pathway, whereas lowering of nuclear POP-1 levels is regulated primarily by the MAP kinase pathway. The MAP kinase LIT-1, the *C. elegans* NLK homolog, phosphorylates POP-1, its only known substrate, promoting its nuclear export, the main mechanism for lowering nuclear POP-1 levels. We and others have shown previously that phosphorylation of POP-1 by LIT-1 requires a diverged β-catenin, WRM-1, although the precise molecular function of WRM-1 remains unclear. WRM-1 does not bind to the N-terminal domain of POP-1, function as a POP-1 coactivator, or function in cell adhesion. We demonstrate here two independent and distinct molecular functions for WRM-1: it serves as both the substrate-binding subunit and a regulatory subunit for the LIT-1 kinase. In addition, we present here a molecular mechanism by which a high nuclear level of SYS-1 can be maintained in E while its binding partner POP-1 is simultaneously exported out of nucleus. We show that WRM-1 binds to the C-terminus of POP-1, and that this binding is essential for all LIT-1-mediated POP-1 phosphorylation. The POP-1 C-terminus resembles the N-terminal SYS-1-binding domain in primary sequence. Computer-based structural analyses provide an explanation for the observed specificities in POP-1/WRM-1 and POP-1/SYS-1 binding. It has been shown that the POP-1/SYS-1 interaction primarily utilizes only one (extended strand) of the two TCF structural modules employed in the vertebrate TCF/β-catenin interaction. Structural modeling predicts that the POP-1/WRM-1 interaction primarily utilizes the other structural module (alpha helix). Finally, we show that the SYS-1/POP-1 (N-terminus) and WRM-1/POP-1 (C-terminus) interactions are mutually inhibitory, providing a molecular mechanism by which levels of the two interacting proteins, SYS-1 and POP-1, can be regulated in opposite directions in the E nucleus. Mutual inhibitory binding would result in two populations of POP-1: one bound by WRM-1 that is LIT-1 phosphorylated and exported from the E nucleus, and the other bound by SYS-1 that remains in the nucleus and transcriptionally activates Wnt target genes.

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Evolution of X-Chromosome Dosage Compensation and Sex Determination Revealed by Targeted Gene Disruption Across Species. **Te-Wen Lo[1]**, Andrew Wood[1,2], Bryan Zeitler[3], Catherine Pickle[1], Andrew Lee[3], Caitlin Schartner[1], Jeffrey Miller[3], Lei Zhang[3], Edward Rebar[3], Philip Gregory[3], Fyodor Urnov[3], Barbara Meyer[1]. 1) Dept MCB-Genetics, UC Berkeley/HHMI, Berkeley, CA; 2) King's College London, UK; 3) Sangamo BioSciences, Inc., Richmond, CA.

Genome sequencing has facilitated research beyond traditional model organisms, but the paucity of broadly effective reverse genetic tools has limited cross-species comparisons of gene function needed to explore biological mechanisms. To overcome this limitation for nematodes, we developed a strategy for heritable, targeted gene disruption using engineered nucleases: fusions between custom-designed DNA binding domains of either the C_2H_2 zinc-finger motifs (ZFNs) or transcription activator-like effector (TALE) repeat motifs and the endonuclease FokI. ZFNs and TALE nucleases (TALENs) induce a double-strand break at a desired locus that can be imperfectly repaired to yield small insertions and deletions. Procedures were optimized for *C. elegans (Ce)* using ZFNs to recover mutant lines without reliance phenotype. TALENs proved equally effective, yielding the first TALEN-induced animal gene knockouts.

We applied this technology to *C. briggsae (Cbr)* to study the evolution of sex determination (SD) and X-chromosome dosage compensation (DC). The DC machinery and key components of the genetic hierarchy that regulate SD and DC proved to be functionally conserved over 15-30 Myr. In contrast, recruitment elements that target the DC machinery to X chromosomes have diverged. The *Ce* X motifs that are enriched on X relative to autosomes and pivotal for recruiting the *Ce* DCC to X are not enriched on the *Cbr* X. Moreover, all DCC recruitment elements imported from *Ce* into *Cbr*, fail to bind the *Cbr* DCC. ChIP-seq confirmed that DNA target specificity has diverged, and on going experiments will identify sequence specificity for *Cbr* DCC binding.

Like many developmental regulatory proteins (e.g. Twist, Dorsal), the DCC controls hundreds of genes through its action on cis-acting target sites. However, the evolution of DCC recruitment sites followed a very different pattern from that of binding sites for regulatory proteins that control multiple, unrelated developmental and cellular processes. Pleitropy of Twist and Dorsal, caused the proteins to accumulate few functionally significant changes to their DNA binding domains or their cognate DNA binding motifs. In contrast, DCC complex with multiple targets but lacking the constraints of pleiotropy exhibited a divergence of binding sites. Such divergence could have been an important driver for nematode speciation.

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X:A Signal Assessment to Determine Sex. **Behnom Farboud**, Margaret M. Jow, Paola Nix, John M. Gladden, Barbara J. Meyer. HHMI/MCB, UC Berkeley, Berkeley, CA.

C. elegans sexual fate is determined by a chromosome counting mechanism that distinguishes one X chromosome from two. It assesses the number of X chromosomes relative to the sets of autosomes. Genes encoded on the X chromosome, called X signal elements (XSEs), communicate X-chromosome dose by functioning in a cumulative, dose-dependent manner to repress the master switch gene *xol-1*. *xol-1* repression in XX embryos results in hermaphrodite development and activation of dosage compensation. Autosomal genes, called autosomal signal elements (ASEs), communicate autosome dose and counteract XSEs to activate *xol-1* in XO embryos, triggering male development. How XSEs and ASEs oppose each other was a mystery.

We had previously characterized three XSEs. *fox-1* encodes an RNA binding protein that binds *xol-1* transcripts, resulting in production of inactive *xol-1* splice variants. The XSEs, *sex-1* (a nuclear hormone receptor) and *ceh-39* (a homeobox protein) encode transcription factors that associate with the *xol-1* promoter *in vivo* and repress *xol-1* transcription. To understand how these XSEs and ASEs regulate *xol-1*, binding sites were mapped. Five *xol-1* binding sites for each SEX-1 and CEH-39 were identified *in vitro*. For SEX-1, the sites resemble nuclear hormone receptor binding sites, while for CEH-39, the sites resemble typical homeobox domain binding sites. When these sites were mutated in the context of a *xol-1* integrant placed in a *xol-1(-)* hermaphrodite, *xol-1* levels were upregulated, and hermaphrodites displayed phenotypes associated with mutations in XSEs.

We also found the ASEs SEA-1 and SEA-2 associate with the *xol-1* promoter and activate *xol-1* transcription. The T-box transcription factor SEA-1 binds the promoter at five sites. These sites, however, bear little semblance to T-box binding sites. Mutation of the sites in a *xol-1* transgene upregulates *xol-1* transcription and rescues lethality associated with XSE mutants. Also, SEA-2 colocalizes with multiple *xol-1* promoter array constructs *in vivo*, suggesting SEA-2 binding is more ubiquitous than that of other signal elements. While SEA-1 does not appear to compete with XSEs for binding to *xol-1*, since all mapped binding sites are unique and non-overlapping, the possibility still exists that SEA-2 may compete with XSEs for promoter occupancy. Our findings show that antagonistic molecular interactions carried out on a single promoter communicate the X:A signal. The concept of a sex signal composed of zygotic ASEs that oppose zygotic XSEs arose as a hypothesis for fruit flies. Ironically, while the worm sex signal fits this simple paradigm, the fly sex signal does not.

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Ascaroside mediated lifespan increase in *C. elegans* requires the chemosensory neuron ASK, the G-protein coupled receptor DAF-37 and the deacetylase SIR-2.1. Hanno Ludewig[1], **Yevgeniy Izrayelit[1]**, Donha Park[2], Rabia Malik[1], Anna Zimmerman[1], Axel Bethke[1], Bennet Fox[1], Don Riddle[2], Frank Schroeder[1]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 2) Department of Medical Genetics, The University of British Columbia, Vancouver, BC, Canada.

We report that a set of endogenous small molecules, the ascarosides, extend lifespan and increase thermotolerance in *Caenorhabditis elegans*. These investigations led to discovery of a signaling cascade connecting small molecule messengers to sirtuin dependent regulation of longevity.

The ascarosides were originally identified as major components of the *C. elegans* dauer pheromone, and more recently have been shown to function as social signals. We asked whether ascarosides affect adult lifespan in *C. elegans*. Using agar-based plate assays, we show that ascarosides extend lifespan and thermotolerance of wild-type worms without decreasing fecundity or feeding rate. These effects are reduced when nutrients are restricted. We found that ascaroside mediated increases in lifespan and stress resistance (AMILS) require the NAD-dependent deacetylase SIR-2.1. Overexpression of SIR-2.1 confers hypersensitivity to low concentrations of ascarosides, but does not further increase maximal lifespan extension. In contrast to many other lifespan modulating factors, AMILS does not require insulin signalling via the FOXO-homolog DAF-16 or the insulin/IGF receptor homolog DAF-2.

We used ascaroside#2 (ascr#2), one of the most abundantly produced ascarosides, to further probe the mechanism of AMILS. Using a genetically ASK-ablated strain, we showed that ascr#2-mediated AMILS requires the chemosensory neuron ASK. Additionally, DAF-37, a G-protein coupled receptor expressed in the ASK neuron, is required for AMILS. ASK-specific expression of DAF-37 is sufficient to fully restore AMILS in *daf-37(0)* worms. Furthermore, over expression of DAF-37 further increased ascr#2-mediated AMILS.

These findings demonstrate that in *C. elegans* endogenous small molecules regulate lifespan and stress resistance via SIR-2.1 dependent pathways downstream of chemosensory perception. Therefore, ascarosides represent useful small-molecule probes to interrogate conserved pathways connecting chemosensation, aging and sirtuins.

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The microRNA *mir-71* Acts in the Ectoderm to Promote Germline-Mediated Longevity by Regulating DAF-16 Activity. **Konstantinos Boulias**, Bob Horvitz. HHMI, Dept Biol, MIT, Cambridge, MA.

The reproductive systems of worms, flies and mammals control aging of the entire organism. When the germline of *C. elegans* is removed, either by laser microsurgery or by mutations that block germ-cell proliferation, animals live up to 60% longer than controls. This lifespan extension requires the activities of the FOXO family transcription factor DAF-16 and of the steroid hormone receptor DAF-12. In animals lacking germ cells, DAF-16 accumulates specifically in the intestinal nuclei and activates the transcription of stress-related and metabolic genes.

microRNAs, a class of small non-coding RNAs, have emerged as critical post-transcriptional regulators of gene expression in diverse biological processes. *C. elegans* microRNAs have been shown to play critical roles in the control of developmental timing, cell-fate specification, embryonic development, neural synaptic activity and longevity.

We performed a comprehensive search for microRNA genes that regulate *C. elegans* aging by determining the lifespans of deletion mutants for most of the 115 known microRNA genes. We found that the microRNA *mir-71* promotes longevity and stress resistance and mediates the effect of germ cell loss on lifespan. *mir-71* is required for the lifespan extension caused by germline removal, and overexpression of *mir-71* further extends the lifespan of animals lacking germ cells. Genetic epistasis analysis indicated that *mir-71* functions upstream of or in parallel to DAF-16 to promote germline-mediated longevity. Through a series of mosaic analysis and tissue-specific rescue experiments, we found that *mir-71* functions cell non-autonomously in the ectoderm of germline-less animals to promote the activity of DAF-16 in the intestine. Our findings reveal a novel microRNA-dependent mechanism of lifespan regulation by the germline and support a model in which signaling among the ectoderm, the intestine and the gonad coordinates the rate of aging of the whole organism.

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C. elegans SIRT6/7 homolog SIR-2.4 as a novel regulator of DAF-16 localization and function. **Wei-Chung Chiang[1]**, Daniel X. Tishkoff[2], Bo Yang[2], Angela R. Wang[2], Mark Eckersdorff[2], David B. Lombard[2,3], Ao-Lin Hsu[1,3,4]. 1) Department of Molecular & Integrative Physiology, University of Michigan, Ann Arbor, MI; 2) Department of Pathology, University of Michigan, Ann Arbor, MI; 3) Institute of Gerontology and the Geriatrics Center, University of Michigan, Ann Arbor, MI; 4) Department of Internal Medicine and Division of Geriatric Medicine, University of Michigan, Ann Arbor, MI.

FoxO transcription factors and sirtuin family deacetylases/ADP-ribosyl-transferase regulate diverse biological processes, including stress responses, metabolism and longevity. Here we show that the *Caenorhabditis elegans* sirtuin SIR-2.4 - homolog of the mammalian SIRT6 and SIRT7 proteins - is required for responses to multiple stress: heat shock, oxidative insult, and proteotoxicity in *C. elegans*. It is known that multiple stresses promote nuclear translocation and activation of FoxO transcription factor DAF-16, which directs the transcriptional program controlling metabolism, longevity and stress resistance. We found that SIR-2.4 is required for stress-induced DAF-16 nuclear localization and DAF-16 dependent gene expression, indicating that SIR-2.4 may play an important role in modulating DAF-16 function in response to stress. We investigated the mechanism in which SIR-2.4 may regulates DAF-16 activity. We found that SIR-2.4 promotes deacetylation of the DAF-16. Surprisingly, the deacetylase/ADP-ribosyltransferase catalytic activity of SIR-2.4 is not required for DAF-16 deacetylation and nuclear localization, suggesting that SIR-2.4 modulates DAF-16 activity via a mechanism involving other unknown deacetylase or acetyltransferase. These findings reveal a novel role of SIR-2.4 in modulating DAF-16 function and demonstrate that SIR-2.4 is a critical mediator of multiple stress responses through a DAF-16-dependent pathway.

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MicroRNA Predictors of Longevity in *C. elegans*. **Zachary Pincus**, Thalyana Smith-Vikos, Frank Slack. Molecular, Cellular, & Dev Biology, Yale University, New Haven, CT.

Inbred animals in homogenous laboratory conditions often experience a wide spread in lifespans, suggesting that neither genetic nor environmental factors fully determine individual longevity. Here we analyze biometrics of *C. elegans* observed throughout their lives using novel single-animal solid-media culture techniques. We find that early-to-mid-adulthood variability in several measures of homeostatic ability jointly predicts 62% of longevity variation. Though correlated, markers of growth/muscle maintenance and of metabolic by-products ("age pigments") report independently on lifespan. We also identify three microRNAs for which single-gene expression patterns predict up to 47% of lifespan differences. Though expression of each increases throughout early adulthood, *mir-71* and *mir-246* expression in this time range correlates with future lifespan, while *mir-239* expression anti-correlates. With upstream roles in longevity pathways, including insulin/IGF-1-like signaling (IIS), these microRNAs are both biomarkers and determinants of future longevity, and are the first genes shown to predict future longevity in un-perturbed, intact individuals. While it has been long assumed, this work is also the first demonstration that endogenous fluctuations in IIS activity act to determine individual lifespan. Taken together, these findings indicate that individual differences in longevity are at least partially the result of epigenetic states set early in life, and not solely the result of differences in stochastic damage accumulation over time.

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Molecular aging driven by Wnt signaling in *C.elegans*. **Yelena V. Budovskaya**[1], Stuart K. Kim[2]. 1) Swammerdam Institute for Life Science (SILS), University of Amsterdam, Amsterdam, Netherlands, Netherlands; 2) Department of Developmental Biology, Stanford, CA, USA.

We are using a system biology approach to reveal the molecular basis for aging in nematodes *C. elegans* by characterizing gene expression differences between young and old animals and then determining at a molecular level how these changes contribute to aging. This analysis revealed that gene expression differences between young and old animals are under control of a relatively simple gene regulatory network that involves the *elt-3*, *elt-5*, and *elt-6* GATA transcription factors. Expression of *elt-5* and *elt-6* increases in old age, leading to decreased expression of *elt-3*, thus causing changes in the expression of the many downstream target genes. We found no evidence that it is caused by cellular damage or environmental stresses. Rather, we found that *elt-3* expression in the adult is controlled by increased expression of the repressors *elt-5* and *elt-6*, which also guide *elt-3* expression during development. These results suggest that age-regulation of *elt-3* is caused by age-related drift of an intrinsic developmental program that becomes imbalanced in old age. This *elt-3/elt-5/elt-6* transcriptional circuit as the first genetic mechanism that is responsible for some of the age-related changes that occur as the worm grows old.

A key unanswered question is what causes *elt-3/elt-5/elt-6* transcriptional drift during aging? In *C. elegans*, Wnt/Wingless signaling pathways activate *elt-5* and *elt-6* expression during development. Here we demonstrated that Wnt signaling is responsible for the increased expression of *elt-5* and *elt-6* GATA transcription factors during aging. Mutations in one of the β-catenins, *wrm-1*, decreases expression levels of the *elt-5* and *elt-6* GATA transcription factors throughout life, leading to increased *elt-3* GATA expression. We demonstrated that mutation in *wrm-1*/ β-catenin, extends lifespan of other wise wild type animals by ~50%. These results indicate that changes in Wnt signaling - a regulator of the *elt-3/elt-5/elt-6* GATA transcriptional circuit during normal development - plays an important role in the age-related regulation of the *elt-3/elt-5/elt-6* transcriptional circuit and possibly many others.

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Spontaneous Age-Related Neurite Branching in *C. elegans*. **Elizabeth Tank**, Cynthia Kenyon. Dept. of Biochemistry and Biophysics, UCSF, San Francisco, CA. USA.

The analysis of morphological changes that occur in the nervous system during normal aging could provide insight into cognitive decline and neurodegenerative disease. Previous studies have suggested that the nervous system of *C. elegans* maintains its structural integrity with age despite the deterioration of surrounding tissues. Unexpectedly, we observed that neurons in aging animals frequently displayed ectopic branches, and that the prevalence of these branches increased with age. Within age-matched populations, the branching of touch neurons correlated closely with decreased touch responsiveness. The incidence of branching was influenced by two pathways that can affect the rate of aging, the Jun kinase pathway and the insulin/IGF-1 pathway. Loss of Jun kinase signaling, which slightly shortens lifespan, dramatically increased and accelerated the frequency of neuronal branching. Conversely, inhibition of the *daf-2* insulin/IGF-1-like signaling pathway, which extends lifespan, delayed and suppressed branching, and this delay required DAF-16/FOXO activity. Both JNK-1 and DAF-16 appeared to act within neurons to influence branching, and, through their tissue-specific expression, it was possible to uncouple the rate at which branching occurred from the overall rate of aging of the animal. Old age has generally been associated with the decline and deterioration of different tissues, except in the case of tumor cell growth. To our knowledge, this is the first indication that aging can potentiate another form of growth, the growth of neurite branches, in normal animals.

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Inhibition of mitochondrial respiration extends lifespan via reactive oxygen species that increase HIF-1 activity. **Ara B. Hwang**[1], Seung-Jae Lee[1], Eun-A Ryu[1], Cynthia Kenyon[2]. 1) Division of Molecular and Life Science, School of Interdisciplinary Bioscience and Bioengineering, and World Class University Information Technology Convergence Engineering, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Kor; 2) Department of Biochemistry and Biophysics, University of California, San Francisco, California 94158, USA.

A mild reduction of mitochondrial respiration extends the lifespan of many species including yeast, *C. elegans*, Drosophila and mice. However, until recently the molecular mechanisms by which impaired respiration promotes longevity are poorly understood. Hypoxia (low oxygen) is an environmental condition that reduces rates of respiration. Thus, it is possible that mechanisms that sense oxygen levels play a role in the longevity response by reduced respiration. By performing a genome-wide RNAi screen to identify genes that affect the activity of the hypoxia inducible factor HIF-1, we found that knockdown of several respiratory-chain genes can activate HIF-1. This increased HIF-1 activity seems to play a crucial role in the longevity induced by reduced respiration, since we found that the long lifespan of respiration-defective *clk-1* and *isp-1* mutants was suppressed by *hif-1* mutations. We also showed that the longevity conferred by down-regulation of *vhl-1* (ubiquitin ligase) or *egl-9* (prolyl hydroxylase), which normally stabilizes HIF-1 protein, was not additive to the extended lifespan caused by impaired mitochondrial respiration. These data suggest that the reduced respiration and the mutations in the *vhl-1* and *egl-9* genes act in the same pathway. As a potential mechanism by which conditions that reduce respiration increase HIF-1 activity, we hypothesized that reactive oxygen species (ROS) levels rise in the long-lived mutants with defective respiration, and that this ROS, in turn, may activate HIF-1. We showed that the respiratory *clk-1* and *isp-1* mutants displayed slightly but significantly elevated levels of ROS. We found that elevating mitochondrial ROS levels chemically, using paraquat, can up-regulate HIF-1 activity. Furthermore, we demonstrated that low levels (e.g. 250 μM and 125 μM) of paraquat can extend the lifespan of C. elegans in a partially *hif-1*-dependent manner. Together our data imply that the longevity caused by *clk-1* and *isp-1* respiration mutations is due to HIF-1 activation and that this process is mediated by ROS. Since the lifespan-extending effect of paraquat was partially independent of *hif-1*, we are currently identifying other genes that contribute to paraquat-induced longevtiy.

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Physiological IRE-1-XBP-1 and PERK/PEK-1 Signaling in C. elegans Intestinal Homeostasis and Immunity. **Claire E. Richardson**, Stephenie Kinkel, Dennis H. Kim. Biol, MIT, Cambridge, MA.

Endoplasmic reticulum (ER) stress activates the Unfolded Protein Response (UPR), a compensatory signaling response mediated by the IRE-1, PERK/PEK-1, and ATF-6 transmembrane proteins. Genetic studies have implicated roles for UPR signaling in animal development, but the function of the UPR under physiological conditions, in the absence of chemical agents administered to induce ER stress, is not well understood. Here, we show that in Caenorhabditis elegans, XBP-1 deficiency results in dramatically increased activities of IRE-1 and PEK-1, reflecting constitutive ER stress under basal physiological conditions. Innate immune activation and elevated physiological temperatures exacerbate this basal ER stress and necessitate IRE-1- XBP-1 and PEK-1 signaling for development and survival. Our data suggest that the negative feedback loops involving the activation of the IRE-1-XBP-1 and PERK/PEK-1 pathways serve essential roles not only at the extremes of exogenously imposed ER stress, but also in the maintenance of ER homeostasis under physiological conditions.

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The serine/threonine kinase SGK-1 promotes longevity by activating DAF-16/FoxO. Chunfang Guo[1], Kathleen J. Dumas[1,2], Travis W. Williams[1], Kaveh Ashrafi[5,6], **Patrick J. Hu[1,3,4]**. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA; 2) Cellular and Molecular Biology Program, University of Michigan Medical School, Ann Arbor, MI, USA; 3) Division of Hematology/Oncology, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA; 4) Department of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, USA; 5) Department of Physiology, University of California, San Francisco, San Francisco, CA, USA; 6) UCSF Diabetes Center, University of California, San Francisco, San Francisco, CA, USA.

The serum- and glucocorticoid-induced kinases (Sgks) are conserved members of the AGC (cAMP-dependent, cGMP-dependent, and protein kinase C) family of serine/threonine kinases. Sgk is highly similar to Akt in amino acid sequence and substrate specificity, and both kinases are activated by insulin and insulin-like growth factors (IGFs) in a phosphoinositide 3-kinase (PI3K)-dependent manner. In cultured mammalian cells, Sgk and Akt mediate IGF-dependent inhibition of FoxO transcription factor activity by directly phosphorylating FoxO. Accordingly, in *C. elegans*, SGK-1, AKT-1, and AKT-2 are thought to control lifespan by inhibiting the FoxO transcription factor DAF-16. Recently we and others showed that, in contrast to *akt-1* null mutation, which extends lifespan in a DAF-16/FoxO-dependent manner, *sgk-1* null mutants have a shortened lifespan compared to wild-type animals. We now find that *sgk-1(ft15)* animals, which harbor a gain-of-function mutation in *sgk-1*, live ~18% longer than wild-type animals. This lifespan extension is fully suppressed by *daf-16/FoxO* null mutations. Isoform-specific knockdown of either *daf-16a* or *daf-16d/f* reduces lifespan in *sgk-1(ft15)* animals, suggesting that SGK-1 extends lifespan by activating both DAF-16A and DAF-16D/F isoforms. Thus, in contrast to AKT-1 and AKT-2, which shorten lifespan by inhibiting DAF-16/FoxO activity, SGK-1 promotes longevity by activating DAF-16/FoxO. These results are inconsistent with prevailing models of concordant FoxO regulation by Sgk and Akt and highlight a previously unappreciated complexity in the regulation of FoxO transcription factors by Sgk.

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Gut microbiota as a therapeutic target of biguanides to extend *C. elegans* lifespan. **F. Cabreiro**, C. Au, C. Slack, P. Aston, L. Partridge, D. Gems. Inst healthy ageing & G.E.E, Univ College London, London, United Kingdom.

The biguanide drugs metformin and phenformin have been used to treat type 2 diabetes since the 1950s. Metformin can also extend lifespan in worms[1] and in short-lived mice[2]. Biguanides activate AMP-dependent kinase (AMPK), but their exact mode of action remains unclear. One possibility is that they might affect mammalian physiology via their effects on intestinal microbiota. Notably, metformin causes gastrointestinal upset. We are studying the role of *E. coli*, which plays a dual role as the worm's food source and microbiota[3], in effects of biguanides on lifespan. Phenformin is active in humans at lower concentrations than metformin, and we find the same to be true of its effects on worm lifespan. For both drugs, the concentrations optimal for life extension on *E. coli* OP50 also retard bacterial growth. By contrast, *E. coli* HT115 are resistant to such growth inhibition, and on this strain metformin does not increase worm lifespan. Moreover, on UV-irradiated OP50 metformin does not extend lifespan either. This suggests that *E. coli* mediate the effects of metformin on worm lifespan. In the absence of *E. coli*, metformin only shortens lifespan, suggesting drug toxicity. Metformin increases lifespan in *daf-16* but not *aak-2* or *skn-1* mutants, perhaps because AMPK and SKN-1 protect against metformin toxicity. Consistent with this, larval development in *aak-2* but not *daf-16* mutants was retarded by metformin (50μM, the optimal dose for life extension). Thus, metformin seems to have a dual effect on C. elegans, extending lifespan via their effect on *E. coli*, and shortening lifespan via their direct effect on the worm. How do biguanides affect E. coli to increase worm lifespan? One possibility is that they reduce E. coli pathogenicity. However, metformin does not increase lifespan in the cilium structure mutants *che-3*, *daf-10* and *osm-5*. This could suggest that metformin increases lifespan by changing perceived food quality; moreover, metabolic defects in *E. coli* can increase worm lifespan. Thus, effects of biguanides on the metabolic status of the worm's *E. coli* microbiota may increase its lifespan. In fruitflies, metformin treatment activates AMPK and disturbs gut microbiota but does not increase lifespan. One possibility is that flies' microbiota do not influence their lifespan. Alternatively, concentrations of metformin high enough to sufficiently alter microbiota may not outweigh direct toxicity to the fly, as is the case in *C. elegans* cultured on E. coli HT115. 1. PLoS One 5 (2010). 2. Cell Cycle 7, 2769 (2008). 3. J. Gerontol. 63, 2422 (2008).

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A heterodimeric *C. elegans* G-protein coupled receptor specific for the dauer pheromone Ascr#2/C6. **Donha Park[1]**, Rishi Somvanshi[2], Inish O'Doherty[3], Frank Schroeder[3], Ujendra Kumar[2], Don Riddle[1]. 1) Michael Smith Laboratories, University of British Columbia,Vancouver, BC, Canada; 2) Faculty of Pharmaceutical Sciences, Division of Pharmacology and Toxicology, UBC, Vancouver,Canada; 3) Boyce Thompson Institute, Cornell University, Ithaca, New York.

The G-protein coupled receptors DAF-37 and DAF-38 are required for perception of dauer-inducing ascaroside, ASCR#2/C6. The *daf-37* mutant does not form dauer larvae in response to ASCR#2/C6, whereas it responds normally to ASCR#3/C9 or ASCR#5/C3. The *daf-38* mutant form less dauers in response to ASCR#2/C6, ASCR#3/C9 and ASCR#5/C3. By contrast, animals over-expressing *daf-37* exhibiteded hyper-responsive dauer formation in ASCR#2/C6. Ascr#2/C6-induced male attraction and hermaphrodite repulsion behaviors are absent in the *daf-37* mutant. *daf-37* is expressed in the sensory neurons ASI, ASK, IL2 and male specific CEM neurons, and the protein is localized to the neuronal cilia. In transfected human cells, DAF-37 is observed in the plasma membrane as well as in some endogenous organelles. DAF-37 forms homodimers and heterodimers with DAF-38, as judged by a pbFRET (photo-bleaching Fluorescent Energy Transfer) assay. Heterodimerization is associated with localization at the cell surface. ASI-specific expression of DAF-37 exhibited hyper-sensitive dauer formation and a ASK neuron specific expressor confers a hyper-sensitive hermaphrodite repulsion phenotype in response to Ascr#2/C6. Our results suggest that DAF-37 is a specific receptor for Ascr#2/C6. Its localization at the cell surface is facilitated by heterodimerization with DAF-38. DAF-37 regulates larval dauer development in ASI neurons and adult behavior in ASK neurons.

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Steroid secrets revealed - Identification of novel endogenous ligands of the nuclear hormone receptor DAF-12 using NMR based comparative metabolomics. **Parag Mahanti**[1], N. Bose[1], A. Bethke[1], J. Judkins[1], J. Wollam[3], P. J. Hu[2], A. Antebi[3], Frank C. Schroeder[1]. 1) Boyce Thompson Institute and Dept of Chemistry & Chemical Biology, Cornell Univ., Ithaca, NY; 2) LSI, Univ. of Michigan, Ann Arbor, MI; 3) Max Planck Institute for Biology of Ageing, Cologne.

Small-molecule hormones control metazoan development as ligands of nuclear hormone receptors (NHR's) that regulate gene expression in a ligand-dependent manner. In the nematode *C. elegans*, more than 280 NHR's have been identified, which have been shown to serve diverse physiological roles. Ligands for only one of these have been identified: DAF-12, a homolog of vertebrate vitamin D and liver X receptor, which constitutes a central switch determining the developmental fate of *C. elegans* larvae. Two steroidal hormones called dafachronic acids (Δ-4 and Δ-7 DAs) have been predicted to serve as endogenous DAF-12 ligands, promoting reproductive development and suppressing entry into larval diapause (dauer)[1]. However due to their very low rates of biosynthesis, unambiguous detection of the dafachronic acids in *C. elegans* has not yet been accomplished.

Here, we describe the use of NMR-based metabolomics (DANS) to determine the structures of endogenous DAF-12 ligands. DANS constitutes an unbiased analytical approach that can be used to identify novel types of signaling molecules by comparing the metabolomes of different mutant strains.[2]

Using DANS to compare the wild-type metabolome with that of hormone-deficient *daf-9:daf-12* mutant worms, we detected only one of the two predicted DAF-12-ligands, Δ-7 DA. In addition we have identified two novel DAF-12 ligands that are differentially produced in specific mutants. Our findings further suggest that Δ-7 is not the most abundant endogenous DAF-12 ligand and that Δ-4 DA is not present in physiologically significant concentrations. The newly identified ligands show strong bioactivity and exhibit unexpected structural features suggesting that existing models of steroid hormone biosynthesis in *C. elegans* are incomplete.

1. Motola DL et. al. (2006) *Cell* 124, 1209-12232. **2**. Pungaliya C et. al. (2009) *Proc. Natl. Acad. Sci. USA* 106, 7708-13.

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A defensive escape behavior reveals xenobiotic surveillance pathways in *C.elegans*. **Justine Melo**[1,2], Gary Ruvkun[1,2]. 1) Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept. of Genetics, Harvard Medical School, Boston, MA.

Synthesis of xenobiotic compounds obstructing fundamental cellular processes in foreign species is a common tactic employed by microbes and fungi against potential predators or hosts. Animals have evolved a range of detoxification responses in self-defense, but little is known of how xenobiotics are detected or coupled to adaptive responses. We have found that when *C. elegans* is exposed to a broad range of exogenously applied xenobiotics, including those targeting the genome, ribosome, mitochondrion, and endoplasmic reticulum, they evacuate an otherwise benign bacterial food source. A similar escape response is triggered when feeding-based RNAi is used to inactivate genes that encode the same proteins or pathways targeted by xenobiotics. Both xenobiotic agents and essential gene inactivations induce specific combinations of stress-responsive GFP reporters that constitute a signature for each xenobiotic chemical or gene inactivation. The coincidence of self-protective behavior with adaptive changes in gene expression suggests that surveillance pathways for xenobiotics monitor the integrity of core cellular components to coordinate multiple survival-directed responses. To discern the cellular processes subject to surveillance, we screened for gene inactivations that induce the escape response from otherwise innocuous bacteria. Of ~4000 essential and metabolic genes examined, approximately 400 gene inactivations induced an escape response. Many of these inactivations affect highly conserved eukaryotic processes, including protein translation, mRNA splicing, oxidative respiration, secretion, ion transport, cytoskeletal integrity, and protein homeostasis. Disruption of these processes in isolated tissues induced robust escape behavior, revealing an endocrine axis of control originating from multiple tissues that act as "sentinels" for the presence of poisons. Specific nuclear hormone and insulin-like endocrine pathways were required for engagement of the escape behavioral program. In humans, xenobiotic surveillance systems underlying behavioral and endocrine responses may explain the counterproductive or undesirable side effects of many drugs, including nausea and appetite suppression.

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Shining light on behavior: An optogenetic dissection of neural circuits in freely behaving *C. elegans*. **Andrew Leifer**[1], Christopher Clark[2], Quan Wen[3], Christopher Fang-Yen[4], Mark Alkema[5], Aravinthan Samuel[3]. 1) Program in Biophysics, Harvard University, Cambridge MA; 2) Program in Neuroscience, University of Massachusetts Medical School, Worcester, MA; 3) Department of Physics and Center for Brain Science, Harvard University, Cambridge, MA; 4) Department of Bioengineering, University of Pennsylvania, Philadelphia, PA; 5) Department of Neurobiology, University of Massachusetts Medical School, Worcester, MA.

Optogenetics provides a promising new platform for conducting behavioral neuroscience investigations in the nematode *Caenorhabditis elegans*. The ability to precisely manipulate neural activity in individual neurons in a moving worm allows one to pinpoint the contribution of individual neurons to animal behavior. Previously we developed a high-resolution optogenetic illumination system capable of delivering arbitrary light patterns to targeted cells in a freely moving C. *elegans* (Leifer and Fang-Yen et al, *Nature Methods*, 2011). The system, called CoLBeRT, uses a high speed camera and custom computer vision software to monitor the motion of an unrestrained worm. As the worm swims or crawls, the system instructs a digital micromirror device to reflect patterns of blue or green laser light onto specific cellular targets expressing Channelrhdopsin-2 or Halorhodopsin. The system has sufficient accuracy and resolution to stimulate individual mechanosensory neurons while a worm swims.

Here we have expanded the CoLBeRT system's capabilities to dissect the motor circuit, mechanosensory circuit and investigate the roles of command interneurons. The system is now able to generate illumination patterns with arbitrary amplitude waveforms and we have adapted the system to work with microfluidic devices. We have used the system to differentiate models of wave propagation in the motor circuit, to study habituation in the mechanosensory circuit and to begin an exploration of the command interneurons. Preliminary evidence suggests that stimulation of the command interneuron AVA modulates the worm's backward velocity.

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C. elegans selects distinct crawling and swimming gaits via dopamine and serotonin. **Andrés G Vidal-Gadea**[1], Stephen Topper[1], Layla Young[1], Ashley Crisp[1], Leah Kressin[2], Erin Elbel[1], Thomas Maples[1], Abram Axelrod[2], Martin Brauner[3], Karen Erbguth[3], Alexander Gottschalk[3], Dionicio Siegel[2], Jonathan Pierce-Shimomura[1]. 1) Section of Neurobiology, The University of Texas at Austin, Austin, TX, USA; 2) Department of Chemistry and Biochemistry, The University of Texas at Austin, TX, USA; 3) Institute of Biochemistry, Johann Wolfgang Goethe-Universität, Frankfurt am Main, Germany.

The ability to select and switch between alternate locomotory gaits is shared among many animals that are required to transition between different environments. Failure to achieve motor transitions is often devastating to the organism as exemplified by patients with advanced Parkinson's disease. The nematode *C. elegans* is well adapted for locomotion on land and in water and is likely required to transition between these sub-niches in nature. Despite recent advances, it remains controversial if swimming and crawling are distinct gaits in *C. elegans*, and if so, how transitions between them are accomplished (1-4). Answering these questions could enable the use of this powerful model system to study the mechanisms underlying locomotory transitions. We used a multifaceted approach spanning in depth behavioral assays, mechanic and optogenetic stimulation, laser ablations, mutant analysis, and in vivo photolysis of caged amines to test whether the worm uses distinct gaits and to determine how they transition between them. We show through a series of behavioral assays that in *C. elegans* crawling and swimming are distinct gaits. Dopamine released by ADE and PDE neurons is necessary and sufficient to initiate and maintain crawling by a pathway activating D1-like receptors. Conversely, serotonin is necessary and sufficient to initiate and maintain transition from crawling to swimming and to inhibit a set of crawl-specific behaviors. We found these transitions to be modulated by the balance between dopamine and serotonin. These amines have been found to play crucial roles in transition between alternate locomotory forms in diverse species stressing the importance locomotor transitions have for survival. Further study of locomotory switching in *C. elegans* and its dependence on dopamine may provide insight into comparable motor deficits observed in Parkinson's disease. 1) Boyle H *et al.* N. Front. Behav. Neurosci. 2011 5:10 doi: 10.3389/fnbeh.2011.00010. 2) Mesce KA, Pierce-Shimomura JT. Front. Behav. Neurosci. 2010 4:49 doi: 10.3389/fnbeh.2010.0004 3) Vidal-Gadea AG *et al.* 2009 Neuroscience 366:17 Calabrese R: Faculty of 1000 Biology, 11 November, 2009. Conference 2009 October 16-21. Available at: http://f1000biology.com/article/id/1182956/evaluation 4) Fang-Yen C *et al.* 2010 PNAS 107, 20323-20328.

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Axonal regeneration proceeds through axonal fusion in *C. elegans* neurons. **Brent Neumann**[1], Ken C.Q. Nguyen[2], David H. Hall[2], Adela Ben-Yakar[3], Massimo A. Hilliard[1]. 1) Queensland Brain Institute, The University of Queensland, Brisbane, Australia; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY; 3) Department of Mechanical Engineering, The University of Texas at Austin, Austin, Texas.

Invertebrate axons and those of the mammalian peripheral nervous system are able to regenerate in the adult. Functional neuronal recovery following injury arises when severed axons reconnect with their targets. In *C. elegans*, following laser axotomy, the regrowing axon still attached to cell body (proximal) is able to regrow and reconnect with its separated distal segment through unknown mechanisms. Using the mechanosensory neurons ALM and PLM as a model system, we have found that reconnection of separated axon fragments during regeneration occurs through a mechanism of axonal fusion, which prevents Wallerian degeneration of the distal fragment. Through electron microscopy analysis and imaging with the photoconvertible fluorescent protein Kaede, we show that the fusion process re-establishes membrane continuity and repristinates anterograde and retrograde cytoplasmic diffusion. Through the use of dual colour labeling of adjacent axonal pairs, we also provide evidence that axonal fusion occurs with a remarkable level of accuracy, with the proximal re-growing axon specifically reconnecting with its own separated distal fragment. Finally, from a candidate screening approach, we have identified several molecules as being necessary for successful regeneration and specifically involved in the process of axonal fusion.

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Insulin signaling directly regulates age-dependent axon regeneration. **Alexandra Byrne**, Marc Hammarlund. Department of Genetics and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT.

Adult worms lose the ability to regenerate damaged axons with increased age[1]. We find that the insulin/IGF factor receptor DAF-2 directly regulates this age-dependent decline in regeneration. Axons in young adult *daf-2* and wild type animals regenerate at the same frequency. However, regenerative ability is downregulated in wild type animals as they age and maintained in aged *daf-2* animals as they age. The high regeneration in aged *daf-2* mutants is dependent on the downstream transcription factor DAF-16/FOXO, as aged *daf-2; daf-16* animals exhibit decreased regeneration compared to aged *daf-2* animals. Thus, *daf-2* signals at least in part through the canonical insulin/IGF-1 pathway to inhibit regeneration in older animals.

Surprisingly, regulation of regeneration by *daf-2* is separable from *daf-2*'s function in determining lifespan. We found that *daf-2* mutants fed *daf-16* RNAi, which have normal lifespan, still display increased regeneration in aged animals. In this experiment, *daf-16* RNAi knocks down *daf-16* in the intestine, where it regulates lifespan, but allows *daf-16* to remain active in neurons[2]. Similarly, *daf-2; daf-16* double mutants that express wild type *daf-16* in the intestine have extended lifespan but do not display increased regeneration in aged animals. These experiments demonstrate that regulation of regeneration by insulin signaling is not a secondary consequence of insulin signaling's effect on lifespan, and define a novel, neuron-specific function for *daf-16*.

The MAP kinase kinase kinase *dlk-1*/MAPKKK is necessary for axon regeneration in both aged and young animals[1,3]. Several lines of preliminary evidence suggest that insulin signaling regulates regeneration by directly modulating levels of *dlk-1*: 1) *dlk-1* transcript levels are elevated in aged *daf-2* mutants compared to aged wild type animals; 2) Expression of the transcription factor *cebp-1*, which is dependent on activity of the *dlk-1* pathway[3], is increased in aged *daf-2* animals relative to aged wild type animals; 3) The transcription factor *daf-16*/FOXO binds to the promoter region of *dlk-1*[4]. Together, our findings suggest that insulin signaling directly regulates regeneration in aged animals by acting in neurons to control expression of *dlk-1*. Understanding the mechanisms that control age-related decline in axon regeneration may aid in the treatment of spinal cord injury and neurodegenerative diseases, particularly in older patients. [1]Hammarlund, M., *et al*., *Science* (2009). [2]Libina, N., *et al*., *Cell* (2003). [3]Yan, D., *et al*., *Cell* (2009). [4]Schuster, E. *et al*., *Mol Syst Biol* (2010).

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Understanding the molecular mechanisms underlying the age-dependent nerve regenerative response in adult *C. elegans*. **T. Hubert[1]**, Y. Jin[1,2]. 1) Division of Biological Sciences, UC San Diego, La Jolla, CA 92103, USA; 2) Howard Hughes Medical Institute.

Aging is often associated with declines in cognitive function and neuronal plasticity and an increased risk of neurodegenerative disease. However, the factors causing age-dependent changes in the nervous system remain largely unknown. We have used laser axotomy on PLM axons to study the age-dependent responses to axonal injury. We axotomized PLM axons of animals aged from L4 stage to day 10 of adulthood and then measured the regrowth 24 hours after the surgery. As previously reported (Wu *et al.*, 2007), PLM axons of L4 or young adult animals show robust regenerative regrowth in the first 24 hours. We find PLM regrowth significantly declines in adult animals after 3 days of adulthood (A3). In addition, regrowing axons of older animals show a higher percentage of growth cones and secondary neurites formation, as well as an increased rate of proximal to distal process fusion events compared to L4 animals. Calcium and cAMP play critical roles in the neuronal regenerative response. Genetic mutations that increase neuronal calcium or cAMP improve regeneration in L4 animal (Ghosh-Roy *et al.*, 2010). However, we find that simply elevating calcium or cAMP has little effect on the regrowth extent in older animals. Mutations increasing the lifespan of *C. elegans*, such as *daf-2* or *eat-2* can delay some age associated phenotypes and improve learning and memory performance in aging animals (Kauffman *et al.*, 2010). However, we find that these long-lived mutants show the same regeneration profile as wild type animals. Our results suggest that the adult decline in regenerative axonal regrowth is independent of organismal aging or health, and that mechanisms intrinsic to the neuron itself are likely responsible for the regenerative decline. In our screen of stress resistance and metabolism genes, we find several mutants in which the L4 to A3 regeneration decline is abolished. Preliminarily, we find that loss of function in *akt-2*, *rsks-1* or *isp-1* attenuates the age-dependent regeneration decline. We are currently investigating whether they act cell-autonomously as well as whether an age-dependent decline in neuronal mitochondrial respiration is responsible for the decline in adult axon regeneration.

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Sleep-dependent Changes in the C. elegans Neural Network. **Julie Cho**, Alon Zaslaver, Paul Sternberg. Biology, California Institute Tech, Pasadena, CA.
*equal contribution

Sleep is a physiological state essential for human and animal health. This state is behaviorally characterized by inactivity and a decreased arousal in response to external stimuli. Despite the fact that this conserved behavior has been studied across various mammalian and non-mammalian species, the modulation of the neural network that governs this quiescent state is largely unknown. C. elegans offers a uniquely tractable system to study sleep regulation on the neural-wide level.

Here we use optogenetic techniques to probe the dynamics in individual circuits during sleep. We targeted each level of information processing from sensory neurons through interneurons and conversion of this information to coordinated behavior through the motor circuit and muscles. Using a genetically-encoded calcium indicator, we find that sensory response to external stimuli is dampened in the sleeping worm as compared to the wake worm. To elucidate activity of downstream components of the circuit, we expressed the light-activated channelrhodopsin in specific cells or tissues. Response was followed with behavioral assays. Targeted activation of individual sensory neurons in the sleeping worms caused a delayed response when compared to wake worms. However, targeted activation of interneurons or motor neurons did not result in a delayed response. Furthermore, targeted activation of body wall muscles did not awake the worms, although the muscles did contract. These results show that inhibition occurs only in a subset of the sensory-motor circuit and suggest that the sleep state reflects modulation of the top levels of the neural network spanning the sensory and inter-neurons. This study offers the first step toward network-wide analysis of the sleep state, where targeted activation of specific neural components is achieved, and may shed light on the essential components that regulate sleep in animals in general.

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Cyclin-dependent kinases control the axon-dendrite polarity of presynaptic components. **Chan-Yen Ou**, Vivian Y Poon, Andrew B Hellman, Celine I Maeder, Kang Shen. Biology, Stanford University, Stanford, CA.

The neuron is the basic unit of the nervous system, which allows organisms to sense, memorize, think, and respond to the world. The function of the neuron relies on its proper polarization and compartmentalization in order to receive signals from the right field, process information, and transmit it to the correct target. To understand the molecular mechanisms underlying how presynaptic components like synaptic vesicles (SVs) and active zone proteins are localized to defined axonal domains, an unbiased visual forward genetic screen was performed to identify genes controlling the localization of GFP::RAB3, a specific synaptic vesicle marker, in the cholinergic motor neuron DA9 in C. elegans. Two cyclin-dependent kinases (CDKs), CDK-5 and PCT-1, and their specific activators p35/CDKA-1 and CYY-1 respectively were identified as key regulators for axon-dendrite polarization of presynaptic components. The loss of both PCT-1 and CDK-5 activity caused nearly all synapic vesicles and active zone proteins ectopically accumulated in dendrites. To identify downstream components, the *cdk-5* modifier screen was performed that revealed the dynein-mediated retrograde transport and clathrin-mediated endocytic machinery were necessary for the polarity phenotype. Further characterization of CDK-5 and PCT-1 indicated that CDK-5 is mainly localized at the presynaptic terminals, while CYY-1, acting as the activator of PCT-1, is enriched in dendrites. Overexpression of PCT-1, but not CDK-5, eliminated proximal presynaptic clusters. In contrast, the cdk-5 mutant, but not the pct-1 mutant, has imbalanced polarized SV trafficking at the proximal axon. Based on these data we propose that CDK-5 acts in the presynaptic domain to guide polarized SV trafficking and PCT-1 is activated by CYY-1, which prevents SV clusters from stabilizing in dendrites.

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Direct visualization and functional modulation suggest a role for specific heparan sulfate motifs in cholinergic synaptic transmission in *C. elegans*. **Matthew Attreed**, Hannes Buelow. Department of Genetics, AECOM, Bronx, NY.

Heparan sulfates (HS) are unbranched, variably modified polysaccharide chains in the extracellular space that are invariably attached to proteins. The modification process of the sugars results in HS chains harboring different motifs of specifically arranged modifications. We and others have shown that individual HS modifications are crucial for various aspects of neural development in metazoans. Yet, little is known about the dynamics and function of defined HS motifs in the nervous system. In order to address this question experimentally, we have developed a tool that allows for live imaging of HS sugar motifs. By transgenically expressing secreted single chain variable fragment (scFv) antibody::GFP fusions, which recognize different HS motifs, we can visualize for the first time HS sugars with distinct modification patterns in living animals. We detect a highly refined distribution of HS motifs in both neuronal and non-neuronal tissues that often exhibit dynamic changes during development. Specifically, we find different HS motifs associated with the nervous system from late embryonic stages into adulthood. Intriguingly, our data suggest the existence of neuron-specific HS motifs. Genetic experiments and colocalization studies show that some HS motifs are associated with synaptic markers in the nerve cords. The possibility of functional neutralization of the HS motifs recognized by different scFv antibodies prompted us to study the nervous system of the transgenic animals in more detail. We find that the gross neuroanatomy in transgenic animals expressing different scFv antibody constructs is indistinguishable from wild type animals. However, based on aldicarb and levamisole assays, we detect defects in cholinergic synaptic transmission in animals expressing certain antibodies, possibly as a result of functional neutralization by the antibody fusions. These findings suggest that specific HS motifs serve a role in cholinergic transmission. In conclusion, we have developed a novel tool for the visualization and modulation of HS sugar motifs *in vivo*. Our experiments indicate that HS motifs are expressed dynamically and, possibly, in a neuron-specific manner. Moreover, distinct HS motifs may serve a role in modulating synaptic transmission.

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A novel RAB-2 effector complex regulates sorting of dense-core vesicle cargos at the Golgi. **Michael Ailion**[1], Mandy Hannemann[2], Susan Dalton[1], Andrea Pappas[1], Shigeki Watanabe[1], Jan Hegermann[2], Hsiao-Fen Han[1], Mingyu Gu[1], Kim Schuske[1], Patrick Hullett[1], Stefan Eimer[2], Erik Jorgensen[1]. 1) Department of Biology, HHMI, University of Utah, Salt Lake City, UT; 2) European Neuroscience Institute, Goettingen, Germany.

Release of neuromodulators from dense-core vesicles is of critical importance in neuronal signaling. However, dense-core vesicle biogenesis, trafficking and release are not well understood, primarily because few molecules specific to dense-core vesicle function have been identified. By screening for genetic suppressors of an activated Gαq mutant in *C. elegans*, we identified at least ten genes that appear to regulate dense-core vesicle trafficking or release. One of the genes we identified (*rund-1*) encodes a novel conserved protein with a putative small GTPase effector domain (the RUN domain). Three pieces of data suggest that RUND-1 acts with the small GTPase RAB-2 to regulate sorting of proteins into dense-core vesicles. First, *rab-2* and *rund-1* mutants exhibit similar defects in trafficking soluble and transmembrane dense-core vesicle cargos to axons, and double mutant analysis suggests that *rab-2* and *rund-1* act in the same genetic pathway. Second, RUND-1 physically interacts with RAB-2; this interaction requires the RUN domain of RUND-1 and the active GTP-bound form of RAB-2. Third, RUND-1 and RAB-2 colocalize in neuronal cell bodies. Using super-resolution correlative fluorescence electron microscopy, we demonstrate that RUND-1 localizes specifically to the Golgi. Thus, RUND-1 may function as a new RAB-2 effector in the maturation of dense-core vesicles at the trans-Golgi network. However, unlike typical Rab effectors, RUND-1 localization is not dependent on RAB-2, and does not require its RUN domain. A second gene identified in our screen encodes a novel conserved protein with coiled-coil domains that may act as another novel RAB-2 effector. Mutants of the coiled-coil protein have similar behavioral and cargo-trafficking phenotypes as *rund-1* and *rab-2* mutants, and double mutants do not have enhanced phenotypes, indicating that the coiled-coil protein also functions in this genetic pathway. Furthermore, like RUND-1, the coiled-coil protein interacts with the active GTP-bound form of RAB-2, but does not interact with inactive GDP-bound RAB-2. Finally, the coiled-coil protein shows similar localization to RAB-2 and RUND-1 in neuronal cell bodies. Although trafficking of soluble and transmembrane cargos is defective in these mutants, morphologically normal dense-core vesicles are transported to axons. These data suggest that RAB-2 along with two novel effectors may act as a complex to regulate sorting of dense-core vesicle cargos at the Golgi.

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HIF-1 Independent Glutamate Receptor Trafficking as a Novel Neuroprotective Response to Hypoxic Insult. Eun Chan Park[1], **Piya Ghose[1,2]**, Zhiyong Shao[3], Lijun Kang[4], Jo Anne Powell-Coffman[3], Shawn Xu[4], Christopher Rongo[1], (ECP and PG contributed equally to this work). 1) The Waksman Institute, Department of Genetics, Rutgers University, Piscataway (NJ), USA; 2) Graduate Program in Neuroscience, Rutgers University, Piscataway,(NJ), USA; 3) Department of Genetics, Development and Cell Biology, Iowa State University, Ames (Iowa), USA; 4) Life Sciences Institute, University of Michigan, Ann Arbor (Michigan), USA.

Ischemic stroke results in excitotoxicity via hypoxia, glutamate receptor (GluR) overactivation and deregulated calcium homeostasis. Changes in GluR trafficking can occur in response to hypoxia, but the nature of those changes, whether they protect or exacerbate excitotoxicity, remains controversial. Here we examine how hypoxia and the known hypoxia response pathway alter the trafficking of the *C. elegans* GluR GLR-1. GLR-1 acts in the command interneurons mediating an escape response by triggering reversals in locomotion. Synaptically localized GLR-1 receptors can be observed using a GLR-1::GFP chimeric protein. Recently, we observed a change in GLR-1 trafficking in response to hypoxic treatment: receptors become trapped in internal endosome-like accretions and animals behave similar to *glr-1* knockouts. Loss of function mutations in *egl-9*, a prolyl hydroxylase that normally inhibits the cellular response to hypoxia by hydroxylating key proline residues on the transcription factor HIF-1, mimics the effects of hypoxia on GLR-1 trafficking and depresses both glutamate-activated currents and GLR-1- mediated behaviors. HIF-1, the canonical substrate of EGL-9 activity, is not required for this effect; instead, we find that EGL-9 physically interacts with LIN-10, a PTB/PDZ-domain protein that promotes GLR-1 membrane recycling. We find that normal oxygen levels induce a specific EGL-9 isoform to colocalize with LIN-10 in dendrites, where it prevents the phosphorylation of LIN-10 by the proline-directed kinase CDK-5. Un-phosphorylated LIN-10 is recruited to endosomes, where it mediates GLR-1 recycling. By contrast, under hypoxic conditions, EGL-9 is inhibited by lack of oxygen, allowing CDK-5 to inhibit LIN-10 by directly phosphorylating its proline-rich N-terminus. This results in LIN-10 delocalization and the trapping of GLR-1 in endosomes. Mutations in either *cdk-5* or the proline residues of the CDK-5 phosphorylation sites in LIN-10 block the effects of hypoxia and *egl-9* mutations, suggesting a novel kinase regulatory mechanism in which EGL-9 regulates CDK-5 kinase activity by hydroxylating its substrate prolines, thus precluding substrate phosphorylation by CDK-5. We propose that neurons use this novel neuroprotective mechanism to respond to hypoxia by inhibiting receptor membrane recycling so as to reduce GluR synaptic abundance.

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Inter-axonal interaction defines synaptic tiling in *C. elegans*. **Kota Mizumoto[1,2]**, Kang Shen[1,3]. 1) Biology, Stanford University, Stanford, CA; 2) HFSP; 3) HHMI.

Cellular interactions between neighboring axons are essential for the stereotyped positioning of individual axons and topographic map formation. So far however, it is not known how axons communicate with each other at the level of synapse formation. To answer this question, we focused on two closely related cholinergic motor neurons, DA9 and DA8. Both DA8 and DA9 neurons extend their axons through a commissure into the dorsal nerve cord, where they proceed anteriorly to form a series of *en passant* synapses. Although those axons largely overlap in the dorsal cord, they form synapses only at the specific area. While DA9 forms synapses onto the dorsal muscles in its axon in the posterior-most domain (DA9 synaptic domain) of dorsal muscle, DA8 axon has no synapses in DA9 synaptic domain, and starts to form synapses just anterior to DA9 synaptic domain. Therefore, DA8 and DA9 form 'tiled' synaptic domains leaving there is no apparent gap or overlap between DA8 and DA9 synaptic domains. Therefore, these two motor neurons have 'tiled' synaptic domain along dorsal nerve corthemd.

We first tested whether such 'synaptic tiling' is dependent on axon contact between DA8 and DA9 using the mild axon guidance mutants, *unc-34* and *unc-129*. In approximately half of mutant animals, either DA8 or DA9 axon turns prematurely before it reaches the dorsal nerve cordis misguided and completely miss the dorsal cord. In such circumstances where DA8 and DA9 axons fail to contact one another, the DA8 synaptic domain expands posteriorly while DA9 synaptic domain expands anteriorly, causing significant overlap of their synaptic domains along the A-P axis. In contrast, synaptic tiling is normal in animals where both axons are correctly guided to the dorsal nerve cord, suggesting that contact between DA8 and DA9 axons appears critical.

From a forward, visual-based genetic screening, we identified that two transmembrane Semaphorins (s*mp-1, smp-2*) and their receptor Plexin (*plx-1*) are essential for the synaptic tiling between DA8 and DA9. Interestingly, cell specific rescue and mosaic experiments suggested that they both ligands and receptor function in cis in DA9. We also found that PLX-1::GFP is localized at the anterior edge of the synaptic domain of DA9 in a Semaphorin and axon-axon interaction dependent manner. Deletion and mutational analyses of PLX-1 indicate that PLX-1 cytoplasmic RasGAP domain is essential for its function. Consistently, constitutive active Ras mimicked *plx-1* loss of function phenotype. We propose that contact-dependent PLX-1 subcellular localization sets up the synaptic boundary between DA8 and DA9 by restricting the synaptic domain via inactivation of Ras.

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Optogenetics dissection of the nociceptive PVD network: RNAi of PVD-specific genes reveals TRP channels as signal amplifiers. **Steven J. Husson[1]**, Wagner Steuer Costa[1], Jeffrey N. Stirman[2], Joseph D. Watson[3], W. Clay Spencer[3,4], Millet Treinin[5], David M. Miller III[3,4], Hang Lu[2], Alexander Gottschalk[1,6]. 1) Institute of Biochemistry, Goethe-Univ Frankfurt, Frankfurt, Germany; 2) Interdisciplinary Bioengineering Program, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA; 3) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN, USA; 4) Program in Neuroscience, Vanderbilt University, Nashville, TN, USA; 5) Department of Medical Neurobiology, Hadassah Medical School, Jerusalem, Israel; 6) Frankfurt Molecular Life Sciences Institute, Goethe-University Frankfurt, Germany.

Higher animals display myriads of neurons contributing to pain sensation, making it extremely difficult to study the neuronal circuits underlying nociception. In contrast, only a handful of neurons mediate nociception in *C. elegans*. Nociceptive neurons contain highly branched dendrites and trigger the sensation of pain in response to noxious insults. The FLP and PVD neurons adopt similar complex dendritic arbors and have been implicated in harsh mechanical touch responses, while other touch receptor neurons (TRNs) detect gentle mechanical stimuli. Studying the behavioral output of the mechanosensory modality of PVD or FLP cells has so far been complicated, because a harsh touch inevitably agitates the whole body of the animal, thus making it impossible to prevent contributions of other cells. In contrast, optogenetic tools like the blue light-activated depolarizing channelrhodopsin-2 (ChR2), allow non-invasive stimulation of the sensory neuron of interest. Expression of ChR2 in the PVD neurons, FLP neurons or downstream command interneurons and subsequent photoactivation, allows us to mimic the harsh touch response, without interfering signaling from the TRN neurons. We show that behavioral responses to harsh touch are shaped by both the FLP and PVD sensors to jointly determine the output response as forward or reverse escape. Molecular players required for PVD function were identified by microarray profiling of mRNAs specifically isolated from PVD. Candidate nociceptive genes were knocked down by RNAi and effects on photo-triggered, ChR2-mediated escape responses were quantified. We found the VGCC α- and β-subunits UNC-2 and CCB-1 to be required for chemical synaptic output from PVD. Targeting synaptobrevin by the Tetanus toxin in PVD to impair synaptic output abolished PVD-dependent behavior. Knockdown of regulators of PVD differentiation severely impaired PVD function, while a DEG/ENaC channel and a TRP channel act cell-autonomously in PVD to shape its dynamic range and amplify the output signal, uncovering a novel role of TRP channels downstream of primary sensor molecules.

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Two endocytic pathways revealed by capturing channelrhodopsin induced synaptic transmission. **Shigeki Watanabe**, Qiang Liu, Wayne Davis, Gunther Hollopeter, Mingyu Gu, Erik Jorgensen. Dept Biol, Univ Utah, Salt Lake City, UT.

To sustain release of neurotransmitters at high rates of stimulation, synaptic vesicles need to be recycled locally at a synapse. Two major pathways for endocytosis were identified nearly 40 years ago: clathrin-scaffolds[1] and kiss-and-run[2]. Clathrin-mediated endocytosis is a slow process, which seems to take place distant from release sites. On the other hand, kiss-and-run occurs at release sites immediately after neurotransmission. The existence of either pathway at synapses has not been satisfactorily demonstrated[3]. Moreover, molecular mechanisms behind these pathways are poorly understood[3]. To address these issues, we need to observe synaptic vesicles as well as their associated proteins in action at nanoscale resolution. We developed two techniques that allow such an observation. First, we combined optogenetics with electron microscopy to induce synaptic transmission and capture rapid events at nanoscale resolution. Specifically, we expressed a variant of channelrhodopsin, ChIEF, in the C. elegans acetylcholine neurons, and stimulated them at various times before the high-pressure freezing. Using this technique, we identified two endocytic pathways that act at different times and in different parts of the synapse: a slow process distant from the dense projection and a fast process near the dense projection. Second, we developed a correlative super-resolution fluorescence microscopy and electron microscopy (fEM) imaging technique[4] to identify molecules that act in the processes. We found that the adaptor protein AP2 is localized to both identified endocytic sites, suggesting that AP2 may be functioning in both processes. By combining these two techniques, we are beginning to understand how endocytosis takes place at synapses. References: [1]Heuser, J.E. & Reese, T.S. Evidence for recycling of synaptic vesicle membrane during transmitter release at the frog neuromuscular junction. J. Cell Biol 57, 315-344 (1973). [2]Ceccarelli, B., Hurlbut, W.P. & Mauro, A. Depletion of vesicles from frog neuromuscular junctions by prolonged tetanic stimulation. J. Cell Biol 54, 30-38 (1972). [3]Royle, S.J. & Lagnado, L. Clathrin-mediated endocytosis at the synaptic terminal: bridging the gap between physiology and molecules. Traffic 11, 1489-1497 (2010). [4]Watanabe, S. et al. Protein localization in electron micrographs using fluorescence nanoscopy. Nat. Methods 8, 80-84 (2011).

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Lateral facilitation between primary mechanosensory neurons controls nose touch perception in *C. elegans*. **Marios Chatzigeorgiou**, Ithai Rabinowitch, William Schafer. Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

The nematode *C. elegans* senses head and nose touch using multiple classes of mechanoreceptor neurons that are electrically-coupled through a network of gap junctions. Using modelling, *in vivo* neuroimaging, and genetic tools capable of modifying neural circuits we have found that one sensory neuron class, the multidendritic FLP nociceptors, respond to harsh touch throughout their receptive field but respond to gentle touch only at the tip of the nose. Whereas the FLP harsh touch response depends solely on cell-autonomous mechanosensory channels, gentle nose touch responses require the activities of additional mechanoreceptor classes, OLQ and CEP, which are electrically-coupled to FLP through a gap junction network. Conversely, FLP activity is required to indirectly facilitate nose touch and harsh head touch responses in the OLQs, demonstrating that information flow across the network is bidirectional. Thus, nose touch perception involves a hub-and-spoke network that allows individual sensory neurons to integrate information from multiple interconnected mechanoreceptors. A model of a hub-and-spoke network predicts that inactive neurons will inhibit other cells in the network through shunting; artificial rewiring experiments using ectopic connexin expression support this hypothesis. Thus, the nose touch circuit uses electrical connectivity to detect and generate responses to coincident mechanoreceptor activity.

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Regulation of myosin synthesis, cytosolic protein degradation, and muscular dystrophies by the kinome, phosphatome, and "muscle genes" in *C. elegans*. Susann Lehmann[1], Freya Shephard[1], Tim Etheridge[1], Beth Oczypok[1,2], Ademola A Adenle[1], Lew Jacobson[2], **Nate Szewczyk[2]**. 1) University of Nottingham, School of Graduate Entry Medicine and Health, Royal Derby Hospital, Derby, DE22 3DT, UK; 2) University of Pittsburgh, Department of Biological Sciences, Pittsburgh, PA, 15260, USA.

Over the last 15 years our laboratories have developed *C. elegans* as a system for studying the intramuscular signals regulating protein degradation. We study degradation as it is required for muscle atrophy to occur and because very little is known of how extramuscular signals regulate the four key proteolytic systems within muscle. We have recently analyzed 762 genes for those that have a role in maintaining muscle homeostasis. 168 genes previously identified to regulate muscle function (*unc, egl, eat, exp, pbo, dec, aex, mua, mup, pat* and *rol* genes) were analyzed using mutants and RNAi and 402 kinases and 192 phosphatases were analyzed by RNAi alone. A comparison of the behavioural/developmental defects we observed with past reports suggests a false positive rate of < 1% and a lower limit of the false negative rate of 3%. The variation between our RNAi observations and past observations appears to be ~10% in each set of genes analyzed (e.g. muscle, kinase, phosphatases). Together these results suggest that RNAi can be used very effectively (if proper quality control and replicate procedure are used). Utilizing various transgenic strains, we analyzed RNAi effects on muscle proteostasis (balance of protein synthesis and degradation), protein degradation and myofibrillar and mitochondrial structure. Proteostasis was most commonly affected (254 genes), followed by mitochondrial morphology (178 genes) and myofibrillar morphology (135 genes). These observations fit with the hypothesis that proteostasis provides a buffer against individual gene disruption; we do frequently observe alterations in proteostasis in the absence of developmental/behavioural phenotypes. RNAi treatment of young adults reveals that the majority of genes identified appear to be important in maintaining terminally differentiated muscle: RNAi against 72%, 65%, and 61% of muscle, kinase, and phosphatase genes yields muscular defects (e.g. protein degradation or dystrophy) within 48-72 hours (e.g. early/mid adulthood). We have completed functional classification of the muscle genes that regulate protein degradation (57 genes) into groups acting via common pathways/mechanisms. This classification suggests at least three regulatory networks impinging upon proteasomes, lysosomes, and calpains with genes regulating proteasomes and lysosomes infrequently affecting myofibrillar and/or mitochondrial morphology. This work is funded by US NIH NIAMS (AR054342) and UK MRC (G0801271).

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A High-Resolution C. elegans Essential Gene Network Based on Phenotypic Profiling of a ComplexTissue. **Rebecca A. Green[1,8]**, Huey-Ling Kao[4], Anjon Audhya[3], Arur Swathi[2,9], Jonathan Mayers[3], Heidi Fridolfsson[6], Monty Schulman[4], Siegfried Schloissnig[7], Sherry Niessen[5], Kimberley Laband[1,8], Shaohe Wang[1,8], Daniel Starr[6], Anthony Hyman[7], Tim Schedl[2], Arshad Desai[1,8], Fabio Piano[4], Kristin Gunsalus[4], Karen Oegema[1,8]. 1) Ludwig Institute for Cancer Research, San Diego, CA; 2) Washington University, St. Louis, Missouri; 3) University of Madison, Wisconsin, WI; 4) New York University, New York, NY; 5) The Scripps Research Institute, La Jolla, Ca; 6) University of California, Davis, CA; 7) Max Planck Institute of Molecular Cell Biology and Genetics, Germany; 8) University of California, San Diego, CA; 9) University of Texas, Houston, TX.

High-content screening for gene profiling has generally been limited to single cells. Here, we explore an alternative approach— profiling gene function by analyzing effects of gene knockdowns on the architecture of a complex tissue in a multicellular organism. We profile 554 essential *C. elegans* genes by imaging gonad architecture and scoring 94 phenotypic features. To generate a reference for evaluating methods for network construction, genes were manually partitioned into 102 phenotypic classes, predicting functions for 106 uncharacterized genes across diverse cellular processes. Using this classification as a benchmark, we developed a robust computational method for constructing gene networks from high-content profiles based on a network context-dependent measure that ranks the significance of links between genes. Our analysis reveals that multi-parametric profiling in a complex tissue yields functional maps with a resolution similar to genetic interaction-based profiling in unicellular eukaryotes— pinpointing subunits of macromolecular complexes and components functioning in common cellular processes.

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Two-step evolution of a hybrid operon with both ubiquitous and tissue-specific promoters. Alisson Marques Gontijo[1,2], Fabiana Heredia[2], Sylvie Aubert[1], Diana M. Vallejo[2], Veronica Miguela[2], Maria Dominguez[2], **Bernard Lakowski[1]**. 1) Nematode Genetics Group, Department of Neuroscience, Institut Pasteur, Paris, France; 2) Instituto de Neurociencias de Alicante - CSIC/UMH, Unidad de Neurobiología del Desarrollo, Campus de Sant Joan, Alicante, Spain.

About 15% of all *Caenorhabditis elegans* genes are found within operons of between two to eight genes. Most *C. elegans* operons contain a single promoter at the 5' end which drives the expression of all genes within the operon. However, the existence of a relatively small number of hybrid operons, containing a 5' promoter and an internal promoter, has been demonstrated recently. These structures are relatively poorly studied and their evolution has not been addressed. We provide multiple lines of evidence that the operon CEOP4312, containing the genes *oma-1, spr-2* and C27B7.2 (in that order) is a hybrid operon with a promoter in front of *oma-1* driving germline expression of all three genes and a promoter in front of *spr-2* that drives expression of *spr-2* and, presumably, C27B7.2 in all cells of the soma. Consequently, *spr-2* is both SL1 and SL2 trans-spliced. The *spr-2(ar199)* deletion deletes elements within the *spr-2* promoter essential for somatic expression. Surprisingly, although the *oma-1(tm1396)* deletion deletes the promoter in front of *oma-1* and virtually eliminates SL2 splicing of *spr-2*, *tm1396* does not drastically affect SPR-2 protein expression in the germline. This suggests that there maybe cross talk between the two promoters in the germline and that the use of the promoter in front of *oma-1* may prevent use of the *spr-2* promoter. We also show that the CEOP4312 operon evolved within the Caenorhabditis genus with the association first of *spr-2* and C27B7.2, before *oma-1* inserted in front of these two genes. As these genes were assembled into an operon, their intron/exon structures were altered. Finally, within the Caenorhabditis genus this operon contiues to evolve and has even split into two operons in *C. remanei*.

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A LIN-35/pRb network controlling pharyngeal development through PHA-1. **David S. Fay**, Kumaran Mani. Dept Molec Biol, Univ Wyoming, Laramie, WY.

Genetic screens have previously implicated a role for the C. elegans pRb tumor suppressor ortholog, LIN-35, in pharyngeal development and morphogenesis (1, 2). We find that LIN-35, together with UBC-18/UBCH7-ARI-1/ARIH1, a conserved E2-E3 ubiquitin-ligase complex, cooperate to repress the expression of the Zn-finger protein, SUP-35/ZTF-21 (3-5). Whereas LIN-35 acts in conjunction with the conserved E2F pathway members EFL-1 and HCF-1 to regulate *sup-35* transcription, UBC-18-ARI-1 appears to regulate SUP-35 at the level of protein stability. SUP-35 in turn acts to repress the expression of PHA-1, a cytoplasmic protein of unknown function that is required for embryonic and pharyngeal development (6-8). Thus, in *lin-35; ubc-18* double mutants, PHA-1 expression is strongly reduced due to increased levels of the SUP-35 transcriptional repressor. Furthermore, we find that inhibition of PHA-1 expression by SUP-35 requires two additional factors, SUP-37/ZTF-12, a Zn-finger protein, and SUP-36/F38A5.7, a novel protein. Genetic epistasis and expression data suggest that SUP-35-37 are likely to act a common step, possibly within a single complex, in order to repress pha-1 transcription. Additionally, SUP-37 carries out an essential role in larval pharyngeal pumping that is separable from its role in PHA-1 regulation. Our findings also suggest a potential role for the microtuble cytoskeleton in the activity of SUP-35-SUP-37 given that (1) SUP-36 can physically associate with PTL-1, a member of the tau family of microtuble-binding proteins, (2) SUP-35 contains an RMD domain that places it within a conserved family of regulators of microtubule dynamics, and (3) *pha-1* mutants show strong synthetic lethality with *tbb-1*, which encodes for a homolog of beta-tubulin. Finally, a genome-wide RNAi suppressor screen has identified a number of additional factors that contribute to the regulation of this developmental network.

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Core promoter T-blocks correlate with gene expression levels in *C. elegans*. **Uladzislau Hryshkevich**, Tamar Hashimshony, Itai Yanai. Technion Israel Institute of Technology, Haifa, Israel.

Core promoters mediate transcription initiation by the integration of diverse regulatory signals encoded in the proximal promoter and enhancers. It has been suggested that genes under simple regulation may have low-complexity permissive promoters. For these genes, the core promoter may serve as the principal regulatory element; however the mechanism by which this occurs is unclear. We report here a periodic poly-thymine motif, we term T-blocks, enriched in occurrences within core promoter forward strands in *C. elegans*. An increasing number of Tblocks on either strand is associated with increasing nucleosome eviction. Strikingly, only forward strand T-blocks are correlated with expression levels, whereby genes with ≥6 T-blocks have five fold higher expression levels than genes with ≤3 T-blocks. We further demonstrate that differences in T-block numbers between strains predictably affect expression levels of orthologs. Highly expressed genes and genes in operons tend to have a large number of T-blocks, as well as the previously characterized SL1 motif involved in trans-splicing. The presence of T-blocks thus correlates with low nucleosome occupancy and the precision of a trans-splicing motif, suggesting its role at both the DNA and RNA levels. Collectively our results suggest that core promoters may tune gene expression levels through the occurrences of T-blocks, independently of the spatio-temporal regulation mediated by the proximal promoter.

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A quantitative RNA code for mRNA target selection by the germ line fate determinant GLD-1. **Jane E. Wright[1]**, Dimos Gaidatzis[1], Mathias Senften[1], Brian M. Farley[2], Eric Westhof[3], Sean P. Ryder[2], Rafal Ciosk[1]. 1) Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; 2) Department of Biochemistry and Molecular Pharmacology, University of Massachusetts Medical School, Worcester, USA; 3) Institut de biologie moléculaire et cellulaire du CNRS, Strasbourg France.

RNA-binding proteins (RBPs) are critical regulators of gene expression and comprehensive analysis of their interaction with RNA is required to understand and predict their effect on transcriptome expression. The STAR family of RBPs includes developmental regulators and tumor suppressors such as C. elegans GLD-1, which is a key regulator of germ cell development. To obtain a comprehensive picture of GLD-1 interactions with the transcriptome, we identified GLD-1-associated mRNAs by a ribonomic approach. Based on computational analysis of these mRNAs we generated a predictive model, where GLD-1 association with mRNA is determined by the strength and number of 7-mer GLD-1 binding motifs (GBMs) within UTRs. We verified this quantitative model both in vitro, by competition GLD-1/GBM binding experiments to determine relative affinity, and in vivo, including by transplantation experiments, where weak and strong GBMs imposed translational repression of increasing strength on a non-target mRNA. This study demonstrates the feasibility and the importance of a computational approach to quantify RBP/mRNA interactions, which may be used in the future to predict biological outcomes of posttranscriptional regulatory networks.

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The TransgeneOme of *C. elegans*: a platform for *in vivo* analysis of protein function. **Mihail S. Sarov[1]**, John Murray[2,7], Kristin Schanze[1,5], Andrei Pozniakovski[3], Karolin Angerman[1,5], Susanne Ernst[3], Andrea Zinke[3], Tina Teichgraber[3], Judith Janette[4], Siegfried Schloissnig[1], Susanne Hasse[1], Matthew Tinney[1], Elizabeth Vinis[1], Michaela Rupprecht[1,5], Stuart Kim[6], Valerie Reinke[4], Francis Stewart[5], Michael Snyder[6], Robert Waterston[2], Anthony Hyman[3]. 1) TransgeneOmics, Max Planck Institute for Molecular Cell Biology and Genetics, Dresden, Germany; 2) University of Washington School of Medicine, Seattle, Washington; 3) Hyman lab, Max Planck Institute for Molecular Cell Biology and Genetics, Germany; 4) Yale University, New Haven, CT; 5) University of Technology Dresden, Germany; 6) Stanford University Medical Center, Stanford, CA; 7) University of Pennsylvania School of Medicine.

We have developed a genome wide tag based platform for rapid *in vivo* function analysis of any *C. elegans* protein of interest. We used high-throughput recombineering to systematically engineer a "synthetic genome" collection of large genomic fragments caring fluorescent and affinity tagged alleles for each protein coding gene in the *C. elegans* genome. Upon genome integration these large transgenes behave like additional alleles expressing a tagged version of the protein of interest, which can be localized, biochemically purified or quantified using generic, tag based assays. Because all *cis* acting elements are typically included in a construct of this size the endogenous regulation mediated by regulatory proteins and RNAs at the level of transcription, splicing, message turnover and translation are maintained and can reveal fine-grained expression pattern dynamics that are not possible with the traditional cDNA based transgenes or promoter reporter constructs. The resource was successfully utilized in the modENCODE project to describe transcription factor localization at both molecular level (by ChIP-seq) and cellular level (by *in vivo* fluorescent microscopy).

The availability of the TransgeneOme resource opens the possibility to uncover the function of thousands of previously unstudied proteins, and would eventually allow us to build a systems wide atlas of protein localizations and interactions within the context of a multicellular model organism. Distribution of the effort throughout the community can significantly cut down the time required to achieve this goal. The use of a standard tag based techniques would make independently obtained data more comparable and easily reproducible. Finally, many of the transgenes would prove useful as reporters for loss of function studies, which will dramatically expand the accessible phenotypic space for both classic and reverse genetic approaches.

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Dissecting *C. elegans* insulins using a systems approach. **Ashlyn Ritter**[1], Heidi Tissenbaum[1,2], Marian Walhout[1,2,3]. 1) Program in Gene Function & Expression, Univ. of Massachusetts Medical School, Worcester, MA; 2) Program in Molecular Medicine, Univ. of Massachusetts Medical School, Worcester, MA; 3) Program in Bioinformatics and Integrative Biology, University of Massachusetts Medical School, Worcester, MA.

The insulin/IGF-1 signaling pathway plays a fundamental role in the aging process. This important pathway has been extensively studied in humans and a variety of model organisms. In *C. elegans*, the insulin/IGF-1 signaling pathway modulates fat storage, development, stress resistance in addition to life span, and these roles are conserved across phylogeny. Remarkably, while vertebrates have only a few insulin genes, the *C. elegans* genome encodes 40 insulin genes. Whereas the downstream effects of the *C. elegans* insulin/IGF-1 signaling pathway have been studied extensively, the upstream insulin ligands remain largely uncharacterized.

We used a systems biology approach to gain insight into the functional significance of the dramatic expansion of this important gene family. We first asked where and when each insulin gene is expressed during development. We identified four main classes of expression; the nervous system, the reproductive system, the digestive system and the muscle. Each of these expression patterns exemplifies tissues that play important roles in the function of mammalian insulin and insulin-like peptides.

Since the insulin/IGF-1 pathway is involved in numerous physiological processes, we further investigated the changes in expression of each of the insulin genes under a variety of relevant conditions and insults, including oxidative stress and heat stress, aging and exposure to glucose. We found dramatic changes in some, but not all insulins under most conditions, and identified a single insulin gene that responds to most conditions. By modeling these observations into an insulin gene expression network, we uncovered principles of redundancy and specificity, and derived numerous detailed hypotheses of the function of individual insulin genes. Altogether, our results indicate a "division of labor" by which multiple insulin genes act during the lifetime of the animal and under different developmental, physiological and environmental conditions.

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Distinct functional constraints partition sequence conservation in a *cis*-regulatory element. **Kacy Gordon**[1], Antoine Barrière[2], Ilya Ruvinsky[1,2]. 1) Department of Organismal Biology and Anatomy, The University of Chicago; 2) Department of Ecology and Evolution and Institute for Genomics and Systems Biology, The University of Chicago.

Different functional constraints contribute to different evolutionary rates across genomes. To understand why some sequences evolve faster than others in a single *cis*-regulatory locus, we investigated function and evolutionary dynamics of the promoter of the *C. elegans unc-47* gene. We found that this promoter consists of two distinct domains. The proximal promoter is conserved and is largely sufficient to direct appropriate spatial expression. The distal promoter displays little if any conservation between several closely related nematodes. Despite this divergence, sequences from all species confer robustness of expression, arguing that this function does not require substantial sequence conservation. We showed that even unrelated sequences have the ability to promote robust expression. A prominent feature shared by all of these robustness-promoting sequences is an AT-enriched nucleotide composition consistent with nucleosome depletion. Such AT-rich, nucleosome-depleted tracts are common upstream of many *C. elegans* genes, suggesting that robust expression of many genes may be ensured by regions of open chromatin. Because general sequence composition can be maintained despite sequence turnover, our results explain how different functional constraints can lead to vastly disparate rates of sequence divergence within a promoter.

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Chromosome-biased binding and gene regulation by the *C. elegans* DRM complex. Tomoko M. Tabuchi[1], Bart Deplancke[2], Naoki Osato[2], Lihua J. Zhu[2], M. Inmaculada Barrasa[2], Melisa M. Harrison[3], H. Robert Horvitz[3], Albertha J.M. Walhout[2], **Kirsten A. Hagstrom**[1]. 1) Program in Molecular Medicine, UMass Medical School, Worcester, MA; 2) Program in Gene Function and Expression, UMass Medical School, Worcester, MA; 3) Howard Hughes Medical Institute, Department of Biology, MIT, Cambridge, MA.

X chromosomes differ in number between the sexes, and differ from autosomes in their associated proteins and gene regulatory properties. In *C. elegans* both X chromosomes are partially silenced in hermaphrodite germlines. Germline-expressed and essential genes are autosome-enriched, and thought to have fled the X chromosome during evolution because silencing of these genes would result in sterility or lethality. Here we show that the *C. elegans* DRM transcription factor complex is autosome-enriched yet regulates X chromosome gene expression in the germline. Using genome-wide chromatin immunoprecipitation and gene expression profiling, we find that DRM regulates genes involved in cell division, development, and reproduction. DRM promotes activation of reproduction genes in the germline but prevents ectopic expression of germline-specific genes in the soma. Strikingly, the DRM binding motif, the genes bound by DRM, and the embryonic genes regulated by DRM are all under-represented on the X chromosome. DRM is autosome-enriched, yet its loss of its function in the germline down-regulates many X-linked genes. This is reminiscent but opposite of the histone methyltransferase MES-4, which is autosome-enriched yet its loss of function up-regulates X-linked gene expression. To test the hypothesis that DRM and MES-4 may counteract each other to provide the right balance of X chromosome gene expression, we analyzed double mutants. Indeed, we find that X-linked genes down-regulated in the DRM mutant *lin-54* are restored to more normal levels of expression in *lin-54; mes-4* double mutant germlines. Moreover, mutations of *mes-4* suppress the sterility of *lin-54* mutants. We speculate that DRM and MES-4 are each affecting the X chromosome versus autosome distribution of an unknown transcriptional regulator, but in opposite directions, so that in double mutants balanced X chromosome gene expression is restored.

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Transcriptome and proteome profiling during oocyte-to-embryo transition in *Caenorhabditis elegans* suggests paternal contribution to early development. **Marlon Stoeckius**[1], Dominic Grün[1], Marieluise Kirchner[2], Nadine Thierfelder[1], Wei Chen[1], Matthias Selbach[2], Fabio Piano[3], Nikolaus Rajewsky[1]. 1) Systems Biology of Gene Regulation, BIMSB, Berlin, Germany; 2) Cell Signalling & Mass Spectrometry, MDC-Berlin, Berlin, Germany; 3) Genomics & Systems Biology, NYU, New York, USA.

The oocyte-to-embryo transition is a fundamental event in early development and one of the most dynamic processes in biology. During this transition the fusion of two highly specialized cells, sperm and oocyte, triggers the development of a mitotically-dividing totipotent embryo. This entire process is thought to be driven by post-transcriptional gene regulation in most animals. In *C. elegans* not only the oocyte-to-embryo transition, but also the first two cell divisions are thought to be entirely post-transcriptionally driven. During these divisions stereotypical lineage and axis specification is already occurring. It is believed that these processes rely on maternally provided mRNAs and proteins. Potential critical functions of sperm components are still a matter of debate. Although a number of key players are known, the oocyte-to-embryo transition remains poorly understood. Therefore, we sought to obtain a global characterization of changes on coding and non-coding transcriptome and proteome during this fundamental developmental process in *C. elegans*. While oocytes can be obtained in large quantities, collecting many precisely staged embryos has been impractical. Thus, *C. elegans* embryogenesis has not been amenable to most high-throughput genomics or biochemistry assays. We devised a method to collect staged *C. elegans* embryos by fluorescence-activated cell sorting (eFACS). A single eFACS run routinely yields tens of thousands of almost perfectly staged one-cell stage embryos. To this end, we developed a method that uses stable isotope labeling by amino acids to quantify changes in the abundance of thousands of proteins between any two *C. elegans* samples. We combined these techniques to profile the expression of coding and non-coding RNAs, small RNAs and the amount of up to 4,800 proteins in oocytes, one-cell-, two-cell stage embryos and sperm. We discern highly orchestrated and decoupled changes of proteins and mRNAs, indicating a high level of post-transcriptional regulation upon fertilization. We also discovered dynamic expression between and within almost all classes of small RNAs. We have evidence for a large number of putatively sperm-derived mRNAs, 21U-RNAs and proteins in the zygote, suggesting an unexplored substantial paternal contribution to early development. Moreover the data challenges the notion that the early *C. elegans* embryo is transcriptionally silent. We will report on the currently ongoing analysis and validations of our data.

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A Core C. elegans Transcription Factor Regulatory Network. **John S. Reece-Hoyes**[1,2], Amanda Kent[1,2], Shaleen Shrestha[1,2], Alos Diallo[1,2], Jiali Zhuang[1,2,3], Amelie Dricot[4], Zhiping Weng[3], David Hill[4], Chad Myers[5], Albertha J.M. Walhout[1,2,4]. 1) Program in Gene Function and Expression, UMass Medical School, Worcester, MA; 2) Program in Molecular Medicine, UMass Medical School, Worcester, MA; 3) Program in Bioinformatics and Integrative Biology, UMass Medical School, Worcester, MA; 4) Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Boston, MA; 5) Department of Computer Science and Engineering, University of Minnesota-Twin Cities, Minneapolis, MN.

Differential gene expression is controlled by a complex regulatory network of interactions between proteins, DNA, and microRNAs. Transcription factors (TFs) are primary modulators within this network, largely by binding to specific sites within a promoter and activating or repressing nearby genes. Some of these downstream genes encode TFs that subsequently also regulate a combination TFs and non-TFs. By focusing on TFs and their TF targets, the central core of the greater gene regulatory network will be revealed. Yeast one-hybrid (Y1H) assays provide a gene-centered approach for detecting interactions between genomic loci such as promoters and TF proteins. We comprehensively screened interactions between 650 TF-encoding gene promoters and 834 TF proteins using our novel enhanced Y1H (eY1H) pipeline that yields a quantitative readout of protein-DNA interactions. We will present this novel pipeline as well as insights we obtained from the core TF network that consists of more than 4500 interactions.

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Towards molecular understanding of gene localization. **Peter Meister**, Benjamin Towbin, Sabine Rohner, Susan Gasser. Functional Implications of Nuclear Organization, FMI-Novartis Research Foundation, Basel, Switzerland.

To understand whether the spatial organization of the genome reflects the cell's differentiated state, we have examined whether genes assume specific subnuclear positions during C. elegans development. Monitoring the radial position of developmentally controlled promoters in embryos and larval tissues, we found that small integrated transgenes bearing three different tissue-specific promoters have no preferential position in nuclei of undifferentiated embryos. However, in differentiated cells they shifted stably towards the nuclear lumen when activated, or to the nuclear envelope when silent. We uncovered two additional parameters which influence subnuclear positioning. First, high copy number repeats targets arrays to the nuclear rim, even in the presence of active housekeeping promoters. Tissue-specific activation of promoters in the arrays overrides perinuclear anchoring. Second, some promoters contain sequences which target them to the nuclear periphery, both in their active and inactive form. Using genetic and biochemical methods, we are now using our system to uncover the molecular machinery involved in subnuclear positioning of genes.

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Evidence that cAMP signaling in the somatic gonad transduces the MSP signal for oocyte meiotic maturation via translational regulation by OMA ribonucleoprotein particles. **Seongseop Kim**, Caroline Spike, Todd Starich, J. Amaranath Govindan, Saravanapriah Nadarajan, David Greenstein. Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN USA.

Oocyte meiotic maturation is a conserved developmental transition, defects in which are the major cause of human birth defects and infertility. The meiotic maturation processes in *C. elegans* and mammals share a number of biological and molecular similarities. Major sperm protein (MSP) and luteinizing hormone, though unrelated in sequence, both trigger meiotic resumption using somatic cAMP signaling and gap-junctional communication pathways. Here we describe evidence that transduction of the MSP signal by somatic $G\alpha_s$-adenylate cyclase-protein kinase A (PKA) signaling involves the regulation of translation in the germline by the TIS11 zinc finger proteins OMA-1 and OMA-2. To identify effectors of MSP signaling, we conducted a genetic screen for mutations that suppress the sterile phenotype caused by a null mutation in *acy-4*. We recovered 63 suppressor of adenylate cyclase (*sacy*) mutations, defining at least sixteen genes. Thus far, we have identified two groups of *sacy* mutations. The first encodes components of the CoREST repressor complex. CoREST appears to function downstream of PKA, and the suppression requires OMA-1 and OMA-2 function. CoREST may set the context for MSP signaling by preventing the expression of factors that alter germline translational control. The second group includes a highly conserved DEAD-box helicase shown to be required in translational regulation in *Drosophila*. Interestingly, a biochemical purification of OMA ribonucleoprotein particles (OMA RNPs) also identified this helicase. OMA RNPs contain a large number of germline-expressed RNA-binding proteins including translational activators and repressors. OMA RNP components include multiple subunits of the GLD-2 poly(A) polymerase and CCR4/NOT1 deadenylase complexes and many RNA-binding proteins, including MEX-3, MEX-1, and LIN-41. We used microarray analysis to define mRNA components of OMA RNPs. This analysis identified a set of approximately 400 germline-expressed mRNAs that are highly enriched in OMA RNPs. Several tested mRNA targets, including *cdc-25.3*, are regulated by OMA-1/2. The genetic and biochemical identification of protein and mRNA components of OMA RNPs provides a unique opportunity to determine how reception of the MSP signal by the somatic gonad is transduced into the meiotic maturation response.

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The sperm activation protease TRY-5 is transferred to hermaphrodites during mating. **Joseph R. Smith**, Gillian Stanfield. Dept Human Gen, Univ Utah, Salt Lake City, UT.

What is expressed in males does not always stay in males. During mating, males transfer both sperm and seminal fluid factors that promote male fertility through effects on sperm and on female physiology. In *C. elegans*, little is known about the composition of seminal fluid or its roles in fertility. We have identified a seminal fluid protease that regulates sperm activation, the step of sperm differentiation at which nematode sperm become motile and competent for fertilization. Normally, male sperm are stored in an immature form until mating, when they become activated. *try-5* is required for males to transfer activator to hermaphrodites during mating. However, *try-5* is not required for fertility, likely due to a separate pathway that functions in hermaphrodites to activate their self sperm. Indeed, animals mutant for both the hermaphrodite *spe-8* group pathway and *try-5* are completely infertile, suggesting that all pathways to activation have been removed. We have found that *try-5* is expressed in the male somatic gonad in cells of the seminal vesicle, valve, and vas deferens. Within secretory cells in this region, TRY-5::GFP localizes to vesicle-like structures lining the lumen. During mating, it is transferred to hermaphrodites. Different cell types release TRY-5::GFP in a specific order, revealing discrete steps of seminal fluid and sperm transfer. After spicule insertion, TRY-5::GFP is released first from the vas deferens and rapidly transferred to the hermaphrodite; it is then released and transferred from the valve region; and finally sperm are transferred from the seminal vesicle. In older males, and in males lacking the protease inhibitor SWM-1, TRY-5::GFP spreads into the seminal vesicle and sperm are prematurely activated, indicating that regulation of its release is crucial for male fertility. Taken together, our results suggest that TRY-5 is the male sperm activation signal provided during mating to couple the onset of sperm motility to entry into the hermaphrodite. TRY-5 represents the first *C. elegans* seminal fluid factor with a role in gamete function. In addition, TRY-5::GFP should provide a useful tool for dissecting the process of seminal fluid transfer during the mating behavior program.

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NRDE-1/MRT-4 defects result in a heritable toxic stress that can remodel germ cell development. **Jacinth Mitchell**[1,2], Ashley Hedges[2], Alicia Simmons[2,4], Malik Godwin[2], Yan Liu[2,5], Aisa Nakashima[2], Kirk Burkhart[6,7], Scott Kennedy[6,7], Shawn Ahmed[1,2,3]. 1) Curriculum in Genetics and Molecular Biology, University of North Carolina at Chapel Hill; 2) Department of Genetics; 3) Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, N.C; 4) Department of Toxicology, N.C. State University, Raleigh, N.C; 5) Laboratory of Biochemistry and Genetics, NIDDK, NIH, Bethesida, M.D; 6) Department of Pharmacology, University of Wisconsin; 7) Program in Molecular and Cellular harmacology, University of Wisconsin, Madison, WI 53706.

Immortal germ cell lineages contain the capacity for unlimited proliferative ability, through successive generations. To better understand how proliferative aging is prevented in the germ line, *mortal germline* mutants were isolated, which show progressive sterility when propagated for multiple generations. Two alleles of *mortal germline-4* (*mrt-4*), *yp4* and *yp5*, were identified, which initially display normal levels of fertility and become sterile after growth for 8 to 14 generations at 20°C. As sterile *mrt-4* mutants develop, L4 larvae with apparently normal germ lines mature into adults with few to no germ cells. To investigate the mechanism of this germline remodeling, double mutants of *mrt-4* with *ced-3*, a caspase that is essential for apotosis, were constructed. Although alteration to the apoptotic cell death pathway does not suppress the progressive sterility phenotype of *mrt-4*, *ced-3* abolished the germline restructuring phenotype observed in most sterile *mrt-4* adults. Our results suggest that a heritable form of endogenous stress builds up in the absence of *mrt-4*, ultimately resulting in a caspase-mediated remodeling of the germline, which may correspond to a form of Adult Reproductive Diapause (1). *mrt-4* was cloned and corresponds to *nrde-1*, a gene that is required for nuclear RNA interference (2). Although not all nrde genes are required for germ cell immortality, nuclear silencing defects may underlie the heritable form of toxic stress that causes sterility in *mrt-4*/*nrde-1* strains.

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Sensory control of germline differentiation via TGFβ. **Diana Dalfó**, E. Jane Albert Hubbard. Developmental Genetics Program, Helen and Martin Kimmel Center for Stem Cell Biology, Skirball Institute of Biomolecular Medicine, NYU Cancer Institute, Department of Pathology, NYU School of Medicine, NY.

The balance between proliferation and differentiation is critical for stem and progenitor cell populations. Defects in this balance during development can be deleterious to the establishment of adult stem cells. However, the mechanisms that influence this balance are poorly understood. GLP-1/Notch signaling is important for maintaining the proliferating population of *C. elegans* germ cells: loss of *glp-1* activity causes all germ cells to differentiate. We have shown that Notch-independent signaling pathways such as insulin/IGF contribute to the robust proliferation of the larval germ line, and that this is required for optimal fecundity. In large-scale RNAi screens for genes affecting early germline proliferation and/or differentiation, we identified genes encoding TGFβ "dauer" pathway components. Further analysis indicates a role for this TGFβ pathway in the regulation of proliferation versus differentiation in the *C. elegans* germ line. Like its role in the dauer decision, this role is dependent on *daf-3*/Co-SMAD and *daf-5*/SNO/SKI. Unlike dauer, however, it is *daf-12*/NHR-independent. We find that the TGFβ ligand-producing ASI sensory neurons are required for TGFβ-mediated germ cell accumulation, and that the TGFβ receptor and downstream transcription complex acts in the distal tip cell, in parallel with GLP-1/Notch signaling. Our results implicate the TGFβ pathway as a mediator between environmental cues perceived by specific sensory neurons and a stem cell niche to influence the proliferation/differentiation balance.

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Noncanonical control of germline apoptosis by the insulin/IGF-1 signaling pathway. Andrew J. Perrin[1,2], Bin Yu[1], Kelvin Yen[3], Shu Ito[1,2], Heidi Tissenbaum[3,4], **W. Brent Derry[1,2]**. 1) Developmental & Stem Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Ontario, Canada; 3) Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America; 4) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, United States of America.

We previously demonstrated that *akt-1* regulates DNA damage-induced apoptosis of germ cells by antagonizing the activity of CEP-1, the sole p53 family member (Quevedo *et al*., *Curr Biol*, Vol. 17: 286-92. 2007). Because the anti-apoptotic activity of Akt is induced by receptor tyrosine kinases and PI3K in mammals, we hypothesized that the insulin-like/PI3K pathway would fulfill this function in *C. elegans*. When we examined the response of multiple *daf-2(lf)* and *pdk-1(lf)* mutants to IR, however, we were surprised to observe that they were resistant to damage-induced germline apoptosis. Furthermore, germ cells in *pdk-1(gf)* mutants were hypersensitive to IR, confirming that *daf-2* and *pdk-1* functionally oppose *akt-1*. To determine whether *akt-1* could still function downstream of the insulin-like receptor, we inhibited *daf-2* by RNAi and found that this suppressed apoptosis in *akt-1(lf)* mutants. The CEP-1 pro-apoptotic target gene *egl-1* was induced by IR in *daf-2* and *pdk-1* mutants, suggesting that these components of the insulin-like pathway bypass *cep-1* to promote apoptosis. In addition, the *pdk-1(gf)* mutation was not able to restore apoptosis sensitivity in *daf-2(lf)* mutants, indicating that *akt-1* and *pdk-1* likely function independently of *daf-2* in the control of germline apoptosis. Consistent with these findings, DNA damage did not affect phosphorylation of the PDK-1 site in AKT-1 (Thr350/Thr308) but increased phosphorylation at Ser517/Ser473 by ~2-fold. *daf-16* does still does mediate some of the apoptotic effects of *daf-2*, however, since loss of this transcription factor partially rescued apoptosis in *daf-2(lf)* mutant worms. This structural fragmentation of the worm insulin-like pathway is reinforced by different tissue-specific requirements for *akt-1* and *daf-2*. Whereas, *akt-1* functions autonomously in the germline to modulate the apoptotic activity of CEP-1, *daf-2* is required in both the soma and germline to promote apoptosis in parallel to, or downstream of, *cep-1*. Our data implies that differing functional modules of the insulin-like/PI3K signaling pathway can be selected in a stimulus-specific manner *in vivo*.

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P granules extend the nuclear pore complex environment in the C. elegans germ line. **Dustin Updike**, Susan Strome. MCD Biol, Univ California Santa Cruz, Santa Cruz, CA.

C. elegans germline development relies heavily upon post-transcriptional regulation in the germ plasm. Within the germ plasm, ribonucleoprotein aggregates called P granules overlie nuclear pore clusters and receive mRNAs as they are exported from the nucleus. A number of P-granule components, including the Vasa-related proteins GLH-1, GLH-2, and GLH-4, contain phenylalanine-glycine (FG) repeat domains, which are a common feature of nuclear pore complex (NPC) proteins. Within the NPC, FG-rich domains form a cohesive meshwork of filaments through hydrophobic interactions involving the phenylalanines in the FG motifs, creating a size-exclusion barrier that prevents diffusion of molecules larger than 45 kDa between the nucleus and the cytoplasm. We demonstrated that P granules, like NPCs, are held together by weak hydrophobic interactions and that they also establish a size-exclusion barrier similar to that of NPCs within the germ plasm. By expressing P-granule components in heterologous (intestinal) cells, we show that GLH-1 and its FG domain are not sufficient to form granules, but require factors like PGL-1 to nucleate the localized concentration of GLH proteins. GLH-1 is necessary but not sufficient to target intestinal PGL granules to the nuclear periphery. Our results provide insights into the roles of the PGL and GLH families of proteins and suggest that P granules extend the NPC environment in the germ line to create a specialized hydrophobic microenvironment that may facilitate post-transcriptional processing events while selectively excluding large protein complexes from gaining access to mRNAs and endogenous siRNAs.

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Complementary mechanisms to specify hermaphrodite development during evolution. **Yiqing Guo**, Ronald Ellis. Dept Molec Biol, UMDNJ, Stratford, NJ.

To learn how novel traits are created, we are studying the origin of self-fertile hermaphrodites in the nematode genus *Caenorhabditis*. Although most species in this group are male/female, three separate lineages have acquired the ability to make self-fertile hermaphrodites.

Throughout the genus, spermatogenesis is initiated by *fog-1* and *fog-3*, which are themselves repressed by the master transcription factor TRA-1. Thus, the activity of TRA-1 has to be limited in *XX* hermaphrodites to a range that allows *fog-1* and *fog-3* to be turned on during larval development, and turned off in adults.

We have shown that *C. briggsae* and *C. elegans* use unrelated F-box proteins to direct hermaphrodite spermatogenesis. The *C. briggsae* SHE-1 and *C. elegans* FOG-2 proteins work by distinct mechanisms to control the receptor TRA-2, which regulates TRA-1 activity.

But how is TRA-1 activity kept at a level where these genes can alter germ cell fates effectively? In *C. elegans*, the FEM proteins promote the ubiquitinylation and degradation of TRA-1, and are required for spermatogenesis in both sexes. In *C. briggsae*, however, mutations in the fem genes do not block spermatogenesis (Hill et al. 2006), except in special genetic backgrounds.

We found that *trr-1* and other components of the Tip60 Histone Acetyl Transferase complex are essential for spermatogenesis in both sexes of *C. briggsae*. Moreover, *trr-1* acts just upstream of TRA-1, at the same regulatory level as the *fem* genes. Further, if *trr-1* activity is lowered, the FEM proteins become essential for spermatogenesis in *C. briggsae*.

By contrast, mutations in *C. elegans trr-1* do not block spermatogenesis. However, if the activity of the *C. elegans fem* genes is decreased, the animals need TRR-1 to make sperm.

Thus, the Tip60 HAT complex and the FEM proteins play partially conserved roles in sex determination. However, each hermaphroditic species has adapted a unique mix of their regulatory activities to control the master transcription factor TRA-1.

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Sexual plasticity and behavior in a sexually polymorphic species. **J. Chaudhuri**, V. Kache, A. Pires da Silva. Department of Biology, The University of Texas at Arlington, Arlington, TX.

Statement of purpose:

Nematodes are ideal for studying the evolution of mating systems because the phylum includes both a large range of reproductive modes and large numbers of evolutionarily independent switches. Our experimental model to study the evolutionary plasticity of reproductive modes is the free-living and lab-culturable nematode *Rhabditis* sp. SB347 (SB347). Like the model nematode *Caenorhabditis elegans*, SB347 can reproduce either by self-fertilization or outcrossing. However, unlike *C. elegans*, the XX animals exhibit reproductive plasticity as they can develop into either females or selfing hermaphrodites. Under standard conditions, SB347 XX females and XO males molt through four molting feeding larval stages, whereas XX hermaphrodites develop through an obligatory non-feeding stage, the dauer larva. We show that passage through dauer is necessary and sufficient for hermaphrodite development in *Rhabditis* sp. SB347. We also report that secreted molecules by females make them more attractive to males than hermaphrodites.

Methods:

To test the role of dauer formation for hermaphrodite development, we suppressed dauer development in SB347 by administering hormones named dafachronic acids (DAs). DAs are known to be nematode steroid hormones that serve as ligand for the DAF-12 receptor, a nuclear receptor that regulates dauer formation. We also forced female larvae to become dauers by exposing them to dauer-inducing conditions. Using laser ablation, we identified the cells in females that secrete male-attracting pheromones.

Results and conclusions:

Our experiments show that dauer inhibition redirected larvae that usually develop into hermaphrodites to develop into females. We could also redirect female larvae (L1 stage) to develop as hermaphrodites by inducing them through the dauer stage. These findings clearly indicate that the dauer pathway is coupled to germline sex determination. In contrast to hermaphrodites, SB347 females secrete pheromones that attract males. Overall, our results report a species of free-living nematode that uses pheromones and DAs to regulate sexual differentiation and behavior.

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Update on *Caenorhabditis* species discovery, biogeography, phylogeny and evolution. **Karin C. Kiontke**[1], Marie-Anne Félix[2], Michael Ailion[3], Christian Braendle[4], Jean-Baptiste Pénigault[2], Matthew V. Rockman[1,5], David H.A. Fitch[1]. 1) Dept Biol, New York University, New York, USA; 2) CNRS-Institut Jacques Monod, Paris, France; 3) Dept Biol, University of Utah, Salt Lake City, USA; 4) Inst Dev Bio & Cancer, CNRS-University of Nice, Nice, France; 5) Center for Genomics and Systems Biology, New York University, New York, USA.

Since the 17th International *C. elegans* meeting, 7 more *Caenorhabditis* species were discovered. There are now 26 species in culture. Most of these new species were isolated from rotting fruit, supporting the previous observation that *Caenorhabditis* are "fruit worms", not soil nematodes. All species are reproductively isolated, but partially or fully sterile hybrids are produced in crosses between *C. briggsae* and *C.* sp. 9 and between *C. angaria* and *C.* sp. 12. Several species can co-occur in the same location or even the same fruit. At the global level, some species have a cosmopolitan distribution: *C. briggsae* is particularly widespread, while *C. elegans* and *C. remanei* are found mostly or exclusively in temperate regions, and *C. brenneri* and *C.* sp. 11 exclusively in tropical zones. Other species have limited distributions, for example *C.* sp. 5 appears to be restricted to China, *C.* sp. 7 to West Africa and *C.* sp. 8 to the Eastern United States.

We used sequence data from two rRNA genes and 9 protein-coding genes to elucidate the phylogenetic relationships of 25 *Caenorhabditis* species in culture (the 26th species will be added). The phylogeny shows two monophyletic groups, the *Elegans* super-group with 17 species and the *Drosophilae* group with 7 species. *C.* sp. 1 and *C. plicata* are positioned outside of these groups as the first two branches of the tree. The *Elegans* super-group contains one well-supported clade which comprises 9 species, including *C. elegans, C. briggsae* and 5 other genome-sequenced species. Two further species with sequenced genomes are members of the *Elegans* super-group and *C. angaria* belongs to the *Drosophilae* group. We still do not know of a sister species of *C. elegans*. We scored phenotypic characters such as reproductive mode, mating behavior and male tail morphology, and tested congruence of their distribution on the phylogeny. A notched terminal end of the fan evolved once in the *Elegans* super-group; a narrow fan and spiral copulation evolved once in the stem species of *C. angaria, C.* sp. 8 and *C.* sp. 12. We find that several other character changes occurred convergently. For example, hermaphroditism evolved three times independently in *C. elegans, C. briggsae* and *C.* sp. 11. We also developed ITS barcodes for easy species identification.

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Molecular phylogeny and divergence time estimates for beetle-associated Pristionchus nematodes. **Ralf J. Sommer**. Dept Evolutionary Biol, MPI Developmental Biology, 72076 Tuebingen, Baden-Wuertemberg, Germany.

Pristionchus pacificus has been established as a model system in evolutionary developmental biology (evo-devo) and for comparison to *C. elegans*. Recent studies expanded the evo-devo work to ecology and population genetics. *P. pacificus* and related species have a well-defined association with scarab beetles: Sampling of more than 20,000 scarab beetles from around the world resulted in the isolation of more than 8,000 *Pristionchus* isogenic female lines. Mating experiments and molecular sequence analysis identified 26 species to date. We provide a molecular phylogenetic framework based on nearly 11,000 characters. Studies on La Réunion, an Island with a unique *P. pacificus* biodiversity hotspot, provide a case study to link population genetics with ecology and evo-devo. We obtained more than 400 wild isolates of *P. pacificus* and carried out microsatellite analysis of 21 markers. These studies indicate four major biogeographic clades of *P. pacificus*. La Réunion represents the only geographic area with *P. pacificus* strains being present in all four clades. This finding is best explained by *P. pacificus* invading the island independently with different beetle vectors, such as *Oryctes*, *Amneidus* and *Maladera* (Herrmann et al., 2010). We assessed the molecular phylogeny of *P. pacificus* and used mutation accumulation line approaches to provide divergence time estimates. Mutation rates were assessed first, by mitochondrial DNA analysis and second, from representative microsatellite markers. Mitochondrial DNA analysis suggests a minimal divergence time for *P. pacificus* of 105 to 106 generations (Molnar et al., 2011). Similarly, microsatellite markers are used to robustly provide minimal divergence time estimates for closely related strains from La Réunion. This includes a first attempt for a serious analysis of the population size of *P. pacificus*.

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Natural variation in a glutamate-gated chloride channel subunit confers resistance to avermectins. **Rajarshi Ghosh**, Justin Gerke, Erik Andersen, Leonid Kruglyak. Lewis Sigler Institute of Integrative Genomics,Ecology and Evolutionary biology, Princeton University, Princeton, NJ.

Avermectins, secondary metabolites of the ubiquitous soil bacterium *Streptomyces avermitilis*, are widely used for management of agricultural and parasitic nematode infestations. Although resistance to avermectins is a major problem worldwide, genes conferring natural resistance to avermectins are unknown. We found that CB4856, a wild strain from Hawaii, was more resistant than the laboratory strain N2 to the prevalent sources of avermectins, including abamectin (mixture of avermectin B1a and B1b), ivermectin (a synthetic derivative of avermectin B1), and the avermectin-producing bacterium *S.avermitilis*. Using a quantitative and population genetic approach we discovered that allelic diversity resulting in loss-of-function of the gene encoding alpha-subunit of a glutamate-gated chloride channel(*glc-1*) shapes the global distribution of resistance to all the above mentioned sources of avermectins.

Quantitative trait loci (QTL) mapping of 210 advanced intercross recombinant inbred lines made from N2 and CB4856 revealed a significant loci on chromosome V. Using a combination of introgression strains, complementation tests and transgenic rescue, we identified *glc-1* as the causative gene under this QTL. Comparison of *glc-1* sequences in N2 and CB4856 revealed dramatically elevated levels of divergence relative to the genome average.

To find important genetic determinants of avermectin resistance in the broader population of *C. elegans*, we quantified the resistance to abamectin in 97 wild isolates of distinct haplotypes. Genome-wide association analyses identified a 28 kb genomic region that included *glc-1*, suggesting allelic variation at this locus is a major determinant of global variation in abamectin resistance. Analyses of sequences of *glc-1* from several wild isolates revealed unusually elevated levels of polymorphism at *glc-1* that was positively correlated with abamectin resistance. Population genetic analyses suggested that the divergent haplotypes segregate at intermediate frequency consistent with maintenance of the N2-like and CB4856-like haplotypes by balancing selection. Thus, we have identified one mechanism by which a naturally varying quantitative trait gene contributes to generation of avermectin resistance.

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The genetic basis of natural variation in food preference behavior in *Caenorhabditis elegans*. **Elizabeth E. Glater**, Cornelia I. Bargmann. Neural Circuits & Behavior, HHMI/Rockefeller University, New York, NY.

Our research focuses on the genetic basis of differences in the food preference behavior between two wild-type strains of the nematode *Caenorhabditis elegans*. We have found that *C. elegans* strains isolated from different locations around the world have distinct bacterial preferences. Specifically, in a bacterial choice assay between *Serratia marcescens*, a pathogenic soil bacteria, and *Escherichia coli* HB101, a common laboratory food source for *C. elegans*, the N2 Bristol strain had a stronger preference for *Serratia* than the CB4856 Hawaii strain did. Using N2-CB4856 recombinant inbred advanced intercross lines (RIAILs) (Rockman and Kruglyak, 2009), chromosome substitution strains (constructed by Man-Wah Tan and colleagues) and introgression lines (Doroszuk et al., 2009), we found that this variation in food preference behavior has a complex genetic basis, involving at least five quantitative trait loci (QTL). Based on genetic mapping and transgenic rescue, one of the QTLs appears to represent a divergent member of the F-box protein family. F-box proteins function as adapters between the E3 ubiquitin ligase complex and its ubiquitinated substrates. F-box proteins comprise one of the largest protein families in *C. elegans*, but few have known functions. In addition, we have begun to characterize the neural circuit that underlies food choice and found that one chemosensory neuron, AWC^{on}, is important for discrimination between these two bacterial species. Understanding the genetic basis of this natural variation will provide insights into the mechanisms by which *C. elegans* tunes its responses to different complex stimuli, and more generally, into the relationship between the genome and behavior.

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What eQTLs won't tell you about natural variation in gene expression: *cis* and *trans* acting variants, dominance and epistasis. **Daniel A. Pollard**[1], Matthew V. Rockman[2]. 1) Biological Sciences, UCSD, San Diego, CA; 2) Biology, NYU, New York, NY.

Variation in when, where and how much each gene is expressed potentially underlies much natural phenotypic variation. Expression QTL analysis can reveal the number, genomic location, and effect size of genetic variants affecting mRNA levels. However, it does not distinguish between *cis* and *trans* acting variants closely linked to genes. It also fails to provide dominance information, and is inefficient at capturing highly polygenic effects and the non-additive effects of epistatic interactions. To distinguish between *cis* and *trans* acting variation, we compared genome-wide expression levels in young adult *C. elegans* N2 and CB4856 hermaphrodites with the expression level of each strain's allele in F1 hybrids of the two strains. When the expression ratio of the two parental alleles in the F1 matches the ratio observed between the parental strains, we attribute the difference in expression level to *cis* acting effects. Genes whose allelic expression differs between parental strains but is equal in the F1s are considered to be governed by *trans* acting effects. We evaluated the degree of dominance for *trans* acting effects by measuring deviation in the F1 from the mean of parent strain expression levels. Finally, we investigated the role of epistatic interactions between *cis* and *trans* acting variants by modifying the genetic background for the above experiment and measuring how this affected F1 allelic expression. Our results on the proportion of *cis* and *trans* acting variants, the degree of dominance of *trans* acting variants, and the extent of epistatic interactions between variants, combined with previous results from eQTL studies, provide great insight into the complexity of the genetic basis for natural variation in gene expression.

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Host-parasite Coevolution selects against Males in *Caenorhabditis elegans*. **Leila EL Masri**[1,2], Rebecca Schulte[3], Nadine Timmermeyer[1], Stefanie Thanisch[1], Nico K Michiels[1], Hinrich Schulenburg[1,2]. 1) Institute of Evolution and Ecology, University of Tuebingen, Germany; 2) Institute of Zoology, , Christian-Albrechts-University of Kiel, Germany; 3) Department of Behavioral Biology, University of Osnabrueck, Germany.

Although *Caenorhabiditis elegans* is known as a self-fertilizing hermaphrodite, a low number of males is present in natural populations. Various theories predict that the presence of males facilitates adaptation to changing environments in general and coevolving parasites in particular. We tested this in two independent host-parasite coevolution experiments using *C. elegans* and its microparasite *Bacillus thuringiensis* for 28 and 48 host generations respectively. Contrary to our expectations, we observed a loss of males in the coevolution treatment and a maintenance of males under pathogen-free conditions. A subsequent comparison of males and hermaphrodites showed that in the presence of parasites, males exhibit reduced pathogen avoidance, are more easily infected and mate less frequently. The presence of males reduced population growth rate under parasite stress. In contrast to hermaphrodites, males also did not coevolve with *B. thuringiensis*. Microsatellite analysis demonstrated that, although the coevolved populations showed decreased male ratios, they produced a higher genetic diversity than the controls. In conclusion, our findings demonstrate that males are differentially affected by pathogens relative to hermaphrodites. Moreover, a low proportion of males seems to be sufficient to maintain high levels of genetic diversity. This implies that the male proportion *per se* may not be a good indicator of outcrossing rates.

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Sensing Salt: Plasticity in Sensory System and Behavioral Responses. **Yuichi Iino**. Univ. of Tokyo, Japan.

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let-70, an E2 ubiquitin-conjugating enzyme, promotes the non-apoptotic death of the linker cell. **Jennifer A. Zuckerman**, Shai Shaham. Laboratory of Developmental Genetics, Rockefeller University, New York, NY 10065.

Cell death is an essential developmental process.Many observations suggest that apoptosis does not explain all cell death that occurs in development.The *C. elegans* linker cell is an important regulator of male gonad morphogenesis.It directs gonad development and its death allows fusion of the *vas deferens* with the cloaca.Previous studies from our lab show that linker cell death is independent of known *C. elegans* cell death genes and is characterized by non-apoptotic features, including nuclear membrane invagination, open chromatin, and swelling of cytoplasmic organelles. These features are characteristic of non-apoptotic cell death that occurs during normal development of the vertebrate nervous system and in polyQ-related neurodegeneration. An RNAi screen for genes important in linker cell death identified the gene *let-70*, encoding an E2 ubiquitin-conjugating enzyme. Wild-type L1 males fed *E. coli* expressing *let-70* dsRNA show a strong block in linker cell death.A block in the final stage of linker cell migration is also observed, but is genetically separable from the cell death defect.Linker cell-specific RNAi, using *rde-1* rescue, blocks its death but does not perturb migration, supporting that these processes are separately controlled by *let-70* and that *let-70* functions cell autonomously to kill the linker cell. *let-70*::GFP fusions are only expressed in the linker cell as the cell begins to die. PQN-41, a polyQ-repeat protein also required for linker cell death, has a similar expression pattern (E.Blum, unpublished), suggesting the existence of an inducible cell death program in the linker cell. *let-70*::GFP is not expressed in surviving linker cells in *sek-1(ag1)* mutants, suggesting that both genes function in the same pathway. Consistent with this observation, *let-70*(RNAi); *sek-1(ag1)* double mutants have as many surviving linker cells as single mutants alone.Similarly, RNAi against the linker cell death genes *tir-1*(E.Blum, unpublished), or *set-16* and *swd-2.2* (M.Kinet, see abstract this meeting), blocks *let-70*::GFP expression, suggesting that these genes also function upstream of *let-70*. By contrast, *nhr-67*(RNAi) does not affect *let-70*::GFP expression and RNAi against both *nhr-67* and *let-70* results in a synergistic increase in linker cell survival, suggesting that these genes act in parallel.A similar result is seen in *pqn-41* mutants. To identify LET-70 targets, we screened for E3 ligases required for linker cell death.We found that RNAi against the seven-in-absentia homolog *siah-1*, and the ring-box E3 *rbx-1* blocked linker cell death in 10% of animals. Our studies begin to uncover a molecular pathway controlling a novel, conserved form of cell death, with potential relevance to neurodegeneration.

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Cell Extrusion Is a Caspase-Independent Mechanism for Programmed Cell Elimination in *C. elegans*. **Dan Denning**, Victoria Hatch, Bob Horvitz. HHMI, Dept Biol, MIT, Cambridge, MA.

Programmed cell death plays critical roles in metazoan development and in the removal of damaged, virus-infected or cancerous cells. Most developmental cell deaths in *C. elegans* require the caspase CED-3. However, some cells die in mutants lacking *ced-3*. We observed that *ced-3* (but not wild-type) embryos contain on average six "shed cells" that detach from the animal and die in the egg's extra-embryonic space. To test if other caspases mediate the appearance of *ced-3* shed cells, we generated a strain lacking all four *C. elegans* caspase genes (*ced-3*, *csp-1*, *csp-2* and *csp-3*). These embryos also contain shed cells, indicating that caspases are not required for the shed cell deaths. Surprisingly, the caspase-independent shed cells exhibit apoptotic characteristics like TUNEL-reactive DNA fragmentation and phosphatidylserine exposure, demonstrating that apoptosis can occur in the absence of caspases in *C. elegans*.

Using time-lapse microscopy, we determined the cellular identities of the shed cells in *ced-3* embryos and established that these are cells fated to die in wild-type embryos. Normally, these cells undergo *ced-3*-mediated cell death followed by engulfment. However, in the absence of *ced-3*, they can be eliminated by a caspase-independent extrusion mechanism. To identify factors required for extrusion, we screened for mutations that block cell shedding in *ced-3* embryos. We observed that a mutation of *pig-1*, which governs the asymmetry of many cell divisions, reduces the number of shed cells by 75%. *pig-1* encodes an AMPK-related Ser-Thr kinase homologous to the mammalian protein MELK. Most mammalian AMPK-related kinases are activated via phosphorylation by the LKB1:STRAD:MO25 tumor suppressor complex. Inactivation of *par-4/LKB1* or *strd-1/STRAD* also blocks cell shedding in *ced-3* animals, suggesting that PIG-1 is a substrate of the PAR-4 kinase. Mutations in human *LKB1* cause Peutz-Jeghers syndrome (PJS), which is characterized by the appearance of intestinal polyps. Epithelial cells are normally extruded from the mammalian small intestine at a rate of ~1400 enterocytes per villus per day. Based on our observations of *C. elegans*, we suggest that LKB1 activates MELK in a kinase pathway that makes enterocytes competent for elimination by extrusion and that *LKB1* mutations cause a cell-extrusion defect that contributes to polyp formation in PJS patients. The similarity between cell shedding in the *C. elegans* embryo and in the mammalian gastrointestinal tract also suggests that caspase activity is not required for physiological rates of cell shedding in the mammalian intestine or the apoptotic appearance of shed enterocytes.

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CCPP-1 Is A Putative Tubulin Deglutamylase That Regulates The Function And Stability Of Neuronal Sensory Cilia. **Robert O'Hagan[1]**, Brian P. Piasecki[2], Malan Silva[1], Prasad Phirke[2], Ken C.Q. Nguyen[3], David H. Hall[3], Peter Swoboda[2], Maureen M. Barr[1]. 1) Dept of Genetics, Rutgers, The State University of NJ, Piscataway, NJ; 2) Karolinska Institute, Center for Biosciences at NOVUM, Dept of Biosciences and Nutrition, Hälsovägen 7, S-141 83 Huddinge, Sweden; 3) Center for C. elegans Anatomy, Albert Einstein College of Medicine,1410 Pelham Parkway, Bronx NY 10461.

When assembled into microtubules (MTs), tubulins have protruding C-terminal tails that accumulate post-translational modifications (PTMs) such as detyrosination and polyglutamylation. PTMs have long been known to mark stable MTs. Although their cellular function and significance are not yet clear, PTMs have been proposed to act as signposts that guide various kinesin and dynein motors to transport their cargoes to specific destinations. For example, trafficking of KIF5C, a mouse kinesin-1 motor, is affected by detyrosination, and KIF1A, a mouse kinesin-3, is affected by polyglutamylation. In cilia, axonemal MTs are especially prone to PTMs, which we propose regulate MT stability as well as the function of motors that drive intraflagellar transport. We have found that perturbation of a PTM profoundly affects cilia in sensory neurons in *C. elegans*.

We identified a mutation in *C. elegans* that affects *ccpp-1*, which encodes a cytosolic carboxypeptidase that deglutamylates microtubules in a subset of neuronal sensory cilia. Loss of CCPP-1 function causes defective localization and abundance of the polycystin PKD-2 and a kinesin-3, KLP-6, in male-specific neurons. Loss of CCPP-1 also causes increased velocity of motile kinesin-2 OSM-3::GFP puncta in the cilia of male-specific neurons. Loss of CCPP-1 had cell-specific effects on the level of polyglutamylation detected by a monoclonal antibody. Mutations in *ccpp-1* cause defective dye-filling of sensory neurons that becomes progressively more severe with age, suggesting that sensory neurons construct cilia that degenerate over time. Ultrastructural analysis of mutants showed that some cilia had defective MT structures and some cilia were fragmented or absent.

Others have shown that loss of CCP1, a homolog of CCPP-1, in mice causes degeneration of brain neurons such as Purkinje neurons in the cerebellum, olfactory mitral cells, and retinal photoreceptors, and also causes sperm immotility. Although all affected cells are ciliated, the impact of loss of CCP1 on cilia has not been explored in mutant mice. We propose that neurodegeneration caused by loss of CCP1 in mammals may represent a novel ciliopathy in which cilia are formed normally but degenerate, depriving the cell of cilia-based signal transduction that is necessary for maintenance of neurons.

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Novel axon regeneration pathways identified by systematic genetic screening in *C.elegans*. **Lizhen Chen**[1], Zhiping Wang[1], Anindya Ghosh-Roy[1], Thomas Hubert[1], Dong Yan[1,2], Sean O'Rourke[3], Bruce Bowerman[3], Zilu Wu[1,2], Yishi Jin[1,2], Andrew Chisholm[1]. 1) Dept Biol, Univ California,San Diego, La Jolla, CA; 2) Howard Hughes Medical Institute; 3) Institute of Molecular Biology, University of Oregon, Eugene, OR.

The mechanisms underlying the ability of axons to regrow after injury remain little explored at the molecular genetic level. We use a recently established laser injury model in *Caenorhabditis elegans* mechanosensory neurons to screen 654 conserved genes for novel regulators of axonal regrowth. We uncover several unexpected functional clusters of genes that promote or repress regrowth, including genes classically known to affect membrane excitability, neurotransmission, and synaptic vesicle endocytosis. We find that the conserved Arf Guanine nucleotide Exchange Factor (GEF), EFA-6, acts as an intrinsic inhibitor of regrowth. By combining genetics and in vivo imaging we show that EFA-6 inhibits regrowth via microtubule dynamics, independent of its Arf GEF activity. Among the newly identified regrowth inhibitors, only loss of function in EFA-6 partially bypasses the requirement for DLK-1. Identification of these pathways significantly expands our understanding of the genetic basis of axonal injury responses and repair.

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The P38/JNK MAP kinase pathway regulates forgetting in *Caenorhabditis elegans*. **A. Inoue**, T. Ishihara. Dept Biol, Kyushu Univ, Fukuoka, Japan.

An acquired short-term memory is usually disappeared within hours if not consolidated into a stable long-term memory. However, the mechanisms of the forgetting processes remain largely unknown. Since *C. elegans* possesses a simple nervous system and their memories usually do not persist more than hours, it is suitable to study the mechanisms of forgetting processes at the molecular level. To identify the gene that regulates those processes, we isolated a mutant *qj56* whose memories for the olfactory adaptation and the salt chemotaxis learning retained longer than those in wild type animals. In the *qj56* mutant, the memory for the olfactory adaptation was extended from a few hours to more than one day, and the memory for the salt chemotaxis learning was also prolonged. We found that *qj56* is an allele of *tir-1*, which encodes an ortholog of human SARM, a Toll interleukin 1 receptor (TIR) domain protein. In *C. elegans*, TIR-1 regulates innate immune responses and asymmetric expression of STR-2 in the AWC sensory neurons via the P38/MAPK pathway. We found that mutants of CaMKII(UNC-43), p38/JNK MAPKKK(NSY-1), MAPKK(SEK-1), and the JNK-1 show defects in forgetting processes.

In the salt chemotaxis learning, the forgetting defect of *tir-1* mutants was rescued by wild type *tir-1* gene with *zig-5* promoter, which drives expression in some sensory and inter-neurons. In the olfactory adaptation, the forgetting defect of *tir-1* mutants could be rescued by the expression of the wild type TIR-1 only in the AWC sensory neurons. In addition, *ceh-36* mutants, in which the functional AWC sensory neurons were lost, also showed prolonged retention of memory for the olfactory adaptation, suggesting that P38/JNK signals in AWC sensory neurons regulate forgetting processes for the olfactory adaptation in *C. elegans*. Although, TIR-1 regulates both forgetting and left-right asymmetry of AWC neurons, both *nsy-5* mutants, which did not express STR-2 in both AWC neurons ($2AWC^{off}$), and *nsy-5* mutants overexpressing *olrn-1* gene, which express STR-2 in both AWC neurons ($2AWC^{on}$), did not show prolonged retention of memory, suggesting that AWC^{on} or AWC^{off} cells can regulate the forgetting process adequately. These results indicate that the P38/JNK MAP kinase pathways in the AWC sensory neurons actively regulate the forgetting processes for the olfactory adaptation, probably by secreting signals that induce forgetting.

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Behavioral quiescence is regulated by the FRPamide neuropeptide encoding genes *nlp-22* and *-23*. **M. Nelson**, C. Smith, J. George-Raizen, N. Trojanowski, D. Raizen. Dept Neurology, University of Pennsylvania, Philadelphia, PA.

Sleep is an ancient behavioral state required for the maintenance of an animal's physiological health. Neuropeptide signaling has been shown to regulate sleep. During larval stage transitions, *C. elegans* larvae undergo a state of behavioral quiescence called lethargus, which exhibits sleep-like properties (Raizen et al, 2008). We find that behavioral quiescence is regulated by *nlp-22* and *-23*, which encode for FRPamide neuropeptides. Microarray analyses have shown that *nlp-22* is upregulated prior to lethargus and *nlp-23* is upregulated during lethargus. Over expression in young adulthood of *nlp-22* or *nlp-23* causes an inhibition of pharyngeal pumping, locomotion, and egg-laying. Animals over-expressing *nlp-22* or *-23* are less responsive to olfactory and photic stimuli yet normally responsive to strong mechanical stimuli, demonstrating an elevated arousal threshold, a property of sleep. Interestingly, *nlp-22* (OE) worms woken by strong mechanical stimuli display a curious behavior of nearly continuous backwards movements for several minutes. *nlp-22* induced quiescence requires an intact signal sequence, suggesting that secretion of the peptide is required. *nlp-22* induced quiescence requires *egl-4*, a gene required for quiescent behavior during lethargus (Raizen et al, 2008), but does not require *ceh-17*, a gene required for the normal function of the ALA lethargus-regulating neuron (Van Buskirk and Sternberg, 2007). This suggests that *nlp-22* functions upstream of *egl-4* but downstream of or in parallel with ALA signaling during the regulation of lethargus.

Using a transgenic RNAi approach, we show that *nlp-22* is required for the proper induction of lethargus and for completion of the molts. We hypothesize that the molting-defective phenotype is a consequence of impaired lethargus regulation since, in separate experiments in our lab, we found that worms deprived mechanically or genetically of lethargus show varying degrees of molting defects and larval lethality (Unpublished). Our preliminary results indicate that *nlp-22*(RNAi) worms enter lethargus but show a lower arousal threshold, suggesting impaired quality of sleep-like behavior. *nlp-22* fluorescent reporters containing the *nlp-22* 3'UTR are expressed in a small set of neurons, most prominently in the pair of RIA interneurons. We are currently testing the hypothesis that *nlp-22* function is required in the nervous system and specifically in the RIA neurons. We will report additional experiments testing the neuroanatomical locus of *nlp-22* action and on our efforts to identify the *nlp-22* receptor.

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Internal state and sex regulate an ambivalent circuit for pheromone responses. **Heeun Jang**[1,4], Kyuhyung Kim[2,4], Rebecca Butcher[3], Piali Sengupta[2], Cori Bargmann[1]. 1) Rockefeller University, New York, NY; 2) Brandeis University, Waltham, MA; 3) University of Florida, Gainesville, FL; 4) equal contributions.

Animals respond flexibly to environmental cues based on internal and external context. Cocktails of several ascarosides, the components of *C.elegans* pheromones, are repulsive to wild-type hermaphrodites from the solitary strain N2 but are less repulsive or even mildly attractive to social hermaphrodites with reduced activity of the *npr-1* neuropeptide receptor gene. We have examined the circuit mechanisms underlying these alternative behavioral responses to pheromones using behavioral assays and *in vivo* imaging with genetically encoded Ca^{2+} sensors. We found that the C9 ascaroside (also called ascr#3) evokes strong avoidance behaviors in N2 hermaphrodites. This avoidance is mediated by the ADL chemosensory neurons, which directly sense C9. Interestingly, pheromone attraction in *npr-1* mutant hermaphrodites also requires ADL, which cooperates with the previously described ASK sensory neurons to detect blends of ascarosides C9 and C3 (also called ascr#5) that are not individually attractive. ADL and ASK both belong to a hub-and-spoke circuit of neurons connected through gap junctions to the central hub neuron RMG, which is regulated by *npr-1*. Attraction to C9 and C3 requires RMG synaptic activity, but avoidance of C9 does not. Conversely, avoidance of C9 requires ADL synaptic activity, but attraction to C9 and C3 does not. Our results suggest that the balance between avoidance and attraction can be regulated by changing the relative strengths of ADL chemical synapses compared to the ADL/ASK-RMG gap junction circuit. A parallel mechanism generates sexual dimorphism in pheromone responses. Males exhibit reduced avoidance of high C9 concentrations, and their ADL sensory neurons are less responsive to C9 than those of hermaphrodites. The effects of sexual dimorphism are additive with the effects of *npr-1*, suggesting that these pathways function independently. Modulation of neuronal responses by neuropeptide signaling and sex may permit small neuronal circuits to maximize their adaptive responses and behavioral outputs.

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Axonal transport of a new DAF-2 isoform governs associative learning. **Hayao Ohno**[1], Masahiro Tomioka[2], Shinya Kato[1], Yasuki Naito[1], Hirofumi Kunitomo[1], Yuichi Iino[1]. 1) Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Tokyo, Japan; 2) Molecular Genetics Research Laboratory, Graduate School of Science, The University of Tokyo, Tokyo, Japan.

It is of great survival advantage for animals to adapt to their environment by altering their behavior. Recently, we have found that *C. elegans* is attracted to the NaCl concentration at which food has been previously provided, whereas it learns to avoid the experienced NaCl concentration if it has been starved.

The ASER salt-sensing sensory neuron has a critical role in this behavioral plasticity. In ASER, the insulin/PI3K pathway and CASY-1 act for the avoidance of the NaCl concentration experienced during starvation.

Here, we show that a novel isoform of DAF-2/insulin receptor, which is produced by alternative splicing (AS) and named DAF-2c, regulates starvation-induced learning. The AS reporter assay using fluorescent proteins (Kuroyanagi *et al.*, 2010) suggested that *daf-2c* is almost exclusively produced in head neurons. DAF-2c was localized to the axon of the ASER neuron, whereas DAF-2a, an isoform identified previously, was not. Moreover, the axonal localization of DAF-2c increased in response to starvation. The expression of DAF-2c rescued the learning defect of *daf-2* mutants much more effectively than that of DAF-2a, although both isoforms rescued the dauer-constitutive phenotype of *daf-2* mutants to a similar extent.

CASY-1 is the *C. elegans* homolog of Calsyntenins/Alcadeins, which are type I transmembrane proteins of the cadherin superfamily highly expressed in mammalian brain. In a suppressor screen of *casy-1* mutants, two missense mutations of *daf-18*, the homolog of PTEN phosphatase that negatively regulates the insulin/PI3K pathway, were identified. Furthermore, we found that the axonal localization of DAF-2c was abolished in *casy-1* mutants. These data imply that CASY-1 regulates starvation-induced learning through the axonal transport of the DAF-2c isoform.

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Multiplex sensorimotor encoding in RIA interneurons. **Michael Hendricks**, Heon-Ick Ha, Nicolas Maffey, Yun Zhang. Harvard University, Cambridge, MA.

The *C. elegans* nervous system is a compact, highly interconnected network with considerable overlap between circuits mediating different sensorimotor behaviors. Describing how neurons integrate signals at the physiological level is necessary for understanding neural circuit function and for constructing biologically relevant models of network dynamics. We therefore chose to investigate the physiological properties of the RIA interneuron, which receives convergent polymodal inputs from sensory networks and has reciprocal contacts with motor neurons. Furthermore, RIA is required for experience-dependent modulation of several complex behaviors, suggesting that it is a key component of these networks.

Here, we show that RIA simultaneously encodes sensory input and motor activity through physiological partitioning of its single axonal process. Compartmentalized calcium signals in the axon spatially encode motor behavior; simultaneously, sensory inputs are temporally encoded by synchronous calcium transients throughout the axon. We used mutants in specific transmitter systems and transgenic silencing of transmitter release in RIA's synaptic partners to dissect the circuit elements that drive these dynamics. Compartmentalization of the RIA axon and motor mapping require cholinergic input from motor systems, while both glutamatergic and cholinergic transmission are required for normal sensory-evoked responses. Therefore, we propose that RIA modulates locomotory behaviors by integrating motor dynamics with sensory input on a subcellular scale.

Our results suggest that information flow in neural circuits can be multiplexed, with individual neurons receiving and distributing signals of different types and modalities via distinct compartments and encoding mechanisms. This provides a physiological basis for understanding sensorimotor integration at the single neuron level and suggests an intriguing new layer of network complexity within the *C. elegans* nervous system.

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Functional dynamics in the *C. elegans* sensory system. **Alon Zaslaver**, Lisa Yee, Paul Sternberg. Howard Hughes Medical Institute and California Institute of Technology, Pasadena, CA, USA.

A major goal of systems neuroscience is to understand how the brain encodes the external world. Here we use *C. elegans* to study how a myriad of environmental signals are identified and integrated by its sensory system. We have constructed a comprehensive library of transgenic animals, where each line expresses the genetically-encoded calcium indicator GCaMP in a different individual sensory neuron. This library contains the vast majority (>80%) of the sensory system in the hermaphrodite. Using microfluidics, we measured neural activity from individual neurons as animals were subjected to various stimuli. These experiments reveal that the external world is intricately mapped onto the sensory neurons, where some neurons respond to a broad range of signals, while other neurons are activated by a narrow spectrum of signals. Response dynamics also varies among the different sensory neurons: Some neurons remain active for the duration of the stimulation, whereas other neurons oscillate or exhibit a single brief 'spike-like' response. We next studied the behavioral outcome following activation or inhibition of individual sensory neurons. We generated an additional library of transgenic animals where each animal expresses a light-activated channel (either Channelrhodopsin or Archaerhodopsin) in individual sensory neurons. Behavior analysis (e.g., locomotion) reveals that only a selected set of sensory neurons directly modulate locomotion. The nature of the modulation as well as the response time varied among the different sensory neurons. In summary, using a comprehensive library of optogenetically-encoded transgenic animals, we revealed key design features in the *C. elegans* sensory system. To our knowledge, this is the first functional dynamics study performed at the level of the entire sensory system of an animal.

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Mechanisms that regulate UNC-6/netrin mediated axon guidance in *C. elegans*. **Joseph Culotti**. Samuel Lunenfeld Research Institute, Toronto, Canada.

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Control of one-carbon cycle genes by SBP-1 links SAMe production, PC biosynthesis and lipogenesis. **Amy K. Walker[1]**, René Jacobs[2], Jennifer L. Watts[3], Veerle Rottiers[1], Malene Hansen[4], Anne Hart[1], Anders Näär[1]. 1) Cancer Center, MGH, Charlestown, MA. 02149; 2) Dept. of Agricultural, Food and Nutritional Science, University of Alberta, Canada; 3) School of Molecular Biosciences, Washington State University, Pullman, WA; 4) The Sanford Burnham Institute for Medical Research, La Jolla, CA.

To understand regulation of lipogenesis, we have examined the SBP-1/SREBP transcription factors necessary for fatty acid, phospholipid and cholesterol biosynthetic gene expression and lipid accumulation *in vivo*. Inactive SREBPs reside cytoplasmically in the ER and are activated by transit to the Golgi, where proteases release transcriptionally active forms to the nucleus. We have found a new set of SBP target genes in the 1-carbon cycle (1CC), including *sams-1*, an enzyme which produces s-adenosylmethionine (SAMe), the major methyl donor. We also found a feedback loop increasing SBP-1-dependent transcription and lipogenesis in *C. elegans*, murine liver and human cells when SAMe is limited. Deficiencies folate or other 1C metabolites predispose human fatty liver disease; thus, our studies linking SAMe production to SBP-1-dependent lipogenesis in *C. elegans* are likely relevant to metabolic disorders common in Western populations.

sbp-1(RNAi) animals are lean and several SBP-1 targets, such as *fat-7* are also required for lipogenesis. However, *sams-1(RNAi)* animals have increased lipid accumulation and lipogenic gene expression which depend on *sbp-1*, suggesting a feedback loop activating SBP-1 when SAMe is limiting. Metabolomic profiling after *sams-1* RNAi revealed defects in phosphatidylcholine (PC) biosynthesis, which can require multiple methylation steps. Genetic ablation of PC biosynthesis coupled with dietary rescue experiments show that SBP-1 activity is sensitive to PC levels. We also found that murine liver or human cells with diminished SAMe or PC synthesis had increases in lipogenesis and SREBP-1 activity. Because PC is a major membrane component, we reasoned that SBP-1 activation was mechanistically linked to ER or Golgi function. We found that ER stress responses are strongly activated after *sams-1* RNAi, but are not contributors to SBP-1 activation. However, Golgi markers were disrupted. Therefore we examined localization of Golgi-resident, SREBP-activating proteases in human cells and found that limiting SAMe or PC caused these proteases to relocalize to the ER, allowing processing of SREBP-1 and activation. Our observations show SBP-1/SREBP-1 may be over activated when SAMe and PC are low and may also reveal how methyl-deficient diets contribute to human fatty liver disease.

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Adult Reproductive Diapause (ARD) Protects Both Germline Stem Cells and Somatic Cells in *C.elegans*. **Giana Angelo[1]**, Alex Lin[1], Wayne Van Voorhies[2], Marc Van Gilst[1]. 1) Basic Sciences Division, FHCRC, Seattle, WA; 2) Molecular Biology Program, NMSU, Las Cruces, NM.

In response to starvation encountered during the L4 stage of development, *C.elegans* establish a specialized dormant state termed Adult Reproductive Diapause (ARD). Worms in ARD can persist a minimum of 30 days without food and fully recover their lifespan and reproductive activities upon refeeding. In the reproductive tract of 'arrested adults', the adult germline is progressively killed off until only 35 mitotic germ cells remain. Unexpectedly, the adult soma, which consists solely of post-mitotic cells in worms, also undergoes massive downsizing and reorganization during ARD. As a result of these starvation-induced adaptations, a functional germline is regenerated and somatic tissue is restored upon refeeding, even after very prolonged starvation in ARD. We have begun performing transmission electron microscopy on arrested adult worms at different points during maintenance and following recovery from starvation-induced ARD. We observe many tissue and cell-specific alterations occurring in both the adult germline and soma, implicating unique adaptive responses that may facilitate cell survival during starvation. To further understand the mechanisms by which germline stem cells (GSCs) are surviving prolonged starvation, we are determining the cell cycle state of the surviving 35 germ cells and visualizing the remodeling of the somatic tissues of the starved adult reproductive tract. Finally, we have found that deficiency of a single essential nutrient, heme, even in the presence of abundant food, induces an ARD-like state in wild-type worms. ARD provides a unique opportunity to study how both adult stem and somatic cells adapt to nutritional stress.

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Questioning Adult Reproductive Diapause. **Hannah Seidel**, Judith Kimble. Howard Hughes Medical Institute and Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706.

Upon starvation, some *C. elegans* L4 hermaphrodites do not 'bag' as adults but instead survive long-term, reportedly via a regulatory program termed adult reproductive diapause (ARD) (Angelo and Van Gilst, 2009). Key features of ARD include degeneration of most germ cells, embryonic arrest *in utero* (as evidenced by early-stage embryos visible 3-5 days after starvation), and the ability to regenerate a functional germline upon refeeding (Angelo and Van Gilst, 2009). We have confirmed most but not all of these observations. We removed food at ~2hr intervals from early- to late-stage L4 hermaphrodites, and over the next several days, we scored these animals for germline shrinkage, oocyte production, and embryo viability. After starvation of early L4s, germlines shrank and a fraction of animals survived long-term, as expected for ARD; also as expected, the depleted germlines regenerated after refeeding. However, two observations lead us to question the proposed embryonic arrest associated with ARD. First, in animals starved from early L4, oogenesis was severely delayed, with many animals producing their first oocyte only 3-5 days into adulthood. This delay in oogenesis suggests that the early-stage embryos visible 3-5 days after starvation in putative ARD animals may reflect recent fertilization events, rather than embryonic arrest. Second, the delayed oocytes made viable embryos in some cases but dead embryos in others. This increase in embryo lethality provides an alternate explanation for the ability of some animals to avoid the bagging fate and survive long-term. Furthermore, we found that germline shrinkage was not unique to animals in putative ARD: hermaphrodites starved from late L4 uniformly bagged as adults, yet prior to bagging, these animals nevertheless possessed shrunken germlines much like the germlines reported for ARD. From these observations, we propose that all L4 hermaphrodites respond to starvation equivalently: the oogenic germline degenerates and shrinks; the animal produces as many embryos as possible, given the nutrients available; and the viability of these embryos determines long-term survival. We propose that when starvation begins in late L4, embryos are typically viable, and the animal dies via bagging; when starvation begins earlier in L4, embryos are often inviable, and this inviability allows some animals to survive long-term. We are now performing additional experiments to test this possibility. [Angelo G, Van Gilst M (2009) Starvation protects germline stem cells and extends reproductive longevity in *C. elegans*. Science **326**: 954-958.].

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Calcium signaling is required for epidermal wound repair in *C. elegans*. **Suhong Xu**, Tiffany I Hsiao, Andrew D Chisholm. Division of Biological Sciences, University of California, San Diego, La Jolla, CA.

Epidermal wound repair in general involves several coordinated responses, including actomyosin based closure of the wound, repair of the permeability barrier, and cutaneous innate immune responses. *C. elegans* has a remarkable ability to repair and respond to epidermal wounds, such as those inflicted by injection needles or nematode-penetrating fungi. Wounding of the epidermis by injection needles or laser damage activates a cutaneous innate immune response via p38/PMK-1 signaling (Refs. 1, 2). This p38 MAPK pathway is important for survival post-wounding (Ref. 3) but does not appear to be involved in wound closure or barrier repair. We are interested in the triggers and effectors of wound closure and barrier repair. Studies of wound repair in single cells have implicated calcium as a key second messenger pathway in response to damage. In *C. elegans* epidermal calcium signaling is involved in epidermal enclosure (Ref. 4), but the role of calcium in the mature epidermis is well known. Using genetically encoded calcium sensors we find that needle or laser wounding triggers a rapid and robust calcium wave that spreads from the injury site through the epidermis. The speed of travel of the calcium wave suggest it results from calcium-induced calcium release from internal stores. Dominant-negative fragments of the inositol trisphosphate receptor ITR-1 ("IP3 sponges") reduce the amplitude of the wound-triggered transient. IP3 sponges and calcium chelators reduce survival post-wounding, suggesting calcium signals are important for wound repair. To identify genes involved in the initiation or transduction of the calcium signal we are screening the ~400 C. elegans genes with domains implicated in calcium binding or flux (the 'calciome'). We find that the TRPM channel GTL-2 is important for epidermal calcium dynamics and survival post wounding. GTL-2 is expressed in the epidermis (T. Stawicki and Y. Jin, personal communication) and can function cell autonomously to regulate epidermal calcium. *gtl-2* mutants display normal induction of epidermal innate immune responses to wounding, suggesting GTL-2 acts in parallel to the p38 MAPK pathway. Conversely, we find that calcium signaling, but not the p38 pathway, is important for actomyosin mediated closure of epidermal wounds. In summary, epidermal wounding appears to trigger multiple signal transduction pathways in the epidermis that must cooperate for effective wound healing. References: 1.Pujol et al, 2008 *Current Biology* 18, 481-489 2.Ziegler et al, 2009, *Cell Host & Microbe* 5, 341-352 3.Tong et al, *PNAS*, 2009, 106, 1457-1461 4.Thomas-Virnig et al, 2004, *Current Biology* 14, 1882-1887.

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Immuno-stimulatory small molecules rescue Caenorhabditis elegans from infection with antibiotic-resistant Gram-positive and Gram-negative bacteria. **Read Pukkila-Worley**, Rhonda Feinbaum, Jonah Larkins-Ford, Natalia V. Kirienko, Kevin Chen, Annie L. Conery, Eleftherios Mylonakis, Frederick M. Ausubel. Massachusetts General Hospital, Boston, MA.

Antibiotic-resistant bacteria pose a major threat to public health. As a means to identify novel antimicrobial therapies, we previously screened 33,931 compounds for those that cured Caenorhbaditis elegans of infection with the Gram-positive bacterial pathogen Enterococcus faecalis. We identified 33 small molecules that rescued infected nematodes at a concentration more than ten-fold lower than required to inhibit bacterial growth in vitro. We therefore postulated that a subset of these compounds cured infection by directly stimulating host immunity. To test this hypothesis, we used C. elegans transcriptional reporters to provide a visual readout of immune gene activation and found that five of the 33 compounds strongly activated at least one immune reporter. We predicted that immuno-stimulatory compounds would cure nematodes infected with diverse and antimicrobial-resistant pathogens. Indeed, we found that two compounds (referred to here as #1 and #2) were able to rescue worms infected with the clinically troublesome pathogens, Pseudomonas aeruginosa (a Gram-negative bacteria) and Vancomycin-Resistant E. faecalis (VRE). Using genome-wide, transcription-profiling analyses, we found that compound #1 induced the expression of genes in a pattern that overlapped significantly with those upregulated after exposure to P. aeruginosa. Interestingly, compound #1-mediated activation of immune response genes and resistance to P. aeruginosa infection was entirely dependent on ATF-7, a conserved transcription factor orthologous to the mammalian ATF2/ATF7 and target of the p38 MAP-kinase PMK-1, a central regulator of immunity in the nematode. In a separate transcriptome analysis, we found that compound #2 induced a strong detoxification response that involved upregulation of defense response genes. In summary, we have identified two compounds that cure nematodes infected with E. faecalis, VRE and P. aeruginosa infection by directly stimulating host immunity. Taken together, these data suggest that pharmacologic manipulation of innate defense responses may be a strategy to combat infection with multi-drug resistant microbes.

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Reciprocal genetics of *C. elegans* - *Bacillus* interactions reveal insight into virulence and resistance mechanisms. **Igor Iatsenko**, Robbie Rae, Ralf J. Sommer. Dep. for Evolutionary Biology, Max Planck Institute for Developmental Biology, Tuebingen, Germany.

Bacillus bacteria are abundant in soil and can serve as a food for free-living nematodes, such as *C. elegans* and *Pristionchus pacificus*. It has been speculated that nematodes may contribute to the evolution and spreading of *Bacillus*. Here, we study the susceptibility of nematodes to *Bacillus* bacteria and identified the molecular biology of virulence and resistance mechanisms by using genetic approaches in bacteria and nematodes. First, we assessed the pathogenicity of 768 naturally isolated *Bacillus* strains to *C. elegans* and *P. pacificus* to investigate whether these nematodes differ in their susceptibility and to discover what proportion of *Bacillus* are virulent to nematodes. We found 20 *Bacillus* strains that were pathogenic to *C. elegans* and *P. pacificus* causing 70-100% mortality. Most pathogenic strains are three *B. thuringiensis*-like strains isolated from dung beetles, which exhibit extreme virulence to *C. elegans* causing 100% mortality in 16 hours, whereas *P. pacificus* is resistant. We picked one of these strains (*B. sp. 27*) for studying both sides of host-pathogen interactions using *C. elegans*-*B. sp. 27* as a model system. *C. elegans bre* mutants are not resistant to *B. sp. 27*, indicating that these bacteria use different virulence mechanism than Cry5B toxin. Also, *daf-2* mutants don't increase resistance, which implies that DAF-16-independent pathways may be involved in *C. elegans* defence against *B. sp. 27*. To discover these possible defense mechanisms against *B. sp. 27* we took forward genetic approach and isolate three preliminary called *btr* (*Bacillus thuringiensis* resistant) mutants that are resistant to *B. sp. 27*. *Btr-1* maps to chromosome II and was cloned by next-generation sequencing technology. Interestingly, *btr-1* mutants appeared to be resistant to *S. aureus* (produces pore-forming toxins) and Cry5B toxin, suggesting that *B. sp. 27* may also use pore-forming toxins to kill *C. elegans*. To identify possible virulence factors we used transposon-mediated mutagenesis, which allowed us to isolate 24 attenuated *B. sp. 27* mutants. Sequencing analysis of mutants showed linkage majority of mutations to B. thuringiensis megaplasmids, which encode Cry and Cyt pore-forming toxins active against insects. Reciprocal genetic and molecular studies of *C. elegans* - *Bacillus sp. 27* interactions provide comprehensive insight into the virulence and resistance mechanisms and the evolution of nematode - bacterial associations. We will present a detailed analysis of the virulence genes and the potential resistance mechanisms of *C. elegans*.

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Neuronal control of stress response and innate immunity in C. elegans. **Varsha Singh**, Jingru Sun, Alejandro Aballay. Dept Molec Gen Microbiol, Duke Univ, Durham, NC.

Heat Shock Protein (HSP) and Unfolded Protein Response (UPR) genes are upregulated in response to various stresses including heat shock, oxidative stress and ER stress. In *C. elegans*, HSP and UPR genes are also required for an adequate immune response to pathogens. Recent studies have shown that thermosensory neurons can regulate HSP gene induction at the organismal level. Increasing evidence also indicates that the nervous system of *C. elegans* controls avoidance to certain pathogens and activation of innate immune pathways. To identify neurons capable of controlling the activation of HSP or UPR genes as well as innate immune responses to pathogens, we screened mutants, in genes involved in the function of sensory neurons, for expression of stress proteins in response to bacterial pathogens and their pathogen susceptibility. Mutation in thermosensory neurons affected not only the expression of stress proteins but also pathogen susceptibility. Additional sensory neurons suppressed innate immunity while others enhanced innate immunity.

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Resistance is futile: two Leucobacter strains have complementary action as virulent surface pathogens of Caenorhabditis. **Jonathan Hodgkin[1]**, Marie-Anne Félix[2], Maria Gravato-Nobre[1], Delia O'Rourke[1], Frederick Partridge[1], Dave Stroud[1]. 1) Dept Biochem, Univ Oxford, Oxford, United Kingdom; 2) Institut Jacques Monod, Paris, France.

Natural pathogens of Caenorhabditis can reveal novel modes of pathogen attack and host innate immune defense. Two related bacterial strains have recently been isolated from a wild isolate of *Caenorhabditis n. sp. 11* collected in the Cape Verde islands, both of which are able to kill Caenorhabditis worms but use different mechanisms. The two strains belong to the Gram-positive genus Leucobacter, and are referred to as Verde1 and Verde2. Verde2 resembles *Microbacterium nematophilum* in its pathogenic effects on *C. elegans*: it targets the rectum and induces tail-swelling. Verde2 causes lethal disease, in contrast to *M. nematophilum*, which is deleterious but not usually lethal to *C. elegans*. Many Bus (Bacterially Un-Swollen) mutants have previously been isolated and studied on the basis of their resistance to *M. nematophilum* infection, and most of these prove to be also resistant to Verde2.

Verde1 bacteria adhere densely over the surface of wildtype worms and impair growth and fertility, but the infection is not lethal. However, most of the Bus mutants resistant to Verde2 are lethally hypersensitive to Verde1 and unable to survive in its presence, apparently dying from surface coat destruction. This effect shows that numerous *bus* genes, which affect the cuticle surface but are dispensable under lab conditions, are naturally essential for resistance to pathogens such as Verde1.

Most of the >20 *bus* genes identified from *M. nematophilum*-based screens have now been cloned and found to encode proteins involved in surface glycosylation or related processes. The reciprocal lethal effects of Verde1 and Verde2 allow powerful selections for additional *bus* genes and for extragenic suppressors of existing *bus* mutants. These suppressor mutants define still more genes with effects on surface properties. Molecular characterization and interaction studies of this large group of genes will assist analysis of the nematode surface coat, the worm's major interface with the environment.

Different wild isolates of *C. elegans* are similar in susceptibility to Verde1 and Verde2, but *C. briggsae* is polymorphic: the genome-sequenced reference strain AF16 is resistant to Verde2 and hypersensitive to Verde1, whereas most other *C. briggsae* strains exhibit the reciprocal phenotypes. Bacterial surface disease may be an important selective agent for natural polymorphism and diversification within Caenorhabditis species.

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An HDA-4-MEF-2 complex couples DKF-2A (protein kinase D) to regulation of innate immunity. **Ya Fu**, Charles Rubin. Dept Molec Pharm, Albert Einstein Col Med, Bronx, NY.

The intestinal innate immune system of *C. elegans* opposes bacterial infections by producing proteins that kill or neutralize pathogens. Knowledge of signaling pathways and mechanisms underlying induction of immune effectors is incomplete. DKF-2A, a protein kinase D (PKD) that transmits signals downstream from EGL-8 and TPA-1, promotes resistance to *P. aeruginosa* (PA14) by (a) inducing ~85 mRNAs that encode antimicrobial proteins and (b) activating PMK-1 (p38 MAP kinase), a key regulator of immunity. A complex containing HDA-4, a type IIa histone deacetylase (HDAC), and MEF-2, a transcription factor, is a potential target of DKF-2A and PMK-1. HDACs repress transcriptional activity of binding partners by chromatin remodeling, direct deacetylation and allosteric inhibition. Thus, we determined if activation of MEF-2, via dissociation and nuclear export of HDA-4, links DKF-2A to the immune response. DKF-2A phosphorylated HDA-4 on serines 162 and 251. This creates binding sites for 14-3-3 adapter proteins, which promote export of HDA-4 to cytoplasm. HDA-4-GFP shuttled between cytoplasm and nuclei in transgenic animals. DKF-2A depletion or substitution of regulatory Ser with Ala (HDA-4-(AA)-GFP) targeted HDA-4 exclusively to nuclei. An hda-4 mutation (*hda-4(oy57)*), which should de-repress MEF-2, generated animals highly resistant to PA14. Expression of HDA-4-GFP in the mutant background restored WT sensitivity to PA14; animals expressing HDA-4-(AA)-GFP were hypersensitive to PA14. DKF-2A was indispensable for nuclear-cytoplasmic translocation of HDA-4, and PA14 resistance in the absence of functional HDA-4. Animals lacking MEF-2 (WT or *hda-4(oy57)* background) were hypersensitive to PA14, revealing that MEF-2 operates downstream from HDA-4. Expression of MEF-2-GFP in a *mef-2(gv1)* null background restored WT resistance to PA14. PMK-1 phosphorylated MEF-2 on serines 104 and 287. When PMK-1 target sites were mutated to Ala, expression of MEF-2-(AA)-GFP in transgenic animals did not rescue hypersensitivity in the *mef-2* null mutant. A mutation in a 14-3-3 protein (*ftt-2(n4426)*) caused PA14 hypersensitivity, suggesting that export of phospho-HDA-4-14-3-3 complexes to cytoplasm is critical for DKF-2A/MEF-2-mediated immune responses. The results indicate that DKF-2A catalyzed phosphorylation of HDA-4 triggers dissociation from MEF-2, binding with 14-3-3 and export of phospho-HDA-4 to cytoplasm. Phosphorylation of MEF-2 by PMK-1 is also essential for PA14 resistance. DKF-2A mediates activation of PMK-1, which explains why the PKD functions both downstream and upstream from HDA-4. DKF-2A is coupled to transcriptional regulation of immunity by its dual actions on the HDA-4-MEF-2 complex.

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HLH-30 is a novel transcription factor important for the C. elegans host response to S. aureus. N. Lhuegbu[1], **O. Visvikis[2]**, L.G. Luhachack[2], G.D. Stormo[1], J.E. Irazoqui[2]. 1) Department of Genetics, Center for Genome Sciences, Washington University Medical School, St Louis, MO; 2) Department of Pediatrics, Program of Developmental Immunology, Massachusetts General Hospital, Boston, MA.

The human pathogen *Staphylococcus aureus* can infect and kill *Caenorhabditis elegans*. Previous microarray analysis showed that *S. aureus* triggers a pathogen-specific transcriptional host response, which appears to be regulated by Toll-like receptor-independent sensing of pathogen-associated molecular patterns (PAMPs). BAR-1/β-catenin and EGL-5/HOX were shown to be important for triggering part of this response. However, because many host defense genes are triggered independently of BAR-1 and EGL-5, it is likely that additional signaling pathways are important for the *C. elegans* response to *S. aureus*. To identify new components that orchestrate the host response, we performed bioinformatic analysis and identified evolutionarily conserved DNA motifs that are over-represented in the promoters of S. aureus induced transcripts (SAITs). One such motif, the M-box/E-box, is known in mammals to be bound by the Microphthalmia-TFE (MiT) transcription factor family. Phylogenetic analysis revealed that *hlh-30* is the unique MiT homologous gene in *C. elegans*. Therefore, we hypothesized that *hlh-30* is important for the expression of host defense genes during *S. aureus* infection. To test this hypothesis, we first analyzed the expression of *hlh-30* by RT-qPCR and found that it was up-regulated by 2-fold upon infection. We then monitored the expression of a subset of SAITs in *hlh-30(-)* strain relative to wild type. We found that HLH-30 is required for the expression of 15 out of 17 SAITs tested in infected animals, 7 out of these 15 SAITs being regulated by hlh-30 in uninfected animals as well. Thus HLH-30 controls expression of a large number of genes implicated in the host response. To understand the role of HLH-30 at the global level, we performed RNA-Seq to determine the downstream target genes of HLH-30. Of 989 SAITs identified in wild-type animals, 619 SAITs were not induced in *hlh-30(-)* animals, confirming that HLH-30 plays a key role in the C. elegans host response to S. aureus. Consistently, we found that *hlh-30(-)* mutants exhibited enhanced susceptibility to S. aureus-mediated killing. Therefore, we suspect that the transcriptional defect observed in *hlh-30(-)* animals is biologically significant for host defense. We are currently investigating potential mechanisms of regulation of HLH-30 during infection. Altogether, these data indicate that HLH-30 is a major transcription factor controlling a biologically significant transcriptional host response to infection.

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A novel RHO-1 signaling pathway is required during the *C. elegans* immune response. **Alexandra Anderson**, Rachel McMullan. Division of Cell and Molecular Biology, Imperial College London, London, United Kingdom.

In order to survive in their natural environment, *C. elegans* respond to pathogens using both behavioral and immune responses. Infection of *C. elegans* with the coryneform bacterium *Microbacterium nematophilum* results in avoidance behavior, as well as tail swelling known as the dar phenotype that forms part of the innate immune response. This swelling is associated with changes in the morphology of cells in the anal region, including the rectal epithelial cells. We have used the interaction between *C. elegans* and *M. nematophilum* as a model for studying the behavioral and innate immune response to infection. Here we describe a role for the EGL-30-RhoGEF UNC-73-RHO-1 signaling pathway in mediating both these responses. Upon infection with *M. nematophilum* neuronal EGL-30 signaling increases neurotransmitter release to mediate avoidance behavior, while EGL-30 signaling in the rectal epithelium cell autonomously alters cell morphology resulting in the dar phenotype. Reduction in the activity of the Rho guanine nucleotide exchange factor (GEF) activity of UNC-73 (unc-73(ce362)) or inhibition of RHO-1 with C3 transferase significantly reduces the dar response following *M. nematophilum* infection. In contrast, activation of RHO-1 phenocopies the dar response in the absence of *M. nematophilum* infection. Reduction-of-function mutations in the ERK/MAPKinase pathway have been shown to suppress the bacterially-induced dar phenotype. Our data demonstrates that the EGL-30-RhoGEF UNC-73-RHO-1 pathway converges with LET-60 signaling to activate the LIN-45-MEK-2-MPK-1 pathway during the immune response. Cross talk between RHO-1 and LET-60 signaling pathways are known to contribute to vulval development in *C. elegans* and cell transformation during cancer in mammals, though the effectors acting between RHO-1 and LIN-45 remain unclear. Using a candidate approach we have screened known Rho effectors for their involvement in the immune response, as well as carrying out an RNAi screen for novel mediators. Known effectors, including LET-502, UNC-120, PKN-1 and CYK-1 appear to have negligible involvement in the immune response. An RNAi screen of chromosome I revealed several novel effectors of the immune response, including components involved in the electron transport chain, ubiquitin-mediated proteolysis and trafficking. Our data identify a role for RHO-1 signaling in both the avoidance and immune responses to infection in *C. elegans* and highlight the complex cross-talk involved in innate immunity.

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Pathogenesis of microsporidian infection in *C. elegans*. **Malina A. Bakowski**, Emily R. Troemel. Biological Sciences, UCSD, La Jolla, CA.

Nematocida parisii is a natural intracellular pathogen of *C. elegans* that infects intestinal cells and leads to premature death of its host. *N. parisii* defines a new species of microsporidia, which are fungal-related parasites that are clinically and agriculturally significant but poorly understood. Therefore, we are using the *N. parisii/C. elegans* model to dissect molecular mechanisms of microsporidian pathogenesis.

To identify *C. elegans* genes required by *N. parisii* for infection, we took advantage of the fact that infection with high doses of *N. parisii* causes larval arrest of *C. elegans*. We screened three RNAi sub-libraries of *C. elegans* genes (leucine rich repeat proteins, transcription factors, and kinases) and found 30 RNAi clones that impair infection-induced larval arrest. Because we are most interested in genes needed by *N. parisii* to establish a productive infection (as opposed to genes involved in larval arrest), we are currently determining which of the identified genes affect pathogen load and survival of infected worms.

In collaboration with the Broad Institute and as part of the Microsporidian Genomes Consortium we have also recently sequenced the genome of *N. parisii* and are performing RNA-seq analysis to determine the worm and microsporidia transcriptomes during distinct stages of infection. In addition to yielding valuable information about the *C. elegans* response to microsporidian infection this work will also help us identify pathogenesis effectors expressed by *N. parisii*. Accordingly, we are currently testing the effects of overexpressing specific *N. parisii* genes in the *C. elegans* intestine.

The above approaches will identify both *C. elegans* and microsporidian genes involved in the infection process. Because of the similarities between *C. elegans* and mammalian intestinal epithelial cells, these studies may also lead to greater insight into the molecular interactions of clinically relevant species of microsporidia with human intestinal cells.

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The *C. elegans* Early Response to *P. aeruginosa* Infection: Defining a Novel Pathway. **T.L. Dunbar**, Z. Yan, E.R. Troemel. Biological Sciences, University of California, San Diego, La Jolla, CA.

We are investigating how intestinal cells discriminate between pathogenic and non-pathogenic microbes by looking at the *C. elegans* transcriptional response to infection by the Gram-negative bacterial pathogen *P. aeruginosa*. Intestinal epithelial cells are regularly exposed to microbes, and consequently must be able to distinguish between innocuous and harmful microbes in order to avoid a hyper-inflamed state and unnecessary energy expenditure. The intestinal epithelial cells of *C. elegans* and vertebrates are morphologically similar and provide a major line of defense against infection. *P. aeruginosa* causes a lethal intestinal infection in *C. elegans* that requires some of the same virulence factors required for killing mammalian hosts. To investigate the early response to infection we performed microarray profiling and defined a set of genes specifically induced by pathogenic *P. aeruginosa*. A GFP reporter for one of these genes, called *irg-1* (infection response gene 1), is robustly induced by infection independently of previously identified immune signaling pathways. The *irg-1::GFP* reporter is induced specifically by pathogenic *P. aeruginosa*: it is not induced by other pathogens so far tested, nor by attenuated *P. aeruginosa*. This reporter provides a convenient read-out for the early events in intestinal cell response to *P. aeruginosa* infection *in vivo*. To define the pathway that mediates induction of *irg-1* in response to infection we have used an RNAi screening approach. Pilot RNAi screening identified a bZIP transcription factor called *zip-2* that is required specifically for *irg-1* induction and provides defense against *P. aeruginosa* infection. Recent *in vitro* analysis of interactions between ZIP-2 and other bZIPs has identified additional bZIPs as possible members of this infection response pathway. Our preliminary data suggests that one of these bZIPs may form a heterodimer with ZIP-2 in the early infection response to *P. aeruginosa*. To identify additional genes required for *irg-1::GFP* induction we have also screened a full genome RNAi library and have confirmed 19 candidates that have impaired induction of *irg-1* but still induce *irg-3*, another infection response gene. Most of these hits correspond to genes that appear to be important for mitochondrial function, nuclear import/export, or splicing. We are examining these hits for their effects more broadly on infection response genes. We also are investigating their effect on ZIP-2 expression and localization. Altogether our studies should shed light on the mechanisms by which animals detect and respond to pathogenic attack in the intestine.

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C. elegans orthologs of LKB1, STRAD and MO25 regulate asymmetric cell division of the Q.p lineage. **Shih-Chieh Chien**, Gian Garriga. Dept Molec & Cell Biol, Univ California, Berkeley, Berkeley, CA.

Many *C. elegans* neuroblasts, including the Q.p neuroblasts, divide to produce a larger neuronal precursor and a smaller cell that dies. Mutations in some of the genes that regulate these divisions result in daughter cells that are more equivalent in size and transform the fate of the apoptotic cell to that of its sister, resulting in the production of extra neurons. One such gene is *pig-1*, which encodes a protein orthologous to vertebrate MELK and belongs to a family of serine/threonine kinases that include PAR-1, SAD-1 and AMPK [1]. This group of kinases can be phosphorylated and activated by the polarity-regulating kinase LKB1. LKB1 kinase, along with its binding partners STRAD and MO25, are master regulators of polarity in many different contexts, and we find that *C. elegans* orthologs of LKB1 (PAR-4), STRAD (STRD-1) and MO25 (MOP-25.2) regulate the Q.p division.

Mutations in *par-4* or *strd-1* generated Q.p daughter cells that are more symmetric in size, and in a sensitized background, *par-4* or *strd-1* mutations or *mop-25.2(RNAi)* resulted in extra neurons, suggesting that PAR-4/STRD-1/MOP-25.2 activates PIG-1. Consistent with this hypothesis, *par-4* and *strd-1* mutations enhance the extra-neuron phenotype of a weak but not a null *pig-1* mutant. LKB1 activates AMPK family kinases by phosphorylating a conserved threonine residue in their activation loops [2]. By contrast, MELK is autophosphorylated at this residue [3], and mutating it to alanine abolishes kinase activity [2, 3]. To test whether the threonine residue in the activation loop (T169) of PIG-1 is equally essential for its activity, we generated a non-phosphorylatable form, PIG-1(T169A), and a phosphomimetic form, PIG-1 (T169D). Transgenes expressing PIG-1(T169A) failed to rescue the extra-neuron phenotype of a *pig-1* mutant, indicating that the threonine residue is important for PIG-1 activity. We observed a partial rescue of the extra-neuron phenotype from transgenes expressing PIG-1(T169D). PIG-1(T169D) also induced extra neurons in the wildtype background, suggesting that the phosphomimetic form possessed deregulated PIG-1 activity.

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A combinatorial code of transcription factors defines the terminal differentiation of dopaminergic neurons. **Maria Doitsidou[1,2]**, Nuria Flames[1,2], Terry Felton[1], Oliver Hobert[1]. 1) Dept Biochem, Columbia Univ, New York, NY; 2) Equally contributing.

Dopaminergic neurons control various aspects of behaviour and their loss leads to Parkinson's disease. There are 8 dopaminergic (DA) neurons in the C. elegans hermaphrodite that fall into 4 morphological classes. All 4 classes display very distinct developmental histories, but nevertheless express the core pathway genes responsible for the biosynthesis and transport of dopamine. We call these genes the dopamine pathway genes. It was previously shown (Flames, 2009) that *ast-1*, an ETS transcription factor, is necessary for the expression of all dopamine pathway genes in all dopaminergic cell types. AST-1 exerts its action through binding to the dopaminergic motif, present in the promoters of the dopamine pathway genes. However, neither AST-1 nor its corresponding binding sites are sufficient to activate the dopamine pathway genes. We used a combination of genetic and promoter analysis approaches to identify the transcription factors as well as the corresponding regulatory elements that act in concert with *ast-1* for terminal differentiation of DA neurons. Extensive analysis of the dopaminergic motif revealed the presence of functional homeodomain and PBX/Hox binding sites in all DA pathway genes. Automated COPAS forward genetic screens combined with Whole Genome Sequencing, uncovered a homeodomain transcription factor of the Distal-less family, *ceh-43*, and a Hox domain/MEIS transcription factor, unc-62, that are necessary for terminal differentiation of DA neurons. We find that these transcription factors are required for proper expression of all dopamine pathway genes in all DA neuronal classes. Phenotypic analysis, ectopic expression studies and epistatic relationships between *ceh-43*, *unc-62* and *ast-1* will be presented. In addition, candidate approach revealed that a PBX transcription factor, *ceh-20*, is also involved in terminal differentiation of DA fate. In conclusion, we have uncovered a combinatorial code of transcription factors, as well as the corresponding cis-regulatory elements that control terminal differentiation of dopaminergic neurons.

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Coordinated regulation of cholinergic motor neuron traits through a C.elegans terminal selector. **Paschalis Kratsios**, Oliver Hobert. Howard Hughes Medical Institute, Department of Biochemistry, Columbia University, New York, NY.

Motor neurons in the spinal and ventral cords of vertebrates and invertebrates generally use the neurotransmitter acetylcholine (ACh) to communicate with their target muscles. Cholinergic neurons are defined by the co-expression of a battery of genes ("cholinergic gene battery") that include proteins that act sequentially to synthesize, package and break down ACh and reuptake its breakdown product choline. How expression of this gene battery, the most basic identity determinant of motor neurons, is coordinated is not sufficiently understood in any system studied to date. We show here that in the nematode Caenorhabditis elegans all members of the cholinergic gene battery (*unc-17, cha-1, cho-1, ace-2*, and *acr-2*) are co-regulated by a shared cis-regulatory signature and a common trans-acting factor, the phylogenetically conserved COE transcription factor UNC-3. Mutant analysis revealed that UNC-3 controls expression not only of the cholinergic gene battery, but many other markers of differentiated cholinergic motor neurons, such as *trp-1, nca-1, unc-8, unc-129*, and *del-1*. UNC-3 is continuously expressed throughout the life of these neurons and is not only required to initiate but also to maintain expression of the cholinergic gene battery. Lastly, UNC-3 is sufficient to induce cholinergic fate in other neuron types, such as the glutamatergic sensory neurons ASE and AWC. In conclusion, UNC-3 is a terminal selector of cholinergic fate whose function may be conserved across phylogeny.

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Insulin/IGF-1 signaling and endogenous RNAi affect HSN migration and axon guidance in *C. elegans*. **Lisa Kennedy**[1], Alla Grishok[2]. 1) Genetics & Development, Columbia Univ, New York, NY; 2) Biochem & Mol Biophys, Columbia Univ, New York, NY.

We are investigating the biological roles of endogenous RNA interference in *Caenorhabditis elegans*. Specifically, we have identified migration and axon guidance defects in the hermaphrodite-specific neurons (HSNs) for a subset of RNAi pathway mutants. The HSNs are a pair of bilaterally symmetric serotonergic motor neurons that migrate during embryogenesis, undergo axonal outgrowth during the larval stages and release serotonin to stimulate egg laying in the adult. Mutations in the following RNAi-promoting factors exhibit HSN defects: a chromatin-binding PHD Zinc Finger Protein 1 (ZFP-1); a dsRNA-binding protein that is part of the Dicer complex, RDE-4; a Dicer-related helicase (DRH-3) and a nuclear Argonaute, CSR-1, which is required for proper chromosome segregation. DAF-16, a highly conserved FOXO family transcription factor, is negatively regulated by insulin/IGF-1 signaling in *C. elegans* and is most well studied in the context of aging. We have observed phenotypic similarities between null *daf-16(mu86)* and loss-of-function *zfp-1(ok554)* mutants. Among these shared phenotypes are HSN under-migration and axon guidance defects. DAF-18, the C. elegans PTEN homolog negatively regulates the insulin/IGF-1 signaling pathway by dephosphorylating the AGE-1-activated PIP3. We observed HSN migration and axon guidance defects in the *daf-18(ok480)* null mutant and no enhancement of these defects in a *daf-16(mu86)*; *daf-18(ok480)* double null mutant, indicating that the HSN phenotype in the *daf-18(ok480)* mutant arises solely from an increase in Insulin/IGF-1 signaling and a decrease in DAF-16 activity during development. We have also observed that enhanced DAF-16 activation by both removing PI3K signaling and inducing the heat stress response simultaneously leads to HSN over-migration in a DAF-16-dependent manner. Moreover, the loss-of-function *zfp-1(ok554)* and null *rde-4(ne299)* mutations enhance the axon guidance and HSN under-migration defects in the null *daf-16(mu86)* mutant, suggesting that *daf-16* and RNAi components work in parallel genetic pathways to regulate HSN development. Our data points to novel roles for both insulin/IGF-1 signaling and RNAi in the regulation of cell migration and axon guidance in *C. elegans*. We are currently investigating downstream candidate targets of both endogenous RNAi components and DAF-16 that may regulate neuronal genes required for proper migration and axon guidance.

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Genetic and Molecular Characterization of VAB-1 Eph Receptor Signaling in Axon Guidance. **Ahmed M. Mohamed**, Jeffery Boudreau, Jun Liu, Ian D. Chin-Sang. Department of Biology, Queen's University, Kingston, ON, Canada.

The *vab-1* gene encodes for the only *C. elegans* Eph receptor tyrosine kinase (RTK), and is involved in various aspects of neuronal and epidermal development. Mutations in *vab-1* result in abnormal cell migration and axon guidance [1-4]. Unfortunately, we still lack knowledge on the molecular mechanisms of how VAB-1 regulates cell migration and axon guidance in *C. elegans*. To identify components of VAB-1 RTK signaling, we utilized a hyper active form of VAB-1 (MYR-VAB-1) that caused penetrant axon guidance defects in the mechanosensory neurons [3], and screened for suppressors of the MYR-VAB-1 phenotype. Using a candidate gene approach, we identified NCK-1 and WSP-1/N-WASP as downstream effectors of VAB-1 Eph RTK. Analysis of *nck-1* and *wsp-1* mutants revealed PLM axon defects similar to *vab-1* animals, where PLM axons over extended beyond their target region. Double mutant experiments indicate that both *nck-1* and *wsp-1* function in the same pathway with *vab-1* in PLM axon guidance. In addition, we found that activation of the Arp2/3 complex via the VCA domain of WSP-1 induced defects similar to the MYR-VAB-1 phenotype. We also found that VAB-1, NCK-1 and WSP-1 are able to form a complex in vitro. Interestingly, protein binding experiments revealed that the actin-regulator UNC-34/Ena, known to function in axon extension, can physically interact with NCK-1. Genetic experiments to investigate the nature of this interaction suggest that *unc-34* inhibits *nck-1*. Furthermore, genetic and biochemical data show that VAB-1 is able to disrupt the interaction between NCK-1 and UNC-34, and negatively regulate UNC-34. Taken together, our results provide a model of how VAB-1 signaling can induce axon termination. We propose that VAB-1 activation results in the recruitment of NCK-1, and disrupts the interaction between NCK-1 and UNC-34 to relieve NCK-1 inhibition. Consequently, NCK-1 is now able to form a complex with WSP-1 to activate the Arp2/3 complex and form extensive short branched filaments that inhibit growth cone filopodia formation. Simultaneously, VAB-1 activation results in the negative regulation of UNC-34 to prevent further actin filament extension. In summary, our work provides a pathway that connects the VAB-1 Eph RTK directly to the cytoskeleton, and suggests how VAB-1 regulates the actin cytoskeleton for axon termination. [1] Boulin *et al.*, Current Biology 2006 16:1871-83. [2] George *et al.*, Cell 1998 92:633-43. [3] Mohamed and Chin-Sang, Developmental Biology 2006 290:164-76. [4] Zallen *et al.*, Development 1999 126:3679-92.

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An Axon's Journey To Find Its Path: The Rac GEF TIAM-1 Controls Axon Pathfinding Downstream of CDC-42 in the UNC-6/Netrin Attractive Pathway. **Rafael S. Demarco**, Erik A. Lundquist. Molecular Biosciences, University of Kansas, Lawrence, KS.

During growth cone migration, guidance receptors signal via Rac GTPases to reassemble the actin cytoskeleton. Such process enables the growth cone to modulate filopodial and lamellipodial protrusions in order to migrate to its final place. The two redundant Rac GTPases MIG-2 and CED-10 are essential for this process, since the abolishment of Rac activity disrupts virtually every PDE axon. Racs shuffle between an active, GTP-bound state, to an inactive, GDP-bound state. Rac activation via binding to GTP can be aided by Guanine-nucleotide Exchange Factors (GEFs). UNC-73 is a GEF for MIG-2 and CED-10 in axon guidance, but mutations in *unc-73* did not cause defects as severe as the double *mig-2;ced-10*. We then suspected other GEFs may also control Rac activity in parallel to UNC-73. In a screen for other Double Homology (DH) containing molecules, TIAM-1 displayed a genetic interaction with the Racs. Like *unc-73*, loss-of-function (lof) mutations in *tiam-1* synergized with lof *mig-2* and *ced-10*. Additionally, a gain-of-function (gof) version of *tiam-1* caused ectopic lamellipodia in PDEs that were suppressed by lof *mig-2* and *ced-10*. These results suggest TIAM-1 acts upstream of Racs in axon pathfinding. We then investigated the mechanism of action of TIAM-1. We purified the GEF catalytic DH/PH domains of TIAM-1 and performed a mant-GTP based exchange assay. The biochemical results suggest TIAM-1[DH/PH] acts as a GEF specifically for Rac, but not Cdc-42 nor RhoA. A point mutation that is predicted to disrupt TIAM-1 GEF activity abolished the ability of TIAM-1[DH/PH] to exchange mant-GTP in Rac. Next we asked whether TIAM-1 localized to the plasma membrane, where Racs are active. *In vivo* TIAM-1 expression in neurons, as well as in NIH 3T3 fibroblasts, suggest TIAM-1 localized at the plasma membrane with CED-10. *In vitro* studies implicated hTiam1 as a downstream effector of hCdc-42. *In vivo*, lof *tiam-1* and *cdc-42* had no increase of defects in PDE guidance. Plus, gof *cdc-42* displayed ectopic lamellipodia that were suppressed by the lof *tiam-1*. These data implicate TIAM-1 as a downstream effector of CDC-42 *in vivo*. Ventrally-guided neurons are attracted to UNC-6 and repelled by SLT-1. We asked if TIAM-1 acts in either pathway. Lof *tiam-1* did not increase defects seen in *unc-6* animals, but increased in *slt-1*. Gof *unc-40* caused ectopic lamellipodia that also required TIAM-1 (but not UNC-73) for its occurrence. Our work revealed the role of the previously unidentified *C. elegans* TIAM-1 as a GEF for MIG-2 and CED-10, downstream of CDC-42, in the UNC-6/Netrin attractive signaling pathway. Future studies will further dissect the mechanisms of action of TIAM-1 in growth cone morphology and dynamics.

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Sensing the environment: novel molecular pathway for the formation of sensory cilia. Chunmei Li[1], Victor L. Jensen[1], Katarzyna Kida[2], Corey L. Williams[3], Bradley K. Yoder[3], Oliver E. Blacque[2], **Michel R. Leroux[1]**. 1) Dept Molecular Biology & Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) School of Biomolecular and Biomedical Science, UCD Conway Institute, University College Dublin, Belfield, Dublin, Ireland; 3) Department of Cell Biology, University of Alabama, Birmingham, AL, USA.

C. elegans perceives its environment mainly by way of sensory neurons which have cilia at the distal ends of dendrites, much like mammals can smell with the use of olfactory cilia or can see with photoreceptor cilia. In studying several human disorders involving cilia dysfunction (ciliopathies), we have uncovered a novel molecular pathway necessary for ciliogenesis. The disorders in question—nephronophthisis (NPHP), Meckel syndrome (MKS), Joubert syndrome (JBTS), Senior-Løken syndrome (SLSN), and Leber congenital amaurosis (LCA)—present with overlapping ailments, such as retinopathy, kidney disease, liver fibrosis and brain malformations. They also show considerable allelism between at least twelve causative genes, suggesting a common molecular aetiology that remains unexplained. We demonstrate using *C. elegans* that the recently-identified MKS-6 and MKS-2 proteins, together with MKS-1, MKSR-1, MKSR-2, MKS-5, NPHP-1 and NPHP-4, collectively function at the base of cilia, in a region termed transition zone (TZ), to orchestrate cilium formation. Specifically, the proteins act as two distinct modules, which we term MKS and NPHP, to facilitate basal body-transition zone anchoring to the membrane; disruption of the TZ proteins results in defects in prominent ciliary TZ and axoneme formation defects, and thus, chemosensory anomalies. This first pathway is independent of a second pathway specifically required for the formation and function of the ciliary organelles, involving intraflagellar transport (IFT) and Bardet-Biedl syndrome (BBS) proteins. Our genetic and cell biology analyses reveal a hierarchical organisation of the TZ proteins, with MKS-5 as the central anchor, followed by B9 domain-containing proteins (MKS-1, MKSR-1, MKSR-2). Together, our findings expand the interaction network of ciliopathy-associated proteins and suggest a two-stage ciliogenic pathway that first involves transition zone proteins, followed by an intraflagellar transport (IFT)-dependent formation of the remaining axoneme.

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A novel regulatory circuit for PtdIns(3)P production on phagosomal surfaces during the degradation of apoptotic cells. N. Lu[1], **Q. Shen[1]**, TR. Mahoney[2], Y. Wang[1], Z. Zhou[1,3]. 1) Verna and Marrs McLean Department of Biochemistry and Molecular Biology; 2) Department of Molecular and Human Genetics; 3) Program in Developmental Biology, Baylor College of Medicine, Houston, TX, 77030.

In metazoan organisms, apoptotic cells are swiftly engulfed by phagocytes and degraded inside phagosomes through phagosome maturation. The defects in the removal of apoptotic cell could result in inflammatory response and autoimmune diseases. Using the nematode C. elegans as a model system, we have established a signaling pathway that triggers the degradation of apoptotic cells through promoting the fusion between endocytic vesicles and maturing phagosomes. Phosphatidylinositol 3-phosphate (PtdIns3P), a lipid second messenger that is rapidly synthesized and transiently presented on the surface of nascent phagosomes, has been implicated in triggering phagosome maturation, yet how it is produced on phagosomal surfaces and how it regulates key molecular events in the degradation of apoptotic cells inside phagosomes remains largely unknown. Recently, we have identified three sorting nexins that act as PtdIns(3)P effectors that facilitate the fusions between organelles of the endocytic pathway and phagosomes. Previously, the Class III PI-3 kinase VPS-34 was the only kinase known to produce PtdIns(3)P on intracellular membranes. Here, we report that PIKI-1, a novel Class II PI 3-kinase, is another important producer of PtdIns(3)P on phagosomal membranes in C. elegans in addition to VPS-34. PIKI-1 and VPS-34 play differential yet complementary roles in the production of PtdIns(3)P on nascent phagosomes. We further identified the novel role of MTM-1, a PI-3 phosphatase in catalyzing the turnover of PtdIns(3)P on phagosomes. Our findings demonstrate that the dynamic presence pattern of PtdIns(3)P on phagosomes is critical for phagosome maturation, and further indicate that this dynamic pattern is precisely controlled by a regulatory circuit composed of two PI 3-kinases and one PI 3-phosphatase.

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A screen for neuroprotective genes uncovers a membrane protein human haplotypes of which are associated with an increased risk of Parkinson's Disease. **N. MASOUDI[1]**, P. IBANEZ[1,2], A. GARTNER[1]. 1) WELLCOME TRUST CENTRE FOR GENE REGULATION AND EXPRESSION, DUNDEE UNIVERSITY, UK; 2) INSERM - Ecole Normale Superieure, France.

Parkinson's disease (PD) is the second most common human neurodegenerative disease. It is associated with a specific loss of dopaminergic neurons of the substantia nigra. While symptomatic treatment based on restoring dopaminergic function is initially effective, debilitating symptoms progressively worsen. The majority of PD cases are sporadic, while only 10% of PD patients display familial forms of the disease. Over the past decade, research on familial PD has identified causative mutations in six major genes, but those genes are linked to a relatively low percentage of PD patients. To eventually cure PD we need to understand the underlying cause of dopaminergic neurodegeneration and to develop drugs that prevent neurodegeneration. The degeneration of dopaminergic neurons can be recapitulated in many models, including C. elegans using 6-hydroxy-dopamine (6OHDA), an oxidized derivative of dopamine (Nass et al. PNAS, 2002). To find genes that protect dopaminergic neurons we searched for mutants exhibiting hypersensitivity to the neurotoxin 6OHDA. We cloned the gene corresponding to the strongest such mutant and found that the mutation is in an integral membrane protein belonging to tetraspanin protein family. 30 members of human tetraspanin protein family have been described to be potentially involved in cell adhesion, motility and tumour metastasis. The hypersensitivity of this mutant is rescued by introducing the wild type allele of tetraspanin and depends on the DAT-1 transporter. Also, based on reporter gene fusion this tetraspanin is expressed in dopmainergic neurons. To our surprise over-expression of mutant and wild type copy of this gene lead to spontaneous degeneration, even in the absence of the neurotoxin. Recently, Genome wide association studies identified variants of human genes that are associated with an increased risk to acquire Parkinson's disease (Singleton et al. Lancet, 2011). One of the few genes uncovered in this study appears to be a homolog of the tertaspanin we uncovered in our screen. The combined data are consistent with a conserved function of the tetraspanin in protecting dopaminergic neurons. At the moment we try to complement the C. elegans mutant with the human gene. Furthermore, we aim to find out how the tetraspanin protect domapninergic neurons. 1) Excessive dopamine levels have been shown to be neurotoxic. We thus aim at determining its epistatic relationship with genes involved in the dopamine biosynthesis, uptake or catabolism. 2) We aim at testing of the tetraspanin is involved in protecting dopaminergic neurons from oxidative stress.

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The Neurodegenerative Disease Protein Progranulin Regulates Programmed Cell Death Kinetics and Stress Resistance. **Aimee Kao**[1,2], A. Nakamura[2], M. Judy[2], R. Eisenhut[2], L. Herl Martens[3], A. Huang[2], A. de Luis[4], J. Cabello[4], R. Farese[3], Cynthia Kenyon[2]. 1) Dept. of Neurology, Univ. of San Francisco, San Francisco, CA; 2) Dept. of Biochemistry and Biophysics, Univ. of San Francisco, San Francisco, CA; 3) Gladstone Institute of Cardiovascular Disease, San Francisco, CA; 4) Center for Biomedical Research of Rioja, Logroño, Spain.

Frontotemporal lobar degeneration (FTLD) is the most common cause of dementia in individuals under the age of 60. Mutations in the progranulin (*Pgrn*) gene resulting in haploinsufficiency are responsible for nearly half of familial FTLD cases and polymorphisms in *Pgrn* may increase the risk for Alzheimer's and Parkinson's Disease. Although progranulin has some growth factor properties and is highly secreted by several aggressive cancer types, the mechanism by which haploinsufficiency leads to neurodegeneration remains unclear. We have characterized the phenotype of a *C. elegans* mutant lacking progranulin (*pgrn-1*). By 4-D Nomarski time-lapse microscopy, we show that *pgrn-1* mutants exhibit accelerated clearance of apoptotic cells. Progranulin appears to be acting through the canonical engulfment pathways (*i.e ced-1* and *ced-2*) to exert its effect on programmed cell death kinetics. Similarly, mouse macrophages cultured from progranulin knock out mice exhibit increased phagocytosis of apoptotic cells. We also find that *pgrn-1* mutants are resistant to osmotic, heat and unfolded protein stress while young but lose this stress resistance with age. The ability of young *pgrn-1* mutants to resist unfolded protein stress is dependent upon *daf-16*, *ire-1* and *pek-1* but independent of *atf-6*. Human progranulin can partially rescue the stress resistance phenotype of *C. elegans pgrn-1* mutants. Although most neurodegenerative diseases are thought to be due to the toxic effects of aggregated proteins, our findings suggest that susceptibility to neurodegeneration may be increased by a change in the kinetics of programmed cell death. We propose a model in which damaged or injured cells that might otherwise recover are prematurely engulfed and removed in situations with inadequate progranulin. This subtle shift in the balance of recovery and repair may serve as a basis for neurodegenerative disease development. Youthful stress resistance may explain the late onset of genetically determined FTLD.

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Entry to dauer state prevents necrotic neuronal degeneration. **Andrea Calixto**[1], Felipe A. Court[2]. 1) Biological Sciences, P. Catholic University of Chile, Santiago, Chile; 2) Millenium Nucleus for Regenerative Biology, P. Catholic University of Chile, Santiago, Chile.

Neuronal degeneration underlies many neurological disorders. Invertebrate neurons under cytotoxic or mechanical stress degenerate like mammalian neurons, sharing the mechanisms by which damaged neurons meet their end. In *C. elegans*, hyperactivated degenerin channels cause neuronal somas to swell and die. Constitutively opened MEC-4 channels (due to a dominant *mec-4d* mutation), cause progressive death of touch receptor neurons. Historically, the study of *mec-4d* mediated degeneration focused on neural somas and their distinctive vacuolization, but degeneration of axons and dendrites has not been examined. Axonal degeneration is key to the pathogenesis of neurodegenerative conditions, so it represents an important target for neuroprotection. To search for novel mechanisms of axonal degeneration, we performed a detailed temporal analysis of the AVM touch neuron ontogeny in *mec-4d* mutants. After birth, neuronal soma swells, then disappears, and axons bead in a centripetal fashion. Following extensive beading, axons become fragmented. These features are reminiscent of axonal degeneration in mammals after pro-degenerative stimuli. In addition, we show here that *mec-4d* dependent axonal degeneration is mechanistically similar to Wallerian degeneration of mammalian axons. Even though the mechanisms of axonal degeneration are not clearly defined, Ca^{2+} dysregulation, enhanced energetic metabolism and elevated reactive oxygen species contribute to axonal destruction. We reasoned that a state that regulates these processes might protect neurons from degeneration. *C. elegans* dauers have enhanced anti-ROS defense systems and oxygen consumption is low. *mec-4d* worms were induced to form dauer by starvation for one week or one month. Strikingly, there was a complete prevention of somatic and axonal degeneration in dauer, independently of the diapause length. Noticeably, AVMs not only survived but were also repaired from signs of degeneration in the population at dauer entry. Dauer larvae express *mec-4::gfp* normally and are touch sensitive, which rules out that MEC-4 is not expressed, or non-functional. The degeneration rate of somas and axons during dauer exit was contrasted to developmental degeneration: While somas degenerate at the same rate in both cases, axons degenerate 3 times slower in post dauer worms.This is the first report showing prevention of neuronal degeneration and activation of repair mechanisms by a change in an organism metabolic state. Like suspended animation, the induction of a dauer state may constitute a window for prevention and even repair of neuronal damage.

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The kinesin-3 family member KLP-4 regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of *C. elegans*. **Michael I Monteiro**[1], Shikha Ahlawat[2], Jennifer R Kowalski[1,3], Emily Malkin[1], Sandhya P Koushika[2], Peter Juo[1]. 1) Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, 150 Harrison Avenue, Boston, MA. 02111; 2) National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bangalore, India; 3) Department of Biology, Butler University, Indianapolis, IN, 46208.

The transport of glutamate receptors from the cell body to synapses is essential during neuronal development and may contribute to the regulation of synaptic strength in the mature nervous system. We previously showed that cyclin-dependent kinase-5 (CDK-5) positively regulates the abundance of GLR-1 glutamate receptors at synapses in the ventral nerve cord (VNC) of *C. elegans* (1). GLR-1 is an AMPA-type glutamate receptor that is expressed in VNC interneurons where it is localized to sensory-interneuron and interneuron-interneuron synapses (2-4). Here, we identify the kinesin-3 family member *klp-4*/KIF13 in a *cdk-5* suppressor screen for genes that regulate GLR-1 trafficking. *klp-4(pz19)* and *klp-4(tm2114)* mutants have decreased abundance of GLR-1 at synapses in the VNC and this effect can be rescued by expression of wild type *klp-4* cDNA in the ventral cord interneurons. Genetic analysis of *klp-4* and the clathrin adaptin *unc-11*/AP180 suggests that *klp-4* functions prior to endocytosis in the VNC. Time-lapse microscopy indicates that *klp-4* mutants exhibit decreased anterograde flux of GLR-1. Genetic analysis of *cdk-5* and *klp-4* suggest that they function in the same pathway to regulate GLR-1 in the VNC. However, GLR-1 accumulates in cell bodies of *cdk-5* but not *klp-4* mutants. Interestingly, GLR-1 does accumulate in *klp-4* mutant cell bodies if receptor degradation in the MVB/lysosome pathway is blocked. This study identifies KLP-4 as a motor that regulates the anterograde trafficking of GLR-1 and reveals a potential cellular control mechanism where the level or availability of a motor determines the fate of its cargo.

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Soulless nematodes: KIN-29 mediates the essential function of the CAN neurons. **Catarina Morck**, Gian Garriga. Department of molecular and cell biology, University of California, Berkeley, Berkeley, CA.

The canal-associated neurons (CANL/R) are two bilaterally symmetric neurons that are born in the head and migrate to the middle of the embryo. Each neuron extends two processes: one process grows to the head and one to the tail (1). The CANs are essential for viability. Sulston and Hodgkin originally discovered that killing the CANs with laser microsurgery resulted in a starved appearance and larval arrest, leading them to propose that the CANs were the nematode soul (2). Similarly, ceh-10(gm58) mutants lack CANs and die as larvae (3). In vab-8(e1017) mutants, the CAN neurons fail to migrate, and their posterior processes only extend a short distance. As a consequence, the part of the body that lacks CAN processes becomes much thinner and the worms exhibit a withered tail phenotype (4). We identified a mutation that suppresses the withered tail phenotype of vab-8 mutants and the larval lethality of ceh-10 mutants. Surprisingly, the double mutants still retain defective CANs, which indicates that the suppressor mutation bypasses the requirement for CAN function. The suppressor encodes kin-29, a serine threonine kinase most homologous to members of the ELKL motif kinase (EMK) family and salt-induced kinase family (5). KIN-29 and its homologs phosphorylate and inhibit class II histone deacetylases. In C. elegans, KIN-29 inhibits HDA-4, which acts with the MADS domain transcription factor MEF-2 to regulate chemoreceptor gene expression (6). Loss of HDA-4 or MEF-2 is not lethal, indicating that KIN-29 must have other targets that mediate CAN function. The pseudocoelom fills with fluid in animals with compromised CAN function. This phenotype and the association of the CAN processes with the excretory canals led us to test the model where the CANs essential function is to regulate the activity of the excretory canals. The analysis of CAN and excretory canal mutants suggest that the CANs regulate other cells, and we are currently attempting to identify CAN target cells by addressing where KIN-29 functions. REFERENCES: 1.Sulston, J.E., E.Schierenberg, J.G. White and Thomson, J.N. (1983) Dev. Biol. 100: 64-119 2.Sulston, J.E. and Hodgkin, J.A. Worm Breeder's Gazette 5(1): 19 3.Forrester, W.C., Garriga, G. (1997) Development 124(9): 1831-43. 4.Manser, J. Wood, W.B. (1990) Dev Gen 11:49-64 5.Lanjuin, A., Sengupta, P. (2002) Neuron 33(3): 369-81 6.van der Linden, A.M., Nolan, K., Sengupta, P. (2007) EMBO. 26(2): 358-70.

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Controlling Gene expression with the Q Repressible Binary Expression System In *C.elegans*. **Xing Wei[1,2]**, Christopher J. Potter[1,2,3], Liqun Luo[1,2], Kang Shen[1,2]. 1) Department of Biology, Stanford Univ, Stanford, CA; 2) Howard Hughes Medical Institute, USA; 3) Current Address: The Solomon H. Synder Department of Neuroscience, The Johns Hopkins University School of Medicine,Baltimore, MD.

The capability to regulate the expression of engineered transgenes has revolutionized the study of biology in multi-cellular genetic model organisms. One popular and powerful strategy is using a binary expression system such as the tetracycline-regulated tTA/TRE system in mammals and the GAL4/UAS system in *Drosophila*. However, so far there has not been any transcription-based binary expression system reported in nematode *C.elegans*. Recently, a novel repressible binary expression system, the Q system, was established in *Drosophila* and mammalian cells based on the regulatory genes from the *Neurospora crassa qa* gene cluster. The transcriptional activator QF binds to a 16bp sequence (named as QUAS) and activates expression of target genes under the control of QUAS sites; the expression can be efficiently suppressed by its transcriptional repressor QS; the transcriptional suppression can be relieved by feeding animals quinic acid, a non-toxic small molecule. So far, we have successfully adapted the Q system into *C.elegans* and proven its high specificity and sensitivity in nervous system. We created transgenic lines that co-express QF in a specific subset of neurons together with the *QUAS::GFP*, and as expected, GFP was only expressed in these neurons. When *QS* was expressed in these neurons with *QF* and *QUAS::GFP*, the expression of GFP was efficiently suppressed. And the suppression can be relieved in 6 hours if feeding these transgenic animals on NGM plates containing quinic acid, suggesting that the Q repressible binary system is as effective in nematode *C.elegans* as in mammalian cells and *Drosophila*. Besides precise temporal control of expression, the Q system can also be utilized to refine spatial control of transgene expression by using combinatorial promoters. Using different promoters to express QF and QS, we can label more specific subset of neuron. Furthermore, we split QF into two halves, binding half and activation half, and we fused a heterodimerizing leucine zipper fragment with each half to enhance the reconstitution efficiency of active QF. When the two halves were expressed using different promoters, the transcriptional activity could be reconstituted within the intersectional subset of two promoters. The newly introduced “split Q system” with intersectional promoters can afford even higher degree of control and achieve expression at the single cell resolution.

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A selective plane illumination microscope for high-speed, long-term C. elegans embryogenesis studies. Yicong Wu[1], Alireza Ghitani[1], Ryan Christensen[2], Anthony Santella[3], Gary Rondeau[4], Zhirong Bao[3], Daniel Colón-Ramos[2], **Hari Shroff[1]**. 1) National Institute of Biomedical Imaging and Bioengineering, NIH, Bethesda, MD; 2) Program in Cellular Neuroscience, Neurodegeneration and Repair, Department of Cell Biology, Yale University School of Medicine, New Haven, CT; 3) Developmental Biology Program, Sloan-Kettering Institute, New York, NY; 4) Applied Scientific Instrumentation, Eugene, OR.

We introduce a high speed selective plane illumination microscope (SPIM) for in toto studies of development in C. elegans. The system allows continuous visualization of fluorescent protein constructs in transgenic nematode embryos, from fertilization through hatching with no detectable photodamage. Volumetric images are collected every two seconds, for a total of ~24,000 imaging volumes over the entire 14 hours of embryogenesis. The high imaging rate afforded by the microscope minimizes artifacts due to motion blur and enables examination of developmental events through twitching without the use of anesthetics or genetic perturbations. The microscope is 30 times faster than spinning disk confocal microscopy, but the signal-to-noise ratio is superior and subcellular resolution is comparable, allowing visualization of cell biological events throughout development. We have combined the system with existing computer-assisted cell identification approaches to generate a module that enables unambiguous identification of individual cells through the use of lineage identity. Our results establish a strategy and pave the way for systematic mapping of the wiring process and extension of the wiring diagram to a four dimensional dynamic atlas. The microscope design employs a straightforward add-on of a SPIM module onto an inverted microscope and uses conventional mounting of specimens on glass slides, making it readily adaptable by other cell- and developmental biology labs.

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Three-dimensional molecular architecture of a cell using photo-activated localization microscopy and electron microscopy. **Shigeki Watanabe**, Gunther Hollopeter, Robert Hobson, Jackson Richards, Pin-An Chen, Christian Frøkjær-Jensen, Erik Jorgensen. Dept Biol, Univ Utah, Salt Lake City, UT.

Mapping the molecular architecture of proteins within a cell is essential for understanding their function. Fluorescence microscopy has been widely used for this purpose. However, the diffraction limit of light limits this approach, since fluorophores that are within ~200 nm of each other cannot be resolved. Several microscopy techniques have been developed to break this limit, including photo-activated localization microscopy (PALM)[1] and related techniques (STORM[2] and fPALM[3]). PALM allows one to precisely pinpoint the location of a single molecule in a cell to within 10 nm. However, precise localization in a field of black is not useful. Where is that protein in the context of cellular structure? PALM images need to be overlaid on the structures visualized by electron microscopy. Therefore, a method to preserve fluorophores in tissues fixed and embedded in plastic needs to be developed. To preserve fluorescence in plastic, three conditions are required: hydration, limited oxidation from fixatives, and neutral pH. We found that use of 5% water, a less oxidative fixative (KMnO4), and hydrophilic plastic (GMA - pH8), were sufficient to preserve signals and morphology. To test the feasibility of fEM using PALM, we tagged histone, TOM-20, and liprin, with photo-convertible fluorescent proteins. We showed that histone, TOM-20, and liprin were successfully localized to the expected organelles - nucleus, mitochondrial membrane, and dense projection, respectively[4]. We also have mapped the $\alpha 2\delta$ subunits of voltage-gated calcium channels in the nerve ring of C. elegans. We found the subunits to be exclusively localized to dense projections, supporting the previous localization pattern by immuno-EM[5]. This result suggests that the fEM is feasible for protein localization, and many proteins can be localized to their cellular structures if they can be tagged by fluorophores. References [1]Betzig, E. et al. Science 313, 1642-1645 (2006). [2]Rust, M.J., Bates, M. & Zhuang, X. Nat. Methods 3, 793-795 (2006). [3]Hess, S.T., Girirajan, T.P.K. & Mason, M.D. Biophys. J 91, 4258-4272 (2006). [4]Watanabe, S. et al. Nat. Methods 8, 80-84 (2011). [5]Gracheva, E.O., Hadwiger, G., Nonet, M.L. & Richmond, J.E. Neurosci. Lett 444, 137-142 (2008).

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Arp2/3 promotes nuclear migration and cell polarity in the C. elegans zygote. Huajiang Xiong[1], William A. Mohler[2], **Martha C. Soto[1]**. 1) Dept. of Pathology, UMDNJ/RWJMS, Piscataway, NJ; 2) Dept. of Genetics and Developmental Biology, UCHC, Farmington, CT.

Dynamic remodeling of the actin cytoskeleton plays an essential role in all organisms. Our laboratory has established that the actin nucleation cassette, encoded by Rac1-WAVE/SCAR-Arp2/3, referred to as the GEX (GTPase/Enhancer of nucleation/actin nucleation eXecution) complex, is essential for embryonic cell migration and morphogenesis in C. elegans. However, 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, which requires distinct complexes composed of two novel nuclear envelope protein families, SUN and KASH proteins. Interestingly, studies in yeast and plants demonstrate that interaction of MTs with F-actin is required for proper nuclear migration. Nevertheless, the role of Arp2/3 in nuclear migration remains unknown. We report that loss of WAVE/SCAR components leads to nuclear migration failure in various tissues at different developmental stages. Upon depletion of WAVE/SCAR molecules, we observed nuclear migration defects in embryonic epidermal cells during enclosure of the embryo as well as in the larval P-cells during differentiation into motor neurons. Further, we studied nuclear migration in the one-cell zygote, in which the female and male pronuclei undergo a stereotypical migration to form the zygote. One-cell zygotes depleted of WAVE/SCAR components display a defective anterior migration of the male pronucleus that is accompanied by altered non-muscle myosin-II dynamics and decreased cortical enrichment of the posterior polarity determinant, PAR-2. Reduced establishment of microtubules and actin at the posterior cortex in Arp2/3 mutants correlates with reduced male pronuclear migration, suggesting that cortical actin affects MT assembly during nuclear migration. Additionally, we noticed that mutation of the SUN protein, UNC-84, which is involved in nuclear migration in embryonic epidermal cells and larval P-cells, leads to defective male pronuclear migration. In conclusion, our studies demonstrate that Arp2/3-dependent actin nucleation contributes to nuclear migration throughout development and provide a novel system to examine the interaction of the MT and actin cytoskeletons.

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Actin binding proteins affect the range of polarizing cortical flows in *C. elegans* zygotes. **Sundar Naganathan[1,2]**, Justin Bois[1,2], Guillaume Salbreux[1,2], Stephan Grill[1,2]. 1) MPI-CBG, Dresden, Germany; 2) MPI-PKS, Dresden, Germany.

Establishment of polarity is essential for conferring different developmental fates to the dividing cells of an embryo. In *Caenorhabditis elegans* one cell embryos, anteroposterior polarization is facilitated by long-ranged flow of the actomyosin cortex. Even though the flowing cortex contains many actin binding proteins (ABPs) that contribute to its structure and dynamics, there are only a limited number of mechanical properties that are important at large length and time scales relevant for polarization, for example contractility and cortical viscosity (Mayer, Bois, Depken, Jülicher, Grill, 2010). Importantly, this suggests that there is only a reduced spectrum of cortical flow phenotypes that one might expect to obtain by modulating these few mechanical properties through different molecular mechanisms. To bridge the gap between molecular and cellular scales, we here sought to investigate which cell-scale mechanical properties are controlled by which ABPs. We devised a candidate RNAi screen of ABPs and found that several ABPs affect cortical flow. This was achieved by analyzing myosin foci size and density and several flow characteristics, such as peak velocities and spatio-temporal velocity-velocity correlations, for each ABP knockdown. The velocity-velocity correlations provided us with an estimation of the characteristic hydrodynamic length of cortical flow, which describes the extent to which flows are long-ranged. Interestingly, all those ABPs that displayed a detectable cortical flow phenotype did so through affecting this hydrodynamic length. RNAi either resulted in short-ranged flows, indicative of a less viscous cortex, or it resulted in flows that were longer-ranged than wild type, indicative of a cortex that is more viscous that under wild-type conditions. Our results suggest that the characteristic hydrodynamic length is a central physical property subject to precise regulation. They also point towards a type of “mechanical redundancy” in animal development, with many molecular mechanisms affecting the same cell-scale physical property.

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The Morphogenetic Mechanisms Mediating Self-Organization and Plasticity during C. elegans Gastrulation. **Christian Pohl**, Anthony Santella, Julia M. Moore, Zhirong Bao. Developmental Biology Program, Sloan-Kettering Institute, New York, NY.

Gastrulation is a key transition in embryogenesis and a complex morphogenetic process that requires self-organized cellular coordination, which has to be both robust - to ensure proper development - and plastic - to allow adaptation to environmental or evolutionary change. We report that cellular rearrangements during Caenorhabditis elegans gastrulation depend on the cooperative action of two cellular processes, contractile flow-dependent protrusion formation and oriented cell divisions. Contractile cortical flows in gastrulating cells organize the extension of polarized lateral protrusions in adjacent cells, which close over the gastrulating cells with radial symmetry thereby creating multicellular rosettes. Radial covering re-establishes a continuous epithelial-like monolayer on the embryo's surface after each cell internalization event and resembles embryonic wound closure. Moreover, we find that the formation of multicellular rosettes represents the major mechanism for scalable local and global tissue patterning. Furthermore, rosette formation can adapt to severe topological alterations, providing a mechanistic explanation for morphogenetic plasticity. We also find that cell divisions polarized during rosette formation release tissue strain generated due to volume constraints during cell internalization. Our findings extend the spectrum of metazoan gastrulation mechanisms and provide insight into self-organization of embryonic morphogenesis.

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Filamin is required for the maintenance of F-actin and calcium signaling in the spermatheca. **Ismar Kovacevic**, Erin Cram. Biology, Northeastern University, Boston, MA.

We are using the *C. elegans* spermatheca as a model system to study how cells respond to mechanical forces *in vivo*. The spermatheca—a simple myoepithelial tube—experiences dramatic stretching forces caused by oocytes during ovulation. Following oocyte entry into the spermatheca, proximalward constriction of the spermatheca propels the fertilized oocyte into the uterus. We identified FLN-1/filamin as being required for the exit of oocytes from the spermatheca. Filamin is a stretch-sensitive structural and signaling scaffold that binds actin, transmembrane receptors, and a variety of intracellular signaling proteins. The *C. elegans* filamin ortholog (FLN-1) has a well-conserved overall structure, including an actin-binding domain, and a series of 20 immunoglobulin-like repeats. FLN-1 is expressed in spermathecal and uterine cells, colocalizes with F-actin, and is required to maintain the actin cytoskeleton in the spermatheca and uterus. Filamin-deficient animals accumulate embryos in the spermatheca, and consequently lay damaged eggs and exhibit reduced brood sizes. PLC-1/phospholipase C-ε is also required for the exit of embryos from the spermatheca, and analysis of double mutant animals suggests that PLC-1 and FLN-1 act in the same pathway. Because PLC-1 is thought to be upstream of intracellular calcium release, we used GCaMP—a genetically encoded calcium indicator—to image calcium during ovulation and spermathecal transit. Using worms expressing GCaMP we show that entry of an oocyte into the spermatheca initiates a distinctive series of autonomous calcium transients, which likely result in spermathecal constriction that propels the fertilized oocyte into the uterus. Interestingly, loss of FLN-1 results in drastically delayed onset of calcium signaling, followed by abnormal calcium oscillations. As expected, loss of PLC-1 abolishes the calcium transients entirely in the spermatheca. Given the effect on calcium signaling and the known mechanosensory role of filamin, we hypothesize that filamin is required in the spermatheca to respond to increased tissue tension, and to initiate the calcium signaling. Understanding how cells sense and respond to mechanical forces has implications for development, cancer metastasis, and normal organ function.

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ERM-1 is a negative regulator of LET-23 EGFR signaling. **Juan M. Escobar Restrepo**[1], Peter Gutierrez[1], Alessandra Bühler[1], David Kradofer[1], Erika Fröhli[1], Attila Stetak[2], Alex Hajnal[1]. 1) Institute of Molecular Life Sciences, University of Zürich, Switzerland; 2) Univeristy of Basel, Switzerland.

Vulval development is induced by activation of LET-23 EGFR in the basal membrane compartment of polarized Vulval Precursors Cells (VPCs) after interaction with the Anchor Cell secreted LIN-3 EGF. LET-23 is retained in this compartment by the conserved tripartite LIN-2/LIN-7/LIN-10 PDZ protein complex (Kaech et al. 1996). This interaction is mediated through a C-terminal PDZ binding domain in LET-23. Mutations in any of the components of this complex cause a loss of retention of LET-23 in the baso-lateral membrane compartment and result in the apical mislocalization of LET-23. This impairs the interaction of LET-23 with LIN-3 and leads to a reduced vulval induction. We have performed a screen to find novel regulators of LET-23 localization in the VPCs and found that LET-23 is miss-localized in *erm-1* mutants. *erm-1* encodes the homologue of mammalian Ezrin, Radixin and Moesin proteins (ERM), which in their open phosphorylated form link plasma membrane proteins to the actin cytoskeleton. Here, we show that *erm-1* acts as negative regulator of the *let-23/let-60/mpk-1* pathway, possibly by sequestering and stabilizing the LET-23 receptor in an inactive compartment at or near the baso-lateral plasma membrane of the VPCs. The following lines of evidence support our model: (1) An *erm-1(lf)* mutant suppresses the Vulvaless phenotype caused by reduction-of-function mutations in the *let-23/let-60/mpk-1* pathway and enhances the Multivulva phenotype caused by a gain-of-function mutation in *let-60 ras*. (2) An ERM-1::mCherry fusion protein as well as a constitutively active, phospho-mimicking ERM-1::T544D::mCherry mutant co-localize with a functional LET-23::GFP protein on the baso-lateral membrane, while the phosphorylation resistant ERM-1::T544A::mCherry mutant remains in the cytoplasm. (3) Recombinant ERM-1::GST interacts with LET-23 from worm extracts, this interaction is independent of LIN-7 and the PDZ binding motif at the LET-23 C-terminus, suggesting that ERM-1 interacts with LET-23 in a complex distinct from the LET-23/LIN-2/LIN-7/LIN-10 complex. (4) *erm-1(lf)* mutants display reduced basal LET-23::GFP staining and Fluorescence Recovery After Photobleaching experiments show a significantly faster recovery of basal LET-23::GFP in *erm-1* mutants compared to the wild-type. In summary, we propose that ERM-1 retains a fraction of LET-23 in an inactive compartment, thereby competing with the activating LET-23/LIN-2/LIN-7/LIN-10 complex. ERM-1 may act as a buffer to prevent the immediate activation of the entire pool of baso-lateral LET-23 and thus allow prolonged LET-23 signaling to occur.

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Functional transcriptomics of the migrating linker cell in *C. elegans*. **Mihoko Kato**, Erich Schwarz, Paul Sternberg. Div. of Biology, HHMI/Caltech, Pasadena, CA.

Dynamically regulated cell migrations are vital for the development of multicellular organisms. The *C. elegans* linker cell (LC) is an individual cell that guides the complex yet highly stereotyped migration of the male gonad, as the LC travels through much of the body length. We have previously demonstrated that stage-specific changes in the migrating LCs of L3 and L4 larvae are regulated by *nhr-67*, an ortholog of *tailless* and NR2E1 in *Drosophila* and mice (Kato and Sternberg [2009], Development *136*, 3907-3915.). To better understand how *nhr-67* controls LC migration, we used RT-PCR and RNA-seq on individually microdissected wild-type LCs from L3 and L4 larvae, and *nhr-67*(RNAi) LCs from L4 larvae. We found 8,011 genes expressed in wild-type LCs, of which 963 are highly LC-specific (being expressed 20x more strongly than in whole larvae, and with ≥0.5 RPKM). Genes with the strongest LC-specific expression preferentially had functions in transcriptional regulation, cytoskeletal protein binding, cell adhesion, and intracellular protein transport. Two other preferential functions were synaptic transmission and axonal components; these might reflect a role for synaptic genes in the LC for secreting and sensing cues or for membrane recycling during migration. 2,051 genes (25%) showed ≥100-fold expression changes between wild-type and *nhr-67*(RNAi) L4 stage LCs. Genes upregulated in LCs from the L3 to L4 stage in an *nhr-67*-dependent manner disproportionately encoded potassium channels and regulators of muscle contraction. To validate our profiling data in vivo, we assayed 200 of our ~1,000 LC-enriched genes for function in migrating LCs with feeding RNAi and Nomarski microscopy. This revealed 29 new LC-enriched genes required for normal migration, including two likely partners of HLH-2 (an E/Da transcription factor specifying LCs) and a seven-transmembrane receptor gene positively regulated by NHR-67 and showing one of *nhr-67*′s four RNAi phenotypes. Unexpectedly, we found that several different SMC subunits of cohesin or condensin are required for persistent attachment of the gonad to the migrating LC. We also found many genes for major sperm proteins (MSPs) are strongly upregulated in L4 LCs from the L3 stage, and that at least some of them are required for LC shape and mobility, demonstrating the use of MSPs for motility in a non-sperm cell type during somatic development.

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NHR-67, a key transcription factor that maintains *C. elegans* anchor cell identity and the invasive phenotype. **David Q Matus**, Adam J Schindler, Qiuyi Chi, David R Sherwood. Biology, Duke University, Durham, NC.

The transcriptional networks that specify and regulate the ability of a cell to traverse the boundaries imposed by basement membranes (BMs) are poorly characterized. This is largely due to the difficulties of studying this complex and dynamic event in vivo. Cell invasion is a critical cell biological process that occurs during normal development, immune surveillance, and is mis-regulated during metastatic cancer. Utilizing the simple model of *C. elegans* anchor cell (AC) invasion, we have previously identified four transcription factors (MEP-1, FOS-1A, EGL-43L, and HLH-2) and several downstream targets (*cdh-3*, *zmp-1*, *hemicentin*, T03F1.8) that function to regulate distinct aspects of AC invasion. We screened by RNAi 698 *C. elegans* transcription factors in a uterine-specific RNAi sensitive background, and identified the vertebrate tailless ortholog, NHR-67, as a potent regulator of AC invasion. Reduction in NHR-67 function by RNAi, loss-of-function alleles, and an AC-specific dominant negative NHR-67 construct suggest that NHR-67 functions at multiple levels to regulate the ability of the AC to initiate a BM transmigration program. NHR-67 is expressed in the gonadal lineage leading to the AC, where it appears to regulate the LIN-12/LAG-2 (Notch/Delta) signaling event that specifies the AC. Following Notch-mediated AC specification, loss of NHR-67 results in the presence of multiple ACs that express markers of an active cell cycle (*rnr::GFP* and *cye-1>GFP*), suggesting that NHR-67 functions downstream of Notch signaling to maintain the AC in a post-mitotic state. Loss of NHR-67 also inhibits AC invasion, where it appears to regulate multiple aspects of the invasion program, including the establishment of a specialized invasive cell membrane domain and the expression of the transcription factor FOS-1A and the guanylate kinase, T03F1.8. Taken together, these data suggest that NHR-67 functions at a key node in the gene regulatory network that both maintains and promotes the ability of the AC to invade.

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The long cellular projections of *C. elegans* pharyngeal gland cells form via retrograde extension under the control of CAM-1/ROR and EGL-15/FGFR signaling. **Jay Kormish**, Wahyu Raharjo, Patricia Rohs, Shinhye Kim, Jeb Gaudet. Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada.

The pharynx (foregut) of *C. elegans* is a powerful model for organ development. As in other organs, the specific morphology of pharyngeal cells is intricately connected to cellular function, with many pharyngeal cells having an unique and intricate form. For example, each of the five pharyngeal glands consists of a cell body in the posterior pharyngeal bulb that sends a long cellular extension to a specific anterior position within the pharynx; these extensions are surrounded by the pharyngeal muscles. Our work has been focused on understanding how these projections form within the context of the developing pharynx. While most cellular projections form by growing out from a cell body (i.e., growth cones in neurons and filopodia in myoblasts), the glands use a strikingly distinct mechanism termed “retrograde extension” (coined by Heiman and Shaham 2009, to describe the behavior of amphid dendrites) - cells are born at the site where projections anchor and migrate to the posterior pharynx resulting in the “drawing out” of a long cellular projection. To date little is known about the signaling pathways that regulate retrograde extension. We have used a genetic candidate approach to define the molecular pathways governing gland cell migration. Standard pathways that regulate neurite outgrowth (slit/robo, netrin/TGFβ, ephrin and semaphorin) do not appear to play roles in gland cell morphogenesis. However, of the many signaling pathways tested, the Wnt/ROR and FGF/FGFR pathways play antagonistic roles in gland cell migration. The Wnt/ROR pathway appears to function in attraction: in *cwn-2*/*Wnt* and *cam-1*/*ROR* mutants, one of the gland cells fail to migrate to its normal position and frequently occupies an anterior position in the pharynx. In contrast, FGFR appears to function in repulsion: in *let-756*/*FGF* and *egl-15*/*FGFR* mutants, glands migrate beyond their normal positions and are often located in the anterior intestine. Surprisingly, studies of cell-autonomy show both pathways function in the non-gland cells of the pharynx. Furthermore, the Wnt ligand, CWN-2, provides a non-directional cue allowing for migration. These finding are unexpected because in neurons CAM-1 appears to act cell-autonomously to regulate cell migration (Kennerdell et al., 2009). In summary, CAM-1 and EGL-15 function antagonistically in the muscle cells of the pharynx to provide a permissive/restrictive signal or substrate to allow gland cells to migrate to their proper positions. We are currently identifying possible points of connection between the CAM-1 and EGL-15 receptor tyrosine kinase.

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K40 α-Tubulin Acetylation Defines Microtubule Quaternary Structure *in vivo*. **Juan Cueva**, Miriam Goodman. Molecular & Cellular Physiology, Stanford University, Stanford, CA.

Microtubules are cytoskeletal components critical for diverse cellular functions including mitosis and meiosis, cellular migration, subcellular transport, and neural function. They are composed of polymers of αβ-tubulin arranged into a tubular quaternary structure. The quaternary structures of microtubules required for different cellular functions are often distinct despite the high level of tubulin sequence conservation both within and across species. Post-translational modifications such as acetylation of α-tubulin are hypothesized to confer structural and thus functional heterogeneity to microtubules as they do to histones. But, the *in vivo* mechanism for this process remains elusive. Here, we demonstrate that K40 acetylation of the α-tubulin MEC-12 by the tubulin acetyltransferases, MEC-17 and ATAT-2, is critical for establishing microtubule quaternary structure *in vivo*. Previously, we established that MEC-17 and ATAT-2 are the tubulin acetyl transferases responsible for acetylating the α-tubulin MEC-12 in *C. elegans* (Shida et al., 2010). MEC-12 is well known as a critical component of the distinctive 15-protofilament microtubules required for touch receptor neuron-mediated mechanosensation. Eliminating MEC-12 K40 acetylation decreases touch sensation. However, it is not known how the loss of this post-translational modification affects microtubule structure. We investigated this question by reconstructing the nanoscale morphology of unacetylated microtubules. In the absence of acetylation, the length of microtubules was reduced by a factor of ten and the total number of microtubules reduced five-fold. These findings confirmed previous speculation that acetylation stabilized microtubules. Intriguingly, many unacetylated microtubules consisted of protofilament numbers not typically observed *in vivo* in *C. elegans*. Microtubules with 9 to 15 protofilaments were observed in TRNs. These structural changes likely account for the partial loss of touch sensitivity, since the neurite morphology and distribution of native mechano-electrical transduction channels are essentially wild type in mutants lacking MEC-12 acetylation. Ultrastructural analysis of microtubules from transgenic animals expressing either an unacetylatable *mec-12*[K40R] or acetyl mimic *mec-12*[K40Q] confirm that acetylation of K40 α-tubulin MEC-12 is critical for defining microtubule quaternary structure *in vivo*.

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ERM-1/ezrin-radixin-moesin interacts with AQP-8/Aquaporin in de novo intracellular lumen and single-cell tube extension. **Liakot A. Khan**[1], Hongjie Zhang[1], Nessy Abraham[1], John T. Fleming[1], David H. Hall[2], Matthew J. Buechner[3], Verena Gobel[1]. 1) Department of Pediatrics, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114; 2) Center for C. elegans Anatomy, Albert Einstein College of Medicine, 1410 Pelham Pkwy, Dept Neuroscience, Bronx, NY 10461; 3) Department of Molecular Biosciences, 1200 Sunnyside Ave., 8035 Haworth Hall, University of Kansas, Lawrence, KS 66045-7534.

Single-cell tubes such as capillaries must intracellularly expand an apical membrane to extend the lumen, a process that is not well understood. Here we show that the cortical organizer ERM-1 is required for de novo apical membrane expansion and single-cell lumen and tube extension of the C. elegans excretory canals. We find that a simultaneous process of vacuole-coalescence and actin-coating builds and directionally expands the C. elegans excretory cell lumenal membrane. ERM-1 removal interrupts this process, while ERM-1 excess (ERM-1[+]) promotes aberrant membrane and cyst formation. An ERM-1[+] suppressor screen identified AQP-8, an aquaporin of previously unknown function, as required for the development of this cystic phenotype. We show here that AQP-8 localizes to vesicles/canaliculi; physically interacts with ERM-1; is recruited by ERM-1; and colocalizes with ERM-1 in concentric rings spaced at intervals along the expanding lumenal membrane. AQP-8 cooperates with ERM-1 in canal morphogenesis in a mercury-sensitive manner, implying water channel activity, and excess ERM-1 increases the canaliculi-lumen connection in an AQP-8-dependent fashion. Our findings suggest that (1) ERM-1 is strictly required for intracellular lumenal membrane expansion and (2) the ERM-1/AQP-8 interaction supports lumen extension by intralumenal flux, providing evidence for a direct tissue-shaping force of water-channel-regulated fluid pressure in metazoan morphogenesis.

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Genetic architecture of male tail tip morphogenesis. Matthew D. Nelson, Karin C. Kiontke, R. Antonio Herrera, **David H.A. Fitch**. Department of Biology, New York University, New York, USA.

A major goal of research in morphogenesis is to delineate all steps that connect spatiotemporal regulation to the cellular machinery which affects changes in cell shape and migration. The four-celled tip of the *C. elegans* male tail is a simple but powerful model for studying morphogenesis. It also presents a model for sexual dimorphism at the cellular level. Through a genome-wide post-embryonic RNAi-feeding screen, we identified 211 genes that regulate or participate in male tail tip morphogenesis. Indeed, we found regulatory roles for well-characterized developmental pathways: the posterior Hox genes, the TGF-beta pathway, nuclear hormone receptors, the heterochronic pathway (for which we have also identified new components, see abstracts by Vuong et al. and Herrera, Kiontke et al.) and GATA transcription factors. Based on gene ontology terms and information mined form WormBase, we have clustered all tail tip morphogenesis genes into 24 developmental pathways or general cell biological processes. Using expression epistasis, we have constructed a genetic interaction network between representatives of these 24 clusters underlying morphogenesis. We have found these pathways to converge at the transcription factors DMD-3, MAB-3 and possibly NHR-25, which then appear to coordinate several modules of the cellular machinery, including cell fusion, endocytosis, and the cytoskeleton. Based on these data, we hypothesize that male tail tip morphogenesis is governed by a gene regulatory network that has a bow-tie architecture. We are currently testing for additional interactions within unresolved parts of this network.

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Cell polarity genetic network revealed by suppressor RNAi screens. **Bruno Fievet**, Josana Rodriguez, Julie Ahringer. Gurdon Inst, Univ Cambridge, Cambridge, United Kingdom.

Cell polarity is crucial for the function of most cell types. Polarity genes have been identified in screens using single gene loss of function approaches. Due to partial redundancy and built in robustness of biological processes many polarity players and functional links between them are still missing. Some genes might have additional loss of function phenotypes masking their polarity role and making identification of genetic interactions between essential genes challenging. We have developed a large scale screening strategy to efficiently detect genetic interactions between essential genes using RNAi suppressor screens. Focusing screening on a sub-library of 2133 genes with embryonic functions, we carried out screens for 17 temperature sensitive (ts) embryonic lethal mutants. These were compromised for essential polarity functions such as actomyosin contractility, PAR protein localization and spindle positioning. We developed a high-throughput protocol to screen for suppressors of a mutant in less than 2 weeks. By modulating the penetrance of RNAi knockdown we have doubled the sensitivity of the suppressor screens for detecting essential genetic interactions. Overall, we tested over 50,000 interactions. Reproducible suppressors were considered specific if they failed to suppress five ts mutants not involved in polarity. The functional analysis of our interactions shows that genes involved in ribosomal functions and mitochondrial respiratory chain non-specifically suppress a broad variety of ts mutants and therefore have been excluded from our analysis.

We have built a cell polarity network with 186 genes connected by 229 different genetic interactions. The network shows a 3-fold enrichment for known cell polarity players and, for each mutant screened, confirmed interactions predicted from the literature. We observed that ts genes sharing polarity functions cluster with each other and we have identified new hubs linking actomyosin, PAR proteins and spindle positioning machinery. Importantly, specific profiles of interactions found in our network suggested functional roles for suppressors that we have been able to confirm experimentally. We have also used our network to predict functions for unknown proteins that share similar interactions with known cellular components (see Rodriguez et al., abstract).

Applying our screening strategy in other contexts should be a powerful way to identify key players and uncover novel mechanisms in other essential biological processes.

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Identification and validation of *C. elegans* permeability mutants as a tool for drug screening and chemical genetics. **Frederick A. Partridge**[1], Steven D. Buckingham[2], Michael L. Youdell[1], Delia O'Rourke[1], Dave Stroud[1], Creg Darby[3], David B. Sattelle[4], Jonathan Hodgkin[1]. 1) Dept Biochem, Univ Oxford, Oxford, United Kingdom; 2) MRC Functional Genomics Unit, Oxford, United Kingdom; 3) School of Dentistry, Univ California San Francisco, CA; 4) School of Life Sciences, Univ Manchester, United Kingdom.

C. elegans is growing in importance as an in vivo screening tool for new drugs. It can be engineered to mimic human disease mutations, and is the only organism in which thousands of candidate compounds can be screened in a high-throughput whole animal model. This is particularly important for complex multi-tissue disease.

Unfortunately, less than 10% of human orally bioavailable drug compounds accumulate in *C. elegans* [1]. This greatly limits the chances of drug discovery using the worm.

Mutants that increase permeability could therefore be useful. We previously identified mutants in *bus-8* that are permeable, but these have additional confounding phenotypes including lethality that limit their use for drug screening [2]. Mutations in a least six other genes can result in increased cuticle permeability.

Here we present the identification and utilization of a new gene, *bus-5,* which encodes a predicted NAD+-dependent epimerase. Some alleles of *bus-5* show greatly increased permeability with few background phenotypes. We are validating the increased permeability of these mutants by directly measuring accumulation via an HPLC-based approach.

We also present proof-of-principle drug screens combining our permeability mutants with existing disease model strains. For these screens we make use of a automated thrashing assay that allows thousands of compounds to be easily screened per day [3].

Permeability mutants are also an important tool for the community as they allow the use of small-molecule inhibitors and other molecular tools that were previously unavailable to *C. elegans* researchers due to poor uptake.

[1] Burns AR *et al,* 2010. Nat. Chem. Biol. **6** 549 [2] Partridge FA *et al,* 2008. Dev. Biol. **317** 549 [3] Sleigh JN *et al,* 2011. Hum. Mol. Genet. **20** 245.

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The WormToolbox: automated quantification of image based *C. elegans* phenotypes. **Carolina Wählby**[1], Lee Kamentsky[1], Zihan Hans Liu[1], Tammy Riklin-Raviv[2], Annie L Conery[3], Eyleen J O'Rourke[3], Katherine L Madden[1], Orane Visviks[4], Vebjorn Ljosa[1], Javier E Irazoqui[4], Polina Golland[2], Gary Ruvkun[1], Frederick M Ausubel[3], Anne E Carpenter[1]. 1) Imaging Platform, Broad Institute of Harvard and MIT, Cambridge, MA; 2) Computer Science and Artificial Intelligence Laboratory, MIT, Cambridge, MA; 3) Dept. of Mol. Biol. and Center for Computational and Integrative Biology, Mass. General Hospital, Boston, MA; 4) Developmental Immunology Program, Dept. of Pediatrics, Mass. General Hospital, Boston, MA.

Changes in worm size, shape, and reporter signal expression patterns in response to chemical or genetic perturbations are visible by microscopy. However, the number and scale of such experiments is increasing, making visual analysis a challenge. Quantifying phenotypes by image analysis could increase throughput, objectivity, and accuracy. Much progress has been made in automating the analysis of some types of *C. elegans* experiments, including high-resolution, 3-D, and time-lapse images, or assays that rely on population-averaged measurements. However, high-throughput chemical and RNAi screens typically result in static images of adult worms in liquid culture and many assays require that the worms, which can be overlapped and clustered, are individually identified and measured. Otherwise, population heterogeneity is hidden in averages, the shape of individual animals is difficult to extract, and reporter signal expression patterns cannot be related to the anatomy of the worm. To improve the throughput and capabilities of *C. elegans* phenotype scoring, we developed a WormToolbox enabling individual worms to be detected and measured regardless of crossing or clustering. The worm detection, or “untangling”, searches for worms in brightfield images using a worm model. Once individual worms are identified, a large number of measurements such as size, shape, intensity, texture, and spot counts can be made on a per-worm basis in multiple image channels. Worms can also be digitally straightened and mapped to a common low-resolution worm atlas, allowing quantification of expression patterns in relation to worm anatomy. We show how the WormToolbox enables quantification of a variety of *C. elegans* phenotypes ranging from live/dead and fat accumulation screening to differentiating between worms based on subtle variations in GFP reporter expression patterns. We provide it free and open-sourced to the *C. elegans* community through the CellProfiler project (www.cellprofiler.org).

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Sequencing of five *Steinernema* genomes and transcriptomes illuminates parasitism, mutualism, and changes in expression timing in nematodes. **Adler R. Dillman**[1], Ali Mortazavi[1], Xiaojun Lu[2], Heidi Goodrich-Blair[2], Paul W. Sternberg[1]. 1) Howard Hughes Medical Institute, Division of Biology, California Institute of Technology, Pasadena, CA 91125; 2) Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706.

Nematodes form one of the largest invertebrate phyla with an estimated one million species occupying every conceivable niche. Besides the free-living model organism *Caenorhabditis elegans*, studied nematodes include parasites of plants, insects, livestock, and humans. Parasitic species often display great specificity to their hosts. The genus *Steinernema* comprises over 55 well-characterized species that are lethal parasites of insects with differing host ranges. We have sequenced and assembled the genome and staged transcriptomes of five whole genomes spanning the *Steinernema* genus (*S. carpocapsae, S. scapterisci, S. monticolum, S. glaseri*, and *S. feltiae*) using the Illumina platform. Steinernematid genomes prove amenable to Illumina sequencing due to their size (~95 Mb) and high G+C content (~45%). The combination of multiple closely related genomes in a non-*Caenorhabidtis* clade and accompanying deeply sequenced transcriptomes allows for powerful comparisons to other genera such as *Caenorhabditis*. In particular, comparisons in expression at defined stages shows significant plasticity of timing across one-to-one orthologous genes in the 5 genomes plus *C. elegans*. We explore the nematode contribution to mutualism using SSH library comparisons between *S. carpocapsae* grown on its symbiont, *Xenhorhabdus nematophila*, and *S. carpocapsae* grown on an *X. nematophila* colonization defective mutant. We identify nematode genes that are likely involved in colonization and explore their conservation and expression within the genus. We further examine the utility of these five genomes by orthology analysis within Nematoda, assessing the conservation of biological pathways, analyzing regulatory regions, and identify candidate genes involved in niche partitioning, host range, and mutualism within *Steinernema*.

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

To better characterize the *C. elegans* genome, to identify possible cis-regulatory elements, and to define genomic traits associated with hermaphroditism, the *Caenorhabditis* Genome Analysis Consortium <*http://wormgenomes.caltech.edu/consortium.html*> is analyzing and comparing the genomes from seven gonochoristic (dioecious) relatives of *C. elegans* (*C. remanei*, *C. brenneri*, *C. japonica*, *C*. sp. 5 JU800, *C*. sp. 7 JU1286, *C*. sp. 9 JU1422, and *C. angaria* PS1010) versus three hermaphroditic genomes (the published *C. elegans* and *C. briggsae*, and the newly sequenced *C*. sp. 11 JU1373). Gene predictions for newly sequenced or resequenced genomes are being performed with AUGUSTUS guided by RNA-seq; genome sequences and annotations will be available through WormBase. Most of the genomic sequences are nonrepetitive, with only 11-20% being predicted to comprise repetitive elements; these elements include likely vestiges of horizontal DNA transfer to individual species. In nonrepetitive genomic DNA, we find a core set of 8.8K orthologous multi-exon protein-coding genes conserved in all species examined, with 5.5K genes variably lost in different individual taxa. The evolutionary tree for 245 syntenic strict orthologs of protein-coding genes supports the previous consensus phylogeny, though it indicates rapid radiation of some taxa. Both intron length and codon bias vary substantially between orthologous protein-coding genes in different *Caenorhabditis* species. There are three prevalent core promoter motifs (TATA, SL1, and Sp1), along with extensive conservation of gene-specific cis-regulatory motifs such as AIY and DAF-12/M-2. Most sex-determination genes are strictly conserved, with *sdc-2*, *dpy-21*, and *fog-3* showing increased change in hermaphroditic species. We are currently defining which regions of the genome assemblies are most likely to represent minor alleles from unresolved heterozygosity in outbreeding species; given this, we expect to be able to resolve which features of hermaphroditic genomes (ranging in size from 90 to 104 Mb) are being consistently lost with respect to gonochoristic genomes (ranging in size from 130 to 190 Mb).

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Recent chromosome-scale selective sweeps reshaped *C. elegans* genomic diversity. **Erik C. Andersen**[1], Justin P. Gerke[1], Joshua A. Shapiro[1], Jonathan R. Crissman[1], Raj Ghosh[1], Joshua S. Bloom[1], Marie-Anne Félix[2], Leonid Kruglyak[1,3]. 1) Lewis-Sigler Inst, Princeton Univ, Princeton, NJ; 2) Institut Jacques Monod, Paris, France; 3) HHMI.

Even though *C. elegans* is central to research in molecular, cell, and developmental biology, nearly all of the research on this organism has been conducted on a single strain - N2. Comparatively little is known about the genomic and phenotypic variation of the species or its evolutionary history. I will report the characterization of *C. elegans* genetic variation using high-throughput selective sequencing of eight megabases from a worldwide collection of 200 wild strains representing nearly every known collection location. The identified set of 41,188 single nucleotide polymorphisms (SNPs) was used to define the most genotypically diverse strains for future quantitative genetic studies. We used these SNPs and strains to map loci controlling novel quantitative traits to haplotypes of less than 50 kb by genome-wide association studies. Unexpectedly, we found that the genome is dominated by a set of commonly shared haplotypes that each spans many megabases, suggesting recent shared ancestry. Furthermore, the distribution of polymorphism is strikingly non-uniform and correlates with variation in recombination rate found between the N2 and CB4856 strains. Simulations show that these patterns were generated by selective sweeps that have dramatically reduced variation worldwide and suggest that at least one of these sweeps occurred in the past few hundred years. Likely the result of human activity, these sweeps dramatically reshaped the global *C. elegans* population.

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C. elegans Dosage Compensation Regulates Histone H4 Chromatin State and RNA Pol II Stalling on X. **Michael B. Wells**, Martha J. Snyder, Gyorgyi Csankovszki. MCDB, Univ Michigan, Ann Arbor, MI., Michigan.

Dosage compensation equalizes X-linked gene expression between the sexes. Dosage compensation in C. elegans involves two-fold downregulation of both hermaphrodite X chromosomes by the dosage compensation complex (DCC), in order to equal the expression from the single male X chromosome. There is currently no mechanistic insight into how the DCC represses transcription. In order to address this question, we searched for histone modification differences on the hermaphrodite X chromosomes as compared to autosomes, which could indicate changes in chromatin structure capable of affecting transcription. We found that the activating modification H4K16-Ac is specifically reduced on hermaphrodite X chromosomes. Hermaphrodite-specific depletion of H4K16-Ac requires the DCC and the conserved histone deacetylase SIR-2.1. A modification known to antagonize H4K16-Ac, H4K20-Me[1], is strongly enriched on the dosage compensated X chromosomes, and we find that this modification's antagonism of H4K16-Ac is conserved in C. elegans. H4K20-Me[1] enrichment depends on the DCC and the histone methyltransferases SET-1 and SET-4. Both of these chromatin changes are linked to RNA Pol II regulation, as we identified DCC- and SET-4-dependent decreases in RNA Pol II occupancy as well as DCC-, SIR-2.1-, SET-1- and SET-4-dependent increases in RNA Pol II stalling on hermaphrodite X chromosomes. DSIF, an RNA Pol II stalling/elongation factor, and several other RNA Pol II regulators, are genetically required for dosage compensation, further supporting a role for RNA Pol II stalling in dosage compensation. Suprisingly, DSIF appears needed for proper DCC localization to the X chromosomes. DCC and stalled RNA Pol II signals were scattered across the nucleus following DSIF knockdown. Our results highlight a chromatin state unique to the dosage compensated X chromosomes, uncover an integral relationship between RNA Pol II regulators and the DCC, and reveal novel mechanistic insight into the role of the DCC in controlling X chromosome transcriptional efficiency.

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A role for H4K20me1 in X chromosome dosage compensation. Julie Ahringer[1], Abby Dernburg[2], Yan Dong[1], Andrea Dose[2], Thea Egelhofer[3], **Sevinc Ercan[4]**, Chitra Kotwaliwale[2], Jackie Lang[3], Jason Lieb[5], Shirley Liu[6], Tao Liu[6], Andreas Rechtsteiner[3], Susan Strome[3], Anne Vielle[1]. 1) The Gurdon Institute, University of Cambridge; 2) HHMI, UC Berkeley; 3) Dept of MCDB, UC Santa Cruz; 4) Dept of Biology, New York University; 5) Dept of Biology, University of North Carolina; 6) Dept of BCB, Harvard School of Public Health.

In *C. elegans*, the dosage compensation complex (DCC) equalizes X chromosome gene dosage between XO males and XX hermaphrodites by repressing X transcription by two-fold specifically in hermaphrodites. The DCC is comprised of proteins homologous to the condensin complex, and it localizes to the X chromosomes in hermaphrodites but not in males. The mechanism by which the DCC downregulates expression in XX animals remains unclear. A genome-wide map of 19 histone modifications in two developmental stages of C. elegans (Liu et al, 2011) showed that the monomethylation of histone H4 at lysine 20 (H4K20me1) is enriched on the X chromosome of XX embryos and L3 larvae. H4K20me1 is also associated with actively transcribed genes on all chromosomes. The X-enrichment of H4K20me1 suggests a possible role for this modification in dosage compensation. Here, we report that H4K20me1 is enriched on the X chromosomes of XX hermaphrodites, but not in XO males. Enrichment of H4K20me1 on the X occurs after the localization of the DCC to the X and depends on the DCC function. In mammals, PR-Set7 catalyzes monomethylation of H4K20. RNAi knockdown of *set-1*, the likely *C. elegans* ortholog of PR-Set7, results in synthetic lethality with a number of dosage compensation mutations. These results support a function for H4K20me1 in dosage compensation. The X chromosome is also silenced in the germline in a DCC-independent manner. We found that in the mitotically proliferating zone of the germline, H4K20me1 is abundant on all chromosomes, but as nuclei enter meiosis, H4K20me1 levels are globally reduced. In contrast to somatic nuclei, H4K20me1 is specifically absent from the X chromosome during the meiotic prophase. H4K20me1 was recently shown to be important for chromosome condensation in mammals (Oda et al, 2009), possibly by recruiting condensin II (Liu et al, 2010). Our data indicate that the condensin-like *C. elegans* DCC preferentially increases H4K20me1 levels on the active X-linked genes. One plausible model for the role of H4K20me1 in dosage compensation is that preferential increase of H4K20me1 to the X may result in further recruitment of condensins to lower access of the transcription machinery to active X-linked genes. Liu et al (2011) Genome Res. 21, 227-36. Oda et al (2009) Mol Cell Biol. 29, 2278-95. Liu et al (2010) Nature. 466(7305):508-12.

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Characterization of novel alleles of the *C. elegans* microRNA specific Argonaute ALG-1. **Anna Y. Zinovyeva[1]**, Christopher C. Hammell[2], Victor Ambros[1]. 1) Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

MicroRNAs (miRNAs) are small ~22nt molecules that function in many developmental processes by affecting target gene expression at the post-transcriptional level. ALG-1 is one of the two C. elegans argonautes important for miRNA biogenesis and function. We will report isolation of antimorphic (anti) alleles of ALG-1 that arose as suppressors of *lin-28(lf)* precocious developmental phenotypes. These *alg-1* mutations result in single amino acid changes in the mid and PIWI domains of ALG-1. The mutant animals display dosage dependent phenotypes that are more severe than complete loss of ALG-1 function. The *alg-1(anti)* mutants each produce an apparently full-length ALG-1 protein that fails to interact with the miRISC (miRNA induced silencing complex) component AIN-1, but that retains interaction with Dicer and the miRISC component CGH-1. Interestingly, *alg-1(anti)* mutations do not result in the microRNA biogenesis defects seen in *alg-1(null)* animals, suggesting that miRNA processing remains mostly undisturbed in *alg-1(anti)* mutants. This conclusion is further supported by qRT/PCR (mirTaqman) quantitation of the levels of mature miRNAs in *alg-1(anti)* mutants, which are roughly equivalent to the levels in wild type animals. Deep sequencing analysis did not show a dramatic difference in the miRNA* populations between wild type and *alg-1(anti)* mutant animals, suggesting that the *alg-1(anti)* phenotypes are not caused by an inappropriate retention of the passenger (*) strand. We conclude that the *alg-1(anti)* mutations cause antimorphic phenotypes by producing an ALG-1 miRISC that lacks effector function (due to inability to interact with AIN-1), but that nevertheless loads with microRNAs, and thereby competes with ALG-2 for a limiting pool of microRNAs.

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The *C. elegans* HEN1 ortholog, encoded by C02F5.6, methylates and stabilizes select subclasses of germline small RNAs. **A. C. Billi[1]**, T. Han[1], S. Mitani[2], J. K. Kim[1]. 1) LSI and Dept of Hum Gen, Univ Michigan, Ann Arbor, MI; 2) Dept of Physiol, Tokyo Women's Medical Univ, Sch of Med and CREST, JST, Tokyo, Japan.

Maturation of some classes of small RNAs includes 2'-*O*-methylation at the 3'-terminal nucleotide by *S*-adenosylmethionine-dependent RNA methyltransferase protein HEN1. Fly, zebrafish, and plant studies indicate that methylation protects small RNAs from terminal polyuridylation/polyadenylation, which induces exonucleolytic degradation (1-3). Thus, methylation by HEN1 increases the perdurance of a small RNA, enabling persistence of target silencing beyond the period of biogenesis. In *C. elegans*, methylation of several classes of small RNAs has been reported (4), but the methyltransferase catalyzing this modification has not yet been identified. C02F5.6 encodes the sole *C. elegans* HEN1 ortholog. Here, we report that this protein, which we refer to as *H*EN1-*l*ike *m*ethyltransferase (HLM-1), terminally methylates both 21U RNAs (worm piRNAs) and the ERGO-1 class of primary 26G endo-siRNAs, which are enriched in female germline and embryo. ALG-3/4 class primary 26G endo-siRNAs, enriched in spermatogenic cells (5,6), are not methylated, suggesting that HLM-1-mediated methylation may selectively stabilize female germline-derived 26G endo-siRNAs to direct their inheritance. While 2'-*O*-methylation has not been convincingly linked to small RNA stability in some animal models (7), accumulation of methylated worm small RNAs is impaired in the *hlm-1* mutant. Consequently, loss of HLM-1 results in some defects in secondary siRNA production and target silencing downstream of methylated primary endo-siRNAs. Because loss of HLM-1 impairs function of worm piRNA and ERGO-1 class endo-siRNA pathways, the *hlm-1* mutant exhibits phenotypes that approach those observed in mutants lacking these small RNAs. Due presumably to decreased 21U RNA levels, the *hlm-1* mutant shows a modest fertility defect at 25°C. Similarly, the *hlm-1* mutant exhibits enhanced sensitivity to somatic RNAi, mirroring the enhanced RNAi phenotype observed in mutants defective for production of ERGO-1 class 26G RNAs. Together, these data provide the first example of germline-specific methylation of a subclass of endo-siRNAs and may further our understanding of a germline small RNA regulatory mechanism that is widespread in higher eukaryotic organisms. *N.B.* C02F5.6 has recently been named *rem-1*. 1. Ameres, S. L. et al., Science 328, 1534 (2010). 2. Kamminga, L. M. et al., EMBO J 29, 3688 (2010). 3. Li, J. et al., Curr Biol 15, 1501 (2005). 4. Ruby, J. G. et al., Cell 127, 1193 (2006). 5. Conine, C. C. et al., PNAS 107, 3588 (2010). 6. Han, T. et al., PNAS 106, 18674 (2009). 7. Saito, K. et al., Genes Dev 21, 1603 (2007).

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A memory of developmental experience regulates small RNA populations. **Sarah E. Hall**, Gung-wei Chirn, Nelson C. Lau, Piali Sengupta. Department of Biology, Brandeis University, Waltham, MA.

Early environmental and developmental experiences have a significant impact on adult phenotypes. We have established *C. elegans* as a model system to investigate the mechanisms by which a memory of early developmental experiences modulates adult behavior and physiology. We previously showed that wildtype adult animals that had transiently passed through the dauer stage (postdauers) exhibit different gene expression patterns, genome-wide chromatin structure, and behaviors when compared to adults that bypassed the dauer stage (controls). To determine whether small RNA (sRNA) populations are also differentially affected upon passage through the dauer stage, we deep-sequenced sRNA libraries cloned from growth-synchronized control and postdauer adults. To investigate the developmental progression of sRNA populations, we also sequenced sRNA libraries cloned from the L3 larval stage as well as animals that spent one day in the dauer stage. We found that while control and postdauer libraries contain comparable overall proportions of multiple sRNA classes, the abundance of individual sRNAs within a class could be markedly different. For instance, some 21U-RNAs and miRNAs exhibited 2 to 30-fold differences in abundance in postdauer animals compared to control adults. Unexpectedly, we observed a depletion of miRNAs in our dauer library as compared to the L3 larval stage, such that miRNAs comprise only 30% of total sRNAs in dauers as compared to 74% in L3 animals. Preliminary results suggest this result is reflective of a reduction in the number of miRNAs present in dauers and not an increase in endo-siRNA production. To determine whether endo-siRNAs play roles in the regulation of gene expression, we first mapped them with respect to predicted coding regions. Remarkably, we found that more than 50% of genes whose expression is significantly altered in both dauer and postdauers as compared to control animals, exhibit a >2-fold difference in siRNA levels mapping to their 5' UTR or coding sequences in postdauer animals. These observations suggest that gene expression changes as a consequence of early developmental history may be regulated via endo-siRNA-mediated alterations of chromatin, or via post-transcriptional regulation of mRNA levels. Together, these results indicate that developmental experience can have a profound long-term effect on sRNA populations in adults, and that sRNA-mediated pathways may play critical mechanistic roles in the regulation of gene expression and contribute to phenotypic diversity as a consequence of early experience.

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C. elegans PRG-1 and piRNAs silence target transcripts through a secondary 22G-RNA pathway. **Alexandra Sapetschnig**, Marloes Bagijn, Leonard Goldstein, Eva-Maria Weick, Nicolas Lehrbach, Eric Miska. Wellcome Trust/Cancer Research UK Gurdon Institute, Cambridge, United Kingdom.

The Piwi proteins of the Argonaute superfamily and their associated Piwi-interacting RNAs (piRNAs) are required for normal germline development in all organisms studied so far. In *C. elegans*, the Piwi protein PRG-1 associates with 21U-RNAs (the piRNAs of *C. elegans*) and is required for Tc3 transposon silencing. *Prg-1* mutant worms display germline defects and a reduction in brood size. The molecular mechanism of PRG-1/21U-RNA mediated silencing of target genes has not been unravelled yet. To analyse piRNA-dependent gene silencing, we generated a transgenic *C. elegans* strain carrying a germline-expressed GFP reporter with a sequence antisense to an endogenous 21U-RNA. The GFP transgene is silenced in a wild-type background and becomes de-silenced in *prg-1* mutant animals. High-throughput sequencing revealed *prg-1* dependent 22G-siRNAs (secondary endo-siRNAs) that are synthesised from the GFP transgene in close proximity to the target site. In a candidate gene approach, we identified additional factors required for GFP silencing and *prg-1* dependent 22G-RNA synthesis including the dicer-related helicase *drh-3* and genes of the mutator class like *mut-7* and *mut-2/rde-3*. We postulate that 22G-siRNAs are the effector molecules generated downstream of a primary piRNA trigger. The gene silencing appears to be achieved by both transcriptional and post-transcriptional mechanisms. Since PRG-1 does not localise to the nucleus, transcriptional gene silencing might be achieved through a nuclear 22G-siRNA pathway. We have identified several worm-specific Argonaute family members ("Wagos") that could act redundantly to silence piRNA targets both transcriptionally and post-transcriptionally through secondary siRNAs. PRG-1 has a conserved catalytic centre (amino acid triad DDH) that is required for "slicing" (cleavage) of target mRNAs in other Argonaute proteins. No slicing activity has been reported for Piwi proteins so far. To elucidate the role of the putative slicer activity in PRG-1 dependent gene silencing, we generated transgenic strains expressing wild-type or a slicer-dead mutant of PRG-1 in the germline. Both transgenes rescue the abnormal phenotype observed in *prg-1* mutant worms indicating that slicer activity is not required for PRG-1 function.

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A novel role for Argonaute in the auto-regulation of let-7 miRNA biogenesis. **Dimitrios Zisoulis**, Zoya Kai, Roger Chang, Amy Pasquinelli. Division of Biology, UCSD, La Jolla, CA.

MicroRNAs (miRNAs) are small RNA molecules (~22 nt) that post-transcriptionally regulate the expression of protein-coding genes by mRNA degradation or translational repression. The functional miRNA form is derived from long primary transcripts via a series of maturation steps: miRNAs are transcribed as long primary transcripts (pri-miRNA) that undergo Drosha processing, releasing short hairpin precursor miRNAs (pre-miRNAs) which are subsequently cleaved by Dicer forming mature miRNAs that are loaded onto Argonaute. The mature miRNA guides Argonaute-containing miRNA induced silencing complexes (miRISC) to specific target sequences in protein-coding mRNAs via imperfect base-pairing interactions and this association results in translational repression and destabilization of the target mRNA.

Here we show that not only protein-coding mRNA transcripts, but also the primary transcript of let-7 miRNA is bound and regulated by Argonaute-containing miRISC complexes. We produced a genome-wide map of interactions between Argonaute Like Gene 1 (ALG-1) and target transcripts in *C. elegans* and discovered an Argonaute-binding site downstream of the let-7 precursor hairpin, towards the 3' end of the primary transcript. We validated these results with RNA immunoprecipitation assays, demonstrating that ALG-1 physically associates with let-7 primary transcripts and that the ALG-1-binding site is essential for this interaction. Moreover, direct ALG-1 interaction with primary let-7 leads to lower primary let-7 levels, as indicated by the accumulation of let-7 primary transcripts in animals lacking ALG-1 protein or its binding site. Interestingly, the mature let-7 can pair to and regulate its own primary transcript: in worms harboring a point mutation in the mature let-7 sequence, which disrupts pairing with the target site, the let-7 primary transcripts no longer associate with ALG-1 and their expression levels are elevated. Furthermore, disruption of ALG-1 binding to let-7 primary transcripts results in reduced mature let-7 levels. Deletion of the ALG-1 binding site from primary let-7 or a weakened pairing capacity of mature let-7 with its own primary transcript is also associated with less pre-let-7, suggesting a novel role for Argonaute in the maturation step from primary to precursor let-7. Together, our results show that ALG-1 and let-7 are involved in a positive feedback loop that promotes let-7 biogenesis. This is the first report of Argonaute regulating a primary transcript guided by its own mature miRNA and reveals a new paradigm for the role of Argonaute in gene regulation, as well as a new model of direct miRNA auto-regulation.

Saturday, June 25 4:45 PM–6:30 PM

Royce Hall

Chairs: Oliver Hobert and Meera Sundaram

4:45 pm WormBase Workshop - Facing New Challenges

WormBase is entering a new decade and facing new challenges of integrating ever increasingly complex and numerous genomic data. We strive to continue to widely capture the fruits of Nematode research, transforming and making information more accessible. This workshop will highlight hands-on tours of use cases, exploring the new WormBase Web design and accessing datasets of large scale.

Speakers:
Next generation WormBase Web GUI, **Todd Harris**
SPELL: a query tool for large-scale, quantitative gene expression data, **Wen Chen**
WormMart: broad integration of WormBase data, **Ruihua Fang**

5:10 pm Worm modENCODE workshop

The modENCODE project is an NHGRI-funded consortium dedicated to the comprehensive identification of functional elements encoded in the worm and fly genomes. The *C. elegans* modENCODE project recently completed its fourth year. During this time, five multi-lab groups have generated and made public over 230 genome-wide datasets, including binding site profiles for many transcription factors and chromatin regulatory proteins, as well as histone modification and histone variant profiles. Additionally, the transcriptomes of many different developmental stages and conditions have been collected and analyzed, including those focusing on small RNAs and 3' UTRs. These data have resulted in significant improvements to the *C. elegans* genome annotation, including the discovery of many new genes, as well as providing global insights into genome organization and the mechanisms regulating gene expression. This work has recently been published in a consortium paper (Gerstein et al., 2010 Science 330: 1775), in conjunction with a series of companion papers analyzing individual datasets in more depth. The goal of this workshop is to provide to the *C. elegans* community an overview of these datasets and the resulting global observations, and demonstrate some of the available tools to access and analyze the data in Wormbase and the modENCODE websites (www.modencode.org and intermine.modencode.org).

Speaker:
Valerie Reinke on behalf of the *C. elegans* modENCODE consortium

5:35 pm Whole Genome Sequencing For Mutant Identification

In last few years Whole genome sequencing (WGS) has transformed the foundation of genetic analysis: the discovery of phenotype-causing lesions in the genome. In this workshop, we will discuss how WGS can be used to identify such lesions in *C. elegans*. We will provide a review of this revolutionary technology, with emphasis in the most recent advances. We will present a complete from mutant-to-mutation methodological guide, that includes sample preparation, experimental design concerns, tools for data analysis, methodologies for simultaneous mapping and mutant identification, RAD mapping and chromosomal region pull-downs. Finally, we will discuss strategic choices when dealing with entire mutant collections or complicated cases, such as multigenic trait mutants.

Speakers:
Maria Doitsidou, Columbia University Medical Center
Sophie Jarriault, IGBMC
Sean O'Rourke, University of Oregon
Kate Weber, Medical Research Council

6:00 pm Engineering the Genome

We will review techniques to engineer genomic loci in nematodes.

One approach uses strains carrying the transposon *Mos1*, which causes a DNA double-strand break when excised from the genome by the Mos transposase. A transgene containing sequences homologous to the broken locus can then be used as a repair template to create desired changes in the genome, including knock-ins (*Mos*TIC, *Mos1* excision-induced transgene-instructed gene conversion), large deletions (*Mos*DEL), and single copy integrants (*Mos*SCI). Resources to obtain *Mos1*-containing strains in loci of interest will be reviewed.

A second approach achieves heritable, targeted disruption of endogenous genes and *cis*-acting regulatory elements in diverged nematode species using nucleases with engineered target specificity. These site-specific nucleases contain fusions between the DNA cleavage domain of Fok1 and a custom-designed DNA binding domain: C_2H_2 zinc-finger motifs for zinc finger nucleases (ZFNs) and truncated transcription activator-like effector domains for efficient TALE nucleases (TALENs). ZFNs and TALENs induce a double-strand break at a desired locus that can be repaired by error-prone non-homologous end-joining to yield small insertions and deletions (InDels) at the break site. Efficient procedures were developed in *Caenorhabditis elegans* to permit recovery of mutant lines without selection or reliance on mutant phenotype and were applied to *Caenorhabditis briggsae* with equal efficacy. An individual treated animal can yield numerous mutant offspring, all with independent mutations.

Speakers:
Jean-Louis Bessereau, Institute of Biology of the Ecole Normale Supérieure
Te-Wen Lo, University of California, Berkeley

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Early C. elegans development: a systems view. **Fabio Piano**. New York University, NY.

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Condensin II is required for RNAi-mediated transcriptional gene silencing. James F. Carey, Weifeng Gu, Craig C. Mello, **Kirsten A. Hagstrom**. Program Molecular Medicine, UMass Medical School, Worcester, MA.

Condensin protein complexes are conserved regulators of chromosome architecture and are essential for chromosome segregation. Here we define a new role for condensin in RNA interference. We first establish that exogenous double-stranded RNA triggers both transcriptional and post-transcriptional silencing of the complimentary target gene during "classic RNAi" in *C. elegans*. In condensin II mutants, RNAi-mediated transcriptional silencing is not effective and dsRNA exposure fails to reduce target pre-mRNA and mRNA levels. Nevertheless, deep sequencing revealed that condensin II mutants produce robust levels of secondary small interfering RNAs (siRNAs) in response to dsRNA. Thus condensin II mutants produce siRNAs but these siRNAs cannot execute transcriptional silencing. Similarly, RNAi-mediated silencing of repetitive transgenes and of certain germline transposons is defective in condensin II mutants yet siRNAs are produced. We show that condensin is required for RNAi in non-mitotic cells, and gene expression profiling suggests that condensin does not act by mis-regulating RNAi pathway genes. Together, our results demonstrate that classic exogenous RNAi in *C. elegans* has a transcriptional component that requires condensin II downstream of secondary siRNA production. We speculate that secondary siRNAs feed back to the target locus and regulate transcription by creating repressive chromatin architecture in a condensin-dependent manner.

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Small RNAs and chromatin modification are required to promote long-lasting changes in odor-induced neuronal plasticity. **Bi-Tzen Juang**, Chen Gu, Noelle L'Etoile. Ctr Neuroscience, Univ California Davis, Davis, CA.

Epigenetic changes have been observed in models of depression and addiction. How do environmental stimuli direct such epigenetic changes to specific genes? In the fission yeast, *S. pombe*, gene silencing can be induced by small RNAs derived from the mRNA. These small RNAs were shown to direct a histone H3 methylation mark, which silenced the gene. This down regulation by a gene's own product provides the potential for an elegant feedback inhibition of a specific mRNA by its own transcription. We present evidence that this type of feedback is important in the process of odor adaptation within the AWC sensory neurons.

C. elegans is inherently attracted to a variety of AWC-sensed odors one of which is butanone. This attraction can be dampened by prolonged exposure of the animals to butanone in the absence of food. We term this decreased attraction odor adaptation. Previously, we showed that prolonged odor-exposure induces translocation of a cGMP-dependent protein kinase (EGL-4) into the AWC nucleus. The nuclear accumulation of EGL-4 is both necessary and sufficient to promote odor adaptation. Here we show that once in the nucleus, EGL-4 activates both the chromatin assembly factor HPL-2 and MUT-7, a member of the endogenous RNAi biosynthetic pathway. We demonstrate that HPL-2 requires tri-methylated Histone H3 Lysine 9 in order to promote adaptation. We also identify most of the members of the 22G RNA biosynthetic pathway as well as the nuclear RNAi pathway members NRDE-2 and 3 as being required for odor adaptation. Further, we have identified a specific target of the 22G RNA. This target encodes a member of the odor signal transduction pathway, the guanylyl cyclase, ODR-1. The *odr-1* mRNA not only produces 22G RNAs but also is itself negatively regulated by odor-dependent increases in its 22G RNA. The extent of the decrease in message levels is directly correlated with the strength of odor adaptation as assessed by behavioral assays.

Thus, we show that an environmental signal acting via a kinase can enhance a 22G RNA directed chromatin process. Similar small RNA-directed chromatin changes may serve as the basis of many other forms of plasticity.

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Induction, maintenance and transvection of heritable germline gene silencing in *C. elegans*. **Heng-Chi Lee**, Masaki Shirayama, Craig Mello. Molecular Medicine, U Mass Medical School, Worcester, MA.

Organisms exhibit a fascinating array of gene-silencing pathways, which have evolved, in part, to confront a constant onslaught of parasitic RNA and DNA invaders. One such mechanism, which has also been a vexing problem for *C. elegans* researchers attempting to transform the germline, is a potent germline silencing pathway that silences multi-copy transgenes. Although transient co-suppression of a homologous chromosomal locus is sometimes observed, the primary mode of silencing within a multicopy array is thought to act in cis. Once established, silencing may be inherited for multiple generations without evidence of reversion.

Methods such as biolistic transformation and Mos1-mediated Single Copy Insertion (MosSCI) can bypass germline silencing of transgenes, presumably by avoiding an unknown mechanism that senses DNA copy-number. We were therefore surprised to observe a high frequency of apparently intact but never-the-less silent, single-copy insertions obtained by MosSCI. In one MosSCI experiment using an extrachromosomal, high-copy array as the donor for the inserted single-copy DNA, all of the resulting single-copy insertion lines were completely silent in the germ line. The silent state of these single-copy transgenes might reflect persistence of copy-number-dependent silencing that was initiated during assembly of the high-copy array. To test this idea, we generated a donor array with fewer copies of the transgene by diluting only the targeting vector in our injection cocktail. Strikingly, nearly all of the resulting single-copy insertions exhibited robust germline expression.

Next we explored the consequences of placing a silent locus in trans to an active locus. Remarkably, the silent allele exhibited complete dominance over the active allele; all of the F1 cross progeny failed to exhibit germ-line expression of the transgene. Furthermore, the silent state was transmitted at 100% penetrance in subsequent generations. Because silencing does not appear to spread to the endogenous chromosomal copy of the corresponding gene (co-suppression), we suspect that pairing of the parental chromosomes is necessary for transmission of the silent state, a phenomenon known as transvection.

Our findings imply that initiation of silencing is sensitive to copy number, while persistence of silencing is mediated by a cis-acting mechanism that can be transferred to an active allele by transvection. We are currently investigating the genetic requirements for the establishment, maintenance and transvection of this remarkable gene-silencing phenomenon.

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A novel chromatin associated protein is required for nuclear RNAi and germ cell immortality. **Kirk B. Burkhart[1]**, Shouhong Guang[1], Bethany Buckley[1], Jacinth Mitchell[3,4], Shawn Ahmed[3,4,5], Scott Kennedy[1,2]. 1) Laboratory of Genetics, Univ Wisconsin, Madison, WI; 2) Dept of Pharmacology, Univ Wisconsin, Madison, WI; 3) Curriculum in Genetics and Molecular Biology, Univ North Carolina, Chapel Hill, NC; 4) Dept of Genetics, Univ North Carolina, Chapel Hill, NC; 5) Dept of Biology, Univ North Carolina, Chapel Hill, NC.

In plants and *S. pombe*, small RNAs function in the nucleus to establish repressive chromatin states. The role of small RNAs in metazoan nuclei is largely unknown. To better understand how small RNAs silence gene expression in metazoan nuclei, we conducted a forward genetic screen for factors that were nuclear RNAi defective (*nrde*). From this screen we recovered 22 mutant alleles of the novel gene *nrde-1*. *nrde-1* mutants fail to silence nuclear localized transcripts, and *nrde-1* is required for small RNA-mediated transcription inhibition. We hypothesized that NRDE-1 functions at the level of chromatin. To test this hypothesis, we performed chromatin immunoprecipitation (ChIP) assays with the NRDE-1 protein. We detect a ~8-fold enrichment in NRDE-1 on chromatin at genomic sites targeted by RNAi. This RNAi driven NRDE-1/chromatin association requires the Ago NRDE-3. In addition, we observe ~40-fold enrichment of methylated histone 3 lysine 9 (H3K9me) at the site of RNAi. This H3K9 methylation requires NRDE-1. We also observe H3K9 methylation at genomic sites that exhibit sequence homology to ERI/DCR-dependent endo siRNAs. H3K9 methylation at these loci requires NRDE-1. In addition to the role of NRDE-1 in nuclear RNAi, we find that nrde-1 mutants display a progressive sterility or germline mortal (Mrt) phenotype. After outcrossing, the brood size of nrde-1 mutants is similar to that of wild type animals. However, the brood size of *nrde-1* mutant animals steadily decreases over multiple generations until *nrde-1* mutants become completely sterile after 8-14 generations. Interestingly, nrde-2 and nrde-4 (additional nuclear RNAi factors identified in our screen) also have the Mrt phenotype. These data support a model in which the *nrde* pathway promotes germ cell immortality by maintaining epigenome stability.

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The Ago-class Argonautes ALG-3 and ALG-4 and 26G-RNAs regulate spermatogenic gene expression to promote thermotolerant male fertility in C. elegans. **Colin Conine[1]**, James Moresco[2], John Yates III[2], Craig Mello[1,3]. 1) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA; 2) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA; 3) Howard Hughes Medical Institute.

Gametogenesis is an inherently thermosensitive process in numerous metazoa ranging from worms to man. In C. elegans a variety of germ-line nuage- (P-granule) - associated RNA-binding proteins have been implicated in temperature-dependent fertility. We have previously shown that two AGO-class paralogs, T22B3.2 (ALG-3) and ZK757.3 (ALG-4) are required for male fertility at elevated temperatures. alg-3/4 mutants are completely sterile at 25o and lack a subgroup of small RNAs, named 26G-RNAs that target hundreds of spermatogenesis-expressed mRNAs. alg-3/4 sterility can be rescued by mating with wild type males, suggesting that the infertility results from defects specific to the male germline. A rescuing GFP::ALG-3 transgene is localized in P-granules beginning at the late pachytene stage of male gametogenesis. ALG-3/4 mutant spermatids are defective in spermiogenesis and fail to form motile pseudopods. In order to understand how a molecular deficit in a small-RNA pathway leads to such striking morphological defects in sperm development, we have undertaken several parallel investigations. First we have screened available mutants with similar ts-sterile phenotypes for loss of 26G-RNAs. We have shown that the RdRP involved in the ALG-3/4 pathway, rrf-3, is allelic to fer-15 and that the un-cloned fer genes, fer-2,3,4 and 6, all exhibit small RNA defects identical to those of alg-3/4 mutants. Transmission Electron Microscopy on alg-3/4 mutants and previous EM studies on the fer mutants reveal defective Fibrous Body-Membranous Organelles (FB-MOs), which leads to aberrant arrangement of Major Sperm Proteins (MSPs). Whole-sperm proteomic analysis performed with the Yates lab, along with our transcriptomic analysis reveals striking ALG-3/4-dependent regulation of specific targets at the level of both mRNA and protein accumulation. For example, five non-canonical MSP proteins including the essential gene dct-9 are upregulated more than 20 fold in ALG-3/4 mutants. We are now exploring the possibility that misregulation of specific targets in alg-3/4 mutants underlies the temperature-dependent abnormalities in sperm morphology and function.

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Multi-tiered transcriptional regulation of the miRNA lsy-6 ensures left-right asymmetry in the ASE neurons. **Luisa Cochella[1,2]**, Oliver Hobert[1,2]. 1) Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY; 2) Howard Hughes Medical Institute.

The ASE neurons are a pair of chemosensory neurons in the head of the worm that are bilaterally symmetric by a number of criteria (position, morphology of their projections, connectivity and gene expression patterns). However, they display a directed functional asymmetry in that they each sense different environmental cues. In the postmitotic ASE neurons, a genetic bistable feedback loop adopts one of two possible conformations that ultimately defines the asymmetric gene battery responsible for the functional lateralization. In ASE left (ASEL) the miRNA *lsy-6* represses a transcription factor (TF) *cog-1* and thus allows for derepression of another TF, *die-1*. In ASE right (ASER), absence of *lsy-6* allows *cog-1* to accumulate and efficiently repress *die-1*. Presence of either *cog-1* or *die-1* is mutually exclusive and determines the ASER and ASEL fate respectively. How the bistable feedback loop is biased to adopt the cognate state in each of the ASEs was until now unknown. The lateralization of ASE function has also been tied to early embryonic asymmetric divisions. ASEL derives from the ABa blastomere while ASER comes from ABp, and the early distinction between these two AB daughters, provided by a Notch signal to ABp, is necessary to correctly specify the two ASE subclasses. How that early asymmetry is relayed to the postmitotic ASE neurons nine cell divisions later has remained elusive. Here we show that the miRNA *lsy-6* provides the link between the early embryonic asymmetry and the later functional asymmetry in the postmitotic ASEs. Using fosmid-based fluorescent reporters we have uncovered that expression of *lsy-6* is exclusive to ASEL and is controlled by two separate enhancers, one located upstream and the other downstream of the hairpin region. Each of these enhancers is sufficient for expression in ASEL, however, both are necessary for early onset of expression and avoidance of ectopic expression in ASER. The two enhancers control at least two temporally distinct phases of *lsy-6* expression resulting in activation of the *lsy-6* locus early in the ASEL lineage (but not in the ASER lineage) and further amplification of the expression by bilateral TFs with concomitant repression of the expression in ASER. Overall, analysis of the complex *cis*-regulatory architecture of this miRNA locus has provided key insight into the mechanism by which symmetric and asymmetric features can be combined to increase cellular diversity, as is the case of the ASE neurons. In addition, it has prompted us to evaluate the prevalence of multiple enhancers on the regulation of other miRNA loci.

209

Two classes of silencing RNAs move between *C. elegans* tissues. **Antony M. Jose**, Giancarlo Garcia, Craig P. Hunter. Dept Molec & Cell Biol, Harvard Univ, Cambridge, MA.

Organism-wide gene silencing is readily observed in plants and in some animals even when only a few cells encounter double-stranded RNA (dsRNA). Such systemic RNA interference (RNAi) triggered by dsRNA is due to the transport of mobile silencing signals throughout the organism. In animals, these mobile silencing signals are likely RNA since their import into *C. elegans* cells requires the RNA transporter SID-1. Here we present genetic evidence that both the initial dsRNA and at least one dsRNA intermediate produced during RNAi can act as or generate mobile RNA in *C. elegans*. This dsRNA intermediate is likely a modified form of double-stranded short-interfering RNA (ds-siRNA) since its production requires the dsRNA-binding protein RDE-4, which recruits the endonuclease Dicer (DCR-1) to cleave long dsRNA, and the putative nucleotidyltransferase MUT-2/RDE-3. However, single-stranded siRNA and downstream secondary siRNA produced upon amplification by RNA-dependent RNA Polymerases (RdRPs) do not generate detectable mobile RNA. Restricting inter-tissue transport to long dsRNA and directly processed siRNA intermediates rather than amplified siRNA may serve to modulate the extent of systemic silencing in proportion to available dsRNA. Furthermore, because a SID-1 homolog can import ds-siRNA into mammalian cells, and because mammals have counterparts of Dicer, RDE-4, and MUT-2, our results suggest that mammals may be capable of generating mobile silencing RNA.

210

Targeting the dosage compensation complex to X and mechanism of transcriptional regulation by dosage compensation. **William S. Kruesi**, Barbara J. Meyer. HHMI / University of California, Berkeley.

The *Caenorhabditis elegans* dosage compensation complex (DCC) reduces X-linked transcript levels in XX hermaphrodites to equalize X-chromosome gene expression with that of XO males. The DCC binds to two types of site on X: *rex* sites that autonomously recruit the DCC in a sequence-dependent manner, and *dox* sites that fail to recruit the DCC when detached from X and lie within the promoters of highly expressed genes. No correlation is apparent between DCC binding in a promoter and the dosage compensation status of that gene. We have examined the *cis* and *trans* features that achieve X-specific targeting of the DCC and the mechanism by which the DCC controls transcript levels. We demonstrated that a subunit of *C. elegans* MLL/COMPASS, a gene-activation complex, acts within the DCC, a condensin complex, to target the DCC to both X chromosomes of hermaphrodites and thereby reduce gene expression. We also showed that sex-specific DCC recruitment to *rex* sites by XX-specific factors greatly elevates DCC binding to *dox* sites in *cis*, which lack intrinsically high DCC affinity on their own, thereby allowing the DCC to disperse along X. Many *rex* sites contain a Motif Enriched on X (MEX) that is required for recruiting the DCC to individual sites, but many *rex* sites lack a MEX motif. Therefore, not all features that contribute to X-specific recognition by the DCC have been identified. We performed ChIP-seq to determine the strongest DCC binding sites and to find additional X-specific features essential for DCC bindng. These experiments allowed us to predict additional *rex* sites and find additional motifs that are enriched on X and function in X-specific recruitment of the DCC. Identification of new motifs revealed a feature of strongly bound sites: the new motifs and MEX often cluster with a constant orientation and spacing. This result suggests that different proteins or domains within the DCC make unique contacts with different DNA sequences, providing clues as to how the DCC assembles on *rex* sites. We recently determined that dosage compensation acts, in part, by controlling transcription of X-linked genes. These studies failed to determine the primary step of transcription controlled by the DCC. To characterize dosage compensation's effect on transcription, we are mapping the position of transcriptionally engaged RNA Polymerase II in wild type and dosage compensation mutants using Global Run-On Sequencing (GRO-seq). This GRO-seq data will not only allow us to determine the stage of transcription controlled by dosage compensation, but will also provide insights into transcriptional processes unique to *C. elegans*, such as trans-splicing and operons.

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Roles of the Nuclear Pore and miRNA specific ALG-2 in *C. elegans* Dosage Compensation. **Emily Crane**, Satoru Uzawa, David Mets, Barbara Meyer. MCB Dept, HHMI, Univ California, Berkeley, Berkeley, CA.

Dosage compensation (DC) equalizes X-linked gene expression between males (1X) and hermaphrodites (2X) of *C. elegans*, through a protein complex(DCC) that is targeted to the X chromosomes of only XX embryos. 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 used FISH to assess the sub-cellular location of strong DCC binding sites in embryonic nuclei. We found pair-wise combinations of *rex* sites separated on X by one to many megabases of DNA co-localized in 3D at a frequency higher in XX and XO animals made to load the DCC , than wildtype XO embryos in the DCC is not loaded onto X. 3C analysis also supports a physical interaction between *rex* sites.

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. IPs revealed an association between DCC components and NPC components. Depletion of individual NPC components disrupts DCC binding to X. The DCC localizes to NPCs, which aggregate in specific nuclear pore mutants, further showing the importance of the nuclear pore in DCC function. The NPC may act as a scaffold to restructure X through DCC binding.

In many organisms non-coding RNAs play a role in dosage compensation. We have found that ALG-2, one of the two argonautes that specifically processes miRNAs, functions in *C. elegans* DC. RNAi to *alg-2* in a weak DCC mutant severly disrupts DCC localization to X, and X gene expression. Surprisingly, mutations in the second C. elegans miRNA specific argonaute *alg-1* do not disrupt DCC localization or function.

212

Dampening of expression oscillations by co-regulation of a microRNA and its target. **Dong hyun Kim**[1], Alexander van Oudenaarden[1,2]. 1) Department of Physics, Massachusetts Institute of Technology, Cambridge, MA; 2) Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Failure to properly execute temporal developmental programs in a multi-cellular organism can result in severe morphological defects and often sterility. In nematode *C. elegans*, the timing of larval stage specific developmental events are tightly controlled by "heterochronic genes" which form dosage temporal gradients during development. This gradient is mainly achieved by negative regulation via short non-coding regulatory RNAs, or microRNA(miRNA)s. During early post-embryonic stages, *lin-4* miRNA mediates the down-regulation of LIN-14 which is required for timely execution of a series of developmental events. A number of reports have demonstrated the dosage dependent activities of these genes. More specifically, LIN-14 is believed to exhibit a temporally decreasing dosage gradient, running from high to low, triggering different stage-specific programs during early larval growth. We have quantitatively measured the transcript levels of *lin-14* and the promoter activity of *lin-4* miRNA in staged *C. elegans* larvae using single-molecule RNA *in situ* hybridization method (Raj et al., 2008). Surprisingly, we found both *lin-14* and its negative post-transcriptional regulator *lin-4* to exhibit an in-phase oscillatory transcription activity which peaks every intermolt throughout the animal body. In wild-type animals, this co-regulation leads to an efficient dampening of the *lin-14* transcript level oscillation and results in a step-wise decreasing *lin-14* mRNA temporal gradient. We have also confirmed this at the individual cell level, including lateral seam cells and tail hypodermis. We have evidence that the underlying oscillation is not larval stage specific and is neither due to increased gene copy number in dividing cells nor regulatory feedback between *lin-14* and/or *lin-4*. Thus, we speculate the source of oscillation is linked to organismal growth. Taking these observations into account, we speculate *lin-4* miRNA and its target *lin-14* form an incoherent feed forward loop (IFFL) modulating oscillatory input signal into discrete graded output and propose a novel functional role of miRNA-mediated IFFL as a developmental clock. Reference - Raj A, van den Bogaard P, Rifkin SA, van Oudenaarden A, and Tyagi S. Imaging individual mRNA molecules using multiple singly labeled probes. *Nat. Methods* **5**, 877-879 (2008).

213A

The Caenorhabditis elegans WRN-1 RecQ helicase participates in both DNA damage signaling and DNA Repair. **Byungchan Ahn**, M Hyun, S Park, E Kim, H Park. Dept Life Sci, Univ Ulsan, Ulsan, Korea.

RecQ helicases play essential roles in maintenance of genomic stability from E. coli to humans. The RecQ helicases interact with proteins involved in DNA metabolic pathways such DNA repair, recombination, and replication. We found that C. elegans wrn-1 mutant is more sensitive to CPT than N2 and that the wrn-1 mutant failed to arrest the S-phase since DNA synthesis was continued following CPT treatment. In addition, more DNA strand breaks accumulate in the wrn-1 mutant. These data suggest that WRN-1 is involved in DSB processing and/or DNA damage signaling. WRN-1 colocalized with CeRPA in nucleus in germ line cells following CPT, but CeRPA foci formation was independent of WRN-1. In addition, WRN-1 foci formation was not affected in atm-1 and atl-1 mutants and WRN-1 was required for phoshorylation of CHK-1 induced by DSBs. WRN-1 also colocalized with RAD51. These results suggest that WRN-1 functions downstream of RPA and upstream of ATM-1/ATL-1 and CHK-1 in the DSB checkpoint pathway and is in involved in a DNA repair pathway with RAD51.

214B

Amyloid-binding compounds extend lifespan in C. elegans. **Silvestre Alavez**, Maithili C. Vantipalli, David J. S. Zucker, Ida M. Klang, Gordon J. Lithgow. Buck Institute for Research on Aging, Novato, CA.

Genetic studies indicate that protein homeostasis (proteostasis) is a major contributor to metazoan longevity. Collapse of proteostasis results in the accumulation of insoluble high molecular weight protein fibrils and aggregates such as amyloids, which adversely influence cell survival. A group of small molecules, traditionally employed in histopathology to stain amyloids in tissues, not only bind protein fibrils but slow aggregation *in vitro* and in cell culture. We hypothesized that treating animals with such compounds would promote proteostasis in vivo and increase longevity. Here we show that exposure of adult Caenorhabditis elegans to some amyloid-binding dyes resulted in a profoundly extended lifespan and slowed aging. ThT also suppressed pathological features of mutant metastable proteins and human β-amyloid-associated toxicity. These beneficial effects of ThT depend on the protein homeostasis network regulator Heat Shock Factor 1 (HSF-1), the stress resistance and longevity transcription factor SKN-1, molecular chaperones, autophagy and proteosomal functions. Our results demonstrate that pharmacologic maintenance of the protein homeostatic network has a profound impact on aging rates, prompting the development of novel therapeutic interventions against aging and age-related diseases.

215C

The role of the sumoylation pathway in the regulation of protein synthesis during ageing. **Artemisia Andreou**, Nektarios Tavernarakis. IMBB-FORTH, Heraklion, Greece.

A significant age-associated reduction in protein synthesis has been observed in many organisms, including humans. However, the molecular mechanisms that bring about this decline and their role in ageing remain unclear. Post-translational protein modification allows for differential and rapid responses by the cell, by modulating protein synthesis and energy requirements for diverse cellular processes. We are studying the role of sumoylation in the regulation of protein synthesis during ageing in *C. elegans*. Sumoylation is the post-translational attachment of a small peptide (SUMO) to a target protein, in a process analogous to ubiquitination, which alters the interaction surfaces between the target and other macromolecules. Sumoylation has been implicated in a number of cellular processes ranging from transcription to protein stability. We find that components of the SUMO pathway have important roles in the ageing process. Although complete lack of SUMO isoforms is embryonic lethal, down-regulation of enzymes that facilitate the SUMO attachment to target proteins extends nematode lifespan. In addition, the sumoylation pathway is required for lifespan extension under conditions of low protein synthesis. Our observations support an important role for sumoylation in pathways that influence the ageing process. Individual enzymes of the SUMO modification pathway may exert their effect on lifespan mostly by affecting specific SUMO-target complexes, rather than global protein sumoylation. In this context, we are investigating the requirement for SUMO components in genetic pathways and regimens that modulate longevity.

216A

5' Fluorourdine (FUDR) is protective against stress and aggregation-related disease phenotypes. **Suzanne Angeli**, David Zucker, Ida Klang, Pedro Rodrigues, Karla Mark, Gordon Lithgow, Julie Andersen. Buck Institute for Research on Aging, Novato, CA.

We recently performed a screen to identify novel inhibitors of various stress responses using a 640 natural product library. Surprisingly, we found that 5' fluorouridine (FUDR), a drug commonly used to sterilize C. elegans, was protective in various models of stress, including inhibiting the mitochondrial unfolded protein response and inhibiting paralysis in a neurodegenerative model of disease. In addition, we found that FUDR extends lifespan. These finding have important implications for the routine use of FUDR and also open new areas of investigation as to the protective mechanism of the drug.

217B

Why do elevated levels of the SOD-1 cytosolic superoxide dismutase increase *C. elegans* lifespan? F. Cabreiro[1], R. Doonan[1,2], D. Ackerman[1], **C. Araiz[1]**, D. Gems[1]. 1) Institute of Healthy Ageing, University College London, UK; 2) Hunter College, City University of New York, USA.

According to the oxidative damage theory, molecular damage caused by the superoxide radical (O_2^-) is a significant contributor to aging. This suggests a role in longevity assurance for superoxide dismutase (SOD), which eliminates O_2^-. In *C. elegans*, over-expression of *sod-1*, the major cytosolic Cu/ZnSOD, does indeed increase lifespan (Doonan, R. *et al. Genes Dev.* 22: 3236, 2008). Yet is this extended lifespan observed really due to enhanced O_2^- scavenging? Elevation of SOD can actually increase levels of reactive oxygen species (ROS), particularly H_2O_2, the product of its activity. Moreover, we previously noted that elevated SOD actually reduces resistance to oxidative stress. We therefore investigated further the consequences of *sod-1* over-expression in *C. elegans.* As predicted, worms over-expressing *sod-1* showed increased levels of H_2O_2. Moreover, their longevity required the DAF-16/FoxO transcription factor. We therefore wondered whether elevated H_2O_2 levels might extend lifespan via a hormetic activation of DAF-16. However, *sod-1*-induced longevity was not suppressed by co-over-expression of catalase, arguing against this. Elevated SOD-1 levels did not reduce markers of oxidation of protein, lipid or glycation; in fact protein oxidation levels were increased. Over-expression of *sod-2*, the major mitochondrial MnSOD, also increased lifespan in a *daf-16*-dependent manner and here again oxidative damage was not reduced. So, why does SOD-1 over-expression increase lifespan? SOD-1 is one of the most abundant cytosolic proteins, and our over-expresser lines show up to a seven-fold increase in SOD-1 protein levels. Potentially, levels of SOD-1 are so high as to challenge protein folding homeostasis, thereby eliciting a stress response. Consistent with this, longevity here is largely dependent upon the heat shock transcription factor HSF-1. Moreover, expression of *hsp-4::gfp*, an ER stress reporter, is induced by *sod-1* over-expression. We are currently testing whether longevity here is the result of an unfolded protein response. Overall, these results imply that the longevity resulting from over-expression of *sod-1* are not attributable to enhanced ROS scavenging, and therefore may not be taken as support for the oxidative damage theory.

218C

A Novel Regulator Linking Insulin/IGF-1 Signaling to Proteasome Activity. O. Matilainen, **L. Arpalahti**, C.I. Holmberg. Molecular and Cancer Biology Program and Institute of Biomedicine, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland.

The ubiquitin-proteasome system (UPS) is the major pathway of controlled protein degradation with vital roles in numerous cellular processes. While regulation of the UPS has remained largely unknown, dysfunctions in the system have been coupled to many severe pathological conditions, including neurological diseases and certain types of cancer. Many of these disorders typically manifest in later stages of life, suggesting that the proteasome plays an important part during aging.

Previously, we have shown that proteasomal activity varies in a cell-type and age-specific manner in C. elegans[1]. Here, we investigate whether lifespan-regulating signaling pathways also modulate proteasomal degradation. By using our *in vivo* UPS reporter system as well as in gel proteasome activity assays, we demonstrate that reduced insulin/IGF-1-signaling (IIS) enhances proteasome function in a DAF-16-dependent manner. Mass spectrometry of proteasome complexes from N2 and *daf-2* animals identified a differentially expressed deubiquitinating enzyme (DUB). This enzyme has a tissue-dependent effect on proteasome activity and its expression is transcriptionally regulated by DAF-16. Knock down by RNAi of the DUB affects lifespan of *rrf-3* animals, but does not further increase the lifespan or thermotolerance of *daf-2*(e1370);*rrf-3*(pk1426) mutants. In conclusion, we have established a link between conserved anti-aging, IIS signaling and proteasome activity, as well as identified a novel regulator of proteasome activity in a multicellular organism.

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219A

Quorum sensing in *Caenorhabditis* starvation. **Alex Artyukhin**, Leon Avery. Molecular Biology, UT Southwestern, Dallas, TX.

Availability of food is always a limiting factor in nature. Periods of food abundance are followed by times of starvation, often in unpredictable patterns. Animals must choose between survival in long starvation, which is achieved by shutting off metabolism to save energy, and a quick recovery once food becomes available, which requires energy-consuming maintenance of housekeeping systems. Reliable information about the environment is a critical ingredient in this decision-making process. One way to improve accuracy is to integrate information communicated by many individuals. To test whether such exchange of information may play a role in choosing starvation survival strategies, we studied L1 starvation in *C. elegans* and other *Caenorhabditis* species. We found that *C. elegans* L1 larvae starved in M9 buffer at high worm density survive up to twice as long as the same worms at low density. Furthermore, medium conditioned with a high density of L1 worms extends starvation survival of worms at low density, indicating that this effect is mediated by substance(s) secreted into the medium. Surprisingly, *C. briggsae* does not have this density effect in L1 starvation. The density dependence (quorum sensing) has a bimodal distribution across *Caenorhabditis* species. Out of eight *C.* species tested so far, only two, *C. elegans* and *C. remanei*, show the density dependence. This dependence has no correlation with group feeding behavior (social vs. solitary), mode of reproduction (hermaphrodites vs. gonochoristic), or proximity in the phylogeny. This brings up intriguing questions about the evolution and physiological significance of this phenomenon. To elucidate pathway(s) responsible for the density dependence, we studied L1 starvation survival in various *C. elegans* sensory mutants. All tested mutants that have impaired chemotaxis to soluble chemicals show significantly reduced density dependence. These results imply that the density dependence is neuronally regulated and its signaling pathway

shares components with the system involved in response and chemotaxis to other soluble substances.

220B

Uncovering the mechanisms for the antioxidant-like properties of divalent manganese in *Caenorhabditis elegans*. **Cynthia Bach**, Chandra Srinivasan. California State University, Fullerton, Fullerton, CA.

Divalent manganese, Mn(II), has been thought to function as an effective superoxide radical scavenger in prokaryotic and eukaryotic organisms. Although previous studies on *Caenorhabditis elegans* have revealed that manganese supplementation increases the organism's tolerance to superoxide and thermal stress, the mechanism through which ionic manganese operates is not yet clearly understood. We hypothesize that Mn(II) provides its antioxidant-like properties through the activation of the DAF-16 forkhead transcription factor, which regulates expression of stress response and longevity genes. Reactive Oxygen Species (ROS) assays were performed, using 2',7'-dichlorfluorescein-diacetate to determine relative levels of ROS within the whole worms. Wild type *C. elegans* grown with Mn(II) supplementation contained decreased levels of ROS when exposed to paraquat (in vivo superoxide generator) treatment in comparison to worms raised on a normal diet, however lower levels were not observed under heat shock conditions. Additionally, TK22(mev-1), a strain known to overproduce superoxide, did not show a reduction in free radical levels when supplemented with Mn(II). Furthermore, a transgenic *C. elegans* strain containing a daf-16::GFP fusion(TJ356), was used to monitor nuclear localization of the transcription factor. Results indicate that prolonged exposure to Mn(II) did not fully alter localization of DAF-16 from the cytoplasm to the nucleus, suggesting that Mn(II) treatment does not induce a stress response. Finally, transgenic strains with a sod-3::GFP fusion(CF1553) and a hsp-16.2::GFP fusion(CL2070) were studied, as sod-3 and hsp-16.2 are downstream targets of DAF-16. Data illustrates that treatment with heat or paraquat stress does not cause an increase in upregulation of these genes. Although hsp-16.2 and sod-3 are both downstream targets of the DAF-16 transcription factor, data suggests that other factors or genes modulated by DAF-16 may be involved in providing manganese's antioxidant-like properties.

221C

Defining healthspan in *Caenorhabditis elegans*. **Ankita Bansal**[1], Kelvin Yen[1], Heidi A Tissenbaum[1,2]. 1) Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA; 2) Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA.

Aging can be defined as the accumulation of changes in an organism with time. Various model systems have been used to study the aging process, which has led to the discovery of both genetic and environmental manipulations that can extend the lifespan of an organism. Despite all the resources put into aging research, thus far, limited efforts have been focused on determining whether these longevity-extending methods enhance and prolong animal health and functional capacities. Since aging is correlated with systemic dysfunction and the onset of multiple diseases, this raises the question whether these life-extending interventions result in an increased length of 'healthy' or 'decrepit' time. We use the nematode *C. elegans* to dissect the relationship between life span and healthspan since worms have many several conserved signaling pathways that have already been shown to regulate lifespan. Healthspan can be defined as the number of healthy, productive disease free years before the onset of age-associated decline. To assay for health, we have performed a battery of tests on wild type and four long-lived mutants in a longitudinal manner. Our preliminary data suggest that though long lived mutants perform better than wild type in terms of actual days they do not show proportionate increase in healthy lifespan, thus, adding onto the years of decrepitude. Therefore, further studies are necessary to continue to dissect the relationship between healthspan and lifespan.

222A

The impact of longevity controlling genes and stress-inducing environmental factors in the functionality of the ASH chemosensory neuron in aging worms. **Daphne Bazopoulou**[1], Trushal V Chokshi[2], Nikos Chronis[1,3]. 1) Mechanical Engineering, University of Michigan, Ann Arbor, MI; 2) Electrical Engineering and Computer Science, University of Michigan, Ann Arbor, MI; 3) Biomedical Engineering, University of Michigan, Ann Arbor, MI.

Understanding the physiology of aged neurons is a particularly challenging task, as aging studies need to be performed in a well-controllable environment and they require a large number of data in order to obtain statistically significantly trends. To address those issues, we have previously demonstrated the use of a microfluidic-based platform for calcium imaging of stimulus-evoked neural responses from single worms in an automated fashion.

Extending that work, we performed ASH calcium imaging experiments on long-lived mutants in order to determine the impact of major aging-controlling pathways in the functionality of the nervous system. We found that the *daf-2(e1370)* mutation, which has already been implicated in lifespan extension and increased stress resistance, has a major effect in the aging of ASH neuron, by preserving the young-like functional characteristics in older ages.

In addition, we quantified the effect of stressful conditions, such as heat shock, osmotic stress and hypoxia, in the functionality of the ASH neuron, during aging. Interestingly, a single treatment including a mild heat-stress early in adulthood was able to significantly increase neuronal responses in older animals. We believe that these enhanced neuronal performances are attributed to the hormetic effects of stressors that increase the resistance, improve the survival and cellular function in low doses but have inhibitory or adverse effects at high doses.

Finally, we present data from a small scale compound screen that includes FDA approved drugs and dietary supplements with proposed neuroprotective function and compounds that have been reported to have beneficial effects in longevity.

223B

Functional studies of Homedomain-Interacting Protein Kinase using *C. elegans* as a model. **S. Berber**, E. Llamosas, H.R. Nicholas. The University of Sydney, Sydney, Australia.

The Homeodomain-Interacting Protein Kinases (HIPK) are a family of serine/threonine protein kinases shown to be critical in regulation of many cellular processes including cell survival, proliferation and apoptosis. Since there are four HIPK family members in mammals that show some redundancy, functional studies of this protein family can be simplified with the nematode Caenorhabditis elegans since it expresses a single HIPK protein (HPK-1).

Both HIPKs and HPK-1 localise to nuclear speckles suggesting that they are likely to be involved in analogous cellular processes. Despite this, the first published observation of *hpk-1* mutants reported no obvious phenotypes (Raich *et al*., 2003). Recently *hpk-1* was reported as one of the genes necessary for the extended lifespan of *daf-2* mutants (Samuelson *et al*., 2007). To expand on this knowledge we performed phenotypic analyses on a worm strain carrying a deletion mutation within *hpk-1* with the global aim of discovering some of the *in vivo* functions of worm HPK-1. Additionally, to gain insights into molecular mechanisms of HPK-1 function, microarray analysis was performed on the *hpk-1* mutant strain. These analyses have revealed that various cellular processes may be regulated by HPK-1 including ageing, metabolism and expression of collagens.

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224C

Regulation of the Xenobiotic Stress Response - Combinatorial Action of Nuclear Receptors? **Leah Blackwell**, Amanda Marra, Tim Lindblom. Division of Science, Lyon College, Batesville, AR.

Worms enjoy a robust detoxification system that allows them to cope with a diverse array of chemical stressors in their environment. This system is complete with members of all the major detoxification enzyme classes in animals. We are studying the mechanisms that allow intestinal cells to upregulate the expression of specific detoxification enzymes in response to specific xenobiotics. Much of our work has centered around the nuclear receptor, NHR-8, which is required for wild type levels of toxin resistance. To probe the role of NHR-8 in the xenobiotic stress response, we have analyzed the expression of genes in animals that lack NHR-8 and are challenged with xenobiotics. Our data indicates that NHR-8 does not mediate the upregulation of detoxification genes but is likely required for basal expression of many intestinally expressed genes. What proteins might mediate the xenobiotic stress response and do they cooperate with NHR-8 for their activity? To answer this question, we have initiated a feeding RNAi screen of the transcription factor loci expressed in the gut to find those that are required for toxin resistance. This screen also probes the RNAi targets in combination with the loss of NHR-8 to look for cooperative control. Of particular interest are the 120+ nuclear receptors with demonstrated expression in intestinal cells. These proteins are ideally suited to mediate chemically induced gene expression through their ligand and DNA-binding domains. This type of nuclear receptor action would help explain why C. elegans contains comparatively many more nuclear receptors than other animals.

225A

Screening of EPA's ToxCast libraries using a *C. elegans* growth assay. **Windy A. Boyd**[1], Majro V. Smith[2], Julie R. Rice[1], Jonathan H. Freedman[1,3]. 1) Biomolecular Screening Branch, National Toxicology Program, RTP, NC; 2) SRA International, RTP, NC; 3) Laboratory of Toxicology and Pharmacology, NIEHS, RTP, NC.

Tox21, an intergovernmental toxicology community, is exploring the use of high-throughput *in vitro* tests and alternative model organisms to screen the toxicity of large numbers of chemicals to prioritize toxicity testing in traditional toxicological models. As part of this effort, the U.S. EPA compiled collections of chemicals known as the ToxCast Phase I and II libraries. The Phase I library contains 309 unique compounds, mainly pesticide active ingredients with well-characterized mammalian toxicities. The Phase II library contains 676 unique chemicals including failed drugs, food additives, and industrial products. Toxicity assays in 96-well plate format have been developed for *C. elegans* including reproduction, growth, and feeding using the COPAS Biosort. In the growth assay, the change in size from L1 - L4 is measured after 48-h exposures. The Phase I library was screened for effects on growth and development at seven concentrations. Chemical activity was evaluated using half-maximal effective concentrations (EC50s) and lowest effective concentrations (LECs). A total of 67 EC50s and 173 LECs were estimated. In order to rank chemical potency of all compounds, an activity score was devised based on decreased size at each concentration and steepness of negative concentration-response trends. Activity scores of 0 were classified as inactive, 1 as inconclusive, and 2 - 9 as active with chemical potencies increasing with score values. Overall, 64% of the chemicals were classified as active. The 67 EC50s were negatively related to activity scores, as a low EC50 and a high activity score both indicated toxicity. Furthermore, these same compounds had activity scores of at least 3, suggesting that the activity score is a good indicator of chemical

potency. The most active compounds affected growth at all concentrations. These were primarily insecticides including several avermectins, compounds designed to control parasitic nematodes. Activity scores were also compared to mammalian endpoints including chronic, sub-chronic, cancer, developmental and reproductive toxicity in mice, rats, and rabbits. In general, the most active chemicals in the *C. elegans* assay corresponded to very low rodent LECs. Initial screening of the ToxCast Phase II library is now underway. Because the Phase II compound set is more chemically diverse, a lower percentage of active compounds has been observed than for Phase I. Information and experience gained during the ToxCast screens are being used to inform the design of future toxicity screens of Tox21 libraries, which contain over 10,000 compounds.

226B

The herbal tonic icariin and its metabolites extend C. elegans healthspan via the insulin/IGF-1 pathway. **Wai Jiao Cai**[1,2], Pankaj kapahi[2], Xin Min Zhang[1], Zi Yin Shen[1]. 1) Huashan Hospital, Fudan University, Shanghai, China; 2) Buck Institute for Research on Aging, Novato, CA.

Compounds that delay aging might also postpone age-related diseases and extend healthy years in humans. Using Caenorhabditis elegans as a model system, we have performed chemical screens to identify compounds that have beneficial effects. Icariin is a flavonol derived from several species of plant in the Epimedium family, which is widely used in Chinese herbal medicine. Although icariin has been shown to have protective effects in neurodegenerative and cardiovascular diseases, bone loss and cancer, the underlying mechanisms of these beneficial effects remain unknown. Since aging is a major risk factor for these diseases, we hypothesize that icariin might slow aging to delay the onset of age-related diseases. Here we show that icariin extends C. elegans lifespan in a dose-dependent manner. Chemical profile of animals treated with icariin using HPLC detects high levels of icariin metabolites icariside II in vivo. Icariside II treatment also extends lifespan in a dose-dependent manner, suggesting it might be the bioactive product in vivo. Icariside II also enhances the healthful lifespan significantly. Icariside II treatment leads to increased thermo tolerance, reduced locomotion decline with age and delayed paralysis in a polyQ-mediated disease model. The lifespan extension effect of icariside II is dependent daf-16, suggesting it may inhibit the insluin like signaling pathway. Consistently, qRT-PCR results indicate that icariside II increases DAF-16 transcriptional activities, as demonstrated by increased mRNA levels of DAF-16 target genes. In conclusion, we have identified novel functions of a commonly used herbal medicine in aging, which will help understand the beneficial effects of icariin in humans.

227C

Young *Caenorhabditis elegans* Battling Stress: the Role of Ethanol and Enhanced Fat Storage in L1 Lifespan Extension. **Paola Castro**, Shilpi Khare, Steven Clarke. Chemistry and Biochemistry, UCLA, Los Angeles, CA.

The nematode *Caenorhabditis elegans* arrests development upon hatching at the first larval stage if exposed to starvation-stress. This 'L1 diapause' is an excellent model for studying stress response in young animals. We have found that nematodes are able to extend their survival in L1 diapause when supplemented with ethanol. To assess whether L1 larvae are metabolizing ethanol to fat by oxidizing it to acetyl-CoA via alcohol dehydrogenase, nematodes were supplemented with two oxidized versions of ethanol: acetate and acetaldehyde. These worms are longer lived than unsupplemented animals but not as long lived as worms supplemented with ethanol, perhaps because ethanol is more efficiently taken up by the worms. L1 larvae supplemented with longer chain alcohols, n-propanol and n-butanol, were as long lived as L1 larvae fed ethanol, but methanol, which cannot be converted to acetyl-CoA, has no beneficial effect. Biochemical analysis of nematode fatty acid content reveals L1 larvae are extending their life span by incorporating ethanol into fatty acids. *C. elegans* L1 diapause larvae may be enhancing their survival through an alternate pathway to the canonical insulin/insulin-like signaling pathway (IIS), as daf-2 mutant L1 larvae are longer in both the absence and presence of ethanol. In conclusion, we have elucidated that low levels of ethanol can be used by *C. elegans* effectively to extend their survival in L1 diapause.

228A

The role of the homeodomain protein CEH-23 in longevity of mitochondrial ETC mutants in Caenorhabditis elegans. **H.W. Chang**, S.S. Lee. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY.

Mitochondrial ETC dysfunctions have long been associated with aging and aging related diseases. Accumulating evidence in various model organisms, including C. elegans, indicates that mutants with a moderate reduction of mitochondrial electron transport chain (ETC) function exhibit lifespan extension. The mechanism accounting for this lifespan extension is poorly understood. Our lab recently identified ceh-23, encoding a homeodomain protein, to be an important factor in mediating the longevity effect of mitochondrial ETC mutants in C. elegans. Inactivating ceh-23 specifically suppresses the lifespan extension of several mitochondrial ETC mutants, but has no effects on the lifespan of other longevity mutants tested. Interestingly, ceh-23 mutation does not affect the developmental and reproductive defects, which are normally associated with mitochondrial ETC dysfunctions. Moreover, ceh-23 expression is induced in long-lived mitochondrial ETC mutants, suggesting that ceh-23 is able to respond to mitochondrial dysfunction. We found that overexpression of CEH-23 extends lifespan in wild type worms, indicating that ceh-23 is a novel longevity modulator. CEH-23 is expressed in the nuclei of a subset of neurons and intestinal cells and has been implicated in the transcriptional cascade regulating thermosensory neuron differentiation in C. elegans, but not much is known about its role in longevity. Recent studies suggest that lifespan extension in some mitochondrial ETC mutants depends on a small increase in reactive oxygen species (ROS) production (1,2), and activation of the mitochondrial unfolded protein response (mtUPR)(3). To further probe the function of ceh-23, I tested whether it may be involved in ROS-mediated lifespan extension and/or mtUPR. My preliminary data suggest that ceh-23 is not required for lifespan extension due to small increase in ROS, and is also not required for downstream components of the mtUPR pathway to be induced in mitochondrial ETC dysfunctioned worms. Together, our data suggest a model in which mitochondrial ETC dysfunction triggers ceh-23 expression, which in turn extends lifespan through a novel pathway. Further study will provide new insights into CEH-23 function and will shed light on the molecular mechanism whereby altered mitochondrial ETC function modulates longevity. References: 1. Yang, W., et al., PLoS Biol, 2011 2. Lee, S., et al., Curr. Biol, 2011 3. Durieux, J., el al., Cell, 2011.

229B

The Checkpoint Protein PCH-2 Regulates Oocyte Quality in *C. elegans* . **Chen-Shan Chen**, Ronald Ellis. Dept of Molecular Biology and GSBS, University of Medicine and Dentistry of New Jersey, Stratford, NJ.

As women age, they have a higher risk of producing a miscarriage or a child with birth defects. One contributing factor is that oocytes arrest development at the end of prophase of meiosis I and remain quiescent for many years, during which their quality declines. A major cause of this decline is the increasing fraction of oocytes with chromosomal abnormalities. To help minimize these failures, meiotic cells have checkpoint mechanisms that trigger cell cycle arrest in response to some chromosome problems, and cull faulty cells by initiating apoptosis. In *C. elegans*, the AAA ATPase PCH-2 monitors synapsis between homologous chromosomes, to ensure accurate meiotic segregation. If PCH-2 detects chromosomes that have not synapsed, it induces apoptosis.

We are investigating whether the rate of asynapsis increases during aging, and if PCH-2 helps alleviate this increase. To see if the frequency of asynapsed chromosomes increases with age, we determined the fraction of *XO* male progeny produced by aging hermaphrodites, since problems with chromosome segregation should lead to a higher fraction of males. We observed a 10-fold increase in the fraction of males produced by older hermaphrodites, as well as an increase in the fraction of defective embryos. Furthermore, both effects were aggravated by a mutation in *pch-2*. Although PCH-2 helped maintain oocyte quality at all ages, its relative importance was greatest in young animals, which raises the possibility that PCH-2 activity does not increase enough with age to cope with mounting problems in chromosome segregation.

230C

Deletion of the ribosomal S6 kinase in *daf-2* mutants synergistically extends *C. elegans* lifespan. **Di Chen**[1], Ben Goldstein[2], Alan Hubbard[2], Simon Melov[1], Pankaj Kapahi[1]. 1) Buck Institute for Research on Aging, 8001 Redwood Blvd, Novato, California 94945, USA; 2) School of Public Health, University of California, Berkeley, California 94720, USA.

The highly conserved insulin/IGF-1 signaling (IIS) and target of rapamycin (TOR) pathways play critical roles in aging in multiple species. Mutations in DAF-2, the *C. elegans* insulin/IGF-1 receptor ortholog, double adult lifespan by activating the DAF-16/FOXO transcription factor. A deletion in the TOR target ribosomal S6 kinase (S6K) encoded by *rsks-1* lead to a moderate lifespan extension in *C. elegans*. IIS and TOR act in a parallel but interactive manner to modulate growth, but whether and how they interact to determine lifespan is not known. Here we show that a *daf-2 rsks-1* double mutant extends lifespan by nearly 5-fold compared to wild-type animals, and this synergistic extension of lifespan depends on DAF-16 activities. Gene expression profile studies indicate that there are large amount of genes differentially expressed in the very long-lived *daf-2 rsks-1* animals. Through RNAi screens, we have identified suppressor genes that mediate the synergistic longevity phenotype of the *daf-2 rsks-1* double mutant. Among these is a γ regulatory subunit of the 5'-AMP-activated protein kinase (AMPK) complex. AMPK activities are elevated in the *daf-2 rsks-1* mutant as indicated by increased transcription of AMPK γ and phosphorylation of AMPK α. Furthermore, a deletion in the AMPK α catalytic subunit gene *aak-2* also abolishes the synergistic longevity phenotype. Since AMPK directly activates DAF-16, our results indicate a positive feedback regulation of DAF-16 by DAF-2, S6K and AMPK. Therefore, our findings identify a critical role for AMPK-mediated interaction between *daf-2* and *rsks-1* that result in synergistic lifespan extension.

231A

Insulin-like Signaling in Nutritional Control of L1 Development in *C. elegans* . **Yutao Chen**[1,2], Nicole Kurhanewicz[1,2], Ryan Baugh[1,2]. 1) Department of Biology, Duke University, Durham, NC; 2) IGSP Center for Systems Biology, Duke University, Durham, NC.

In the wild animals must coordinate development with fluctuating nutrient availability. Insulin-like signaling is conserved among metazoan, and it functions to buffer the physiological effects of nutrient fluctuation. In *C. elegans*, newly hatched L1 larvae remain developmentally arrested until feeding. This phenomenon, called L1 arrest or L1 diapause, provides an opportunity to study regulatory mechanisms mediating nutritional control of growth and development. Insulin-like signaling is a key regulator of L1 arrest (Baugh and Sternberg, 2006; Kao et al, 2007). In spite of great interest in the insulin-like pathway given its function in dauer formation, aging and L1 arrest, the function of specific insulin-like peptide ligands is generally not characterized. The *C. elegans* genome encodes 40 insulin-

like peptides. Although redundancy of these genes is likely, some of them have been shown to have specific phenotypes (Lin et al. 2001, Li et al., 2003, Murphy et al., 2007, Patel et al. 2008, Michaelson et al. 2010,). In addition, over-expression analysis suggests that insulin-like peptides function as either agonists or antagonists of the insulin-like receptor DAF-2, promoting development or arrest, respectively (Pierce et al., 2001). With an mRNA expression analysis platform called nCounter by Nanostring, we comprehensively characterized expression dynamics for all 40 insulin-like genes in response to feeding and starvation. From these expression data we identified several insulin-like genes up-regulated by feeding and several up-regulated by starvation, and we hypothesize that they function as agonists and antagonists, respectively. Destabilized YFP reporter gene analysis confirms nutritional control of transcription, reveals the intestine as the primary site of regulation, and suggests that the putative agonists and antagonists are expressed in different subsets of neurons in L1 worms. We are currently using genetic analysis to study the function of putative agonists during recovery from L1 arrest. By combining expression and phenotypic analysis, we aim to determine the timing and site of action for the insulin-like peptides governing L1 development, dissecting the organismal regulatory network they comprise.

232B

A nucleolar protein regulates xenobiotic detoxification genes via *skn-1*. **Keith P. Choe**, Hyacinth Empinado, Andrew Deonarine, Chi Leung. Biology, University of Florida, Gainesville, FL.

Glutathione S-transferases (GSTs) are evolutionary ancient enzymes that detoxify xenobiotics and promote cellular redox balance. The *C. elegans* genome encodes over 30 GSTs, many of which are transcriptionally induced by xenobiotics and oxidative stress. Using a transgenic reporter, we previously performed a genome-wide RNAi screen for regulators of *gst-4* expression. The WD40 repeat protein WDR-23 was the strongest repressor of *gst-4* and we reported that it functions with the CUL-4/DDB-1 ubiquitin ligase to repress nuclear accumulation and activation of the transcription factor SKN-1. We have now begun to characterize some of the other 9 repressors and 12 activators of *gst-4* that were identified with our RNAi screen. RNAi for most of the other repressors does not have an additive effect on *gst-4* expression when combined with a null allele of *wdr-23* suggesting that they function in common pathways. One exception is F28D1.1, a second WD40 repeat family member. F28D1.1 is an orthologue of the yeast protein UTP7, which functions in ribosome biogenesis, kinetochore organization, and chromosome segregation. Almost nothing is known about F28D1.1 or its orthologues in animals. Real-time RT-PCR data from a deletion mutant confirm that F28D1.1 represses mRNA levels of endogenous *gst-4* and *gst-30* by a mechanism that involves *skn-1* but not *daf-16*. Unlike WDR-23, F28D1.1 protein does not interact with SKN-1 in the yeast 2-hybrid system suggesting that regulation is indirect. GFP reporter analysis suggests that F28D1.1 protein is expressed in the nucleolus of many cells and F28D1.1 loss-of-function increases resistance to the xenobiotic juglone. Multiple *C. elegans* nucleolar proteins are predicted to interact with F28D1.1 based on interactions between homologous yeast proteins (STRING search). Preliminary data indicate that loss-of-function for most of these nucleolar proteins increases *gst-4* expression similar to F28D1.1(RNAi). Taken together, our data suggest that disruption of nucleolar function activates stress-resistance genes via SKN-1. This novel mechanism of SKN-1 regulation appears to function separately from WDR-23.

233C

Characterization of TMEM16 Ca^{2+}-activated Cl^- channels in *C. elegans*. Tashrique Alam[1], Katherine Hill-Harfe[1], Chi Leung[1], Ying Wang[1], Brian Harfe[2], **Keith Choe[1]**. 1) Biology, University of Florida, Gainesville, FL; 2) Microbiology and Molecular Genetics, University of Florida, Gainesville, FL.

Calcium-activated chloride channels (CaCCs) function in transmitter release from photoreceptors, excitability in neuron and myocyte membranes, sensory signal transduction, and epithelial membrane transport. Despite their importance, the molecular identity of CaCCs remained unknown until the TMEM16 transmembrane protein family was recently identified as CaCCs. Two current goals of the field are to characterize the physiological functions of TMEM16 proteins and define the molecular mechanisms that regulate channel activity. There are 10 TMEM16 family members in mammals with overlapping expression and potentially redundant functions that can hinder genetic and physiological analysis; there are only two TMEM16 family members in *C. elegans*, F56A8.1 and Y57G11C.37. To establish a genetic model for TMEM16 function and regulation, we have begun to characterize the expression and function of both *C. elegans* homologues. GFP reporter analysis demonstrates that the F56A8.1 promoter is active in amphid and phasmid neurons and suggest that the protein is enriched in sensory cilia. The Y57G11C.37 promoter is active in the spermatheca, lateral mechanosensory neurons, ventral nerve cord, and a few neurons in the head. Y57G11C.37::GFP fusion proteins are in or near the basal membrane of the spermatheca and in punctate structures with the appearance of synapses in the ventral nerve cord. F56A8.1(RNAi) in a strain of worms sensitized to neuronal RNAi (Calixto et al., 2010 Nat. Methods 7(7):554) reduces osmotic avoidance behaviors suggesting a role in sensory perception or signal transduction. Y57G11C.37(RNAi) in the same strain of worm reduces motility suggesting that the gene plays a role in neuromuscular function. We are currently generating null alleles for both TMEM16 genes in *C. elegans* and designing genetic screens to identify pathway components.

234A

Toxicity of silver nanoparticles *via pmk-1* dependant GST and HIF-1 activation in *Caenohabditis elegans*. **Jinhee Choi**, DongYoung Lim, Jiyeon Roh, Hyunjeong Eom. University of Seoul, Seoul, Seoul, Korea.

In the present study, the involvement of oxidative stress, as a toxic mechanism of silver nanoparticles (AgNPs), was investigated in *Caenorhabditis elegans*, focusing on the p38 mitogen-activated protein kinase (MAPK) pathway. Initially, AgNPs was tested as a potential oxidative stress inducer in *C. elegans* and; subsequently, the potential upstream signaling pathway activated in response to AgNPs exposure was investigated, paying special attention to the AgNPs-induced alteration in the *C. elegans* p38 signaling pathway. The expressions of the downstream genes, known to be regulated by the p38-MAPK, such as glutathione S- transferases (GSTs), and the p38 dependent transcription factors (TFs) were also investigated in *wildtype (N2)* and *pmk-1 (km25)* mutant C. elegans exposed to AgNPs. The overall results indicated that AgNPs exposure lead increased reactive oxygen species (ROS) formation, PMK-1, GST and HIF-1 activations and reproduction failure in *wildtype (N2) C. elegans*; whereas, none of these phenomena were observed in the *pmk-1 (km25)* mutant. These results suggest that oxidative stress is an important toxic mechanism of AgNPs in *C. elegans* and that PMK-1 plays an important role in the response to oxidative stress induced by AgNPs. The results also suggest that GST is a PMK-1 dependent down-stream effecter protein and hypoxia-inducible factor (HIF-1) is a PMK-1 dependent TF on AgNPs exposure in *C. elegans*, which both play an important role in the PMK-1 mediated defense pathway to AgNPs exposure. Acknowledgements : This work was supported by National Research Foundation of Korea (NRF) grant (2010-0016195). Keywords: silver nanoparticles; *Caenorhabditis elegans*; oxidative stress; PMK-1; GST; HIF-1.

235B

Functional toxicogenomic analysis of multi-wall carbon nanotube in *Caenohabditis elegans*. **Jinhee Choi[1]**, Ji-Yeon Roh[1], Maribel Bruno[2], Yue Ge[2]. 1) University of Seoul, Seoul, Seoul, Korea; 2) Environmental Protection Agency , Research Triangle Park, NC 27709, USA.

In the present study, toxicity of multi-wall carbon nanotube (MWCNT) was investigated in *Caenohabditis elegans* using microarray and mutant analyses. Whole genome microarray was conducted to screen the global changes in *C. elegans* transcription profiles 4 and 24 h after MWCNT exposure. Interactome analysis was subsequently conducted on differentially expressed genes using Ingenuity Pathways Analysis (IPA) software. After 4 h exposure to MWCNT, 846 genes were differentially expressed in *C.elegans*, whereas 24 h after exposure, 2247 genes were affected. Interactive gene networks corresponding to Eukaryotic Initiation Factors 4 (EIF4) pathways were highly overexpressed 4 h after MWCNT exposure, whereas NF-kB pathways were downregulated 24 h after exposure. Toxicity of MWCNT was also investigated on 27 potentially stress response *C.elegans* mutants using survival and reproduction as endpoints. Among the tested mutants, *akt-1* (AKT signaling), *nsy-1, sek-1* (p38 MAPK signaling) and *cep-1* (p53) mutants showed more sensitive response to MWCNT exposure than wildtype did, in terms of reproduction potential. Gene expression analysis, subsequently conducted on selected genes in MAPK, AKT, Apoptosis signaling pathways, revealed that expression of *nsy, mpk-2, sgk-1 and ape-1* genes was increased in *C.elegans* exposed to MWCNT. Overall results suggest that MWCNT possess considerable potential of causing toxicity in *C.elegans*, and stress signaling pathways seem to be involved in it. Acknowledgement: This work was supported by National Research Foundation of Korea (NRF) grant (2010-0016195).

236C

Role of endocytosis and ER proteostasis in *C. elegans* longevity. **Ritika Das[1,2]**, Sneha Rath[1], Nam Lee[1], Lindsay Stolzenburg[1], Andrew V. Samuelson[2]. 1) Department of Biology,University of Rochester, Rochester, NY, US; 2) Department of Biomedical Genetics, University of Rochester Medical Center, Rochester, NY, US.

Insulin signaling is a major pathway controlling lifespan in multiple organisms, such as worms, flies and mammals. The insulin signaling pathway initiated by the activation of the insulin receptor (DAF-2) brings about the repression of the downstream transcription factor DAF-16. DAF-16 is one of the key transcription factors that regulate the expression of genes involved in lifespan as well as other functions such as development, immunity and stress resistance. We have previously conducted a genome wide RNAi screen to identify genes that are essential for enhanced longevity in *daf-2* mutant worms {Samuelson, 2007}[1]. This screen led to the identification of 103 gene inactivations which cause premature aging (the “progeric gene panel”, PGP) . Interestingly, 19 genes in the PGP are annotated to be involved in endosomal protein sorting or vesicular trafficking. Similar studies in yeast have also identified genes involved in vacuolar protein sorting to be essential for longevity suggesting a conserved role for proper endosomal function in regulating aging {Fabrizio, 2010}[2]. However, the mechanism by which endocytosis influences longevity is still not clear. To explore this further, our lab has undertaken a systems level analysis to identify the members of the PGP that are involved in endocytosis, the ER unfolded protein response (ER^{UPR}), and protein homeostasis. To this end, transgenic worms for different endocytosis markers such as the Rab GTPases and GFP fusions to those proteins whose proper localization within the worm depends on functional endocytic machinery have been screened. While the endocytosis machinery is critical to protein degradation and proper protein sorting, ER serves as a primary site for protein synthesis and folding and *daf-2* mutant animals exhibit lower levels of the ER^{UPR} marker *hsp-4::GFP* indicating improved ER homeostasis {Korenblit, 2010}[3] . To identify the members of the PGP that influence longevity via the ER stress response pathway, *hsp-4::GFP* transgenic worms have been screened to identify gene inactivations that either induce the ER^{UPR} in the absence of stress or conversely impair the ER^{UPR} after stress. Lastly, we have identified the members of the PGP that cause the premature collapse of protein homeostasis, as measured by foci formation of *Q35::YFP*. Highlights of this comprehensive analysis will be presented.

References 1. Samuelson *et al* (2007) Genes Dev 21(22):2976-94 2. Fabrizio *et al* (2010) PLOS Genet. 6 (7):e1001024 3. Korenblit *et al* (2010) PNAS 107(21):9730-5.

237A

Protein aggregation as an inherent part of aging. **D. David**[1], N. Ollikainen[2], J. Trinidad[3], M. Cary[1], A. Burlingame[3], C. Kenyon[1]. 1) Dept Biochemistry, UCSF, San Francisco, CA; 2) Graduate Program in Biological and Medical Informatics, UCSF, San Francisco, CA; 3) Mass Spectrometry Facility, Department of Pharmaceutical Chemistry, UCSF, San Francisco, CA.

In neurodegenerative diseases such as Alzheimer's disease and Huntington's disease, specific proteins escape the cell's quality-control system and associate tightly together, forming insoluble aggregates. Until this study, little was known about protein aggregation during normal aging in the absence of disease. Using a systematic proteomics approach, we discovered that the aging process itself leads to the insolubilization and increased aggregation propensity of several hundred proteins in *C. elegans*. These aggregation-prone proteins have distinct structural and functional proprieties. An important question is whether this inherent age-dependent protein aggregation impacts neurodegenerative diseases. First, we discovered that known regulators of disease-related protein aggregation are themselves prone to aggregate with age. Second, we showed that higher levels of inherent protein aggregation aggravated the toxicity caused by over-expressing the Huntington's disease polyglutamine motif in worms. Third, we found that many proteins similar to those aggregating in old worms have also been identified as secondary components of human disease aggregates. In addition, we found that mechanisms exist in the organism to protect against inherent protein aggregation and that these mechanisms are activated in longevity mutants. Specifically, we were able to delay the aggregation process by reducing insulin/IGF-1 signaling, whose reduction is known to extend animal lifespan. Overall, we have characterized inherent protein aggregation as a new age-related process. Understanding which pathways modulate this process will bring fresh insight into the mechanisms behind aging and possibly neurodegenerative disease as well.

238B

A model to study drug-induced mitochondrial dysfunction. **Richard de Boer**, Stanley Brul, Hans van der Spek. Molecular Biology & Microbial Food Safety, Swammerdam Institute for Life Sciences, Amsterdam, The Netherlands.

Several studies have shown the occurrence of mitochondrial dysfunction as a consequence of therapeutic drug use, especially the drugs used to treat HIV-1 infected individuals. The cause of these side-effects appears to be a common mechanism: a decreased mitochondrial energy-generating capacity putatively caused by the inhibition of mitochondrial DNA polymerase γ. The generally accepted view is that this leads to a depletion of mitochondrial DNA (mtDNA) and causes the perturbed mitochondrial function. However, the exact mechanisms behind this remain unknown. Since most results have been obtained in patient- or cell culture studies, with limitations on the experiments that can be performed, progress in this field is highly dependent on the development of a good model system. To address fundamental questions concerning the mechanisms of drug-induced mitochondrial dysfunction, *Caenorhabditis elegans* was developed as a model organism.

MtDNA was quantified using the ABS 7300 Real-Time PCR System. Mitochondrial morphology was visualized using mitochondrially localized GFP expression. The redox state of the Quinone pool was studied by HPLC analysis. O_2 consumption rates were measured using a Clark-type electrode and normalized to protein content. ROS formation was studied with a ROS-inducible GFP reporter and was visualized using an Axiovert 40 Fluorescent Microscope or quantified using a plate reader.

Our *C. elegans* model system allowed us to assess respiration by the worms and show a concentration dependent decline in mtDNA copies when the worms are cultured in the presence of various anti-retroviral drugs. This decline was both absolute and relative compared to nuclear DNA. Moreover, exposure to these drugs resulted in increased ROS production and/or disruption of the mitochondrial network. The severity of the observed effects is drug-specific and concentration dependent. Interestingly, the observed biochemical and morphological effects are not necessarily provoked by the same compounds and some of the effects could be alleviated by providing specific supplements that are known to act on the mitochondrial respiratory chain.

Since the side-effects of the anti-retroviral drugs in patients closely resemble the observed effects in *C. elegans*, we conclude that *C. elegans* is a suitable model organism to study drug induced mitochondrial dysfunction and highly expedient to search for compounds to alleviate the induced toxicities. Preliminary results suggest the beneficial effect of supplementation as a way of counteracting and alleviating some of these pernicious side-effects.

239C

Targeting DNA Repair to Enhance Radiosensitivity in Notch-driven Tumor Stem Cells. **X. Deng**[1], D. Rothenstein[1], D. Michaelson[2], SN. Powell[3], Z. Fuks[3], EJ Hubbard[2], RN. Kolesnick[1]. 1) Dept Molec Pharm, MSKCC, New York, NY; 2) Developmental Genetics Program, Skirball Institute of Biomolecular Medicine, New York University School of Medicine, New York, NY; 3) Dept of Radiation Oncology, MSKCC, New York, NY.

The *Caenorhabditis elegans glp-1* mutant *glp-1(ar202)* exhibits constitutive GLP-1/Notch signaling, generating a germ stem-cell tumor. The tumor cell population is resistant to apoptotic stimuli and displays high DNA repair capacity, resulting in resistance to lethal effects of ionizing radiation (IR). Orthologs of genes that regulate DNA damage sensing/repair have been identified in *C. elagans*. To understand how these genes could affect the radiosensitivity of tumor stem cells of *glp-1(ar202),* we knocked down 20 of these genes by feeding RNAi. Efficiency of RNAi was measured by real time PCR. Inactivation of 6 genes including *rad-51, mre-11, mus-101, rad-54, atl-1* and *npp-15* enhanced 210 Gy radiosensitivity significantly, evaluated as inhibition of germ cell proliferation. Except for *npp-15*, these genes are all involved in the homologous recombination (HR) DNA repair pathway of *C. elegans*. In contrast, inactivation of *cku-80* and *lig-4*, core components of the canonical non-homologous end joining (NHEJ) machinery, and of genes involved in DNA damage checkpoints and other repair-related enzymes had no effect on radiosensitivity of the *glp-1(ar202)* germline tumor growth. Knocking down *rad-51* and *mre-11* in *glp-1(ar202)* reduced the IR dose required for killing all tumor cells (LD100) from 480 Gy to 210 Gy, and extended the life span of *glp-1(ar202)* mutants to wild-type levels. Activating mutations in Notch are present in over 50% of patients with T-cell lymphoblastic leukemias and lymphomas. To test if we could sensitize Notch-driven human tumor cells to an IR treatment by inhibition of the HR DNA repair pathway, we employed the T-cell Lymphoblastic Lymphoma cell line CUTLL-1. CUTLL-1 produces a truncated form of a Notch1 receptor resulting in enhanced Notch signaling, which is highly similar to the *C. elegans* mutant of *glp-1*. We showed that silencing rad51 by shRNA or inhibiting the Mre11-Rad50-Nbs1 complex using the small molecule Mirin greatly enhanced radiosensitivity by measuring CUTLL-1 cell clonogenic survival. Our studies suggest that Notch driven tumor cells exhibit stem-cell features, in which the use of the error-free repair pathway of HR is favored and NHEJ is not used. The mechanism of inhibition of NHEJ and the switch to HR is under active investigation.

240A

Distinct DAF-12 activities influence *C. elegans* lifespan. **Kathleen Dumas**[1,2], Chunfang Guo[1], Hung-Jen Shih[1], Patrick Hu[1,3,4]. 1) Life Sciences Institute, University of Michigan, Ann Arbor, MI; 2) Cellular & Molecular Biology Graduate Program, University of Michigan, Ann Arbor, MI; 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/insulin-like growth factor signaling (IIS) pathway promotes reproductive development and controls lifespan by regulating nuclear translocation of the FOXO transcription factor DAF-16 via a conserved PI3-kinase/Akt pathway. Dafachronic acid (DA) signaling through the nuclear hormone receptor DAF-12 also impinges on DAF-16/FoxO, in parallel to the PI3-kinase/Akt pathway, in the control of development and longevity. We explored the role of the DA/DAF-12 pathway in the control of DAF-16/FoxO-dependent phenotypes. We probed the genetic interaction between *daf-12* and *daf-2* in the control of dauer arrest and lifespan, using a null allele of DAF-12 and *daf-2* knockdown via RNAi as well as Class I and Class II *daf-2* mutant alleles. We also investigated the role of the DA/DAF-12 pathway in longevity control by the germline.

We found that knockdown of DA pathway components *hsd-1*, *daf-36*, or *daf-9* enhanced dauer arrest of *daf-2* mutants, while it suppressed longevity induced by reducing IIS. Moreover, the influence of DAF-12 on lifespan depends on the strength of IIS. DAF-12 promotes longevity when IIS is reduced by *daf-2* RNAi. In contrast, DAF-12 is dispensable for increased longevity in the Class II *daf-2(e1370)* mutant. Importantly, in contrast to the synergistic effect of the *daf-12(m20)* mutant allele on lifespan extension in *daf-2(e1370)* mutants, the *daf-12* null allele *rh61rh411* does not extend the lifespan of *daf-2(e1370)* mutants.

We confirmed previously reported findings that DAF-12 promotes longevity of animals lacking a germline, as *daf-12* null mutation significantly reduced the lifespan of germline-ablated animals. Interestingly, *daf-12* null mutation extended the lifespan of germline-ablated animals with defects in DA synthesis, suggesting that DAF-12 shortens lifespan in this context. Thus, distinct DAF-12 activities have opposing effects on lifespan in germline-ablated animals. The DAF-12 coregulator DIN-1 is required for DA deficiency to reduce lifespan in germline-ablated animals, suggesting that DIN-1 cooperates with DAF-12 to shorten lifespan in germline-ablated animals that lack DA. These data shed light on the complex intersection of IIS and steroid hormone signaling in the control of longevity in *C. elegans*.

241B

Cyclophilin D Modulation of Longevity and Mitochondrial Physiology. **Julie C Etzler**, Deborah Holstein, James D Lechleiter. Cellular and Structural Biology, UT Health Science Center, San Antonio, TX.

Cyclophilin D (CyPD), a mitochondrial matrix protein, has been widely studied for its diverse role in mitochondria physiological mechanism. We previously established in this laboratory that overexpression of CyPD in stable HEK cell lines, increased cell survival under oxidative stress conditions. For further investigation, integrated overexpression of CyPD was achieved in Caenorhabditis elegans and lifespan analysis has shown that they are long lived. To understand the mechanism of CyPD in longevity, ROS (reactive oxygen species) production and mitochondrial membrane potential (ΔΥ) were measured throughout the life of the animal using confocal microscopy. ROS was imaged using Mitosox (a superoxide indicator) and ΔΥ was imaged using TMRM (mitochondria potential indicator), in the mitochondria rich organ, the pharynx. Measurements, at various times in the worm's lifespan gave us surprising results. We found that transgenic long lived nematodes have higher rates of superoxide production and have higher mitochondrial membrane potentials. The oxidative stress theory of aging states that more ROS production would limit lifespan as a result of accumulation of damage over time. Our data speaks to the contrary and suggest that ROS production is not an appropriate indicator for lifespan. This new data about ROS corresponds with other aging researchers finding that ROS may have a more complex role in aging than previously suggested. More importantly, the functional

consequences of CyPD overexpression in an organism, have never indicated a role in longevity. CyPD's role in aging pathology will add new understanding to CypD's unique role in the mitochondria.

242C

Steroid Signaling Mediates Longevity Responses to Dietary Restriction in *C. elegans*. **Mindy Farris**, Victor Pineda, Dhaval Patel, Weiqing Li. Biological Structure, University of Washington, Seattle, WA.

Dietary restriction (DR) extends lifespan in a wide range of model organisms, including *C. elegans*. It is an interesting but yet to be addressed question whether hormones serve as a systemic mechanism that mediates this longevity response. We identified a requirement for steroid signaling in lifespan extension induced by DR, by studying two members of the conserved 3-β-hydroxysteroid dehydrogenase (3βHSD) family, HSD-2 and HSD-3. Specifically, we found that lifespan extension in the *eat-2* genetic model of DR is completely suppressed by a deletion mutant of *hsd-2*. This suppression is independent of the decreased brood size or prolonged egglay timing of *eat-2*, as neither was affected by *hsd-2*. This suggests that the *hsd-2*-mediated steroid signal regulates longevity without influencing reproduction. Furthermore, *hsd-2* suppressed the ability of *eat-2* to resist acute heat shock stress, as measured by survival after recovery, but only later in life (day 7, day 11, and day 13 adults). This indicates a role for *hsd-2* in age-dependent responses to DR, consistent with *hsd-2* expression, which is primarily in adults. Since *hsd-2* did not affect DR-induced resistance in day 1 adults, we hypothesized that *hsd-3*, which is only expressed in larvae, might be functioning in that role. Indeed, we found that while a deletion mutant of *hsd-3* does not appear to affect *eat-2* overall adult lifespan, it completely suppressed its ability to resist heat shock stress as day 1 adults. Therefore, although both *hsd-2* and *hsd-3* contribute to stress resistance in *eat-2* adults, only *hsd-2* impacts lifespan, as though steroid signaling in adults is more important for lifespan extension in *eat-2*. Taken together, our results indicate that steroid signaling through these two steroidogenic enzymes, active at distinct times during the worm lifespan, is vital for the health and longevity conferred by DR.

243A

Identification of a Novel Role for Tyrosine Aminotransferase in Insulin Signaling. **Annabel Ferguson[1]**, Yongsoon Kim[2], Kaitlyn Kormanik[1], Patrick Hu[2], Alfred Fisher[1]. 1) Division of Geriatric Medicine, Department of Medicine, University of Pittsburgh, Pittsburgh, PA; 2) Life Sciences Institute, University of Michigan, Ann Arbor, MI.

In *C. elegans*, the *daf-2* insulin-like signaling pathway is linked to determination of lifespan and formation of dauer larvae, which is an alternative larval stage triggered to avoid adverse conditions. The kinases *akt-1*, *akt-2*, and *sgk-1* act within the *daf-2* pathway to prevent the FOXO transcription factor DAF-16 from initiating dauer formation. Inhibition of *akt-1* alone produces dauer formation only at high temperature, while loss of both *akt-1* and *akt-2* always results in dauer arrest. An additional pathway that determines dauer formation includes the *eak* (enhancer of *akt-1*) genes, including *eak-3*, *eak-4*, *hsd-1*, *sdf-9*, and *eak-7*, which augment the effects of *akt-1* on dauer formation. In an RNAi screen, the worm tyrosine aminotransferase homolog *tatn-1* was identified as an enhancer of dauer formation in *eak-4* mutants. Tyrosine aminotransferase is the first enzyme in the tyrosine degradation pathway and has been extensively studied as an insulin target gene. We have found that increased levels of tyrosine enhance *eak-4* dauer arrest as mutations in either *tatn-1* or the immediate downstream gene *hpd-1*, or an increase in dietary intake of tyrosine lead to dauer formation in *eak-4* mutants. We found that tyrosine levels also determine lifespan, as *tatn-1* mutants are longer lived in either a wild type or *eak-7* background. Further, increased tyrosine levels augment the daf-c phenotype of *daf-2* mutants, while *daf-2* itself appears to positively regulate the expression of *tatn-1*. The *tatn-1 eak-4* interaction occurs upstream of *daf-16* and *daf-12*, but downstream of *pdk-1*. Neither an *akt-1* nor *akt-2* null allele interacts with *tatn-1*, but loss of the AMP kinase *aak-2* or *akt-1* gain of function mutants block dauer formation by an *eak-4/tatn-1* double mutant. Together these results suggest that insulin signaling controls the cellular level of tyrosine through modulating the expression of *tatn-1*, and that high levels of tyrosine act through *akt-1* and *aak-2* to antagonize insulin signaling. *eak* genes act to reduce *daf-16* transcriptional activity and inhibit activation of daf-16 target genes. Tyrosine may interact with eak genes by promoting *daf-16* phosphorylation or trafficking by effects on *akt-1* and *aak-2*.

244B

Engineering longevity in *C. elegans*. **Ari E Friedland**. Developmental Biology, Stanford University, Stanford, CA.

New evidence suggests that widely accepted, damage-based theories of aging are insufficient to explain the full spectrum of age-related malfunctions and senescence in animals. Developmental drift is an alternate theory that posits a deeper and more systemic mechanism, one in which genetic pathways that are critical to the proper growth and development of organisms consistently become misregulated over time, causing various failures and ultimately death. Some of these pathways have been identified and characterized in *C. elegans*; if the drift in these gene networks is indeed causing aging in these worms then it should be possible to engineer healthier, longer-lived individuals by repairing or halting the network drift. I aim to develop inducible systems and synthetic gene networks for use in *C. elegans*, first to address this question of developmental drift but also to expand synthetic biology into a multicellular context, using it to manipulate attributes specific to multicellular organisms, such as development and neuronal signaling.

245C

Characterization of the neuromedin U receptor (NMUR) family and its role in the development and lifespan of C. elegans. **Roxani Gatsi**, Bakhtiyor Adilov, Wolfgang Maier, Martin Regenass, Joy Alcedo. Friedrich Miescher Institute , Basel, Switzerland.

The importance of environmental inputs into animal physiology has long been recognized. However, the mechanism(s) behind the processing and transmission of such information by the sensory system to influence different physiological processes remain to be elucidated. Recently, we have shown that the sensory system recognizes the C. elegans's type of food source to affect development and lifespan and that the neuropeptide receptor neuromedin U receptor-1 (nmur-1) is involved in this process1. Since C. elegans has at least three other members of the NMUR family—nmur-2, nmur-3 and nmur-4, we are characterizing the function of these other receptors in development and lifespan. We are also currently testing for any possible genetic interactions among the different members of this receptor family in regulating physiology. 1. Maier et al. (2010).

246A

Investigating the Dynamic Role of Autophagy in *C. elegans* Subjected to Dietary Restriction. **S. Gelino[1]**, M. Hansen[2]. 1) Sanford Burnham Graduate Program of Biomedical Sciences, La Jolla CA; 2) Development & Aging, Burnham Institiute Med Res, La Jolla, CA.

Multiple conserved pathways and processes can modulate lifespan, including dietary restriction (DR). The underlying mechanism for how DR promotes longevity is poorly understood, yet we and others have recently shown that autophagy is required for DR to extend lifespan in the nematode C. elegans. Autophagy is the major cellular pathway for degrading long-lived proteins and cytoplasmic organelles. To learn more about the role of autophagy in aging, we are investigating how autophagy may promote longevity at the cellular and molecular level during DR. Many long-lived mutants, including animals subjected to DR, share a common phenotype of increased stress resistance. Specifically, the heat stress resistance phenotype may contribute to extended longevity and be equally dependent on autophagy. To learn more about the molecular mechanism by which autophagy modulates lifespan, we asked whether autophagy is required for stress resistance in animals subjected to DR. We found that, as in longevity, autophagy is also required for the increase in heat stress resistance observed in animals subjected to DR. To further address the cellular basis of autophagy, we have utilized a tissue-specific RNA interference model to inactivate autophagy in specific tissues in animals subjected to DR. Specifically, we asked if autophagy-dependent lifespan modulation is specific to certain tissues in C. elegans. Knowledge of where in the animal that autophagy appears to have a rejuvenating effect will shed light on the underlying mechanism by which autophagy promotes longevity. In our preliminary studies, we have observed that the longevity function of autophagy is indeed restricted to specific tissues of the animal. Taken together, our studies suggest a broad physiological role for autophagy in C. elegans. Specifically, our findings indicate that certain tissues, in response to DR, specifically engage autophagy to increase stress resistance and promote lifespan in this multi-cellular organism. We propose that such tissues are more effective in removing damaged proteins and organelles that normally accumulate during the aging process, perhaps in response to a higher metabolic load in these specific tissues. The autophagic turn-over of such material, the nature of which is still to be identified, may prolong the youthfulness of a cell, tissue, and organism. This work was supported by the American Federation for Aging Research Foundation.

247B

Investigating the Effect of Anoxia Exposure on Lifespan and the Role of Age in Anoxia Tolerance in *C. elegans*. **Jo M. Goy**, Mary L. Ladage, Pamela A. Padilla. Department of Biological Sciences, University of North Texas, Denton, TX.

An organism's response to stress may depend on its age at the time of exposure. We are investigating how age influences anoxia survival and if the stress of anoxia exposure alters lifespan. Furthermore, we are using cellular markers to determine if increased anoxia survival positively correlates with maintenance of specific processes (ex: Q35::YFP to assay proteome stability, pan-neural *unc-119*::GFP to assay muscle and neuronal tissues). *C. elegans* survive 1 day of anoxia at all stages of development (>90% survival rate, 20C), but survival rate of 1-day old hermaphrodites decreases (<10%) when exposed to long-term anoxia (LTA, >3 days). We hypothesized that age of the adult will influence the ability to survive anoxia. We found that 3-day to 5-day old adults survive LTA better than 1-day old adults. Yet, the ability to survive LTA gradually decreases as the animal ages (1 to 10 day old adults assayed). We are investigating the genetic and physiological (ex: egg production) changes that may have an age-dependent effect on anoxia survival. We have published that LTA survival rate is increased by mutations in genes including *glp-1*, *daf-2* or *fog-2*; this can be suppressed by mutations in *daf-16* and *aak-2*. It is known that several of these factors increase lifespan (*glp-1*, *daf-2* mutations). We tested if alteration in the insulin-like signaling pathway or AMPK function affects how an aging hermaphrodite survives LTA exposure. We determined that genotype (ex: *glp-1(e2141)*) does influence LTA survival rate as the animal ages. The effect of age on LTA survival rate is also being investigated in males and in the Hawaiian wildtype strain. Previously, we showed that LTA survival rate is increased by environmental conditions (ex: temperature). We determined that recurrent bouts of anoxia significantly precondition for LTA survival in wildtype hermaphrodites and is partially dependent on *daf-16* function. We are also investigating the affect of anoxia exposure on lifespan in these genetic mutants. Anoxia induces a state of suspended animation in which biological processes such as development are arrested. We tested whether a 1-day old adult exposed to 1 day of anoxia has an altered lifespan; lifespan is slightly increased if animals are exposed to 1 day of anoxia. These results indicate that this

stress is not detrimental to the organism. In contrast, LTA exposure may negatively affect lifespan suggesting that the duration of anoxia influences lifespan. Work is being conducted to understand how cellular processes are affected by anoxia exposure in the context of an aging organism. These studies are relevant given that human health related issues involving oxygen deprivation occur in the aging population.

248C

A Genetic Regulatory Network that Controls the Heat Shock Response in C. elegans. **Eric Guisbert**[1], Daniel Czyz[1], Klaus Richter[1], Patrick McMullen[2], Richard Morimoto[1]. 1) Molecular Biosciences, Northwestern University, Evanston, IL; 2) Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL.

The regulation of the Heat Shock Response (HSR) in eukaryotes, mediated by the HSF1 transcription factor, has been primarily studied in unicellular organisms or cultured cells as a transient stress response. However, in metazoans, HSF1 is known to be essential from the start of development through the end of aging. Therefore, a genetic and systems-level approach including genome-wide RNAi screens was taken to identify and analyze regulators of the HSR in C. elegans. We identified seven enhancers of the HSR, including HSF1, that when knocked-down prevent full HSR activation, but do not affect the unfolded protein response (a stress response distinct from the HSR). The enhancers cannot act through a hormesis-type (stress preconditioning) mechanism because they do not enhance thermotolerance. Rather, these enhancers likely act at or downstream of chaperone-mediated HSF1 repression since they are epistatic to chaperone depletion. Further, these regulators are functionally conserved through humans as all seven enhance the HSR in HeLa cells. Five of the seven enhancers are splicing factors or associated with splicing. Although splicing has not previously been implicated in regulation of the HSR, it is well-established that heat shock leads to an inhibition of splicing. Systems-level analysis revealed these regulatory circuits form a network motif that functions to maintain a transient, self-limiting response to stress. The final HSR enhancer is a subunit of the NuRD complex. Although not been previously implicated in HSR regulation, NuRD has been shown to bind HSF1 suggesting that this regulation is direct. A second genome-wide screen identified fifty-two suppressors of the HSR, including the HSP70 and HSP90 chaperones, established as HSR regulators in other model systems. HSR suppressors include known regulators of protein synthesis and gene expression, folding, trafficking, and clearance. Our data show that HSR suppressors form interaction networks, function in an HSF1-dependent manner, and confer differential tissue selective patterns of HSR induction. Taken together, the combined genetic and systems level approaches have led to unique insights into HSR regulation. The genetic approaches led to the identification of novel regulators of the HSR. In addition, we have shown that suppressors, but not enhancers of the HSR, create differential, tissue-selective HSR regulation. Finally, systems-level network analysis of our data has revealed new features of the response, such as motifs that contribute to the transient dynamics of HSR induction.

249A

MTL-1, MTL-2 and CDR-1 are not essential in *C. elegans* resistance to cadmium. **Kathryn L Haas**, Julie Hall, Jonathan Freedman. Laboratory of Toxicology and Pharmacology, National Institute of Environmental Health Science, RTP, NC.

Consideration of biochemical detoxification of heavy metals, including cadmium, in animals has focused mainly on two classes of metal-binding peptides: the thiol tripeptide, glutathione (GSH), and a class of diverse cysteine-rich low molecular weight peptides, the metallothioneins. The nematode *C. elegans* contains two metallothionein genes, *mtl-1* and *mtl-2* that have been presumed to play a dominant role in cadmium detoxification. However knockdown of these genes individually or synchronously by RNA interference and deletion mutations does not result in a cadmium hypersensitive phenotype as compared to wild type. In addition to the metallothioneins as a potential metal detoxification pathway, *C. elegans* possesses a cadmium specific response gene, *cdr-1*, which is highly up-regulated upon exposure to cadmium. To test if *cdr-1* is a critical gene in metal detoxification in nematodes lacking metallothionein, a triple mutant, *mtl-1(tm1770),mtl-2(gk125),cdr-1(tm723)*, was constructed and tested for hypersensitivity to cadmium by examining the brood size, embryonic lethality, Bagging phenotype and growth. Growth at low cadmium concentrations was the only endpoint in which the triple mutant displayed more sensitivity than the single and double mutants. Although *mtl-1*, *mtl-2* and *cdr-1* are highly up-regulated in response to cadmium, these data suggest that they are not the only genes required for the response to cadmium exposure. A likely explanation for the surprising cadmium resistance in *mtl-1(tm1770),mtl-2(gk125),cdr-1(tm723)* nematodes is a compensatory mechanism that can overcome loss of other metal detoxification peptides. In addition to glutathione alone being a potential pathway in cadmium resistance, a class of metal-binding peptides, the phytochelatins (PCs), has recently been show to impart cadmium resistance in *C. elegans*. We are now investigating whether the transsulfuration pathway might be a major player in nematode response to cadmium, especially in metallothionein and *cdr-1* deficient strains. Experiments using LCMS analysis of various nematode metabolites, including glutathione and phytochelatins in wild type, double *mtl-1(tm1770),mtl-2(gk125)*and triple *mtl-1(tm1770),mtl-2(gk125),cdr-1(tm723)* nematodes are currently underway in order to determine relative response of the transsulfuration pathway in response to cadmium exposure in these mutant strains.

250B

Identification of a pathway involved in the cadmium-responsive transcriptional regulation of metallothionein expression. **Julie Hall**, Jonathan Freedman. LTP, NIEHS, RTP, NC.

The carcinogenic metal cadmium can induce various intracellular stresses. Analysis of transcriptome data from multiple species indicate that cadmium exposure alters the expression of hundreds of genes that are regulated by multiple signal transduction pathways, many of which remain to be defined. In response to cadmium, cells increase the expression of highly conserved, small, cysteine-rich metal-binding proteins known as metallothioneins (MTs), which function in metal detoxification. The nematode C. elegans has two MT genes: *mtl-1* and *mtl-2*. To identify regulatory factors and pathways that control metal-inducible *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*. Transgenic strains constitutively express GFP in the pharynx and following cadmium exposure, express GFP in the intestine. In a reverse genetic screen, genes involved in various stress response pathways were tested for their potential role in controlling *mtl-1* expression. Knockout of *akt-1* or *akt-2* did not affect GFP expression however, the knockout of both genes simultaneously increased expression. AKT-1 is a serine/threonine kinase involved in the insulin signaling pathway and complexes with AKT-2 to regulate transcription of downstream factors. PDK-1 directly interacts with this complex and the knockout of *pdk-1* resulted in an increase in expression. Interestingly, *mtl-1* transcription was not affected when other insulin signaling pathway genes were knocked out. This suggests that PDK-1 and the AKT-1/2 complex act independently of this pathway to control mtl-1 transcription. To identify other transcriptional regulators, transcription factors involved in various MAPK pathways were tested. Knockout of *atf-7* which is involved in the JNK/p38 pathway resulted in an increase in GFP expression. Pathway analysis and RT-PCR data indicate that ATF-7 regulates cadmium-inducible MT transcription downstream of PDK-1 and AKT-1/2. Quantitative RT-PCR also suggests that PMK-1, known regulator of ATF-7; PAX-2, a transcription factor; and TAX-4, a gated ion channel, may also be involved in *mtl-1* gene regulation. In a forward genetic screen eleven EMS mutagenized p*mtl-1::GFP* lines with an increase in GFP after cadmium exposure were isolated. Six of the lines have been mapped to chromosome regions via SNP mapping. Through NextGen sequencing mutations in candidate genes are being identified and further analysis will identify which gene is involved in *mtl-1* transcriptional regulation. Through both forward and reverse genetics a pathway responsible for the cadmium-induced transcriptional regulation of *mtl-1* is emerging.

251C

Osmotic stress and loss of Notch ligands extend C. elegans lifespan via FOXO and sirtuins. Edward N. Anderson[1], Mark Corkins[1], David Sinclair[2], **Anne C. Hart**[1]. 1) Neuroscience, Brown University, Providence, RI; 2) Dept. of Pathology, Harvard Med. School, Boston, MA.

Moderate environmental stress, such as oxidative stress, dietary restriction, or heat shock, can increase the lifespan of many species. In S. cerevisiae, moderate osmotic stress causes lifespan extension, and this extension requires sirtuin function in the NAD salvage pathway. (Kaeberlein et al. 2002). We find that moderate osmotic stress increases the lifespan of C. elegans. NGM contains 51 mM NaCl as the major osmolyte. Wild type animals reared on NGM with increased NaCl live longer than those reared on standard 51 mM NaCl. This lifespan extension occurs with other osmolytes and is dose-dependent. Maximal lifespan extension of 30% is observed at 300 mM NaCl. Osmotic stress starting after the L4 stage is sufficient to extend lifespan. Currently, we are investigating the underlying genetic pathways. Osmotic stress induced lifespan extension requires members of the sirtuin family and the FOXO transcription factor daf-16. We also find a partial requirement for pnc-1, a component of the NAD salvage pathway. Loss of Notch co-ligands osm-7 and osm-11 mimics osmotic stress and extends lifespan in a daf-16 dependent manner. Dietary restriction by eat-2 does not further extend lifespan under osmotic stress conditions. Overall, our results suggest that osmotic stress likely extends lifespan by activating conserved pathways.

252A

EMS Mutagenesis of Early Embryos: Does checkpoint status affect the mutational spectrum? James Barry[1,2], Whitney Finstad[1,2], Numan Khan[1,2], Dean Williams[1], **Phil Hartman**[1]. 1) Department of Biology, Texas Christian University, Fort Worth, TX 76129; 2) These authors contributed equally to this work.

Mutagenesis protocols typically call for exposure of late-stage larvae or adults to a mutagen with the hope of inducing mutations in a robust germ line. If recessive, these mutations manifest themselves in the F2 generation. In contrast, we have handpicked and EMS-mutagenized early (1-4 cell) *C. elegans* embryos. Because all meiotic products derive from a single progenitor cell at this stage, homozygotes should be obtained in the F1 rather than the F2 generation. Using CB665 [*unc-58(e665)*], a strain in which secondary mutations can relieve a strong Unc phenotype, we hand-selected *ca*. 16,000 early embryos and exposed them to EMS for 50 minutes. We recovered 21 mutants out of *ca*. 6,400 viable embryos, which calculates to a mutation frequency of $3x10^{-3}$. This is considerably higher than the 10^{-4} to $5x10^{-4}$ typical of classical EMS mutagenesis (Anderson, P. 1995. In: Methods in Cell Biology, Vol. 48, Epstein and Shakes, eds, p. 31). This is not surprising given that cell-cycle checkpoints are muted in *C. elegans* embryos (Holway et al., 2006. J. Cell Biol. 172:999). Of the 21 mutants, 17 are intragenic. We have also isolated 26 EMS-induced *e665* revertants using standard EMS mutagenesis, of which 25 are intragenic. We are currently sequencing the *unc-58* gene in both sets of mutants to see if the mutational spectra differ from one another.

253B

The KGB-1 JNK signaling pathway negatively regulates FOS-1 transcription factor in stress response. **Ayuna Hattori**[1], Tomoaki Mizuno[1,2], Naoki Hisamoto[1], Kunihiro Matsumoto[1]. 1)

Grad. Sch. Sci., Nagoya Univ., Nagoya, Aichi, Japan; 2) Molec. & Cell Physi., Tsukuba Univ., Tsukuba, Japan.

The JNK MAP kinase (MAPK) pathway plays a pivotal role in the various stress responses of 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. Previously, we found that KGB-1 negatively regulates FOS-1, a bZIP transcription factor homologous to human Fos, through its phosphorylation of FOS-1 in heavy metal stress response. However, a downstream target of FOS-1 has not been identified. To identify genes whose expression is regulated by the KGB-1 pathway, we executed DNA microarray analysis and the results were confirmed by quantitative PCR. We identified three kreg (KGB-1 regulated gene) genes, *kreg-1~3*, whose expression was induced by copper in a KGB-1-dependent manner. Furthermore, their inductions were increased in *fos-1* knockdown animals. Knockdown of *kreg-1~3* caused a partial sensitivity to heavy metals. Taken together, these data suggest that KGB-1 induces expression of genes including *kreg-1~3* by negatively regulating FOS-1 in heavy metal stress response.

254C

A Biochemical Approach for the Identification of new DAF-16 Binding-partners. **Thomas W Heimbucher**[1], Fonslow Bryan[2], Yates Jonathan[2], Dillin Andrew[1]. 1) Molecular and Cell Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, USA, 92037, Howard Hughes Medical Institute, Glenn Center for Aging Research; 2) The Scripps Research Institute, La Jolla, CA.

The FoxO transcription factor DAF-16 regulates a wide range of organismal functions: it is involved in C. elegans development and reproduction, in stress response and life span regulation. Association of DAF-16 with diverse binding partners might be crucial for mediating these heterogeneous functions (1-4). To identify DAF-16 regulators we established a biochemical approach for the purification of DAF-16 associated proteins. DAF-16 was fused to various epitop-tags. On the basis of a reporter assay it was analyzed whether the tagged DAF-16 versions were transcriptionally active and a dauer assay was used to test for their physiological activity in worms. A functional tagged DAF-16 variant was used for the generation of transgenic worms. DAF-16 protein complexes were isolated by tandem affinity purification and potential DAF-16 binding partners were identified by tandem mass-spectrometry (MudPIT). In an additional, reporter based screen DAF-16 co-regulators are currently validated and their potential roles in dauer formation, stress resistance and longevity are currently being pursued. We will discuss their identity and potential functions in insulin/IGF-1 signaling.

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255A

DAF-12 regulates a connected network of genes to ensure robust developmental decisions. **Daniel Hochbaum**, Alfred L Fisher. Medicine Dept, University of Pittsburgh, Pittsburgh, PA.

The nuclear receptor DAF-12 has roles in normal development, in the decision to pursue dauer development in unfavorable conditions, and in the modulation of adult aging. Despite the importance of DAF-12, target genes for this receptor are largely unknown. To identify DAF-12 targets, we performed chromatin immunoprecipitation followed by hybridization to whole genome tiling arrays. We identified 1175 genomic regions as being bound in vivo by DAF-12, and these regions are enriched in known DAF-12 binding motifs and act as DAF-12 response elements in transfected cells and in transgenic worms. The genes near these binding sites include an extensive network of interconnected heterochronic and microRNA genes as DAF-12 targets. We also identified the genes of the miRISC complex, which are required for the control of target genes by microRNA, as putative DAF-12 target genes. During reproductive development, null alleles of daf-12 produce mis-regulation of many of these genes, but only infrequently result in developmental phenotypes. In contrast, we and others have found that null daf-12 mutations enhance the phenotypes of many miRISC and heterochronic target genes. We also found that environmental fluctuations significantly strengthen the weak heterochronic phenotypes of null daf-12 alleles. Further in diapause, DAF-12 represses the heterochronic and miRISC target genes, and prior work demonstrates that dauer formation can often suppress the heterochronic phenotypes for many of these target genes in post dauer development. Together these data are consistent with daf-12 acting to ensure developmental robustness to ensure commitment to adult or dauer developmental programs despite variable internal or external conditions.

256B

The sexual dimorphic response to dietary restriction. **Sakiko Honjoh**, Yukiko Kajiwara, Eisuke Nishida. Dept Cell & Development Biol, Kyoto Univ, Kyoto, Japan.

The sexual dimorphism in morphology, behavior, and life span is common in animals. In human, it is well known that women tend to live longer than men. The nematode *C. elegans* has two sexes, hermaphrodite and male. Hermaphrodites can produce both eggs and sperm, so they can reproduce by self fertilization. Males produce sperm and mate with hermaphrodites. Adult males are smaller than age-matched hermaphrodites. We found that inhibition of the translation machinery, such as RNAi suppression of ribosomal protein subunits and translation initiation factors, reduced the body length to more extent in hermaphrodites than in males. This may suggest the lower translation rate in males. As the translation rate has been shown to be involved in life span regulation, we then focused on the sexual dimorphism in aging. Males live longer than age-matched hermaphrodites under normal conditions. We then examined how males respond to dietary restriction, which is the most reproducible way to extend organism's life span in divergent species form yeast to mammals. We tested two regimens, intermittent fasting (IF) and calorie restriction (CR), which extend *C. elegans* life span by 60% and 20%, respectively. Surprisingly, IF failed to extend life span of males while it successfully extended that of hermaphrodites. The longevity response to CR is also modulated in males. Thus, the overall response to dietary restriction in males seems to be much weaker. Now we are investigating the molecular mechanisms underlying the sexual dimorphic response to dietary restriction.

257C

C. elegans vang-1 modulates life span and stress resistance via insulin/IGF-1-like signaling. **S. Honnen**[1], C. Büchter[2], V. Schröder[2], A. Kampkötter[3], O. Bossinger[1]. 1) Molecular and Cellular Anatomy, RWTH Aachen, D-52074 Aachen, Germany; 2) Toxicology, Heinrich-Heine-University Düsseldorf, D-40225 Düsseldorf, Germany; 3) Bayer Animal Health GmbH, Research and Development, D-40789 Leverkusen, Germany.

The planar cell polarity (PCP) pathway is highly conserved from Drosophila to humans and a PCP-like pathway has recently been described in the nematode Caenorhabditis elegans (1-3). The developmental function of this pathway is to coordinate the orientation of cells or structures within the plane of an epithelium or to organize cell-cell intercalation required for correct morphogenesis (4-5). Here, we describe a novel role of VANG-1, the only C. elegans ortholog of the conserved PCP component Strabismus/Van Gogh. We show that two alleles of vang-1 and depletion of the protein by RNAi cause an increase of mean life span up to 40%. In addition, vang-1 mutants show enhanced resistance to thermal and oxidative stress and decreased lipofuscin accumulation. Life span extension in vang-1 mutants depends on the insulin/IGF-1 like receptor DAF-2 and DAF-16/Foxo transcription factor. This is the first time that a correlation between a key player of the PCP pathway and the modulation of life span and stress resistance has been established. references: 1.Green, J., Inoue, T., and Sternberg, P. (2008). Opposing Wnt pathways orient cell polarity during organogenesis. Cell 134, 646-656. 2.Wu, M., and Herman, M.A. (2006). A novel noncanonical Wnt pathway is involved in the regulation of the asymmetric B cell division in C. elegans. Dev Biol 293, 316-329. 3.Hoffmann, M., Segbert, C., Helbig, G., and Bossinger, O. (2010). Intestinal tube formation in Caenorhabditis elegans requires vang-1 and egl-15 signaling. Dev Biol. 4.Wang, Y., and Nathans, J. (2007). Tissue planar cell polarity in vertebrates: new insights and new questions. Development 134, 647-658. 5.Wu, J., and Mlodzik, M. (2009). A quest for the mechanism regulating global planar cell polarity of tissues. Trends Cell Biol 19, 295-305.

258A

Investigating the role of *C. elegans* BRAP-2 in regulation of the oxygen radical detoxification response. **Queenie Hu**, Janet Koon, Terry J. Kubiseski. Department of Biology, York University, Toronto, ON, Canada.

Brap2/IMP is a cytoplasmic retention protein for the tumour suppressor gene Brca1. It is also identified as a Ras effector protein and has a known role in negatively regulating the ERK/MAPK pathway through the KSR scaffold protein, limiting the formation of Raf-MEK complexes to ensure that Brap2/IMP facilitates the MAPK cascade only in the presence of stimuli in mammalian cells. Our lab has previously shown that *C. elegans* BRAP-2 (EEED8.16) is involved in the oxidative stress response, and is crucial for preventing inappropriate responses to reactive oxygen species. To further investigate BRAP-2's role in stress response, we show that the *brap-2(ok1492)* mutant has an increased expression of the Phase II detoxification enzyme, GST-4, and that the enhanced GST-4 expression is dependent on the transcription factor, SKN-1. Based on previous results, we hypothesized that the ERK/MAPK is a possible pathway in regulating the detoxifying genes for worms under oxidative stress, in which BRAP-2 serves as a negative regulator of KSR to induce phosphorylation of MAPK to SKN-1 and enhance GST-4 expression. While the exact mechanism of the BRAP-2/SKN-1 regulation requires further investigation, our results from Western blotting reveal that BRAP-2 physically binds to LET-60/Ras and KSR-1/KSR-2. We are currently creating double mutant worms to further confirm the role of the ERK/MAPK pathway in SKN-1 activation using an RNAi approach and qRT-PCR. As the fundamental principles of signal transduction pathways are evolutionary conserved between worms and higher organisms, this project in *C. elegans* aims to provide a solid framework of how BRAP-2 may regulate SKN-1 to relieve stresses in more complex organisms including human.

259B

High throughput toxicity screen using *C. elegans* and propidium iodide. **Piper R. Hunt**, Nicholas Olejnik, Robert L. Sprando. Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD.

The ICCVAM Authorization Act of 2000 was passed by Congress with the goal of reducing, refining, or replacing animal tests that are currently used in toxicology without compromising analyses of human safety and product effectiveness. High throughput assays in *C. elegans* have the potential to provide mammalian-correlative toxicity data in a whole organism with the speed, scope and cost of *in vitro* testing. Microfluidics technology has allowed our lab and others to study the effects of various compounds on larval growth in *C. elegans* utilizing thousands of animals per condition per experiment. Here we report on the usefulness of a high throughput screening assay using the COPAS Biosort with adult *C.*

elegans and propidium iodide (PI) as a fluorescent indicator of morbidity and mortality. Sodium arsenite (NaAs) was selected as a model toxicant to test this method. We found that increasing concentrations of NaAs were associated with increasing red fluorescence levels as indicated by COPAS analysis. The method was very effective at detecting acute toxicity, but was not useful in identifying animals which had been dead for an extended period of time. Several heavy metals with a range of toxicities, as determined by rat oral LD50 values, were selected for further testing. Adult nematodes were exposed to the test compounds at 7.8, 31.25, 125 and 500 ppm for 2 weeks in axenic liquid media and subsequently treated with PI for 24 hrs prior to analysis using the COPAS. Test compounds were ranked in this assay from most toxic to least toxic as follows: mercuric chloride, cadmium chloride, copper chloride and potassium chloride. This ranking, based on fluorescence in *C. elegans* recently dead or dying, corresponds to heavy metal toxicity as determined by oral rat LD50's. These results suggest that the method described in this poster could be used as one of a battery of tests to screen compounds for their relative toxicity in the absence of mammalian data.

260C

CeleST, a sophisticated software package for the simultaneous quantiation of multiple swimming *C. elegans* for aging and locomotion analysis. **C. Ibanez-Ventoso**[1], C. Restiff[2], S. Guo[1], D. Metaxas[2], M. Driscoll[1]. 1) Dept Molec Biol & Biochemistry, Rutgers University, Piscataway, NJ; 2) CBIM Center, Rutgers University, Piscataway, NJ.

We have a strong interest in the biology of healthy aging. Our research is focused on identification of modulators of healthspan, since the maximization of a period of life free of degenerative changes seems more desirable than a mere prolongation of lifespan. For the evaluation of healthspan genetics, our laboratory uses three main methodologies that reflect the quality of aging: analysis of locomotion vigor (Herndon *et al.*, 2002; Tsechpenakis *et al.*, 2008), quantiation of age pigments (lipofuscin and advanced glycation end products (Gerstbrein *et al.*, 2005), and standard measurement of lifespan with an emphasis on mean length of life. Over a specific period of adult life, age-synchronized *C. elegans* widely differ in their physical capacity even when grown under similar environmental conditions. However, all adults suffer an irremediable loss in muscle integrity and locomotion with age. Active physical activity is usually associated with a relative absence of gross degenerative changes. Together, strong physical activity and maintenance of tissue function define a youthful period of life or healthspan. Differences in physical capacity are easily observable on solid media and thus are feasible to measurement, either manual or automatic, using available computer programs of tracking. Our focus has been to analyze *C. elegans* physical capacity in liquid, as animals are challenged to move in a behavior that has more potential for high resolution parameter characterization. Active worms naturally initiate movement (mostly sinusoidal) immediately after being placed into a drop of buffer. We refer to *C. elegans* general motion in liquid as swimming, and to the repetitive movement demarcated by the head and tail extremities as strokes. *C. elegans* swimming can be quantified by an observer as the number of head strokes over a specific period of time, for instance 30 sec. This method still holds subjectivity and is limited (only one measure). In order to overcome these limitations, we recently developed novel software to analyze *C. elegans* swimming, namely CeleST (*C. elegans* Swimming Tracking). Our software CeleST is objective and extracts multiple measures from *C. elegans* swimming, greatly enhancing the resolution of our analysis of locomotion changes with age. Using CeleST, we have defined the swimming of wild type and of mutants of the Ins/IGF-1 signaling pathway over adult life. We will present CeleST in a framework of aging biology however we foresee our software as a valuable tool for the general *C. elegans* community with interests in locomotory behavior.

261A

Intermittent fasting-induced longevity requires cholesterol in *C. elegans*. **Akiko Ihara**, Sakiko Honjoh, Masaharu Uno, Hiroyuki Yoshimura, Eisuke 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, an every 2 days fasting in *C. elegans*. It increases resistance to heat and oxidative stress, and effectively extends the lifespan. We have also shown that it requires the FOXO transcription factor DAF-16 for its maximal effect on lifespan extension. Cholesterol is widely known as the component of plasma membrane, and it also plays an important role in signaling pathways. In *C. elegans*, it is metabolized to various steroid hormones in the body, which function as signaling molecules for molting, dauer formation, and longevity. However, nematodes are unable to biosynthesize sterol de novo, so they require dietary cholesterol. It has been previously shown that cholesterol deprivation induces larval arrest, defective reproduction, and lifespan reduction. It is thought that the signaling pathway involving a nuclear hormone receptor DAF-12 and its ligand dafachronic acid (DA) may be related to the lifespan regulation by cholesterol deprivation. DA is a cholesterol derivative, and its administration extends the short lifespan of the germline-deficient animals that lack the somatic gonad. This lifespan extension requires both DAF-12 and DAF-16. Moreover, it is known that in mammalian cells, the ligand-bound vitamin D receptor, which is homolog of DAF-12, interacts with FOXO transcription factors. Then, we considered the possibility that cholesterol deprivation should affect the IF-induced longevity pathway, and examined whether IF extends lifespan of worms on cholesterol-free NGM plates. Our results have shown that under the cholesterol-free culture conditions that do not affect lifespan of animals in *ad libitum*, IF-induced lifespan extension is significantly suppressed. Thus, cholesterol may have a role in mediating IF-induced longevity. We are now analyzing the underlying mechanisms.

262B

Genes involved in mevalonate and ubiquione metabolism are essential for delayed muscle aging. **Luv Kashyap**, Alfred Fisher. University of Pittsburgh, Department of Medicine, Division of Geriatric Medicine, 3471 5th Ave., Suite 500, Pittsburgh, PA 15260.

Sarcopenia is the progressive generalized loss of skeletal muscle mass, strength, and function which occurs as a consequence of aging. With a growing older population, there has been great interest in developing approaches to counteract the effects of sarcopenia, and thus fostering independence of the elderly population and reducing the personal and financial burdens associated with sarcopenia. Much like humans, C.elegans also experiences sarcopenia during aging. In the work presented here, we demonstrate that genes involved in mevalonate and ubiquinone metabolism play a critical role in muscle aging in the nematode Caenorhabditis elegans. Worms with mutations in the daf-2 insulin/IGF-1-like receptor show delayed sarcopenia, and we find that the mevalonate kinase gene mvk-1 is required specifically in muscle for this delay. Mevalonate kinase is the next enzyme downstream of the HMG-CoA reductase which is the target of the widely used statin medications, in the mevalonate pathway. Consistently, we have also found that treatment of worms with the commonly used cholesterol lowering drug pravastatin also impairs mobility. Interestingly, an important product of the mevalonate pathway is ubiquione, and statin medications have been proposed to produce muscle pain and weakness, termed statin myopathy, via blocking ubiquione synthesis. We find that inhibition of ubiquione production via RNAi leads to accelerated muscle aging and disrupts protein homeostasis in muscles. Together, our work suggests that the genes involved in the mevalonate and ubiquinone metabolism are essential to ensure successful muscle aging.

263C

Characterization of *C. elegans* mutants deficient in base excision repair genes. **Yuichi Kato**, Takahito Moriwaki, Atsumi Ikemoto, Kazunari Hashiguchi, Qiu-Mei Zhang-Akiyama. Kyoto University, Kyoto, Japan.

Base excision repair (BER) plays an important role in repairing small base damage in DNA and maintaining genome stability. In the BER pathway, damaged bases are removed by DNA glycosylases, and then resulting apurinic/apyrimidinic (AP) sites or 3'-α,β unsaturated aldehydes are processed by AP endonucleases. Unrepaired DNA damage may cause mutation or cell death. It is also thought that accumulation of DNA damage is related to aging. In *C. elegans*, two DNA glycosylases (NTH-1 and UNG-1) and two AP endonucleases (EXO-3 and APN-1) have already been identified. In addition, their enzymatic activities have also been characterized. However, comprehensive study of these genes has not been accomplished yet. We do not yet fully understand their relationship and roles in *C. elegans*.

Our purpose is to investigate how these BER genes functions in *C. elegans* and whether they are related to the aging process of *C. elegans*. In this study, we examined a variety of mutants deficient in BER genes. We found that mutation in *exo-3*, one of the AP endonucleases in *C. elegans*, results in decreased self-brood size. The *exo-3* mutant also showed extended lifespan. These results suggest that mutation in the *exo-3* gene is harmful for maintaining the group, even if it may be beneficial for individual worms.

264A

Telomere lengthening in suppressor mutants of telomerase deficient Caenorhabditis elegans. **Chuna Kim**, Beomseok Seo, Junho Lee. Department of Biological Sciences, Research Center for Functional Cellulomics and Institute of Molecular Biology and Genetics, Seoul National university, Seoul, Korea.

Eukaryotic cells have linear chromosomes which cause 'the end-replication problem'. 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, which is a special kind of reverse transcriptase. However, it was also found that telomeres can be maintained without telomerase in yeast and some cancer cells by the **Alternative Lengthening of Telomere (ALT)** mechanism, which is based on a process involving recombination. But, until now, the mechanism that may suppress ALT in telomerase-negative cells have not been known. Therefore, we performed forward and reverse genetic screenings for finding ALT suppressor. In Caenorhabditis elegans it was known that the *trt-1(ok410)* mutant strain, which lacks the telomerase catalytic subunit, becomes sterile (mortal germline phenotype) after several generations as a result of shortening of telomeres. We mutagenized *trt-1(ok410)* mutant animals and isolated suppressor mutants that had re-acquired immortality of germ cells. With Terminal Restriction Fragments(TRF) assay, Single Telomere Length Analysis(STELA) and Fluorescence In Situ Hybridization(FISH), we found that the telomeres of the suppressor mutants were lengthened and had distinct property from normal telomeres. Currently, we are mapping these suppressor mutants by Single Nucleotide Polymorphisms(SNPs) and whole genome sequencing technology.

265B

Caenorhabditis elegans HIM-6 has a 3'-5' helicase activity and is responsible for DNA damage processing. **eunsun Kim**, moonjung Hyun, sojin Park, hyejin Park, byungchan Ahn. life science 19-321, university of ulsan, ulsan, ulsan, Korea.

Mutations in human RecQ genes display various features of premature aging, cancer predisposition, and developmental abnormalities. Bloom syndrome is characterized by severe growth retardation and dramatic cancer predisposition and caused by mutations in BLM gene. A BLM helicase ortholog, him-6, has been identified in Caenorhabditis elegans. However, little is known about the enzymatic activities and roles of the HIM-6 protein. We

show that him-6 is sensitive to CPT and the accumulation of DNA strand breaks in the mutant was detected using RAD-51 foci and Comet assay. We purified the recombinant HIM-6 protein from E. coil. The HIM-6 protein has a 3' -> 5' helicase activity and needs at least 5-nt overhang for its helicase activity. HIM-6 is able to unwind various DNA structures related to intermediates generated during replication and repair. However, HIM-6 shows a limited processivity on forked duplexes, suggesting that the processivity may be regulated by other proteins in vivo. These characterized properties suggest that HIM-6 may play roles in DNA metabolic pathways and its defect may give rise to genomic instability.

266C

Delimiting a polymorphic longevity locus on the left arm of Chromosome IV using traditional and high throughput longevity assays. **Gunnar Kleemann**, Joshua Boom, Leonid Kruglyak, Coleen Murphy. Lewis-Sigler Institute of Integrative Genomics, Princeton University, New Jersey, USA.

A large body of work has been generated describing how aging is altered by changes in signaling pathways. Less well understood is how the known pathways differ across genetically polymorphic natural isolates of C. elegans. In order to explore how the longevity pathways have diverged in wild strains, we have assessed longevity across a panel of CB4856 x N2 recombinant inbred advanced intercross lines (RIAILs) (Rockman and Kruglyak 2009). We facilitated our analysis by developing a high-throughput longevity assay pipeline, "Chronos" to count worms and estimate longevity curves. While N2 and CB4856 longevity were similar, a number of the RIAIL life spans differed significantly from either parent suggesting that parental life span has converged, but hidden genetic variation is revealed when co-adapted alleles are separated. From the linkage data generated in the RIAIL based QTL (quantitative trait loci) analysis we identified a polymorphic region on the left arm of Chromosome IV that confers a longevity difference when the region is isolated on an isogenic N2 background. We have reduced the longevity-linked region from the initial 2.5 Mbp identified in the QTL analysis to <200 kb long region using Near Isogeneic Lines (NILs). The minimal region encompasses likely functional changes in only 8 genes. We are testing the candidate genes using both single gene and fosmid rescue. Additionally, In order to gain a deeper insight into the processes that differ between N2 and the short-lived NIL worms we are conducting a detailed phenotypic analysis of the NIL survival curves. To collect the large number of survival curves required to fully characterize the hazard (instantaneous likelihood of death) function shape, peak time and variation we are using an imaging robot in conjunction with the Chronos image analysis pipeline.

267A

Identification of mutations that delay reproductive or somatic aging of Caenorhabditis elegans. **Sandeep Kumar**[1], Stacie E Hughes[2], Cheng Huang[3], Kerry Kornfeld[1]. 1) Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110; 2) Stowers Institute for Medical Research, 1000 E. 50th St., Kansas City, MO 64110; 3) Department of Organismal Biology and Anatomy, 1027 E. 57th Street, University of Chicago, Chicago, IL 60637.

Aging is an important feature of animal biology characterized by progressive degenerative changes in a wide range of organs and tissues, including the reproductive system. Genetic studies have begun to identify genes that can influence lifespan, but little is known about genes that might affect aging of specific tissues. To identify genes that are important for controlling these degenerative changes, we used chemical mutagenesis to perform forward genetic screens in Caenorhabditis elegans. Using a screen focused on somatic tissues, mutants were identified that displayed extended periods of pharyngeal pumping, body movement, or survival. One of these mutations is a novel allele of the age-1 gene. age-1 encodes a phosphatidylinositol-3-kinase (PI3K) that functions in the insulin/insulin-like growth factor-1 (IGF-1) signaling pathway. age-1(am88) creates a missense change in the conserved PIK domain and causes dramatic extensions of the pharyngeal pumping and body movement spans, as well as a two-fold extension of the lifespan. By conducting screens focused on reproductive aging in mated hermaphrodites, we identified mutants that displayed increased progeny production late in life. To characterize these mutations, we developed quantitative measurements of age-related morphological changes in the gonad. The am117 mutation delayed age-related declines in progeny production and morphological changes in the gonad. We are currently using whole genome sequencing to identify the gene affected by am117. These studies provide new insights into the genetic regulation of age-related degenerative changes in somatic and reproductive tissues.

268B

Translational Control of Tumor Formation in *C. elegans*. **Caroline Kumsta**, Malene Hansen. Sanford-Burnham Medical Research Institute, 10901 N. Torrey Pines Road, La Jolla, CA.

The major risk factor for cancer development is increased age. With age, the likelihood of tumor formation and cancer incidence increases in many organisms, including humans. Recently, *C. elegans* was employed as a model system for tumor formation, and mutations that increase lifespan, e.g., in the insulin/IGF-1 receptor (InR/*daf-2*), decrease tumor growth in this model, suggesting that aging and tumor progression are linked mechanistically. This link creates the opportunity to identify longevity modulators that have effects on tumor formation in *C. elegans*.

Similarly to inhibition of InR/*daf-2*, the reduction of components of the mRNA translation initiation complex was recently found to increase longevity in *C. elegans*. Conversely, increased function of translation initiation factors has been observed in many human cancers, yet the precise mechanisms by which these factors affect tumor formation are unclear. This study aims to address the hypothesis that reduced translation initiation could constitute a tumor suppressor mechanism in *C. elegans*.

Specifically, we investigate the oncogenic function of the translation initiation machinery on the *C. elegans* tumor model. These tumors arise due to increased Notch/GLP-1 signaling in the *C. elegans* germline, which leads to the massive overproliferation of germ cells and their subsequent break out of the gonad, causing the animals to die prematurely. Notably, we have found that the reduction of specific components of the translation initiation machinery rescues tumor growth and the premature death in *C. elegans*. These findings indeed suggest that impairment of the translation initiation complex has tumor-suppressive effects in *C. elegans*. We have now begun to investigate the possible underlying mechanisms by which the translation initiation complex affects the formation of a tumorous germline in *C. elegans*.

This study, which is rapidly carried out in *C. elegans* due to its tractable genetics and short lifespan, has the potential to identify novel tumor-suppressor and oncogenes that might influence tumor formation also in mammalian systems.

269C

The ESRE stress-response network - analysis of an evolutionary conserved pathway. **Aleksandra P. Kuzmanov**, David S. Fay. Department of Molecular Biology, University of Wyoming, Laramie, WY.

All living organisms require a stable internal environment to develop, function and survive. This internal homeostasis is constantly challenged by a variety of potentially harmful stressors. In the attempt to reestablish homeostasis, many cellular processes are adjusted by modulating gene expression. Our laboratory has identified a largely uncharacterized and evolutionarily conserved stress-response pathway in C. elegans that regulates the expression of hundreds of genes following a variety of stress conditions. This network is named after the regulatory sequence that drives stress-induced gene expression; ESRE for ethanol and stress-response element. The ESRE stress-response network in C. elegans includes SLR-2, a C2H2 zinc finger protein, and its downstream target JMJC-1, a jumonji-C domain-containing protein that functions as a histone modifier. Recently we have identified an additional regulator of the ESRE-dependent transcription, C08B11.3/BAF200, which encodes for a conserved subunit of the PBAF chromatin-remodeling complex. Interestingly, the effect of C08B11.3 on the ESRE-gene expression appears to be tissue-specific; RNAi-mediated knockdown of C08B11.3 reduces ESRE-dependent transcription in ectoderm (neural and hypodermal tissue) but has no effect on endoderm expression. Currently, our primary goal is to identify the direct ESRE-binding protein (EBP). We are undertaking two complementary approaches - biochemical affinity purification using the oligonucleotide-trapping method and focused RNAi screening of candidate EPB factors in C. elegans. In vitro and in vivo studies will be used to further evaluate potential EBP candidates. Identifying the EBP, as well as additional ESRE-pathway regulatory components, will enable us to better understand the complex cellular networks that coordinate stress response.

270A

NAD+ Recycling, Sirtuins, and Stress: A Complex Relationship. **Stephanie E. Lange**, Wendy Hanna-Rose. BMB, Penn State Univ, University Park, PA.

Sirtuins are an important class of lysine deacetylases that have been implicated in aging and stress response. The enzymatic activity of SIR-2.1 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 examined SIR-2 overexpression-mediated *Psod-3::gfp* expression in combination with PNC-1 loss-of-function. Consistent with findings in yeast, we found that PNC-1 regulates SIR-2.1 in *C. elegans*. 1) Anderson et al. 2003. Nature 423(6936):181-5 2) Berdichevsky et al. 2006. Cell 125(6):1165-77.

271B

Nucleotide Excision Repair and its regulation in *C. elegans* development. Andrea E. Karambelas[1], Daniel Cupac[1], Özge Z. Aydin[1], Gert Jansen[2], Jan H.J. Hoeijmakers[1], Wim Vermeulen[1], **Hannes Lans**[1]. 1) Dpt Genetics, Erasmus MC, Rotterdam, Netherlands; 2) Dpt Cell Biology, Erasmus MC, Rotterdam, Netherlands.

Nucleotide Excision Repair (NER) is a versatile DNA repair pathway, capable of removing many helix-distorting DNA damages, including those induced by UV-light. In humans, NER-deficiency is associated with severe clinical symptoms such as cancer predisposition and accelerated ageing. Functional analysis of the proteins that make up the core machinery of NER has led to a detailed molecular model of the multi-step NER mechanism. DNA damage is first recognized in a transcription-coupled manner or independent of transcription through global genome NER. Next, the damaged DNA is excised and exchanged for newly synthesized DNA. This model has emerged mainly using single-cell systems such as bacteria, yeast and mammalian cell cultures. Thus, it is largely enigmatic how NER functions in different cell types and tissues of living organisms. Furthermore, how NER is regulated as part of the DNA damage response (DDR) of cells and functions in vivo within chromatin-embedded DNA is largely not understood.

We use *C. elegans* to study the function of NER *in vivo* throughout development in a multicellular organism. Furthermore, we use *C. elegans* to identify and study novel genes involved in the DDR against UV-light. Our previous analysis suggests that NER functions

similarly in *C. elegans* as in mammals. Intriguingly, we find that NER is differentially regulated in different tissues throughout development: growth and survival of UV-exposed germ cells and early embryos mainly depends on functional global genome NER, while postembryonic somatic cells predominantly depend on transcription-coupled NER. Additionally, we find that some NER proteins are involved in multiple repair pathways and are essential for normal development and proper growth of germ cells, embryos and larvae. These findings highlight the importance of DDR mechanisms, including NER, to protect multicellular organisms against the harmful consequences of DNA damage. Finally, using genetic screening, we have identified several ATP-dependent chromatin remodeling factors that function in the UV-induced DDR. Current characterization of their function in *C. elegans* and mammalian cells suggests an evolutionary conserved function during specific early steps of NER.

272C

Autophagy links lipid metabolism to longevity in *C. elegans*. **Louis Lapierre**[1], Sara Gelino[1], Alicia Meléndez[2], Malene Hansen[1]. 1) Development & Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA; 2) Queens College-CUNY, Flushing, NY.

Autophagy is a key biological recycling process with an emerging role in organismal aging, yet the nature of the autophagic cargo recycled during aging is unknown. Recently, autophagy was shown to be able to lipolyze intracellular lipid droplets by a process called lipophagy. We therefore asked whether lipophagy is an important mechanism for longevity. By investigating long-lived *C. elegans* lacking a germline with increased lipase activity, we find that autophagy and lipolysis, possibly via a process similar to lipophagy, coordinately modulate lifespan extension. Specifically, we find that *glp-1* mutants, which lack a germline due to disrupted Notch signaling, displayed increased autophagy, a process that we observe is regulated by the FOXA transcription factor PHA-4. Accordingly, RNAi knockdown of several autophagy genes or *pha-4* specifically suppressed the extended longevity of *glp-1* mutants. Consistent with these observations, we find that the nutrient sensor TOR, a major upstream regulator of autophagy, is downregulated in response to germline removal. Germline-less animals is also known to overexpress a lipase LIPL-4, which was previously shown to be sufficient to extend *C. elegans* lifespan. Importantly, we observe that overexpression of LIPL-4 increased autophagy, and autophagy genes were required for *lipl-4*-mediated longevity. Similar to *glp-1* worms, we find that longevity associated with overexpression of LIPL-4 was dependent on *pha-4* and on autophagy genes. In addition, RNAi knockdown of autophagy genes or *pha-4* in *glp-1* animals reduced lipolytic activity, suggesting a coordinate modulation of lipid metabolism as well as aging by autophagy. Taken together, these observations suggest a novel link between autophagy and lipolysis in *C. elegans* aging. We propose that the extended longevity of germline-deficient animals depends, at least in part, on the redistribution of fatty acids by autophagy. This work was supported by the Ellison Medical Foundation.

273A

Identification of glucose-regulatory genes using a genome-wide RNAi screen. **Dongyeop Lee**[1], Dae-Eun Jeong[1], Seung-Jae Lee[1,2,3]. 1) Division of Molecular and Life Science; 2) School of Interdisciplinary Bioscience and Bioengineering; 3) World Class University Information Technology Convergence Engineering, Pohang University of Science and Technology, Pohang, Kyungbuk, 790-784, South Korea.

Glucose is an essential nutrient. The glucose metabolism should be tightly regulated and inappropriate regulation can cause diseases such as obesity, type 2 diabetes, and cardiovascular diseases. Recently, several studies reported that glucose directly shortens the life span of *C. elegans (1, 2, 3)*. We showed that glucose shortens life span by down-regulating DAF-16/FOXO and HSF-1/heat shock factor 1, which are the life span-extending transcription factors downstream of insulin/IGF-1 signaling pathway *(3)*. In addition, we identified glucose-responsive genes through microarray analysis and found that reduced expression of *aqp-1*/glycerol channel was responsible for the shortened life span *(3)*. To further elucidate molecular mechanisms by which glucose affects life span, we plan to identify and to characterize glucose-regulatory genes by performing a genome-wide RNAi screen. We are using glucose-responsive GFP transgenic animals as a reporter for the screen. We examined other possible causes of the GFP induction and validated that the reporter is suitable for the RNAi screen. Through this genome-wide RNAi screen, we expect to find glucose-regulatory genes that may modulate life span under glucose-enriched condition and/or genes that may regulate glucose metabolism. This study will lead to better understanding of how glucose influences aging and metabolism at the molecular level. *(1) Schulz et al., 2007, Cell Metabolism (2) Schlotterer et al., 2009, Diabetes (3) Lee et al., 2009, Cell Metabolism.*

274B

GTP binding protein Nog1 homologue regulates fat storage, development and lifespan. **Ho-Hyun Lee**, Ju-Yeon Lee, Jeong-Hoon Cho. Dept of Biology Education, Chosun University, Gwangju, Korea.

Nog1 is a nucleolar GTPase protein and it is critical for 60S ribosome biogenesis. Recently, Nog1 is known as one of downstream genes of TOR, target of rapamycin. Rapamycin is an immunosuppressant drug is increase life span in model animals. Here we shows that nog1 homologue(T09A9.9) in C. elegans regulate lifespan, fat metabolism, development. GFP promoter assay revealed ubiquitous expression of the C.elegans nog1 from early embryo through adult. GFP tagged Nog1 protein localized in nucleus and especially concentrated in nucleolus. In fuctional study of Nog1, knock down result shows slow growth, small broodsize, more fat storage, and increased life span. On the contrary to this Nog1 overexpression decrease life span. Taken together nog1 homologue in C.elegans may be one of downstream of TOR and has important roles in fat storage, development and lifespan.

275C

AMP-dependent protein kinase regulates nutrition dependent diapause in *C. elegans*. **Inhwan Lee**, Young-Jai You. Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA.

To survive starvation, animals reduce their energy use by turning off anabolic pathways and burn stored fuel by turning on catabolic pathways. One of the molecular switches doing that is AMP-dependent protein kinase (AMPK). Using L1 diapause, we study how AMPK regulates the long term starvation. We found that starvation during L1 activates AMPK and induces two phenotypes in *aak-2* mutants; (1) 3 day starvation as L1 induces sterility when the starvation-experienced worms become adults. (2) 7 day starvation as L1s kills them. To get fully activated, the threonine residue at 172 in AMPK needs to be phosphorylated. Because L1 starvation increases phosphorylation in AMPK, we tested the mutants of three known upstream kinases of AMPK (PAR-4, MOM-4 and CKK-1) for both the sterility and the lethality phenotypes. None fully phenocopy the phenotypes induced by L1 starvation in *aak-2* mutants; only par-4 mutants partially resemble them, suggesting redundancy among these upstream kinases. Autophagy is self-degradation process for cells to survive starvation and we previously showed insufficient autophagy shortens L1 diapause span due to insufficient energy [1]. Because AMPK directly induces autophagy by phosphorylating ULK-1 [2], we hypothesized that *aak-2* die earlier than wild type because the mutants fail to induce autophagy. When we measured the amount of cleaved LGG-1, a homolog of LC3 and an indicator of active autophagy, it is reduced in *aak-2*, suggesting that the reduction of autophagy contributes to the death of *aak-2*. It has been suggested that metformin, the most widely used drug for type II diabetes acts through AMPK. But its direct target(s) are unknown. To confirm the function of AMPK during L1 diapause by pharmacological approach and to test if L1 diapause can be used to find targets of metformin, we treated worms with metformin during L1 diapause. Based on the its action, we hypothesize that (1) metformin will over-activate AMPK during L1 diapause where AMPK is already activated. Therefore it will shorten L1 diapause span. (2) If metformin acts through AMPK, treatment with metformin would not shorten L1 diapause span any further in *aak-2* mutants. We found both true; treatment with metformin shortens L1 diapause and it doesn't reduce the short L1 diapause span of *aak-2* further. Our study shows that L1 diapause is starvation response regulated by AMPK; AMPK functions for worms to survive the long-term starvation undamaged. 1.Kang, C., Y.J. You, and L. Avery. Genes Dev, 2007. 21(17): p. 2161-71. 2.Egan, D.F., et al. Science, 2011. 331(6016): p. 456-61.

276A

OASIS, an open-access and user-friendly online application for survival analysis. Jae-Seong Yang[1], Hyun-Jun Nam[1], Mihwa Seo[1], Seong Kyu Han[2], Yonghwan Choi[1], Hong Gil Nam[1,2], **Seung-Jae Lee**[1,2,3], Sanguk Kim[1,2,3]. 1) School of Interdisciplinary Bioscience and Bioengineering; 2) Department of Molecular Life Science; 3) World Class University Information Technology Convergence Engineering, Pohang University of Science and Technology, Pohang, South Korea.

C. elegans researchers have been performing various kinds of survival assays such as life span analysis, stress resistance and pathogenesis assays. Appropriate statistical analyses are needed to properly interpret the experimental results obtained from these survival assays. Here, we developed an online application for survival analysis (OASIS) that performs various statistical tasks involved in analyzing survival data in a user-friendly manner. OASIS takes censored survival data and outputs standard survival analysis results, including Kaplan-Meier estimates and mean/median survival time. OASIS performs statistical tests including log-rank test, Fisher's exact test, Kolmogorov-Smirnov test, survival time F-test and others. To illustrate survival data, OASIS generates survival and log cumulative hazard plots that allow researchers to easily visualize their experimental results. Furthermore, we have developed novel statistical tests that can be used to analyze variation among individuals in a population survival data and to quantify factors that stochastically influence survival rate. We believe OASIS may provide a uniform platform that facilitates the efficient statistical analysis of survival data for *C. elegans* researchers. Web application and detailed description of algorithms are accessible from http://sbi.postech.ac.kr/oasis.

277B

Exploring the function of progeroid *WRN* gene homologues in *C. elegans*. **Hayley Lees**, Lynne Cox, Alison Woollard. Department of Biochemistry, University of Oxford, South Parks Road, Oxford, OX1 3QU.

Understanding the molecular basis of ageing is a necessary precursor to developing therapies to counter deleterious aspects of human ageing including frailty and age-related diseases. The widely-studied premature ageing Werner syndrome (WS) phenocopies normal human ageing but is caused by mutation of a single gene, *WRN*, encoding both helicase and exonuclease activities. In order to dissect the distinct roles of WRN helicase and exonuclease throughout development and ageing, we have identified homologues of both the helicase and exonuclease activity of human WRN in *C. elegans.*

Our comparative genomic analysis in *C. elegans* suggests that *wrn-1* is the closest homologue of the human WRN helicase domain and *mut-7* is the closest homologue of the human WRN exonuclease domain. Ageing phenotypes and shortened lifespan have been previously associated with reduction of function of *wrn-1* by RNA interference [1], while mutation of *mut-7* results in genetic instability by activating transposons in the germline [2], but there have been no reported lifespan studies. We suggest that together, *wrn-1* and *mut-7* constitute the same domain architecture as human WRN, as in other invertebrates

(*Drosophila*) and plants (*Arabidopsis*).

We have established out-crossed worm lines mutant for the WRN exonuclease, and for the WRN helicase (*mut-7* and *wrn-1*, respectively), and have used these strains to generate *wrn-1;mut-7* double mutants. Ageing phenotypes and lifespan of the mutant worms will be presented, as will the phenotype of our novel double mutants lacking both WRN helicase and exonuclease. In addition, further data highlighting how this worm model can be used to dissect at the molecular level the relative contributions of the WRN helicase and exonuclease to DNA damage responses and genome stability will also be given.

References 1.Lee, SJ; Yook, JS; Han, SM; Koo, HS. A Werner syndrome protein homolog affects C. elegans development, growth rate, life span and sensitivity to DNA damage by acting at a DNA damage checkpoint. Development, 2004. 131(11): p. 2565-2575. 2.Ketting, RF; Haverkamp, TH; van Luenen, HG; Plasterk, RH. Mut-7 of C. elegans, required for transposon silencing and RNA interference, is a homolog of Werner syndrome helicase and RNaseD. Cell, 1999. 99(2): p. 133-141.

278C

A dominant mutation in *xrep-3* encoding the bZIP transcription factor SKN-1 abnormally induces GST expression in *Caenorhabditis elegans*. Koichi Hasegawa[1], **Chi K. Leung[2]**, Keith P. Choe[2], Johji Miwa[1]. 1) College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto, Kasugai 487-8501 Japan; 2) Department of Biology, University of Florida, PO Box 118525, B30 Bartram Hall, Gainesville, FL 32611-8525.

The phase II enzyme family of glutathione S-transferases (GSTs) is a major player in an organisms' defense against a variety of xenobiotics. To genetically dissect xenobiotic response pathways, we isolated abnormal GST-response mutations from *gst-4::gfp* transgenic animals [1]. A dominant mutation, *xrep-3(k1023)*, displays constitutively expressed GST-4::GFP throughout the whole body in the absence of xenobiotics. The *xrep-3* mutation also affects the expression of several other phase II enzymes such as GST-7, GST-30, GST-38, and C02D5.4. Conventional mapping linked *xrep-3* to LG IV, and SNP mapping narrowed it between the SNP marker T12E12 and haw56365. We amplified a 16.9 kb DNA fragment containing T19E7.1, T19E7.2, and T19E7.3 from the *xrep-3* mutant genome, and injected it into *gst-4::gfp* transgenic animals; this fragment induced GST-4::GFP expression without xenobiotics. The gene T19E7.2 encodes SKN-1, a nematode homolog of the bZIP transcription factor that controls metabolism, development, and stress responses. The gene *skn-1* is reported to encode the three protein isoforms SKN-1a, b, and c, which share the same C terminus with differing N termini. The mutation harbors a missense mutation in exon 3 of *skn-1a*, which is the same as exon 1 of *skn-1c*. We showed that *skn-1c* cDNA from the *xrep-3* mutant constitutively induces GST-4::GFP expression.

Under non-stress conditions, the WD40 repeat protein XREP-1/WDR-23 physically interacts with SKN-1c and suppresses its activity together with CUL-4, DDB-1, and a proteasome [2]. By yeast two-hybrid experiments, we also confirmed that the *xrep-3* mutation suppresses the SKN-1c interaction with XREP-1/WDR-23. These results suggest that SKN-1c(k1023) escapes from XREP-1/WDR-23 suppression, accumulates into cell nuclei, and constitutively induces GST expression in the absence of xenobiotics. We continue to uncover other genes executing pivotal roles in xenobiotic response pathways.

[1] Hasegawa & Miwa, PLoS ONE (2010) 5: e11194. [2] Choe et al., Mol. Cell. Biol. (2009) 29: 2704-15.

279A

Homeostatic regulation of the WDR-23/ SKN-1 stress response. **Chi K. Leung**, Keith P. Choe. Department of Biology, University of Florida, PO Box 118525, B30 Bartram Hall, Gainesville, FL 32611-8525.

The Cap'n'collar (CnC) transcription factor SKN-1 orchestrates the transcriptional response to oxidants and electrophilic xenobiotics. Experimental and clinical studies indicate that the activity of SKN-1 and its mammalian homologue (NRF2) influences acute stress resistance, longevity, and susceptibility to age-related diseases. *wdr-23* encodes a highly conserved WD40 repeat-containing protein. We recently demonstrated that WDR-23 directly interacts with SKN-1 to regulate nuclear abundance and activity of the transcription factor. Elevated SKN-1 activity caused by *wdr-23* loss of function can promote stress resistance and longevity but also inhibits larval growth and reproduction. These results imply that *wdr-23* is required to tightly regulate SKN-1, which is detrimental to growth and reproduction.

Interestingly, microarray and real-time RT-PCR data suggest an auto-regulation loop between SKN-1 and *wdr-23*. *wdr-23* mRNA levels are strongly elevated by xenobiotics that activate SKN-1 and by a deletion allele of *wdr-23(tm1817)*; these increases in *wdr-23* mRNA require *skn-1*. The 5' regulatory region of the *wdr-23* gene contains five SKN-1 binding elements within 1 kb of the start codon and chromatin immunoprecipitation-sequencing data collected by the modENCODE (model organism ENCyclopedia Of DNA Elements) project rates *wdr-23* as the most likely gene promoter to be bound by SKN-1. These observations support a negative auto-feedback loop in which activation of SKN-1 by stress enhances *wdr-23* transcription to produce newly translated WDR-23 that represses SKN-1 and limits detrimental effects on growth and reproduction. In this model, SKN-1 activation is transient unless the stress-induced stimulus and requirement for SKN-1 activity persist. A *wdr-23* transcriptional reporter containing the putative SKN-1 binding sites will be utilized to assay temporal and spatial regulation of *wdr-23* expression during and after stress. Systematic site-directed mutagenesis will also be conducted to define the *bona fide* SKN-1 binding sites that control *wdr-23* expression *in vivo*. Data from *Drosophila* and mammalian cell cultures suggest that CnCs autoregulate via Keap1, a repressor analogous to *wdr-23* that is not present in *C. elegans*. Remarkably, these data indicate that CnC auto-regulation evolved at least twice, once via *wdr-23* and once via Keap1. We will use translational *wdr-23* transgenes lacking SKN-1 regulatory sites to test, for the first time, the physiological function of CnC auto-regulation.

280B

Differential roles of thioredoxin reductases in *C. elegans*. **Weixun Li[1]**, Jeong Hoon Cho[3], Jaya Bandyopadhyay[2], Sunkyung Lee[1], Joohong Ahnn[1]. 1) Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, 133-791, Korea; 2) Department of Biotechnology, West Bengal University of Technology, BF142, Salt Lake City, Sector I, Kolkata, India; 3) Deivision of Biology Education, College of Education, Chosun University, Gwangju 501-759, Korea.

Thioredoxin reductases are members of flavoprotein family of pyridine nucleotide-disulfide oxidoreductases. These enzymes use NADPH to reduce thioredoxin, one of the major protein disulfide reductases serving as electron donors for various biological substrates.In this study, we report that two thioredoxin reductases in *C. elegans*, cytosolic *trxr-1* and mitochondrial *trxr-2*. GFP reporter system revealed that *trxr-1* ismainly expressed in intestine whereas *trxr-2* is expressed in muscle system. *trxr-2* mutants weremore sensitive toparaquatthan *trxr-1*mutants in terms of lethality and developmental delay, and was rescued by overexpression of wild type *TRXR-2*. Interestingly, the activity of V-ATPase, a proton pump residing at acidic intracellular organelles, was reduced in *trxr-1*deletion and *trxr-2*;*trxr-1* double mutants, but not in *trxr-2* mutants. We present evidence that V-ATPase activity is independent of thioredoxin system. These results suggest that spatial separation of *trxr-1* and *trxr-2* confer them with differential roles in oxidative stress defense and regulation of V-ATPase activity.

281C

The affect of chronic hypoxia on developmental trajectory, lifespan, and egg-laying behavior. **B. Little**. University of North Texas, Denton, TX.

Given its natural environment, *C. elegans* is likely exposed to oxygen deprivation during development. To date, most of the research has focused on the response specific developmental stages have to acute oxygen deprivation; much less is understood regarding the effects of chronic hypoxia (>1 day). We hypothesize that chronic hypoxia will affect various biological processes including developmental trajectory and behavior. To test this, embryos were raised to adulthood in hypoxic environments (.5 or 1% O2, 22C) and analyzed survival rate, developmental progression, and altered behaviors. Wildtype hermaphrodites survive chronic hypoxia yet developmental trajectory is slowed. Animals raised in chronic hypoxia had different phenotypes compared to normoxic controls. First, hermaphrodites exposed to chronic hypoxia produced a significantly lower number of embryos and had a slight increase in male progeny. This suggests that chronic hypoxia during development affects the germline. Second, animals raised in chronic hypoxia from embryos to young adults have a slight increase in lifespan when re-exposed to a normoxic environment, indicating that chronic hypoxia does not negatively decrease lifespan. Finally, hermaphrodites that were raised in hypoxia lay the majority of their eggs on the area of the agar plate where the bacterial lawn is not present. This is in contrast to animals in normoxia, which lay the majority of their eggs on the bacterial lawn. One hypothesis for hypoxia-induced egg-laying behavior is that the animal can sense microenvironments in hypoxia. To examine if various pathways are involved with chronic-hypoxia responses we used RNAi and assayed genetic mutants. Specifically, we phenotypically analyzed genetic mutations affecting oxygen sensing (*egl-9*), aerotaxis (*npr-1*), TFG-β signaling (*dbl-1, daf-7*) and predicted oxygen-binding proteins (globin-like genes). We found that mutations in several of these genes (*npr-1, dbl-1*) resulted in a decrease in hypoxia survival rate. A mutation in *egl-9* also had a detrimental affect on the viability of an animal raised in chronic hypoxia. However, a similar phenotype was not observed in the *vhl-1* mutation indicating that the phenotype may not be due to a mere increase in HIF-1 levels. A mutation in the globin-like gene *glb-13(tm2825)* suppressed the hypoxia-induced egg-laying phenotype. That is, the *glb-13(tm2825)* animal raised in chronic hypoxia laid eggs on the bacterial lawn at a significantly higher rate compared to wildtype controls. Thus, suggesting that glb-like molecules may be involved with the sensing of microenvironments. Together, this research lays the foundation for understanding the implications of chronic hypoxia in developing organisms.

282A

Lifespan extension with protection against β-Amyloid toxicity with FDA approved drugs discovered in a two species screen. **Alex Lublin**, Harshil Patel, Isoda Fumiko, Nguyen Linda, Hajje Daher, Swartz Marc, Mobbs Charles. Dept Neuroscience, Mt Sinai Sch Medicine, New York, NY.

There are numerous diseases that show a clear correlation with age (i.e. Alzheimer's disease and diabetes). It has been shown that dietary restriction (DR) can reduce the rate of aging, and in so doing, delay the onset of age-related diseases. In an attempt to mimic the effect of DR and/or treat the underlying cause of diseases of aging we have performed a 2 species screen on a library of approximately 4000 FDA approved drugs. We have previously shown that glucose increases hydrogen peroxide toxicity in a dose dependant manner in mouse primary neurocytes. Utilizing a paradigm where neurocytes are treated with 15mM glucose and 100 uM Hydrogen peroxide, we screened an FDA drug library for drugs that protect against glucose and hydrogen peroxide induced toxicity. Measurement with vital dye and activity of lactate dehydrogenase produced 30 drugs that exhibited protection. These 30 candidates were screened in a C .elegans strain that expresses human β-amyloid (1-42) under control of the unc-54 promoter (CL2006). CL2006 produces a progressive paralysis over a period of 13 days, enabling assessment of the drugs vs. vehicle

by scoring the number of worms paralyzed. Protection from β-amyloid induced toxicity is produced with treatment of 7 drugs (acetaminophen, caffeine, baicalein, Ciclopirox Olamine, bacitracin, tannic acid, and hydralazine HCl,). Furthermore, all 7 drugs produce a significant increase in wild-type (N2) lifespan. However, performing RNAi against either DAF-16 or CBP-1, 2 molecules involved in differing methods of lifespan extension, we found that the mechanism of action varied between the 7 positive candidates, with some showing a dependency on DAF-16, CBP-1, or both.

283B
A large scale small molecule screen identifies a group of structurally related compounds that extend the lifespan of *C. elegans*. **Mark Lucanic**, Ivan Yu, Dipa Bhaumk, Jill Graham, Aaron Miller, Robert Hughes, Gordon Lithgow. Buck Institute for Research on Aging, Novato, CA.

To identify chemical compounds that can extend the lifespan of C. elegans we have performed high throughput screens of small molecule chemical libraries. Enormous effort from numerous research labs has demonstrated effectively that many gene alterations can extend lifespan and suggest that there are several central signaling pathways that coordinate the lifespan of the organism, as well as more peripheral pathways that impinge on and modulate these primary pathways. Collectively this suggests that C. elegans has a plethora of putative targets for studies on aging. Indeed, in the past decade several known as well as some new drugs have been identified that can extend C. elegans lifespan through distinct pathways. These studies have further highlighted the usefulness of C. elegans as a model organism for use in drug discovery. In addition to being possible leads on potential drug interventions in humans, characterization of new drugs can also function to inform on the overall biology of aging by describing endogenous systems that are altered by these exogenous agents. It is our hope that through drug discovery we can identify new biologically active chemical structures as well as molecular pathways that modulate organismal aging. Toward this end we have screened through approximately 30,000 structurally diverse chemical compounds and tested for their ability to extend the lifespan of C. elegans. Retesting of our primary hits has identified over 50 compounds that significantly extend lifespan under standard biological conditions. Here we will describe our results in characterizing several related members of our retest positives. We find that at least one structural class act independently of insulin/IGF signaling as they extend the lifespan of *daf-16* mutants. The compounds also significantly extend the lifespan of *isp-1* mutants suggesting a mechanism distinct from that resulting from perturbation of mitochondrial function. Interestingly the compounds result in many phenotypes that are consistent with acting through a dietary restriction (DR) type pathway. For our presentation we will describe our data examining these compounds genetic interactions with components of the DR signaling pathways.

284C
High Saturated Fat Diet Disrupts Mitochondrial Function via Reactive Oxygen Species Pathways in *C. elegans*. **Maya Magana**, Stephanie Bryner, Lucinda Carnell, Carin Thomas. Chemistry, Central Washington University, Ellensburg, WA., 98926.

We have investigated the role of high-fat diets on mitochondrial function in a well-established model for studying metabolism, *C. elegans*. We were interested in examining the link between diet and oxidative stress and its implication for type 2 diabetes. Therefore, we initiated studies comparing effects of high saturated (18:0) and unsaturated ($18:1^{\Delta 9}$) fatty acid diets in wild-type nematodes and a mutant animal with a deletion in the nicotinamide nucleotide transhydrogenase gene, *nnt-1*. NNT is a mitochondrial inner membrane protein that is important in reactive oxygen species (ROS) scavenger pathways. In addition, NNT gene polymorphisms have been associated with glucose intolerance and decreased insulin secretion in mouse models. Both wild-type and *nnt-1* mutant animals fed saturated fat diets induced high levels of mitochondrial superoxide production as measured using the fluorescent dye, MitoSOX. We also have evidence that superoxide is being converted into hydrogen peroxide and activating oxidative stress pathways. Transgenic animals that contain a glutathione transferase transcriptional fusion (*gst-4::gfp*), showed increased fluorescent intensity indicating induction of transcription when fed a saturated high fat diet. GST-4 is involved in Phase II oxidative stress response. In wild-type worms measurements of oxygen consumption and mitochondrial membrane potential ($\Delta\Psi_m$) were decreased and normal, respectively, compared to control worms fed a normal diet. However, the *nnt-1* mutant animals displayed increased oxygen consumption and lowered $\Delta\Psi_m$, indicating the uncoupling of mitochondrial membranes and oxidative phosphorylation. These results suggest that NNT plays a pivotal role in maintaining mitochondrial respiratory function under conditions of high saturated fat diet-induced ROS generation. Animals fed unsaturated fatty acid diets showed no increase in superoxide production, decreased oxygen consumption and a tendency toward increased $\Delta\Psi_m$ in both wild-type and *nnt-1* mutant animals, suggesting that oleic acid may play a protective role in mitochondrial function.

285A
The Nuclear Receptor NHR-8 Regulates Sterol Metabolism, Development, and Lifespan in *C. elegans*. **Daniel Magner**[1], Joshua Wollam[1,2], Dangling Li[2], Adam Antebi[1,2]. 1) Max-Planck Institute for Biology of Ageing, Cologne, Germany; 2) Baylor College of Medicine, Houston, TX, USA.

Nuclear receptors (NR) are hormone-gated transcription factors that couple metabolic and environmental signals to transcriptional outputs to regulate development, reproduction, homeostasis, and longevity. The NHR-8 nuclear receptor (homologous to vertebrate sterol and lipid regulators LXR/FXR/PXR and Drosophila HR96) was identified as a transcription factor that regulates larval development, dauer formation, and lifespan. The closest relative of NHR-8 in C. elegans is DAF-12, a NR that regulates dauer formation and lifespan in response to its ligands, bile acid-like steroids called dafachronic acids (DA), which are produced by modifications of dietary cholesterol. *nhr-8* mutants are Daf-c at elevated temperatures (27°C), and under cholesterol deprivation have gonadal outgrowth defects (Mig), phenotypes indicative of reduced DA levels. The first step of DA biosynthesis is the conversion of cholesterol to 7-dehydrocholesterol by the *daf-36*/Rieske oxygenase. In *nhr-8* mutants both *daf-36* transcript and DAF-36::GFP levels are decreased, suggesting one mechanism by which NHR-8 affects DA levels. Accordingly, using gas-chromatography/tandem mass spectrometry (GC/MS/MS), we find that levels of DA are markedly reduced in *nhr-8* animals, as are many of the sterol precursors leading to its production, including 7-dehydrocholesterol. Additionally, microarray and qPCR analyses indicate that *nhr-8* regulates a broader range of sterol and lipid metabolism genes, including vitellogenins/apolipoproteins, stearoyl-CoA desaturases (*fat-5* and *fat-7*), and several cytochrome P450s, reminiscent of gene classes regulated by vertebrate LXR. Additionally, transcriptional assays in cell culture indicate that NHR-8 and DAF-12 both directly bind the promoter of at least one cytochrome P450, supporting the idea that these NRs could comprise a transcriptional network. Interestingly, NHR-8 and DAF-12 share similar DNA- and ligand-binding domains, suggesting similar ligands and overlapping transcriptional targets. The *daf-16*/FOXO transcription factor suppresses *nhr-8* dauer formation indicating that NHR-8 also acts in the insulin/IGF pathway. Finally, *nhr-8* animals are short-lived, suggesting that transcriptional targets are required for normal lifespan. Together, these findings indicate that NHR-8 plays a role in a transcriptional network that regulates cholesterol and bile acid metabolism impacting development and lifespan.

286B
The transcriptional changes associated with food type-dependent lifespan effects. **Wolfgang Maier**, Roxani Gatsi, Martin Regenass, Joy Alcedo. Friedrich Miescher Inst, Basel, Switzerland.

The lifespan of an organism is not only a function of its genes, but rather of the interactions between its genes and its environment. We demonstrated previously that the lifespan of wild-type worms is modulated by the bacterial food source, *i.e.* it differs on different strains of *E. coli*. In addition, we identified two genes, *osm-3* and *nmur-1*, that when mutated lead to lifespan extension compared to wild type, but only on certain food sources. These findings show the existence of a food-type influence on lifespan in worms and provide examples of gene-food interactions in the determination of longevity. We have now carried out microarray analysis of wild-type and *nmur-1* mutant worms grown on different strains of *E. coli* to look for three classes of differentially expressed genes: those that show changes in expression in *nmur-1* mutants compared to wild type (gene-dependent changes), those that change in wild type in response to different food types (food-dependent changes) and those that change in *nmur-1* mutants compared to wild type but only on certain food types (changes dependent on gene-food interaction). We present details of this analysis and discuss the overlap of these classes of genes with known regulators of lifespan, with genes known to be regulated in response to pathogenic bacteria, as well as with metabolic genes that might mediate adaptation to the different food sources. With this analysis we hope to provide a starting point for future investigations into the interplay between genes and environment in regulating worm lifespan.

287C
A transgenerational effect on lifespan. **Frederick G. Mann**, Stuart Kim. Genetics, Stanford Univ, Stanford, CA.

We have found that the lifespan of progeny is affected by the age of the mother when the embryo is formed, which is an example of an age-dependent transgenerational effect on lifespan in Caenorhabditis elegans. Progeny produced during the second day of fertility survive 10-25% longer than those produced during the fourth day of fertility. Progeny produced during the fourth day of fertility have a 5-fold higher incidence of mortality during the first 9 days of adulthood. ced-3 mutant animals, which are incapable of programmed cell death, produce progeny of identical lifespan across all days of fertility.

288A
INS-18, one of *Caenorhabditis elegans* insulin-like peptides, is required for the crosstalk of TGF-β signaling with insulin/IGF-I signaling. **Y. Matsunaga**[1], K. Gengyo-Ando[2,3], S. Mitani[2,3], T. Iwasaki[1,4], T. Kawano[1,4]. 1) Department of Bioresource Sciences, The United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan; 2) Department of Physiology, Graduate School of Medicine, Tokyo Women's Medical University, Tokyo, Japan; 3) CREST, JST, Saitama, Japan; 4) Department of Bioresource Sciences, Faculty of Agriculture, Tottori University, Tottori, Japan.

Insulin/IGF-I signaling (IIS) and TGF-β signaling regulate larval diapause in *Caenorhabditis elegans*. Moreover, the IIS is also known to regulate adult lifespan. To date, many of the 40 insulin-like genes have been identified and only a few of genes have been investigated to identify their function. In our previously study, we have identified *ins-18*, one of the insulin-like genes, which product functions as an antagonist toward a sole insulin receptor-like protein DAF-2. Disruption of *ins-18* reduces larval diapause under diapause-inducing conditions. In addition, the disruption shortens lifespan-extension under lifespan-extending conditions. The *ins-18* expression is regulated by a transcription factor DAF-16 which locates downstream of DAF-2, allowing us to propose a feedback model of regulation of the expression. Recently, it has been reported that TGF-β signaling regulates adult lifespan through crosstalk with IIS. Therefore, our interest is whether the TGF-β signaling has crosstalk with the IIS *via* regulating expression of insulin-like molecules. To

address this question, we first attempted to investigate transcript revel of *ins-18* by means of qPCR in *daf-3* and *daf-5* mutants showing a daf-d phenotype through the TGF-β signaling. Now we are observing not only expression of INS-18::GFP but also larval diapause and lifespan of an *ins-18*-disrupted animal under reduction of the TGF-β signaling.

289B

Exploring real-time *in vivo* redox biology in *Caenorhabditis elegans* . Patricia Back[1], Winnok De Vos[2], **Filip Matthijssens[1]**, Bart Braeckman[1]. 1) Laboratory for aging physiology and molecular evolution, Department of Biology, Ghent University, Ghent, Belgium; 2) Bio-Imaging and Cytometry Unit, Department of Molecular Biotechnology, Ghent University, Ghent, Belgium.

Reactive oxygen species are no longer considered as merely toxic by-products of the oxidative metabolism. Tightly controlled concentrations of ROS and fluctuations in redox potential might be important mediators of signaling processes. Understanding the role of ROS and the redox status in physiology, stress response, development and aging requires non-disruptive, spatiotemporal, real-time quantification in a living organism. We established *C. elegans* strains bearing genetically encoded fluorescent probes for the detection of hydrogen peroxide (H_2O_2) and the glutathione redox potential. These specific probes, respectively HyPer and Grx1-roGFP2, are sensitive and the fluorescence measurement is ratiometric and therefore not depending on its expression level. The transparency of the *C. elegans* model organism makes it particularly tractable for these approaches. H_2O_2 treatment induces a dose-dependent, reversible response of both biosensors. The ratio of oxidized to reduced glutathione decreases logarithmically during post-embryonic development. H_2O_2 levels increase with age and this effect is delayed by dietary restriction. In young adults, we detected specific regions with deviating redox biology, likely related to their site-specific function. Our findings demonstrate the unique advantages of genetically encoded biosensors in real-time in vivo studies in multi-cellular organisms.

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Identification of longevity targets in *C. elegans* translation mutants. **Philip McQuary**, Joyce Chu, Malene Hansen. Development & Aging, Sanford-Burnham Medical Research Institute, La Jolla, CA.

How come many organisms, such as the nematode *C. elegans*, display extended lifespan and increased stress resistance in response to reduced levels of key components of the mRNA translational machinery? Interestingly, in response to environmental stress, all eukaryotic cells shut down their biosynthetic activity and mount an integrated stress response. During recovery, certain proteins are produced against the backdrop of general translational expression. We are examining the hypothesis that inhibition of key translational regulatory factors such as S6 kinase (S6K) and/or eukaryotic translation initiation factors (eIFs) similarly induce a differential translational response. We focused on *rsks-1*/S6K, and *ife-2*/eIF4E, one of five isoforms in worms that is an mRNA 5'-cap binding protein, as these two factors may be regulating different pathways relevant to stress resistance and longevity in *C. elegans*. The main question we addressed here is: What are S6K and eIF4E downstream translational targets that need to be synthesized in order to allow for prolonged lifespan and improved stress resistance? To address this question, we have employed systematic approaches such as proteomic profiling to globally identify which proteins are differentially regulated in *rsks-1*/S6K and *ife-2*/eIF4E mutants compared to wild-type worms. Since both *rsks-1*/S6K and *ife-2*/eIF4E are critical for translation initiation, their absence should indeed lead to changes in protein expression. We have confirmed this prediction via a 2DLC-MS/MS analysis of the *C. elegans* proteome. This analysis successfully identified ~60% of the predicted proteins, and showed that very few proteins were differentially regulated between the two mutants and wild-type animals. Importantly, we have confirmed that at least one of these up-regulated targets is important for the lifespan- and stress-resistance phenotypes observed in *rsks-1*/S6K mutants, validating our approach and supporting our hypothesis. To further investigate which targets are differentially regulated in translational mutants, we are currently performing comparative profiling of polysomal mRNA in combination with deep sequencing analysis to globally assess which mRNAs are being translated in *rsks-1*/S6K and *ife-2*/eIF4Emutants compared to wild-type animals. This analysis compliments our proteomic analysis and aims to identify novel differentially regulated targets with potential roles in lifespan and stress resistance. Taken together, our preliminary results highlight systematic approaches that have identified several possible translational targets with potential roles in the mechanism by which the mRNA translational machinery affect aging.

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The role of autophagy in lifespan extension by resveratrol and spermidine. **Evgenia Megalou**, Nektarios Tavernarakis. IMBB-FORTH, Heraklion, Greece.

Autophagy protects organelles, cells, and organisms against several stress conditions. The acetylase inhibitor, spermidine (a polyamine), and the deacetylase activator, resveratrol (a polyphenol), both induce autophagy in *C. elegans*. Induction of autophagy by resveratrol requires the nicotinamide adenine dinucleotide-dependent deacetylase sirtuin 1 (SIR-2.1). We find that the acetylase inhibitor spermidine stimulates autophagy independent of SIR-2.1 in *C. elegans*. Endogenous levels of polyamines, including spermidine, decline with aging. Low doses of exogenously supplied spermidine induce autophagy and increase lifespan, independent of SIR-2.1. Interestingly, resveratrol failed to extend the lifespan of animals that lack spermidine synthase (SPDS-1) the enzyme that synthesizes spermidine. Therefore, endogenous spermidine is required for lifespan extension by resveratrol. We are investigating whether induction of autophagy by resveratrol also requires endogenous spermidine. Given that both spermidine and resveratrol affect cellular protein acetylation, it is likely that their effects on autophagy and ageing are mediated by concerted alterations in the acetylproteome.

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Adaptation to hydrogen sulfide modulates protein homeostasis. Emily Fawcett[1,2], Kate Stoll[1], **Dana L. Miller[1]**. 1) Department of Biochemistry, University of Washington, Seattle, WA; 2) Molecular and Cellular Biology Program.

Hydrogen sulfide (H_2S), which is naturally produced in animal cells, has dramatic effects on physiology that can improve survival in changing environmental conditions. We are using *C. elegans* as a model to define molecular factors that mediate the physiological response to H_2S in animals. We have defined transcriptional changes in mRNA abundance upon exposure to H_2S using a microarray approach. These data reveal a rapid and progressive transcriptional response to H_2S. We show that the *hif-1* and *skn-1* transcription factors coordinate the transcriptional response to H_2S and are each essential to survive exposure to H_2S. Functional analysis of gene products that accumulate in H_2S suggests that adaptation to H_2S alters protein turnover pathways. We are currently exploring the possibility that these factors mediate the effects of adaptation to H_2S on protein homeostasis. Together, our data suggest that H_2S has multiple effects on protein metabolism from translation to turnover. These changes in protein homeostasis may underlie the beneficial effects of H_2S that result in increased lifespan and thermotolerance. Moreover, this work will provide unique insight into the mechanisms by which adaptation to H_2S is integrated with the response to hypoxia at the cellular and organismal level.

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The analysis of mismatch repair in *C. elegans*. **Takahito Moriwaki**, Yuichi Kato, Satoru Ishikawa, Kazunari Hashiguti, Qiu-Mei Zhang-Akiyama. KyotoUniv, Kyoto, Japan.

DNA mismatch repair (MMR) is an important genome caretaker system, and is highly conserved from bacteria to humans. MMR corrects base-base mismatches and small insertion / deletion mispairs generated during DNA replication, recombination and repair. In addition to correcting mismatches, MMR has various roles to maintain genomic stability. Recently, it was found that MMR works in DNA damage response (DDR). Because of generating mismatch in DNA replication, however, it is difficult to analysis the DDR function of MMR in whole-body level by using MMR defect mutants. Our purpose is to evaluate the contribution of MMR in whole-body level. *C. elegans* stop DNA replication after hatching. Hence we selected *C. elegans* to analyze this function. In *C. elegans*, the following four MMR enzymes have been identified; MSH2, MSH6, MLH1 and PMS2. From previous reports in other species, it is predicted that these enzymes form heterodimer, MutSα(MSH2-MSH6) and MutLα(MLH1-PMS2). We obtained mutants of each genes. In drug resistant tests, all mutants are resistant to MNNG. This result fits past report. But to MMS, some mutants are sensitive. This difference implies unknown dimers and pathways. We hypothesize MMR use some patterns of repair to response various damage.

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C. elegans HSF-1 is a dynamic nuclear protein whose activity is affected by sumoylation in response to proteotoxic stress. **Elizabeth Morton**, Todd Lamitina. Department of Physiology, University of Pennsylvania, Philadelphia, PA.

The heat shock transcription factor HSF-1 is an evolutionarily conserved protein that plays critical roles in proteotoxic stress responses, aging, innate immunity, and cancer. Although much of our current understanding of HSF-1 biology has been developed in *C. elegans*, little is known regarding the expression pattern, localization, and regulation of HSF-1 in this system. To address these questions, we generated single-copy transgenic animals expressing HSF-1::GFP under the control of the *hsf-1* promoter. HSF-1 is broadly expressed and localizes to the nucleus under non-stressful conditions. Following an acute heat shock or exposure to sodium azide, HSF-1 rapidly (less than 1 minute) collects into nuclear granules, a phenomenon previously thought to be restricted to primate cells. Like human HSF-1, *C. elegans* HSF-1 granules are dynamic (as assessed by FRAP), reversible (over ~20 minutes), and rapidly reformed upon subsequent stress exposures. To further characterize the differences between human and *C. elegans* HSF-1, we expressed human HSF-1 (hHSF-1) in *C. elegans* under the control of the *Ce-hsf-1* promoter. hHSF-1 localized to the nucleus but did not form nuclear granules following rapid heat shock (35°C or 42°C) and did not rescue multiple *hsf-1* loss of function phenotypes, suggesting that *C. elegans* and human HSF-1 are not functionally interchangeable. To better understand the factors regulating *C. elegans hsf-1*, we conducted a high-throughput RNAi screen to identify regulators of inducible HSF-1-dependent gene expression (*hsp-16.2p::GFP*). Our screen covered 17,540 genes and identified 44 regulators of the HSF-1-dependent reporter, including *smo-1*, the sole *C. elegans* SUMO homolog. SUMO is a small ubiquitin-like molecule used to post-translationally modify proteins. Biochemical data from both mammalian cell culture and *C. elegans* has demonstrated that HSF-1 is directly sumoylated. However, the functional role of HSF-1 sumoylation is controversial. In our screen, *smo-1(RNAi)* led to hyper-activation of HSF-1-dependent gene expression but only following activation of the reporter by heat shock, suggesting that SUMO functions as an activation-dependent attenuator of HSF-1 activity. Sequence analysis of HSF-1 reveals multiple potential sumoylation sites, and we are currently carrying out site-directed mutagenesis to assess the role of these sites in HSF-1 regulation. Our results suggest a model in which SUMO may directly modify HSF-1 in response to specific activating conditions and that such modifications provide a feedback inhibition circuit to regulate HSF-1-regulated gene expression *in vivo*.

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Unexpected antagonistic pleiotropy in the sod-4 extracellular supreoxide dismutase gene in C. elegans. **S. Murakami**, H. Murakami, A. Yeh, B.V. Nia, D. Lim. Dept of Basic Sci, Touro University-California, Vallejo, CA.

An important principle of the evolutionary theories of aging is that the force of natural selection diminishes late in life. Based on the principle, two popular theories of aging predict the genes that contribute to aging. The mutation accumulation theory predicts accumulation of the genes (or mutations) with late harmful effects. The antagonistic pleiotropy theory predicts a similar but more focused set of the genes that have early beneficial effects at the cost of late harmful effects (referred to as pleiotropic genes). Although the theories may result in different age-related genetic equilibriums, pleiotropic genes are more or less covered in both of the theories. Pleiotropic genes have been found as a form of mutations or gene inactivation with increased lifespan, which show early deficits in development and reproduction. However, mutations that cause damage early in life are problematic to test, as early damage may affect lifespan. In addition, the genes, which are protective against intrinsic and environmental stress, were not thought to be pleiotropic genes, since they are beneficial throughout lifespan. Here we show that the sod-4 gene, a gene protective against oxidative stress, is a new type of pleiotropic genes. Using genetic screening combined with in vivo imaging analysis for reactive oxygen species (ROS), we have identified the sod-4 mutation. The sod-4 gene is the only C. elegans gene for extracellular superoxide dismutase (EC-SOD), which can scavenge deleterious reactive oxygen species (ROS). Mutations in sod-4 caused embryonic lethality, delayed development and reduced fertility. Surprisingly, the sod-4 mutant showed increased fitness during adult phase. The results suggest an unusual example of pleiotropy, which is antagonistic early and late in life. Since recent results questioned the role of age-related oxidative stress in aging, this study offers a first step for more detailed understanding of oxidative stress and aging. Another theory of aging ("midlife crisis" theory) is consistent with the finding, which is being discussed elsewhere.

296C

Clarifying misconceptions about age-related memory impairment (AMI). **S. Murakami**. Dept of Basic Sci, Touro University-California, Vallejo, CA.

Age-related memory impairment (AMI) includes mild impairment of the ability to learn new information and to recall previously learned information. The term, age-associated cognitive impairment (AACI), is also used to describe memory impairment during aging. We define AMI (or AAMI) as memory impairment in comparison with young normal controls (young v.s. old comparison); and AACI as in comparison with age-matched normal counterparts (non-AMI v.s. AMI comparison at the same age) (1). We prefer using AMI over AAMI, since AAMI is often confused with the transition state, MCI. Here we clarify some misconceptions we encountered through scientific communication. Firstly, AMI is not a simple decrease in learning and memory. Recent studies suggest that aging causes not only declines but also increases of learning and memory. AMI is not a gradual decay but rather an aspect of age-related alterations in the nervous functions. Importantly, neural loss plays a minor role in AMI. Secondly, AMI is not a disease. AMI is a normal state prior to the disease state. What distinguishes AMI from dementia besides cognitive deficits? A few lines of evidence analysis in humans suggest that reduced metabolic activity may be a hallmark of AMI in humans. Finally, AMI cannot be independent of aging. There is an argument that aging should be separated from AMI. However, aging affects learning and memory, which results in AMI. More precisely, AMI is caused by age-related processes that affect learning and memory. The age-related processes should include age-related changes in neurons as well as elsewhere, including modifiers and pathways for learning and memory. In fact, a type of AMI can be suppressed by serotonin inhibitors, which are good examples for such modifiers. It is unlikely that aging neurons are the sole cause of AMI. Thus, focusing only on neurons would miss important aspects of the mechanisms for AMI. Although the study of aging neurons has been emphasized, it is equally essential to investigate the aging processes. More details are discussed in Ref. 1. The abstract may be presented with the other presentation. Reference: 1. Murakami, S., Cabana, K., Anderson, D. Current advances in the study of oxidative stress and age-related memory impairment in C. elegans. John Wiley & Sons, Hoboken, NJ. In Press.

297A

Characterization of cellular functions of UFD-2 and UFD-3, C-terminal adaptors for CDC-48, in *C. elegans*. **Yuki Murayama**[1], Yohei Sasagawa[1,2], Teru Ogura[1], Kunitoshi Yamanaka[1]. 1) Department of Molecular Cell Biology, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto, Japan; 2) Functional Genomics Unit, RIKEN Center for Developmental Biology, Kobe, Japan.

CDC-48 is a member of the AAA (ATPases associated with diverse cellular activities) protein family, and is proposed to serve as a molecular chaperone in the ubiquitin signaling pathway. CDC-48 participates in a wide range of cellular activities, including organelle membrane fusion, protein degradation, transcriptional activation, cell-cycle control, apoptosis, and DNA repair. The functional diversity of CDC-48 is determined by differential interactions with a variety of cofactors and adaptors. Several adaptors have been identified in *C. elegans*, e.g. NPL-4-UFD-1 and UBXN proteins, which bind to the N-terminal domain of CDC-48, and UFD-2 and UFD-3, which bind to the C-terminal portion of CDC-48. However, their cellular functions have not yet been elucidated, especially in multicellular organisms. To clarify cellular functions of the C-terminal adaptors UFD-2 and UFD-3, we analyzed deletion mutants of *ufd-2* and *ufd-3* of *C. elegans* in terms of sex determination, ER stress response and meiotic chromosome segregation. Brood size of the *ufd-3* mutant dramatically decreased, while that of the *ufd-2* mutant was similar to that of wild type. The *ufd-2* mutation significantly suppressed the reduced brood size of *ufd-3*. On the other hand, the *ufd-2* mutant was more sensitive to an ER-stress inducible reagent, tunicamycin, while the *ufd-3* mutant and the *ufd-2*; *ufd-3* double mutant showed hyper-resistance to it. These results suggest that UFD-2 and UFD-3 functions in an antagonistic manner. We will discuss the functional diversity of CDC-48 through differential binding of UFD-2 and UFD-3 adaptors.

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Studies on Toxicity of Nano Iron Employing Model System *Caenorhabditis elegans*. Supinder Kaur, Shreesh Raj Sammi, Pooja Jadiya, **Aamir Nazir**. Division of Toxicology, Central Drug Research Institute, CSIR, Lucknow, India.

The rapidly evolving field of scientific and medical research is making numerous advances in terms of newer technologies aimed at bettering human health and quality of life. Amongst such advances, use of nanomaterials in various forms and facets has gained a lot of momentum in recent years. Nano particles find use in diverse processes such as drug delivery, bio- sensing, bio-imaging, blood vessel repair, magnetic cell therapy, hyperthermic cancer treatment and water purification. Having a widespread utility, nanomaterials reach humans via various means, thus making it relevant to assess their safety using efficient, sensitive and reliable methods. Amongst various models employed for studying toxicity and safety of chemicals, *C. elegans* is a fairly new addition and is proving to be a model of immense utility considering its powerful genetics, well dissected out developmental biology, homology of gene sequences with that of humans and ease of laboratory use. Of late, *C. elegan*s is also well suited to large scale toxicity screening campaigns. We conducted the present study with a three-fold aim: 1) to use model system *C. elegans* for toxicity studies on nanoparticles; 2) to assess the safety of nano particle iron that finds widespread use in processes like water purification, disease treatment; and 3) to study the effect of particle size on the toxicity of iron (nano vs bulk) in *C. elegans* model. We first studied the absorption of nanoparticles inside the worm by feeding the worms with fluorescent nanoparticles. In subsequent studies nematodes were exposed to three different concentrations (20μM, 200μM and 2mM) of nanoparticle and bulk Iron (in the form of Fe_2O_3). Worms were subsequently studied for effects on locomotion, longevity, fertility, oxidative stress, apoptosis, mitochondrial content, ATP levels and for effects on expression of *sod-1, sod-2, sod-3, mtl-1, mtl-2 and hsp-60*. Our data reveals that nano iron induces significant effect on longevity of the nematodes as the life span was reduced by 17% in case of worms treated with 2mM nanoparticle Fe_2O_3; worms treated with equal concetntration of bulk Fe_2O_3 did not exhibit a reduced longevity as compared to control subjects. Similarly, nano iron exhibited a significant effect on generation of reactive oxygen species thus increasing the amount of oxidative stress within the worms. We conclude that: 1) *C. elegans* could successfully be employed in studying toxicity of nanoparticles; 2) nano iron did exhibit whole-organismal toxic effects at 2mM concentration in nematode *C. elegans*; and 3) nanoparticulate form of iron, at tested concentrations, induces comparatively more whole-organismal toxicity than that of the bulk form.

299C

Dietary Epigenetics and Neurodegenerative Diseases: Effect of Calorie Restriction on Dopaminergic Neurodegeneration in Caenorhabditis elegans. Pooja Jadiya, Shreesh Raj Sammi, Supinder Kaur, **Aamir Nazir**. Division of Toxicology, Central Drug Research Institute, CSIR, Lucknow, India.

The fact, that causal factors of most common diseases often involve both-susceptibility genes and their interactions with exogenous/endogenous environment, is well established. The conceptual approaches to role of such epigenetics in human disease conditions have been well conceived. Amongst various diseases that are modulated by such factors, age associated neurodegenerative diseases are a class of syndromes that have intrigued researchers for decades. C. elegans models of age associated neurodegenerative diseases have provided several meaningful insights into the understanding of these disease conditions. We employ transgenic nematode models towards understanding various mechanistic aspects of such diseases. In the present study we employed the transgenic C. elegans model (Pdat-1::GFP) expressing green fluorescence protein (GFP) specifically in eight dopaminergic (DA) neurons. Selective degeneration of dopaminergic neurons was induced by treatment of worms with 6-hydroxy dopamine (6-OHDA) as previously described. In subsequent studies, we explored the role of calorie restriction in modulating the 6-OHDA induced neuronal damage. Worms were treated with 25mM 6-OHDA to induce degeneration of DA neurons and were raised either on control diet (normal concentration of bacteria OP50) or on 10,000 fold diluted OP50. It was observed that worms raised on the reduced calorie diet exhibited a protective effect on dopaminergic neurons with ADE and PDE neurons exhibiting an enhanced and complete expression of GFP; CEP neurons exhibited a marginal increase in GFP expression. When quantified using Image J analysis, the mean fluorescence (GFP) intensity corresponding to a healthy dopaminergic neurophysiology, was 6.32 ± 0.56 arbitrary units in control worms, 1.92 ± 0.15 arbitrary units in 6-OHDA treated subjects (a 3.3 fold reduction vs. control) and 5.28 ± 0.61 arbitrary units in case of worms fed with a 10,000 fold diluted OP50 (a 2.75 fold increase as compared to 6-OHDA treated subjects). We further studied the effect of 6-OHDA induced dopaminergic neurodegeneration and calorie restriction on mitochondrial content, ATP levels and oxidative stress in the worms. Our studies provide evidence that calorie restriction affords significant protection against the dopaminergic neurodegeneration, that might have implications for neurodegenerative Parkinson's disease.

300A

AMPK activation is protective in models of neuron dysfunction and death in Huntington's

disease. Rafael Vazquez-manrique[1], Karine Cambon[2,3], Nicolas Offner[1], Aurélie Darbois[1], Anne-marie Orfila[1], Nicole Déglon[2,3], **Christian Neri[1]**. 1) Laboratory of Neuronal Cell Biology and Pathology, INSERM U894, 75014 Paris, France; 2) Commissariat à l'Energie Atomique (CEA), Institut d'Imagerie Biomédicale (I2BM), Molecular Imaging Research Center (MIRCen), Orsay, France; 3) Centre National de la Recherche Scientifique (CNRS), Unité de Recherche Associée CEA-CNRS 2210, Orsay, France.

Huntington's disease (HD) is a neurodegenerative disease caused by polyglutamine (polyQ) expansion in the huntingtin protein (Htt). Expression of the first exon of Htt containing an expanded polyQ produces neuronal dysfunction and axonal dystrophy in mechanosensory neurons in C. elegans (Parker et al., 2001). Genetic pathway analysis using this nematode model has emphasized a role for the longevity-promoting factor daf-16/FoxO in the protection of neurons from expanded polyQ toxicity (Parker et al., 2005). Here, we investigated the role of AMP-activated protein kinase (AMPK), a well-known energy sensor involved in lifespan and health span extension, on neuronal dysfunction in expanded-polyQ nematodes and vulnerability to cell death in mutant Htt striatal cells (HdhQ111 knock-in mice). In C. elegans, activating this enzyme (aak-2/AMPK) with metformin reduces the neuronal dysfunction caused by expanded polyQ expression. In contrast, aak-2 mutants show enhanced neuronal impairment. In striatal cells from HdhQ111 knock-in mice, reducing AMPK levels by siRNA treatment enhances the susceptibility to cell death of mutant htt cells, and metformin treatment has the opposite effect. Additionally, overexpressing AMPK reduced striatal neurodegeneration in a rat lentiviral-based fragment model of HD pathogenesis. Collectively, our results suggest that AMPK activation has therapeutic potential to protect from neuron dysfunction and degeneration in HD.

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Neuroprotection by Sirtuin sir-2.1 from Expanded Polyglutamines Requires β-catenin in C. elegans and the Age-at-Onset of Huntington's Disease is Modified by the β-catenin Repressor GSK-3β. J Alex Parker[1], Cendrine Tourette[1], Nicolas Offner[1], Rafael Vasquez-Manrique[1], Arnad Mukhopadhyay[2], Aurelie Darbois[1], Sophie Menet[1], Anne-Marie Orfila[1], Guillaume Dolbeau[3], Susanne Swenke[4], Patrick Maison[5], Michael Orth[4], Alexis Brice[6], Alexandra Durr[6], Anne-Catherine Bachoud-Levi[7], Heidi Tissenbaum[2], **Christian Neri[1,8]**, European Huntington's Disease Network. 1) INSERM, Unit 894, Laboratory of Neuronal Cell Biology and Pathology, 75014 Paris, France; 2) Program in Gene Function and Expression, Program in Molecular Medicine, University of Massachusetts Medical School, Worcester MA 01605, USA; 3) INSERM, Unit 955, Neuropsychologie Interventionnelle, Créteil, France;; 4) University of Ulm, Department of Neurology, 89081 Ulm, Germany; 5) AP-HP, National Reference Center for Huntington's Disease, Henri Mondor Hospital, 94000 Créteil and Pitié-Salpêtrière Hospital, 75013 Paris, France; 6) INSERM, Unit 975 (ex 679), Pitié-Salpêtrière Hospital, 75013 Paris, France; 7) AP-HP, GH Pitié-Salpêtrière, Department of Genetics, 75013 Paris; 8) AP-HP, Department of Neurology, Henri Mondor Hospital, 94000 Créteil, France.

One of the current challenges of neurodegenerative disease research is to determine whether pathways relevant in either development or as a function of age contribute to neuron survival and thereby contribute to the pathogenic process in human disease. Using a C. elegans model that recapitulates an early phase of expanded polyglutamine (polyQ) neurotoxicity, we previously reported that increased dosage of sirtuin sir-2.1/SIRT1 is neuroprotective, and requires the longevity-promoting factor daf-16/FoxO. Here, we report this neuroprotective effect also requires bar-1/β-catenin, a downstream effector of canonical Wnt that also acts as a DAF-16/FOXO partner, and ucp-4, a DAF-16 regulated gene that encodes the sole mitochondrial uncoupling protein (UCP) in nematodes. These results fit with a previously-proposed mechanism in which the SIR-2.1 and β-catenin pathways converge onto DAF-16 to regulate cell survival. Consistent with these effects in nematodes, survival of mutant-huntingtin striatal cells derived from the HdhQ111 knock-in mice was enhanced with SIRT1 overexpression. In addition, siRNAs of β-catenin enhanced cell death, and these effects were accompanied by the modulation of neuronal UCP (UCP2, UCP4) expression levels. Finally, we further extend these findings where we identify single nucleotide polymorphism in GSK-3β, a major β-catenin repressor, modifies the age-at-onset of motor symptoms in two well-characterized HD cohorts. Our results suggest that FOXO interactors regulate early stages of expanded polyQ toxicity and lead to GSK-3β modulation of the pathogenic process in HD patients.

302C

Circadian rhythms in *C. elegans*. **Maria Olmedo**, Martha Merrow. Molecular Chronobiology, University of Groningen, The Netherlands.

The circadian clock is a temporal program that coordinates gene expression, physiology and behaviour according to time-of-day. We think that *C. elegans* might represent an outstanding model organism to investigate alternative models of the molecular mechanism of the clock since canonical clock genes are absent. Furthermore, the ontogeny of the clock may be ideally studied using the defined developmental program. Circadian rhythms have been described in *C. elegans* but the described behaviours are not amenable to expanded molecular and gentic analyses. Starting with the philosophy that the circadian clock regulates many aspects of physiology, we have developed an 'on-plate' population assay to monitor the response to a repellent (1-octanol). Using this assay we have observed that the response of the nematodes to 1-octanol oscillates over the day in a temperature cycle as well as in constant conditions, suggesting regulation by a circadian clock. A second protocol showing circadian behaviour was developed using a constitutively expressed luciferase reporter, suggesting broad circadian regulation of metabolism in *C. elegans*. We present two robust new protocols for investigation of circadian biology in a model genetic organism. Our work furthermore addresses the conundrum of circadian rhythm in subteranean organisms, which experience irregular light but highly predictable temperature cycles as carriers of information about earthly time.

303A

Why Make Fat?: The Impact of de novo Fatty Acid Synthesis on Reproduction and Longevity. **Carissa Perez Olsen[1,2]**, Marc R Van Gilst[1]. 1) Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology Program, University of Washington, Seattle, WA.

An animal must be able to process its diet and allocate the resources appropriately to a number of distinct physiological fates. For some animals, the major sources of energy usage are somatic maintenance and reproduction. Therefore, progeny production and lifespan likely depend on the efficiency of resource partitioning and utilization as well as the ability to adjust to variable diets and environmental stresses. Because, in C. elegans adults, reproduction requires a large amount of resources, a metabolic shift may be an important component for successful progeny production and continued somatic maintenance. We have used a "mixed isotope" labeling strategy in C. elegans to define the changes in fat metabolism in adult animals. This isotope analysis has revealed that, at the onset of adulthood, the relative amount of de novo fatty acid synthesis almost doubles indicating a significant change in how the diet is processed. Further analysis has demonstrated that this increase in synthesis correlates with the reproductive period but is not involved in germ cell production or in germline migration. In order to test if the increased lipogenesis is necessary to provide the developing oocytes with fatty acids, we purified embryos and quantified the amount of synthesized fatty acids in their triglycerides (TAGs) and phospholipids (PLs). We found an enrichment of synthesized fatty acids specifically in the TAGs of the embryo indicating that de novo lipogenesis at this time may be important for TAG synthesis in the germline. Recently, there have been several studies that have implicated changes in fat metabolism in the enhanced longevity of germline minus mutants. In order to test whether fatty acid synthesis plays a role in adult longevity as well, we performed a lifespan analysis on animals treated with fatty acid synthase RNAi. In the fasn-1 RNAi treated animals, we found a reduction in the adult lifespan indicating an important function for lipogenesis in the adult. Taken together, we have established the model that the lipogenic transition in reproductive adults is important for: (1) the generation of mmBCFAs for embryos, (2) provision of fatty acids for TAG synthesis in the germline, and (3) somatic maintenance under the increased demands of reproduction.

304B

Insulin Signaling and Dietary Restriction Differentially Regulate Glucose Metabolism to Impact C. elegans Healthspan. **Brian D. Onken**, Monica Driscoll. Dept Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ.

A major goal of aging research is to understand the underlying relationship between nutritional intake, metabolism, and healthy aging. Low-glycemic index diets have been shown to reduce risk of age-related metabolic diseases such as diabetes and cardiovascular disease, and reduced caloric intake via dietary restriction increases healthspan across species. One potential approach for supporting healthy aging is via interventions that engage healthspan-promoting metabolism. In Caenorhabditis elegans, adding excess glucose to the growth medium shortens lifespan [1, 2], while inhibiting the glycolytic enzyme hexokinase with the glucose analog 2-deoxyglucose increases lifespan [1]. We have shown that disrupting genes encoding two other glycolytic enzymes that catalyze unidirectional, irreversible reactions lengthens C. elegans median lifespan, induces large gains in youthful locomotory ability, and triggers a fluorescent biomarker that distinguishes a healthy metabolic state. Conversely, disrupting counterpart gluconeogenic genes decreases nematode healthspan. In investigating potential longevity-related pathways that might impinge upon glucose metabolism, we found that disrupting glycolytic genes increases healthspan through the FOXO transcription factor DAF-16, which is also required for the increased lifespan seen with lowered levels of insulin signaling, and which is downregulated by increased glucose availability [2]. Strikingly, we also found that gluconeogenic activity is absolutely and specifically required for increased healthspan under dietary restriction. These results provide evidence for an intriguing new paradigm: breakdown of glucose via glycolysis negatively impacts healthy aging through insulin signaling and DAF-16, while dietary restriction engages the reciprocal gluconeogenic pathway to promote healthspan. Our observations support that healthspan might be optimized via dietary, pharmacological, or genetic interventions that increase gluconeogenic activity or decrease glycolysis. 1. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, et al. (2007). Cell Metab 6: 280-293. 2. Lee SJ, Murhpy CT, Kenyon C (2009). Cell Metab 10: 379-391.

305C

The FLH family of transcription factors is epistatic with the insulin/IGF-1 signaling pathway and modulates L1 diapause and stress resistance in *C. elegans*. **Maria Ow**, Victor Ambros. Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA.

Under unfavorable environmental conditions, many organisms are able to suspend development until more favorable conditions are restored. *C. elegans* embryos hatched under conditions lacking any food enter an arrested stage where development ceases during the first larval stage (L1 diapause) and resumes growth only when nutrition becomes available. The insulin/IGF-1 signaling pathway is a key regulator of L1 diapause, aging and stress resistance. Mutations in members of this signaling pathway, such as the insulin-like tyrosine kinase receptor, *daf-2*, or its main effector, the FOXO transcription factor *daf-16*, exhibit aberrant lifespan and responses to environmental and physiological stresses. A severe temperature sensitive *daf-2* mutant, *daf-2(e979)*, arrests at the first larval stage at the

elevated temperature even in the presence of food, suggesting that the developmental pathways for feeding or the sensing of nutritional cues are defective in this mutant.

We have previously shown that a family of Zn-finger transcription factors, FLH-1, FLH-2, and FLH-3, repress the expression of several microRNAs including *lin-4*, *mir-48* and *mir-241* during embryogenesis. Mutations in members of this family result in pleiotropic phenotypes including L1 larval lethality, egg-laying defects, premature expression of post-embryonic microRNAs during embryogenesis, and L1 diapause in the presence of food. Here we report that the FLH transcription factors, along with the insulin/IGF-1 signaling pathway, modulate L1 diapause and stress resistance and are therefore involved in feeding and/or nutrient sensing and in the maintenance of the animal during physiological and environmental stresses.

306A

Characterization of exceptional longevity pathways in *C. elegans*. **Jennifer Paek**[1], Stephanie Chan[2], Xiao Xiao[3], Jacqueline Lo[2], Sean P. Curran[1,2,3]. 1) University of Southern California, Los Angeles, CA. Davis School of Gerontology; 2) College of Letters, Arts, and Sciences Department of Molecular and Computational Biology; 3) Keck School of Medicine Department of Biochemistry and Molecular Biology.

Aging is a universal and inevitable process driven by diverse molecular pathways. Recent work suggests that the genes that most potently influence the rate of aging in *C. elegans* are those that are essential for growth and development. More than 90% of these genes have conserved developmental roles from yeast to man and together may represent an evolutionarily conserved program to modulate lifespan. Many of these genes function independently of the canonical longevity-modulating pathways including insulin/IGF-I signaling, dietary restriction, mitochondrial respiration and reproduction. Inactivation of one group of longevity modulators results in somatic cells with germ cell-like characteristics, increased tolerance of genotoxic stress and increased lifespan. We have developed a new model to explain the exceptional longevity phenotype of some *C. elegans* essential gene mutants. We identified the misexpression of germ cell specific factors in the somatic cells of the insulin-like signaling mutants, in animals RNAi depleted for the cytosolic chaperonin complex, and in animals with diminished protein synthesis. An important question is how this pathway is triggered in *C. elegans* and why some cells are more responsive than others. As a first step, we have examined the key transcriptional regulators of many longevity-promoting pathways and found that the misexpression phenotype requires at least two transcription factors; the worm ortholog of FoxO/DAF-16 and Nrf2/SKN-1. Both of these transcription factors receive inputs from multiple signaling pathways including insulin signaling, JNK and the p38 MAPK pathways, but the specific signal that initiates the misexpression of germline specific genes in somatic tissues remains a mystery. Importantly, both of these transcription factors potentiate the longevity phenotype identified from post-developmental inactivation of many essential genes.

307B

Aluminium Toxicity and Elemental Composition Changes in *Caenorhabditis elegans*. **Kathryn E. Page**[1,2], David W. Killilea[3], Keith N. White[2], Catherine R. McCrohan[2], Gordon J. Lithgow[1]. 1) Lithgow Lab, Buck Institute for Research on Aging, Novato, CA, United States; 2) Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom; 3) Children's Hospital Oakland Research Institute, Oakland, CA, United States.

Environmental metal contamination events such as the alumina sludge spill in Hungary last year highlight the importance of understanding metal toxicity and predicting detrimental effects. Aluminium (Al) is highly abundant in our environment, but can elicit a variety of toxic responses. Here we present the first characterization of the effects of Al exposure in the nematode worm *Caenorhabditis elegans* by identifying phenotypic abnormalities and disruption in whole body metal homeostasis (metallostasis) following Al exposure. Lifespan was decreased in worms exposed to low levels of Al, but was unexpectedly increased when the Al concentration reached higher levels. Interestingly, this bi-phasic phenotype was only observed when Al exposure occurred during development, as adult worm lifespan was unaffected by Al at similar exposures. Al negatively affected *C. elegans* developmental progression and self-fertility when exposed to as little as 30μM Al, and reduced body size when exposed to 1.9 mM Al. Significant developmental delay was observed even when Al exposure was restricted to embryogenesis. Widespread changes to the elemental content of adult nematodes was observed when chronically exposed to Al from L1. Specifically we saw increased Ba, Cr, Cu, and Fe content, and a reduction in Ca levels. Similar changes have been noted in human Al toxicity, suggesting that *C. elegans* may be exploited to understand the mechanisms of Al toxicity in human tissue. Elemental homeostasis is obviously highly regulated, and here we see that changing the levels of only one element can alter the whole elemental profile. This raises the question of how genes affect these elemental profiles. Here we determine how gene knockdown can alter metallostasis and elemental profiles in *C. elegans*. It is notable that these genetic modulators of elemental profiles are not only important for metallostasis, but are key regulators of known stress response mechanisms. Knock-down of these gene products alters the elemental profile of the worm, which suggests novel mechanisms of action for these genes in metal toxicity and general stress response.

308C

Genetic Analysis of Age-Dependent Defects of *C. elegans* Touch Receptor Neurons. **C.-L. Pan**[1,2], C.-Y. Peng[1], C.-H. Chen[1], Steven L. McIntire[2]. 1) Institute of Molecular Medicine, National Taiwan University School of Medicine, Taipei, Taiwan; 2) Ernest Gallo Clinic and Research Center and Department of Neurology, University of California, San Francisco, Emeryville, CA, USA.

While many genes have been implicated in the pathogenesis of common neurodegenerative diseases, the genetic and cellular mechanisms that maintain neuronal integrity during normal aging remain elusive. Here we describe novel age-dependent morphological defects of *C. elegans* touch receptor and cholinergic neurons, including cytoskeletal disorganization, axon beading and defasciculation. Progression of these age-associated neuronal defects is regulated by DAF-2 and DAF-16 signaling, which also modulate adult lifespan. Mutations that disrupt touch-evoked sensory activity or reduce membrane excitability trigger accelerated neuronal aging, indicating that electrical activity is critical for adult neuronal integrity. Disrupting touch neuron attachment to the epithelial cells induces distinct neurodegenerative phenotypes. These results provide a detailed description of the age-dependent morphological defects that occur in identified neurons of *C. elegans*, demonstrate that the age of onset of these defects is regulated by specific genes, and provide experimental evidence for the importance of normal levels of neural activity in delaying neuronal aging.

309A

Detection of DNA Strand Breaks detection in Caenorhabditis elegans using Comet assay. **sojin Park**, moonjung Hyun, eunsun Kim, hyejin Park, byungchan Ahn. life science 19-321, university of ulsan, ulsan, ulsan, Korea.

DNA damage responses (DDR) are important for organisms to maintain genome stability and to survive. DDR is activated in the presence of DNA damage and leads to cell cycle arrest, apoptosis and DNA repair. In C. elegans, double strand breaks (DSBs) induced by DNA damaging agents have been detected indirectly by antibody against DSB recognizing protein, RAD-51. To detect DNA strand breaks induced by IR and CPT, UV at single cells in C. elegans, we developed COMET assay. We treated C. elegans DDR gene mutants (atm-1, brc-1, hus-1) with IR, CPT, and UV. C. elegans atm-1, brc-1 and hus-1 mutants were more sensitive to CPT than N2. DNA strand breaks in a single cell of these mutants atm-1, brc-1,and hus-1 could be detected using COMET assay and more DNA strand breaks accumulate than N2, which corresponds to more RAD-51 foci formation in a single cell in each mutant. This study is the first report in a direct measuring DNA strand breaks in a single cell of C. elegans and this developed assay can be applied to detect DNA strand breaks in different C. elegans mutants which are sensitive to DNA damaging agents.

310B

Insulin receptor signaling prolongs lifespan under high glucose conditions by restoring glyoxalase-1 activity. **Michael Pfeiffer**, Andreas Schlotterer, Youssef Ibrahim, Georgi Kukudov, Thomas Flemming, Angelika Bierhaus, Peter Nawroth, Michael Morcos. Heidelberg University Hospital, Heidelberg, Germany.

Purpose. The insulin/ insulin-receptor pathway elicits signals shortening lifespan in various organisms under standard conditions. However, in patients with diabetes mellitus treatment with insulin prolongs life of patients with diabetes mellitus under standard conditions and prevents late complications. Therefore, we speculated that under high glucose (HG) conditions, insulin receptor dependent signals could target a life prolonging instead of a life shortening pathway. **Methods.** *C. elegans* were maintained under standard and HG conditions, with or without addition of human insulin. Lifespan was analyzed using Kaplan-Meier-survival curves, expression of insulin homologs and *glod-4* was determined by QRT-PCR. Neuronal structure was visualized using a pan-neuronal YFP-expressing strain, motility was measured by video analysis. Nematodes were stained for reactive oxygen species (ROS) using ethidium-staining and for methylglyoxal-derived advanced glycated endproducts (MG-derived AGEs), using a specific antibody. Enzymatic activity of glyoxalase-1 was determined photometrically. **Results.** HG conditions reduced lifespan and correlated with *ins-7* expression. Human insulin, insulin aspart and insulin detemir increased lifespan, exclusively under HG conditions in a *daf-2* dependent manner. Moreover, insulin normalized ROS formation, restored HG-mediated reduction of GLOD-4 activity, and normalized accumulation of MG-derived AGEs, thus resulting in protection of neuronal structure and normalization of lifespan. Lifespan normalization by insulin in HG conditions was dependent on *glod-4*. **Conclusion.** The metabolic status influences downstream effects of *daf-2* dependent signaling. While human insulin elicits DAF-2 dependent signals, resulting in a glyoxalase-1 dependent normalization of lifespan under HG conditions, there is no effect exerted by human insulin on *C. elegans* under standard conditions. Therefore, insulin action might prevent posttranslational modifications of GLOD-4 caused by excess glucose, which in turn prevents further accumulation of MG-derived AGEs.

311C

Caenorhabditis elegans APN-1 plays a vital role in maintaining genome stability. Chadi Zakaria[1], Henok Kassahun[3], Xiaoming Yang[1], Jean-Claude Labbé[2], Hilde Nilsen[3], **Dindial Ramotar**[1]. 1) Maisonneuve, Rosemont Hosp, Univ Montreal/Research Ctr, Montreal, PQ, Canada; 2) University of Montreal, Department of Pathology and Cell Biology, Institute of Research in Immunology and Cancer, Montreal, Quebec, Canada; 3) University of Oslo, The Biotechnology Centre, P.O.Box 1125 Blindern, 0317 Oslo, Norway.

We previously showed that Caenorhabditis elegans APN-1, the only metazoan apurinic/apyrimidinc (AP) endonuclease belonging to the endonuclease IV family, can functionally rescue the DNA repair defects of Saccharomyces cerevisiae mutants completely lacking AP endonuclease/3'-diesterase activities. While this complementation study provided the first evidence that APN-1 possesses the ability to act on DNA lesions that are processed by AP endonucleases/3'-diesterase activities, no former studies were conducted to examine its biological importance in vivo. Herein, we show that C. elegans knockdown for apn-1 by RNAi displayed phenotypes that are directly linked with a defect

in maintaining the integrity of the genome. apn-1(RNAi) animals exhibited a 5-fold increase in the frequency of mutations at a gfp-lacZ reporter and showed sensitivities to DNA damaging agents such as methyl methane sulfonate and hydrogen peroxide that produce AP site lesions and strand breaks with blocked 3'-ends. The apn-1(RNAi) worms also displayed a delay in the division of the P1 blastomere, a defect that is consistent with the accumulation of unrepaired lesions. Longevity was only compromised, if the apn-1(RNAi) animals were challenged with the DNA damaging agents. We showed that apn-1(RNAi) knockdown suppressed formation of apoptotic corpses in the germline caused by an overburden of AP sites generated from uracil DNA glycosylase mediated removal of misincorporated uracil. Finally, we showed that depletion of APN-1 by RNAi partially rescued the lethality resulting from uracil misincorporation, suggesting that APN-1 is an important AP endonuclease for repair of misincorporated uracil.

312A

'Gerontometabolites' Define A Unique Metabolic Signature In Long-Lived Mit Mutants. Jeffrey J. Butler, **Shane L. Rea**. Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX.

The *Caenorhabditis elegans* mitochondrial (Mit) mutants have disrupted mitochondrial electron transport chain (ETC) functionality yet are long-lived. ByBy mutants also have disrupted mitochondrial ETC functionality but are short-lived. In an effort to identify mechanisms responsible for Mit mutant life extension, we recently established a novel approach that monitored the *C. elegans* exometabolism as a surrogate marker for internal metabolic events. Using HPLC-UV-based metabolomics and multivariate statistical analyses, we showed that long-lived *clk-1(qm30)* and *isp-1(qm150)* Mit mutants have a common metabolic profile that is distinct from that of either aerobically- or anaerobically-cultured wild-type animals. Moreover, we showed that two ByBy mutants, *mev-1(kn1)* and *ucr-2.3(pk732)*, also shared a common metabolic signature that is unique (Butler, *et. al*, 2010). In this study we have used gas-chromatography-mass spectrometry (GC-MS) to fully dissect the excretome of these four mutants. We have now obtained single metabolite resolution and identified over 80 compounds that are excreted by worms. From these profiles we have identified a unique metabolic pathway operative in long-lived Mit mutants that involves the production of several carboxylated end-products. We have used genetic methods to recapitulate the Mit profile and find that, accordingly, lifespan is increased. Finally, our findings reveal an unexpected role for miRNAs in the control of the Mit mutant metabolic profile.

313B

Lifespan extension via eIF4G inhibition is mediated by post-transcriptional remodeling of stress response gene expression in *C. elegans*. **Aric N. Rogers[1]**, Di Chen[1], Gregg Czerwieniec[1], Gawain McColl[2,3], Krysta Felkey[1], Simon Melov[1], Bradford W. Gibson[1], Alan Hubbard[1,4], Gordon Lithgow[1], Pankaj Kapahi[1]. 1) Gen Aging, Buck Institute for Research in Aging, Novato, CA; 2) The Mental Health Research Institute of Victoria, Parkville, Australia; 3) Center for Neuroscience, University of Melbourne, Parkville, Australia; 4) Division of Biostatistics, University of California, Berkeley, CA.

Reducing protein synthesis slows growth and development but can increase adult lifespan. We demonstrate that knock-down of eukaryotic translation initiation factor 4G (eIF4G), which is down-regulated during starvation, results in differential translation of genes important for growth and longevity in C. elegans. Genome-wide mRNA translation state analysis showed that inhibition of IFG-1, the C. elegans ortholog of eIF4G, results in a relative increase in ribosomal loading and translation of stress response genes. Some of these genes are required for lifespan extension when IFG-1 is inhibited and are novel determinants of longevity. Furthermore, enhanced ribosomal loading of certain mRNAs upon IFG-1 inhibition was correlated with increased mRNA length. This association was supported by changes in the proteome assayed via quantitative mass spectrometry. Our results support a role for IFG-1 in mediating the antagonistic effects on growth and somatic maintenance by modulating translation of a specific class of mRNA based on transcript length.

314C

Investigating the spatial and temporal dynamics of protein oxidation in *C. elegans*. **Catalina Romero**, Javier Apfeld, Walter Fontana. Systems Biology, Harvard Medical School, Boston, MA.

Increased protein oxidation is a phenomenon common to aging in animals in diverse phyla. Protein oxidation is often associated with loss of protein function, which may, in turn, impair cellular protein homeostasis and contribute to the organism's functional decline with age. We are interested in investigating the temporal and spatial dynamics of protein oxidation in different tissues during aging, a question that is difficult to tackle through biochemical approaches. To achieve this goal, we have created transgenic animals expressing a genetically encoded redox sensor, which reports the level of oxidation of specific cysteine residues via changes in fluorescence. We have validated our approach by showing that: i) the sensor responds quickly and reversibly to exposure of the worm to cell-permeable oxidants and reductants; ii) the sensor correctly reports differences in the oxidative environments of specific cellular compartments (nucleus, cytosol and endoplasmic reticulum). We are currently investigating whether mutations that affect lifespan also affect the level of protein oxidation, under normal and oxidative-stress conditions.

315A

Epidermal Growth Factor Signaling Activates The Ubiquitin Proteasome System To Modulate *C. elegans* Lifespan. Gang Liu[1], Jason Rogers[2], Coleen Murphy[2], **Christopher Rongo[1]**. 1) Waksman Inst, Rutgers Univ, Piscataway, NJ; 2) Lewis-Sigler Institute for Integrative Genomics, Princeton University, NJ.

Epidermal growth factor (EGF) signaling regulates cell growth, proliferation, and differentiation, and has recently been implicated in regulating lifespan, although the mechanism remains unclear. Here we examine the function of EGF signaling in lifespan in adult *C. elegans*. We find that EGF signaling regulates lifespan via the Ras-MAPK pathway and the PLZF transcription factors EOR-1 and EOR-2. We find that as animals enter adulthood, EGF signaling upregulates the expression of genes involved in the ubiquitin/proteasome system (UPS), including the Skp1-like protein SKR-5 (an adaptor between F-box proteins and Cullin scaffolding in SCF ubiquitin ligases), while downregulating the expression of HSP16-type chaperones. Using GFP-based reporters for (1) global UPS activity, (2) protein aggregation, and (3) oxidative stress, we find that EGF signaling alters protein homeostasis in adults, triggering an increase in UPS activity and in the levels of polyubiquitinated proteins, while also increasing the propensity of misfolded proteins to aggregate - critical facets in aging and cellular degeneration. We show that SKR-5 and the components of the E3/E4 ligases that comprise the Ubiquitin Fusion Degradation (UFD) complex are required for the increase in UPS activity observed in adults, suggesting a novel mechanism - regulation of Skp1 adaptor protein expression - by which cells can regulate the activity of multiple E3 ubiquitin ligases. Animals that fail to upregulate UPS activity via EGF signaling, SKR-5, or the UFD have reduced lifespans and indications of oxidative stress, whereas a mutation that overactivates the EGF receptor prematurely accelerates UPS activity and increases lifespan. Taken together, our results indicate that as animals enter adulthood, EGF signaling switches the mechanism for maintaining protein homeostasis from a chaperone-based approach to an approach involving protein elimination via augmented UPS activity.

316B

The R148.3 (OcaB) gene controls longevity and fat accumulation in the nematode *C. elegans*. **Catherine Roy-Bellavance[1,2]**, Évelyne Rondeau[1], Stéphanie Miard[1], Frédéric Picard[1,2]. 1) IUCPQ, Québec, Qc, Canada; 2) Faculté de Pharmacie, Université Laval, Québec, Qc, Canada.

OcaB (Oct co-activator from B cells) is a nuclear cofactor mainly expressed in immune B lymphocytes, where it regulates B cells development and function. Our lab has recently discovered that OcaB is expressed in white adipose tissue (WAT), and that the gene expression in WAT is very low in diet-induced obese mice but is increased upon aging.

We used the genetic model C. elegans to assess the role of OcaB in fat accumulation and define its pathway in aging. GFP-reporter analysis indicated that R148.3 (OcaB closest homolog gene) is mainly expressed in the pharynx and pharyngeal neurons. mRNA quantification by qPCR analysis showed that R148.3 is mainly expressed in adult worm. Knockdown (KD) of the R148.3 gene was performed by RNAi feeding. R148.3-KD worms showed much higher lipid content than their wild-type counterparts, as evidenced by both Nile Red and Oil Red O staining, and this effect on lipid content tended to be age-dependent. In addition, R148.3-KD worms had a 40% shorter lifespan compared to wildtype nematodes. Using genetic mutants, we further found that the effect of R148.3 on longevity was abrogated in daf-2 and age-1 mutants, but not in daf-16 mutants. We also tested the capacity of R148.3KD worms to resist oxidative stress by soaking the worms in paraquat. We found that KD worms were more sensitive to paraquat and died earlier than wildtype nematodes.

Taken together, these results suggest that R148.3 could possibly serve as a node linking fat accumulation to longevity and stress resistance.

Study funded by CIHR (IAP-102233).

317C

The effects of nickel toxicity upon survival, growth, and reproduction in nematodes. C.D. Douglas[1], J.M. Besser[2], C.G. Ingersoll[2], **D. Rudel[1]**. 1) Department of Biology, East Carolina University, Greenville, NC., Germany; 2) CERC, U.S. Geological Survey, Columbia, MO.

Nickel is one of the most prevalent heavy metal contaminants in the dust floating in the air we breath, in soils, and in many waterways. Contamination continues to increase largely due to human activities like mining, manufacturing, and waste management. Ni^{2+} is a carcinogen to plants and animals, and a major concern for the health and safety both of humans and food crops. In addition to mutagenesis and cancer, nickel exposure leads to a variety of other deleterious health conditions. In collaboration with the United States Geological Survey, an assay to evaluate the effects of substrate bound nickel and soluble nickel on survival, growth, and fecundity using *C. elegans* and *Pristionchus pacificus* has been developed. Synchronized L1 (*C. elegans*) or J2 (*P. pacificus*) larvae were added to sediment and water samples in twelve-well tissue culture plates with a defined amount of freshly killed bacteria as a food source. Animals were grown for 96 hours at 20ºC and harvested. Harvested animals were assayed for survival, growth, and fertility. Growth in eight sediment-types collected at discrete uncontaminated sites along the Missouri/Mississippi river basin suggests that both nematodes grow well in organic based sediments and less well in more sandy/inorganic based sediments. Growth in sediments spiked with increasing amounts of environmentally relevant levels of sediment-bound nickel showed that *C. elegans* was highly sensitive to nickel in the environment of the sediment. Treatment was lethal prior to adulthood. Growth in nickel spiked liquid media with freshly killed food and cholesterol, i.e. hard H20 pH 7.5, distilled (DI) H20, SB Media

(hard H20), and SB Media (DI H20), showed that *C. elegans* has a strong tolerance for environmentally relevant levels of soluble nickel. Adult survivors of nickel treatments showed no significant effects on adult length/width measurements or the presence of fertilized eggs in the uterus. As nickel has other known cell stress effects, we will also assay total germline apoptosis as an measure of DNA damage.

318A

SKN-1-independent oxidative stress signaling from mitochondria to the nucleus. **Eva D. Runkel**[1,2], Shu Liu[1], Ralf Baumeister[1,2,3,4,5], Ekkehard Schulze[1]. 1) Bioinformatics and Molecular Genetics (Faculty of Biology III), University of Freiburg, Germany; 2) Spemann Graduate School of Biology and Medicine (SGBM); 3) Center for Biochemistry and Molecular Cell Research (Faculty of Medicine), University of Freiburg, Germany; 4) Center for Systems Biology (ZBSA); 5) FRIAS Freiburg Institute of Advanced Studies, School of Life Sciences (LIFENET).

The survival of aerobic organisms strongly depends on a proper maintenance of functional mitochondria. Given that mitochondria are the major source of cellular reactive oxygen species (ROS), they are readily subjected to oxidative stress conditions. In such cases a specific defense response is triggered. We recognized that treatment with ROS-generating chemical agents leads to a transcriptional induction of the nuclear-encoded mitochondrial chaperone *hsp-6*. Even though the transcription factor SKN-1 is one of the best-established effectors of oxidative stress responses in *C. elegans*, we found that it is not required in this process. We wondered whether ROS accumulation in mitochondria triggers an organelle-specific oxidative stress response. Thus, we performed a genome-scale RNAi screen that aimed to identify essential components of the pathway. We found 54 essential genes out of which two encode for transcription factors that have so far not been placed into the context of oxidative stress signaling. To test the specificity of our screening positives for the ROS-induced signaling from mitochondria, we analyzed their involvement in the SKN-1-dependent oxidative stress response, heat shock response, and the unfolded protein response of the ER. Indeed, none of our screening positives is required for any of these stress responses. Only one gene that emerged in our screen, namely a conserved PI4-kinase, has an impact on ER stress signaling, too. Our current work aims to the reconstruction of the signaling network.

319B

Understanding the emergence of dynamic response of the reproductive system of *Caenorhabditis elegans* to chronic heat stress. Patrick D. McMullen[1], Erin Z. Aprison[2], Peter Winter[1], Luis A. Amaral[1], Richard I. Morimoto[3], **Ilya Ruvinsky**[2]. 1) Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208; 2) Department of Ecology and Evolution, Institute for Genomics and Systems Biology, The University of Chicago, Chicago, IL 60637; 3) Department of Molecular Biosciences, Northwestern University, Evanston, IL 60208.

A major goal of systems biology is to understand how complex emergent properties of organisms arise from interactions of individual components. We developed an analytical model of the reproductive system of *C. elegans* using basic engineering principles. Although the model incorporated only a few of the many known features of nematode reproduction, it provided quantitatively accurate predictions of performance under a variety of chronic heat stress conditions, as verified by detailed time-resolved experimental data. This suggests that dynamic systems behaviors may be determined by only a small number of key components. Our approach is general and can be applied to other biological systems to reveal which processes within organisms give rise to their dynamic behavior. Importantly, we found that whereas under ambient conditions brood size distribution for individual worms was unimodal (indeed normal), under conditions of harsh stress, when the reproductive system was near shutdown, it was best described by a combination of a normal and an exponential distribution. This implies the existence of a hidden heterogeneity in a population - some animals continue to act robustly, while others display loss of control over performance variance. This hints at the existence of a general principle that governs behavior of robust systems as they near complete collapse.

320C

The recruitment of HSR-9 (53BP1) in response to double-strand DNA breaks requires methylation of histone H4 Lysine 20 in *C. elegans*. **Jinsun Ryu**, Sang Jo Kang, Hyeon-Sook KOO. Yonsei University, Seoul, Korea.

53BP1 contributes to the G2/M checkpoint activation in the downstream of ATM and MDC1 in response to ionizing-radiation. The recruitment of 53BP1 at double strand DNA breaks (DSBs) requires methylation of histone H4 lysine 20. Recently, the methylation of H4 Lys20 at DSBs was found to be mediated by the histone methyltransferases MMSET and SET-D8 in mammalian cells. In order to understand the mechanism how 53BP1 is recruited to the DNA damage sites, we analyzed the function of a 53BP1 homolog, HSR-9, in *C. elegans*. The deletion mutant of *hsr-9* showed hypersensitivity as measured by embryonic lethality. However, the deletion did not significantly affect the cell cycle arrest induced by DSBs in mitotically proliferating germ cells. Since it is known that ATM influences the recruitment of 53BP1 to DSBs in mammalian cells, we tested whether HSR-9 activation is affected by ATM and ATR homologs. Interestingly, a single deficiency of ATM and ATR homologs attenuated the nuclear accumulation of HSR-9 after γ-irradiation slightly, and the double deficiency almost abolished the nuclear accumulation. These results show that both of ATM and ATR homologs influence the HSR-9 activation, unlike the case of mammalian cells, where ATM is the major upstream kinase of 53BP1. We also found that the deficiency of a histone H4 Lys20 methylase SET-1 or a PTIP homolog PIS-1 in *C. elegans* abolished the HSR-9 recruitment. Thus, we propose that the recruitment of HSR-9 at DSBs requires the methylation of histone H4 lysine 20 like mammalian cells.

321A

Deciphering HIF-1 regulatory networks: An interdisciplinary approach to diagnosing stress resistant phenotypes. **Jenifer Saldanha**[1], Archana Parashar[2], Qi Ye[1], Santosh Pandey[2], Jo Anne Powell-Coffman[1]. 1) Genetics,Development and Cell Biology, Iowa State University, Ames, IA; 2) Electrical and Computer Engineering, Iowa State University, Ames, IA.

The Hypoxia inducible factor-1 transcription complex mediates most hypoxia-induced changes in gene expression, and recent studies have revealed roles for *C. elegans* HIF-1 in aging, neuronal development and function, and stress resistance. The pathway for oxygen-dependent HIF-1 degradation is evolutionarily conserved. In a reaction that utilizes oxygen as a co-substrate, the EGL-9 enzyme hydroxylates HIF-1 at a conserved proline residue, and this enables VHL-1-mediated proteasomal degradation. Recent studies have shown that EGL-9 also controls HIF-1 activity in a pathway that has little or no requirement for its hydroxylase activity. Of further interest, strong loss-of-function mutations in *egl-9* have been shown to confer resistance to fast killing by the pathogen *Pseudomonas aeruginosa* PAO1. This fast killing is caused by cyanide that the bacteria produce, and *egl-9* mutants are resistant to cyanide. This phenotype is dependent upon *hif-1* function. In recent studies, we have shown that cyanide resistance can also be conferred by other mutations that dramatically increase HIF-1 activity. To further define the phenotypes caused by over-activation of HIF-1, we designed and developed novel microfluidic devices to test the effects of cyanide and other small molecules on *C. elegans*. In initial experiments, the worms were exposed to cyanide in an agarose based medium in the microchannels, and the movement parameters of the worms were recorded and analyzed before and after the exposure. We are also characterizing other genotypes that increase HIF-1 activity. The results provide insight into the nuances of the stress resistance phenotypes and also serve as a basis for further microfluidics-based assays for studying worm stress responses. Long-term project goals include investigating cell-type-specific roles for *egl-9*; identifying additional genes in the HIF-1 regulatory network; and elucidating the mechanisms by which EGL-9 represses HIF-1 transcriptional activity.

322B

Insulin signaling pathway genes facilitating thermotolerance and protein homeostasis. Sara Farrell, Lindsay Stolzenburg, Jesse Llop, David Johnson, **Andrew V. Samuelson**. Biomedical Genetics, University of Rochester Medical Center, Rochester, NY.

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. 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 and induction of heat shock proteins. Additionally, chronic growth at higher temperature shortens lifespan and accelerates aging, presumably due to increased thermal stress that negatively impacts protein homeostasis. 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. The over-arching goal our research is to gain a comprehensive, systems-level understanding of the mechanisms that promote longevity in conditions of reduced *daf-2* insulin-like signaling. We identified the progeric gene inactivations that are necessary for the increased intrinsic and acquired thermotolerance conferred by decreased insulin signaling. Additionally, we tested the progeric gene inactivations for differential lifespan at varying temperature, premature collapse of proteostasis, induction of heat shock proteins, and the suppression of *hsf-1(o/e)* lifespan. This comprehensive functional analysis identifies the subset of progeric gene inactivations that negatively impact protein homeostasis after acute or chronic stress.

323C

Caenorhabditis elegans NDX-1has an 8-oxo-7, 8-dihydrodeoxyguanosine 5'-diphosphate pyrophosphatase activity that contributes to prevent against oxidative stress. **U Sanada**, Qiu-Mei Zhang-Akiyama. Department of Biological Science, Graduate School of Science, Kyoto University.

Reactive oxygen species (ROS) generate various types of oxidative deoxyribonucleotides, such as 8-oxo-dGTP and 8-oxo-dGDP, in the nucleotide pool. 8-oxo-dGTP is generated by both direct oxidation of dGTP and phosphorylation of 8-oxo-dGDP. It is often incorporated into DNA by DNA polymerases during replication, which could result in mutagenic consequences. *Escherichia coli* MutT hydrolyzes 8-oxo-dGTP. In addition, MutT hydrolyzes 8-oxo-dGDP to 8-oxo-dGMP, thereby preventing mutations caused by misincorporation of 8-oxo-dGTP into DNA. In this study, I identified MutT homologs (NDX) of the nematode *C. elegans*. Purified NDX-1 hydrolyzed 8-oxo-dGDP to the

monophosphate form, but did not hydrolyze 8-oxo-dGTP. Expression of NDX-1 potently rescued the spontaneous mutations and high sensitivity to methyl viologen in an *E. coli* mutT mutant. These findings indicate that NDX-1 is involved in sanitization of 8-oxo-dGDP in the nucleotide pool in *C. elegans*. RNAi of *ndx-1* did not affect lifespan compared with that of control worms. However, the sensitivity to the redox-active superoxide-generating agent methyl viologen (MV) and menadione of the *ndx-1*-RNAi worms was higher than that of the controls. These results indicate that *C. elegans* NDX-1 plays a role in defense against oxidative stress.

324A

Gene by environment interaction reveals proteostasis sub network for adult health and longevity. **A.R. Sanchez**, S. Takano, P.L. Larsen. Department of Cellular & Structural Biology, UTHSCSA, San Antonio, TX.

Cool core-body temperature promotes longevity in mammals, fishes, flies and worms. Molecular mechanisms underlying this cool-mediated longevity are not well understood. Mutations in the *daf-2* gene increase adult lifespan. In *C. elegans*, wild-type adult maximal lifespan is doubled at 15°C compared to 25°C, but this pattern is aberrant in adults bearing mutations in the *daf-2* gene. We investigated the gene by environment interaction using microarrays on samples from wild-type or *daf-2*(*m577, m41, e1370, m579* or *m596*) adults acclimated to 15°C or 25°C. Differentially expressed (DE) genes revealed five categories: pro-longevity, pro-aging, context-specific-pro-longevity, context-specific-pro-aging, and irrelevant.

DE genes lower at 25°C than 15°C included particular protein synthesis and lysosomal proteolysis genes. Adult lifespan was increased at 25°C and decreased lifespan at 15°C when treated with pharmacological lysosomal inhibitors. Lysosomal protease RNAi caused paralysis in middle-aged adults only at 15°C. RNAi of protein synthesis genes caused an increased lifespan in wild-type adults only at 25°C and vulval herniation or paralysis in middle-aged adults only at 15°C. Together, these results indicate that temperature-dependent regulation of the proteostatic network is adaptive for long-term healthy adult survival.

Aspects of temperature regulation were retained in *daf-2* adults. These mutants were hyper-responsive to temperature at the molecular level. There were 338 temperature-responsive genes in wild type, whereas the number in the mutants ranged from 902 to 3410 DE genes. Of the wild-type DE protein synthesis genes, 22 of 26 showed temperature regulation in the mutants, with four elevated at 25°C rather than lowered. There were 615 temperature DE genes shared in mild *daf-2* alleles and 653 genes in severe *daf-2* alleles. An additional 38 genes became DE in *daf-2* mutants. Temperature regulation of 10 of 15 lysosomal proteases was lost in *daf-2* mutants with elevated levels at 25°C correlating with allele severity. RNAi of protein synthesis genes in *daf-2* mutants dramatically increased lifespan at 25°C with no increase in lifespan at 15°C. RNAi of a lysosomal protease increased lifespan in the *daf-2* mutants at 15°C and decreased lifespan at 25°C. This suggests one consequence of the dysregulated *daf-2* transcriptome results in a pro-aging liability at both temperatures. In summary, we have identified proteostatis network profiles which favor long-term healthy survival given a genotype and environmental condition.

325B

Variable Pathogenicity Determines Individual Lifespan in *C. elegans*. **Adolfo Sanchez-Blanco**, Stuart Kim. Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA.

Stochasticity is a common property of aging in all animals, as chronologically and genetically identical individuals age at different rates. To unveil mechanisms that influence aging variability, we identified markers of remaining lifespan for *C. elegans*. In transgenic lines, we expressed fluorescent reporter constructs from promoters of *C. elegans* genes whose expression change with age. The expression levels of aging markers in individual worms from a young synchronous population correlated with their remaining lifespan. We identified eight aging markers, with expression of the superoxide dismutase gene *sod-3* being the best single predictor of remaining lifespan. Visualizing the physiological age of chronologically-identical individuals allowed us to show that a major source of lifespan variability involves different responses to pathogenicity from individual to individual. Ingestion of mildly pathogenic *E. coli* activates *daf-16* FOXO activity via the insulin-like signaling pathway, which induces a beneficial stress response that is protective and extends lifespan. The pathogenicity from the ingested bacteria primarily affects the intestine.

326C

MicroRNA Modulation of *C. elegans* aging. **Mitalie Shah**, Mehul Vora, Monica Driscoll. Cell and Developmental Biology, Rutgers University, Piscataway, NJ.

MicroRNAs (miRNAs) are known to regulate a range of biological processes including cell differentiation, cell death, development, oncogenesis, and metabolism, but little is known on how they impact the biology of aging. Our research mainly focuses on identifying how miRNAs, small molecules that target partially homologous transcripts to block their translational expression, influence healthspan and lifespan in *C. elegans*. There are four measures that reflect how well or how poorly the animals are aging: age pigment levels, swimming body bend frequency assays, pharyngeal pumping rates, and lifespan studies. Age pigments are highly cross-linked fluorescent species (lipofuscin and advanced glycation endproducts) that accumulate over time in lysosomes across species. Age pigment levels can indicate healthspan and correlated with whether an animal has aged gracefully (lower levels of age pigments) or poorly (higher levels). Swimming assays evaluate mobility (frequency of body bends during swimming) and are considered to be an indication of muscle health. Mutants with accelerated or delayed sarcopenia of body wall muscle can be identified by this assay. Pharyngeal pumping assays determine miRNA mutants with strong, regular pumping of the pharynx (a model for the heart) in mid/late adulthood, suggesting extended pharyngeal muscle healthspan, while slow pumping would suggest exacerbated decline. Lifespan studies reveal whether mutants that exhibit specific healthspan changes also have changes in mean and maximum lifespan. We are in the process of identifying conserved miRNAs that modulate aging and showing how their manipulation can be used to extend life- and healthspan of *C. elegans*. Deciphering the roles of age-regulated miRNAs in a relatively simple model organism will provide clues to similar processes in higher organisms and may suggest novel ideas for anti-aging therapies.

327A

Regulation of Autophagy by RNF-5, an E3 Ubiquitin Ligase. **S. Sheffy Levin**, L. Broday. Cell and Developmental Biology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

The three main protein degradation pathways in the cell are the proteasome, the lysosome and autophagy. Autophagy is a general term for processes by which cytoplasmic materials including organelles reach lysosomes for degradation. Ubiquitin-E3 ligases are the components of the ubiquitin machinery that confer specificity to the ubiquitination process by recognizing target substrates and mediating transfer of ubiquitin from an E2 ubiquitin-conjugating enzyme to the substrate. The RING finger E3 ligase RNF-5 is an ER-bound protein implicated in ER-associated degradation (ERAD). Using the LGG-1/LC3::GFP reporter [1] we revealed that RNF-5 inhibition caused an increase in autophagy . Our purpose is to understand the mechanism by which RNF-5 regulates autophagy. *rnf-5(tm794)* mutant worms are more resistant to ER stress than wild-type animals, as assessed by exposing them to tunicamycin, an inhibitor of N-glycosylation. This resistance was reduced when different components of the autophagy core machinery were knocked down, suggesting that the activity of RNF-5 in the regulation of ER homeostasis is mediated through autophagy. Life span analysis revealed that elevated levels of RNF-5 in *eat-2(ad465)* mutant worms, but not in wild-type worms, suppressed their longevity phenotype. Since autophagy is required for the longevity of *eat-2* worms [2, 3], we conclude that elevated expression of RNF-5 reduced autophagy in these animals and diminished their longevity. Together these data suggest that RNF-5 is a negative regulator of autophagy.

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328B

Monascus-fermented dioscorea enhances oxidative stress resistance via insulin signaling pathway. **Yeu Ching Shi**[1,2], Tzu-Ming Pan[2], Vivian Liao[1]. 1) Department of Bioenvironmental Systems Engineering, National Taiwan University, Taipei, Taiwan; 2) Department of Biochemical Science and Technology, National Taiwan University, Taipei, Taiwan.

Monascus-fermented products offer valuable therapeutic benefits and have been extensively used in Eastern Asia for several centuries. Monascus spp. produces many types of polyketide secondary metabolites and some of them have been reported to have antioxidant, antiinflammatory, and antitumor-initiating effects. In this study, we investigated Monascus-fermented products-modulated antioxidant effect in C. elegans. We examined oxidative stress resistance of ethanol extract of red mold dioscorea (RMDE) in C. elegans, and found that it responded to 10 μg/mL RMDE with an increased survival rate and reduced intracellular reactive oxygen species (ROSs). The subcellular distribution of the FOXO transcription factor DAF-16 was affected by RMDE. We analyzed genetic determinant that might influence oxidative stress resistance by RMDE. We showed that the antioxidant phenotype was absent from the daf-2, age-1, akt-2, and daf-16 mutants. These finding suggested that RMDE may act as an antioxidant via insulin signaling pathway and DAF-16/FOXO transcription factor.

329C

The *C. elegans* WRN-2 having the exonuclease domain of Werner syndrome protein is required for efficient cell cycle arrest in response to ionizing radiation. **Hyo ju Shin**, Se jin Lee, Hyeon-Sook KOO. Biochemistry, Yonsei University, Seoul, Korea.

Werner Syndrome protein (WRN) is a member of RecQ helicase family, having additional enzymatic activity of exonuclease. The two enzymatic activities of WRN are conserved in two separate proteins of WRN-1 and WRN-2 in *C. elegans*. WRN-1 having the helicase domain was previously shown to participate in the activation of cell cycle checkpoint in response to DNA replication inhibition and double-strand DNA breaks (DSBs). Here, we examined whether WRN-2(ZK1098.3) having the exonuclease domain functions together with WRN-1 in various DNA damage responses. We found that WRN-2 is not required for checkpoint activation induced by DNA replication inhibition. Nevertheless, WRN-2 mutant worms exhibit accelerated growth rate following ionizing radiation (IR) like WRN-1 mutants, suggesting its role in the checkpoint activation induced by DSBs. Moreover, the number of mitotic germ cells was not so much reduced in *wrn-2(tm2546)* gonads as in wild type gonads after IR treatment, suggesting that WRN-2 plays a role like WRN-1 in the cell cycle arrest induced by IR. These results suggest that WRN-2 is involved in the checkpoint

activation in response to DSBs but not in the DNA replication checkpoint. We are now in the process of analyzing the physical and genetic interactions of WRN-1 and WRN-2.

330A

The DAF-2 somatic longevity pathway interfaces with PRG-1-mediated germ cell immortality. **Matt A Simon**[1], Eric Miska[2], Shawn Ahmed[1,3]. 1) Curriculum in Genetics and Molecular Biology, University of North Carolina, Chapel Hill, NC; 2) The Gurdon Institute, University of Cambridge, Cambridge CB2 1QN, UK; 3) Department of Genetics, University of North Carolina, Chapel Hill, NC.

Mutations in the C. elegans Piwi Argonaute orthologue *prg-1* have previously been reported to result in reduced levels of thousands of germline-specific piRNAs as well as temperature-sensitive sterility (1, 2, 3). We find that outcrossing of *prg-1* mutations results in homozygous strains with normal levels of fertility, but that propagation of these strains for 15 to 40 generations results in sterility. *prg-1* mutants have previously been reported to display increased levels of Tc3 transposition. However, stereotypical germ cell development phenotypes occur at sterility, suggesting that progressive sterility is not induced by random Tc3-mediated mutations but rather by another form of heritable stress. Repression of *daf-2* is well known to result in stress resistance and increased somatic lifespan in C. elegans (4). Independent *daf-2* mutations were able to suppress the progressive sterility phenotype of *prg-1*. Thus, reduced DAF-2-mediated insulin/IGF1 signaling can repress post-mitotic aging in somatic cells as well as proliferative aging in the germ line. These observations defy a prediction of the antagonistic pleiotropy theory of aging (5), which suggests that prolonged lifespan might result in compromised fertility.

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331B

Non-monotonic relationship between tissue levels of 4-hydroxynonenal (4-HNE) and lifespan in *C. elegans*. Kevin E. McElhanon[1], Kira C. Bennett[1], Ludwika Zimniak[1], Piotr Zimniak[1,2], **Sharda P. Singh**[1]. 1) Dept Pharm Tox, Univ Arkansas Med Sci, Little Rock, AR; 2) Central Arkansas Veterans Healthcare System, Little Rock, AR.

The lipid peroxidation product 4-hydroxynon-2-enal (4-HNE) is a reactive electrophile able to modify proteins, aminophospholipids and, to a lesser extent, nucleic acids. Derivatization by 4-HNE of Cys, His, and Lys side chains of proteins often changes protein function; both inhibition and activation have been observed. 4-HNE is considered to be a signaling molecule that conveys the information that an oxidative event has occurred. The signal then coordinates an appropriate cellular response. We have previously found that conditions that lower the steady-state 4-HNE concentration, such as overexpression of 4-HNE-metabolizing enzyme gst-10, extend *C. elegans* lifespan, whereas elevated 4-HNE leads to a shorter lifespan. We now report that expression (driven by the *gst-10* promoter) of human GSTA4-4, an enzyme with high catalytic efficiency toward 4-HNE, curtails *C. elegans* lifespan, whereas expression of a GSTA4-4 mutant in which 4-HNE-conjugating activity is selectively abrogated, has no effect on longevity. Together, these results indicate that moderate depletion of 4-HNE extends life, while excessive depletion of 4-HNE by the highly active human enzyme has the opposite effect. This conclusion is consistent with our finding that overexpression of the *gst-10* gene product in a wild-type background prolongs life (as previously reported), but expression of *gst-10* in a *ins-7* null mutant shortens lifespan. Ins-7 is an insulin-like peptide that is a Daf-2 agonist; disruption of Ins-7 expression has been shown by others to extend life. A possible interpretation of our result is that moderate depletion of 4-HNE (by gst-10 overexpression) in wild-type worms has two countervailing effects on lifespan: extension, possibly mediated by limiting Ins-7 activity, and shortening due to loss of 4-HNE signaling. The sum of these two effects is a moderate gain in longevity. In *ins-7* null background, limiting of Ins-7 activity is no longer possible, but a detrimental loss of 4-HNE functions remains, resulting in a net decrease of lifespan. While further work is necessary to confirm this hypothesis, at present, our results are consistent with at least two distinct functions of 4-HNE that have opposing effects on life span. This would result in the existence of an optimal 4-HNE concentration with regard to lifespan; 4-HNE levels either higher or lower than this optimum reduce longevity.

332C

The role of ubiquitin-conjugating enzymes in polyglutamine protein aggregation. **Gregory A Skibinski**, Lynn Boyd. University of Alabama in Huntsville, Department of Biological Sciences 301 Sparkman Drive Huntsville, AL 35899.

The misfolding of soluble proteins and subsequent aggregation is associated with several neurodegenerative diseases and is thought to be involved in the pathological process. Aggregates in multiple diseases, including polyglutamine (polyQ) aggregates, show positive immunostaining for ubiquitin. The ubiquitin-proteasome system, which degrades transient and damaged proteins, may be impaired in these diseases and is thought to be involved in aggregation. To investigate this, we are using a transgenic line of C. elegans developed by Morimoto et al. that expresses an aggregation-prone stretch of glutamine repeats (Q82) fused to GFP (green fluorescent protein). We have shown that knockdown of specific E2 ubiquitin-conjugating (UBCs) enzymes alters the size and frequency of polyQ-GFP aggregates, and that *ubc-1*, *ubc-13*, and *uev-1* are required for ubiquitin colocalization to aggregates (Howard et al., BMC Cell Biology 2007, 8:32). To view this process in real-time, we are using timelapse microscopy to view aggregation of soluble Q82::GFP into punctate aggregates in early-stage C. elegans larvae. We have observed that the initial in vivo formation of polyQ aggregates in the body wall muscle cells occurs over a span of about 45 minutes. RNAi of UBCs, most notably *ubc-22*, affects the rate of this initial aggregation, primarily by increasing the steady-state levels of Q82::GFP protein in the cell. This may indicate that the initial aggregation even is not directly dependent on ubiquitination of aggregating proteins. Beyond this initial phase, aggregation is slower, but RNAi of *ubc-1*, *ubc-22*, or *uev-1* increases this rate. RNAi of *ubc-13* reduces this rate. These studies and experiments using an mCherry::ubiquitin fusion protein suggest that the initial formation of microscopically visible polyglutamine aggregates is independent of ubiquitination and may primarily be a spontaneous, entropy-driven process. These studies, and studies of the localization of an mCherry::ubiquitin fusion protein indicate that ubiquitin may not be directly involved in the initial aggregate formation, and that the more prominent roles of ubiquitin are primarily relegated to the processing and maturation of previously-formed aggregates.

333A

Modeling membrane protein misfolding in *C. elegans*. Liping He, **Jennifer Skirkanich**, Lorenza Moronetti, Todd Lamitina. Department of Physiology, University of Pennsylvania, Philadelphia, PA.

Membrane proteins comprise ~30% of the proteome. Due to their complex topology, these proteins present a unique challenge for protein folding systems, which must integrate folding information within the cytoplasm, membrane, and ER lumen to determine whether substrates are appropriately folded or are misfolded and warrant degradation. These systems play critical roles in many disease states, including Cystic Fibrosis (CF) where a genetically encoded mutation (ΔF508) causes the 12 transmembrane CFTR chloride channel to misfold. Misfolded CFTR is degraded through a process termed endoplasmic reticulum associated degradation (ERAD). Manipulations that improve CFTR folding or inhibit ERAD stabilize mutant CFTR and partially restore chloride secretion to epithelial cells, the underlying defect in CF. A better understanding of ERAD mechanisms could provide therapeutic insights into CF and other related diseases. Yeast genetics and mammalian cell biochemistry have demonstrated that core components of ERAD are evolutionarily conserved. While these studies have provided a cellular perspective to the study of ERAD, genetic approaches for investigating ERAD in a live animal setting have not yet been developed. *C. elegans* has proven to be an outstanding *in vivo* system for the study of misfolded cytoplasmic substrates (e.g. polyQ, SOD-1, TDP-43). To explore mechanisms regulating misfolded membrane proteins in this system, we introduced the human ΔF508 mutation into *C. elegans* PGP-3, a 12 transmembrane ABC transporter closely related to human CFTR. When expressed in intestine and muscle, PGP-3^{wt} and PGP-$3^{\Delta F508}$ show identical levels of mRNA but exhibit striking differences at the protein level. In both tissues PGP-3^{wt} is stable and efficiently trafficked to the membrane, however membrane localization of PGP-$3^{\Delta F508}$ is strongly reduced. In intestinal cells PGP-$3^{\Delta F508}$ exhibits dramatically reduced protein levels, while in muscle PGP-$3^{\Delta F508}$ is stable but accumulates in both intracellular puncta and reticular patterns. Both physiological (adaptation to 200 mM NaCl) and genetic (Osr mutants *osm-7* and *osm-11*) activation of the osmotic stress response pathway post-transcriptionally stabilize PGP-$3^{\Delta F508}$ but not PGP-3^{wt}. To search for additional stabilizer mutants, we performed a forward genetic screen using the COPAS Biosort. We identified several mutants that post-transcriptionally stabilize the misfolded PGP-$3^{\Delta F508}$ protein and are currently working on cloning the affected loci. Our studies introduce *C. elegans* as a new and powerful *in vivo* model for the study of membrane protein misfolding, reveal tissue-specific differences in ERAD, and suggest that these pathways can be influenced by cell-non-autonomous mechanisms.

334B

The Lifespan Machine comes of age: Time-dependent changes in the action of insulin signaling are revealed by high-resolution mortality data. **Nicholas Stroustrup**, Javier Apfeld, Walter Fontana. Systems Biology, Harvard Medical School, Boston, MA.

Aging *C. elegans* populations show characteristic changes in mortality rate and tolerance to stresses including exposure to high temperature and oxidizing agents. We are interested in how individual genes determine the variability and temporal dynamics of these age-dependent phenotypes. We have developed an automated, high-throughput microscopy platform (the “lifespan machine”) capable of assaying several thousand worms for movement on agar plates every ten minutes over several weeks. In addition to acquiring high-resolution lifespan distributions at 20°C and 25°C, our method also produces consistent survival curves under stress regimes including 35°C thermotolerance assays and *tert*-butyl hydroperoxide resistance assays. By subjecting mutant populations to these stressors starting at different times of adulthood, we probe how individual genes affect the age-dependency of stress resistance.

In isogenic populations, thermotolerance and oxidant resistance can be quite variable between individuals, reminiscent of the wide variation observed in lifespan under standard conditions. We find that genetic determinants of the mean and variation in lifespan also affect the temporal dynamics of stress resistance as animals age. Strikingly, we find that mutations in several pathway components of insulin signaling known to affect lifespan only affect thermotolerance and resistance to oxidative stress at certain times during adulthood.

These findings indicate that worms experience age-dependent changes in the action of insulin signaling.

335C

Identification of DNA maintenance genes specifying the extended longevity of the Mit mutants. **Alessandro Torgovnick**[1], Alfonso Schiavi[1], Roberto Testi[1], Shane Rea[2], Natascia Ventura[1]. 1) Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Rome, Italy; 2) Barshop Institute for Longevity and Aging Studies, University of Texas Health Science Center at San Antonio, San Antonio, Texas, USA.

The Caenorhabditis elegans mitochondrial mutants (Mit) live longer and are more robust and active then wild-type animals or animals with severely affected mitochondrial respiratory chain (MRC) functionality. These observations suggest that mild suppression of MRC proteins extends longevity not simply by reducing animal metabolic rate but rather by an active induction of compensatory, protective pathways. Utilizing different biochemical and genetic approaches, our work is exploiting C. elegans to uncover stress response pathways required in the Mit mutants to optimize mitochondrial functions, protect from cellular damage accumulation and ultimately live longer. Our recent findings show that the C. elegans ortholog of the human tumor suppressor p53, cep-1, uniquely modulates lifespan and stress response of different Mit mutants, including isp-1 and frh-1. Frh-1 is a nuclear encoded mitochondrial protein involved in Fe-S cluster proteins biogenesis. Isp-1 encodes the Rieske-iron-sulfur subunit of complex III of the MRC and the qm150 allele contains a missense point mutation that reduces protein function. isp-1(qm150) is one of the only available mitochondrial genetic mutant and similar to mild suppression of isp-1 and of other mitochondrial proteins by RNAi knock-down, is slow growing and lives longer thus being a good representative for this class of longevity mutants. We crossed the isp-1(qm150) mutant with a GFP reporter strain where the GFP was cloned under the promoter of the antioxidant gene gst-4 and observed that, similar to frh-1 RNAi, the gst-4 is induced in response to isp-1 suppression and its induction is regulated by cep-1/p53. Maintenance of genome integrity and fidelity is essential for the proper function and survival of all organisms. We therefore utilized the isp-1(qm150);Pgst4::GFP strain to screen a library of 300 RNAi clones, derived from the Arhinger whole genome RNAi library, against DNA repair and replication, chromatin organization and remodeling, cell cycle and checkpoint control genes. We identified few genes (e.g. ung-1, xpf-1, brd-1) specifically affecting viability, development, fertility and gst-4 induction of the isp-1 mutant relative to wild type worms. We are currently assessing their ability to modulate Mit mutants longevity.

336A

Studies towards understanding the function of the transcription factor DAF-16. **Jennifer M A Tullet**, Caroline Araiz, Eugene Schuster, David Gems. Institute of Healthy Ageing, University College London, United Kingdom.

The DAF-16/FoxO transcription factor (TF) mediates the effects of by insulin/IGF-1 signalling on metabolism, stress resistance and aging in C. elegans. Yet how DAF-16 works, including the topology of the transcriptional network that it crowns, remain unclear. Using a combination of chromatin profiling (DNA adenine methyltransferase identification, or DamID) and transcriptional profiling (microarrays) we previously identified a small set of genes where DAF-16 is predicted to both bind to the promoter and regulate transcription (Schuster et al., 2010 Mol. Syst. Biol.). This set is enriched for genes encoding signalling proteins and TFs, including known determinants of longevity, but not somatic maintenance functions (e.g. detoxification, repair). Thus, DAF-16 seems to act within a relatively small transcriptional sub-network activating other regulators of stress resistance and aging, rather than directly regulating terminal effectors of longevity. We are now further defining, validating and functionally testing the predicted DAF-16 transcriptional sub-network.

337B

The mechanisms underlying an age-dependent reversal in the protective capacities of the non-neuronal JNK-homolog KGB-1. **Kwame Twumasi-Boateng**[1], Tim Wang[2], Linda Tsai[2], Lianqun Wu[2], Michael Shapira[1,2]. 1) Graduate Group in Microbiology, UC Berkeley, Berkeley, CA; 2) Department of Integrative Biology, UC Berkeley, Berkeley, CA.

More than five decades ago, the theory of Antagonistic Pleiotropy proposed that positive selection of gene variants with early-life beneficial effects, but late-life detrimental effects is what drives the evolution of aging. Supporting this theory are trade-offs frequently observed between traits affecting early-life features, such as accelerated development or increased fecundity and traits affecting late-life features, such as stress resistance and longevity. However, very little is known about the molecular mechanisms underlying antagonistic pleiotropy and their contribution to aging. The p38 and JNK stress-activated protein kinases have conserved roles in orchestrating stress responses, but the outcome of their activation is highly contextual. Here, we describe apparent antagonistic pleiotropy in the function of the non-neuronal Caenorhabditis elegans JNK-homolog KGB-1. During development KGB-1 confers resistance to heavy metals and protein folding stress. However, we found that in early adulthood *kgb-1* stops contributing to heavy metal protection and instead becomes detrimental, decreasing resistance to heavy metals, infection and also shortens general lifespan. Genetic analyses coupled with fluorescent imaging linked this phenotypic switch to age-dependent antagonistic modulation of DAF-16/FOXO: developing animals showed *kgb-1*-dependent enhancement of DAF-16 nuclear localization, but adults showed a *kgb-1*-dependent decrease, both in nuclear localization and in DAF-16 protein levels. Disruption of Insulin signaling in *age-1* mutants prevented the *kgb-1*-dependent decrease in DAF-16 nuclear localization in adults, but did not inhibit the decrease in DAF-16 protein levels. This suggests both IIS-dependent and IIS-independent effects of KGB-1 on DAF-16 regulation. Whole-genome gene expression analyses delineating the age-dependent transcriptome downstream to KGB-1 activation will elaborate on the gene networks involved in the phenotypic switch.

338C

A conserved JNK/AP-1 module is a key mediator of intermittent fasting-induced longevity in *C. elegans*. **Masaharu Uno**, Sakiko Honjoh, Eisuke Nishida. Kyoto Univ, Kyoto, Japan.

Dietary restriction extends lifespan and delays the age-related physiological decline in many species. Intermittent fasting (IF) is one of the most effective dietary restriction regimens that extends lifespan in *C. elegans* and mammals. In *C. elegans*, the FOXO transcription factor DAF-16 is implicated in fasting-induced gene expression changes and the longevity response to IF; however, the mechanisms that sense and transduce fasting-stress stimuli have remained largely unknown. Here we show that a KGB-1/AP1 (activator protein 1) module is a key signalling pathway that mediates fasting-induced transcriptional changes and IF-induced longevity. Our promoter analysis coupled to genome-wide microarray results has shown that the AP-1-binding site, together with the FOXO-binding site, is highly over-represented in the promoter regions of fasting-induced genes. We find that JUN-1 (*C. elegans* c-Jun) and FOS-1 (*C. elegans* c-Fos), which constitute the AP-1 transcription factor complex, are required for IF-induced longevity. We also find that KGB-1 acts as a direct activator of JUN-1 and FOS-1, is activated in response to fasting, and, among the three *C. elegans* JNKs, is specifically required for IF-induced longevity. Our results demonstrate that most fasting-induced upregulated genes, including almost all of the DAF-16-dependent genes, require KGB-1 and JUN-1 function for their induction, and that the loss of *kgb-1* suppresses the fasting-induced upregulation of DAF-16 target genes without affecting fasting-induced DAF-16 nuclear translocation. These findings identify the evolutionarily conserved JNK/AP-1 module as a key mediator of fasting-stress responses, and suggest a model in which two fasting-induced signalling pathways leading to DAF-16 nuclear translocation and KGB-1/AP-1 activation, respectively, integrate in the nucleus to coordinately mediate fasting-induced transcriptional changes and IF-induced longevity.

339A

A genome-wide RNAi screen identifies alternative pathways for activating phase 2 detoxification gene expression in response to arsenite. Helen M Crook, Monika Oláhová, **Elizabeth A Veal**. Inst Cell & Molecular Biosciences, Newcastle Univ, Newcastle Tyne, United Kingdom.

The conserved role of the phase 2 detoxification system in limiting the damage caused by xenobiotics and reactive oxygen species underlies its importance in stress resistance and longevity. In *C. elegans* the ortholog of the mammalian bZIP transcription factor Nrf2, SKN-1, activates the expression of phase 2 genes such as *gcs-1*, which encodes an enzyme important for glutathione synthesis and arsenite resistance. Several signalling pathways regulate levels of active SKN-1. For example, phosphorylation of SKN-1 by the stress-activated p38-related PMK-1 MAPK plays a critical role in promoting SKN-1 activity to increase phase 2 gene expression and stress resistance (1). However, the increased *gcs-1* expression and arsenite stress resistance associated with loss of the 2-Cys peroxiredoxin PRDX-2 is only partially dependent on SKN-1, suggesting the existence of other mechanisms for regulating phase 2 detoxification gene expression and arsenite resistance (2). Hence, to identify new regulators of phase II detoxification, we have performed a genome-wide RNAi screen for genes required for the elevated expression of a *Pgcs-1::GFP* transcriptional reporter in *prdx-2* mutant worms. 39 of the 170 genes identified by this screen were also found to be required for the arsenite-induced expression of *Pgcs-1::GFP* in wild-type animals. These genes include *tir-1* and *rack-1*, which encode signaling proteins that have been suggested to act upstream of the PMK-1 MAPK to promote wound healing and innate immunity (3). However, our data suggests that both RACK-1 and TIR-1 act to increase *gcs-1* expression and arsenite resistance independently from PMK-1. We will present studies addressing the mechanisms by which RACK-1, TIR-1 and other regulators identified in this screen promote phase 2 gene expression in *prdx-2* mutant animals and in response to arsenite. Our improved understanding of these mechanisms provides important insight into how PRDX-2 and phase II detoxification systems contribute to stress resistance and longevity. 1.Inoue, H., Hisamoto, N., An, J. H., Oliveira, R. P., Nishida, E., Blackwell, T. K., and Matsumoto, K. (2005) Genes Dev 19, 2278-2283 2.Olahova, M., Taylor, S. R., Khazaipoul, S., Wang, J., Morgan, B. A., Matsumoto, K., Blackwell, T. K., and Veal, E. A. (2008) Proc Natl Acad Sci U S A 3.Ziegler, K., Kurz, C. L., Cypowyj, S., Couillault, C., Pophillat, M., Pujol, N., and Ewbank, J. J. (2009) Cell Host Microbe 5, 341-352.

340B

Electron transport chain disruption extends lifespan and reduces fat accumulation through p53-dependent induction of autophagy. A. Schiavi[1], A. Torgovnick[1], E.V. Megalou[2], R. Testi[1], N. Tavernarakis[2], **N. Ventura**[1]. 1) Dept Exp Med & Bioch Sci, Univ Rome,Tor Vergata, Rome, Italy; 2) IMBB, Foundation for Research and Technology, Heraklion 71110, Crete, Greece.

Friedreich's Ataxia (FRDA), the most frequent inherited recessive ataxia, is ascribed to severely defective expression of frataxin, a nuclear-encoded mitochondrial protein. Since frataxin plays a role in the biogenesis of Fe-S cluster containing proteins, which mostly are subunits of the Electron Transport Chain (ETC), frh-1 RNAi-suppressed animals are good representatives of the C.elegans class of mitochondrial mutants. In human, the residual level of frataxin protein is critical for disease onset, progression and severity of symptoms. Noteworthy, non-pathological levels of frataxin deficiency in different species is associated with alteration in genes and proteins expression profiles, suggesting that animals attempt to cope with frataxin decrease by inducing adaptive mitochondrial stress response pathways. Accordingly, we had previously found that RNAi-suppression of the C. elegans frataxin

homolog (frh-1), similar to mild suppression of different ETC components, extends longevity and induces stress responses in a p53-dependent manner. Unraveling molecular mechanism underlying life-extension in response to mitochondrial protein deficiency may reveal novel potential strategies to prevent or postpone the established disease in human. FRDA is characterized by progressive degeneration of sensory neurons. We now found that frh-1 RNAi-suppressed animals display behavioral alterations ascribed to deficits in longevity-modulating sensory neurons and speculated this could trigger a caloric restriction like response. Consistently, we also found that frh-1 RNAi animals have decreased levels of ATP and fat accumulation, and increased autophagy compared to control animals. Autophagy is a form of cellular self-digestion that has been associated both with prevention and causation of neurodegenerative diseases in human, and it is regulated by p53. Autophagic genes are required in C. elegans for normal development and to increase lifespan in different genetic backgrounds, including cep-1 (C. elegans p53 ortholog) mutant animals. Interestingly we now found that frh-1 RNAi prevents maximal induction of autophagy elicited by cep-1 KO. On the other hand, cep-1 is required for frh-1 RNAi induction of autophagy and fat decrease. Moreover, contrary to our expectation, although the extended longevity and decreased fat accumulation following frh-1 RNAi depend on an intact core autophagic pathway (bec-1 and atg-1), they are largely independent from a caloric restriction-like response.

341C

Reproductive Aging in C. elegans Males. I. Chatterjee[1], **P. Vijay[1]**, C. Ibanez-Ventoso[3], J. Bair[1], M. Driscoll[3], A. Singson[1,2]. 1) Waksman Institute, Rutgers, Piscataway, NJ; 2) Dept. of Genetics, Rutgers, Piscataway, NJ; 3) Dept. of Molecular Biology and Genetics, Rutgers, Piscataway, NJ.

Reproductive fitness is key to survival of species. Although much is known about female reproductive aging, fairly little is known about male reproductive senescence. Recent research indicates that advanced paternal age (over 35), similar to advanced maternal age is associated with an increased risk of miscarriages, birth deformities, cancer, and neurodevelopmental disorders. The molecular reasons for this decline are unclear. In today's society it has become increasingly common and often more favorable for couples to wait until later in life to have children, and so, the risks of advanced paternal age are worrisome. Consequently, research in male reproductive aging is warranted. Using Caenorhabditis elegans as a model organism, we have developed assays that measure male mating efficiency with age and its contributing factors. Mating efficiency of wildtype N2 and him-5 males was found to decline after 2 days post L4 (day 2) and reach 0%; by day 7. In other words, fertility of C. elegans males declines with age. Vigor, tail morphology, spermatid morphology, and pharyngeal pumping contribute minimally to reproductive aging. Although mating interest remains high, the ability to mate decreases with age, paralleling the decline in fertility. We also studied mating efficiency using mutants in the insulin/insulin-like growth factor-1 (IGF-1) signaling pathway, and discovered that the longer living mutants age-1;him-5 and daf-2;him-5 have a longer and more robust reproductive span than wildtype him-5. That is, the longer living mutants are able to reproduce for a greater number of days and sire more progeny at each day than wildtype. An interesting methodological fact that allowed us to collect the large number of males necessary for these experiments and age them in isolation from hermaphrodites is that we applied a ring of garlic extract around the edges of our worm plates. This dramatically reduced the number of males lost due to mate searching behavior.

342A

Investigating the function of NAD^+ salvage biosynthesis in energy production and storage. **Wenqing Wang**, Kelsey J. Krebs, Marisa J. Pacella, Wendy Hanna-Rose. Dept of Biochemistry and Molecular Biology, Penn State University, University Park, PA.

NAD^+ is a vital molecule in cellular redox reactions and acts as a cosubstrate for NAD^+ consuming enzymes, which are critical to a variety of biological processes. In *C. elegans*, NAD^+ salvage biosynthesis recycles the nicotinamide (NAM) liberated by NAD^+ consumers to rebuild the NAD^+ reservoir. *pnc-1* is a nicotinamidase in the NAD^+ salvage pathway. It catalyzes the deamination of NAM to nicotinic acid (NA). Our previous studies have shown that NAD^+ biosynthesis contributes to the development and function to multiple muscle types in *C. elegans*. We have also found muscle type-specific response to perturbations of NAD^+ or NAM levels [1]. Muscle is a major energy consuming tissue, and NAD^+ is actively involved in ATP production. Moreover, there is accumulating data on the regulatory roles of the NAD^+ consumer sirtuins in metabolism [2]. Thus we hypothesized that the *pnc-1* mutants may be defective in energy production and/or storage. To address this hypothesis, we treated wild type and *pnc-1* mutant with sodium azide, which inhibits complex IV of the electron transport chain. We found that the *pnc-1* mutant paralyzes faster than wild type upon azide treatment. After removing the worms from azide, *pnc-1* mutants took longer than wild type to begin to recover from paralysis but then recovered to a full muscle capacity at the same rate as wild type. Our observation indicates that *pnc-1* mutant is sensitive to ATP depletion, and is slow in recovery of ATP production. We also examined fat storage in wild type, *pnc-1* mutant and *pnc-1* over-expression strains. At young adult stage, the *pnc-1* mutant stores more fat than wild type, and the *pnc-1* over-expression strain stores less fat than wild type. Thus NAD^+ salvage biosynthesis regulates fat metabolism, which contributes to energy production and storage.

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[2]Imai, S. and Guarente, L. (2010). "Ten years of NAD-dependent SIR2 family deacetylases: implications for metabolic diseases." Trends Pharmacol Sci 31(5): 212-20.

343B

The effects of altered omega-3 fatty acid composition on lifespan and stress. Joel Greggain, Christopher Webster, **Jennifer Watts**. Sch Molec Biosci, Washington State Univ, Pullman, WA.

Polyunsaturated fatty acids play vital roles in the regulation of membrane fluidity, cellular integrity, and cellular communication. Humans require omega-6 and omega-3 fatty acids in their diet (essential fatty acids), because they lack some of the enzymes required to synthesize these fatty acids de novo. While omega-6 and omega-3 fatty acids are both essential for health, it is recognized that Western diets do not contain optimal omega-6:omega-3 fatty acids ratios, and that excess omega-6 fatty acids promote certain cancers and heart disease. Because C. elegans contains all of the enzymes necessary to produce a range of omega-3 and omega-6 fatty acids de novo, and the E. coli diet does not contribute polyunsaturated fatty acids, we can use genetic mutations that lead to altered ratios of omega-3 and omega-6 fatty acids to study the roles of these fatty acids in reproduction, lifespan, and stress responses. In this study we compare brood size, lifespan, and stress responses in three strains: wild type; fat-1 mutants lacking omega-3 fatty acids; and RB969, a strain which overexpresses fat-1, leading to decreased omega-6:omega-3 ratios. Our results demonstrate that changes in omega-6:omega-3 ratios do not have drastic phenotypic effects in worms grown in laboratory conditions, but that worms with both increased and decreased omega-6:omega-3 ratios show slightly shorter lifespans and less robust stress responses, indicating optimal ratios of omega-6:omega-3 ratios have evolved in wild type worms.

344C

Regulation of oocyte quality by the level of germ cell death. **Qing Wei**, Sara Andux, Ronald Ellis. Molec Biol, UMDNJ-SOM, GSBS, Stratford, NJ.

As women age, the quality and quantity of their oocytes declines, resulting in a lower chance of getting pregnant and a higher chance of birth defects. We are using *C. elegans* to study this problem. Previous work showed that oocyte quality decreased with age in *C. elegans* females, and that physiological germ cell deaths maintain oocyte quality, possibly by reducing the number of oocytes that compete for resources.

Although blocking all germ cell deaths leads to poor oocyte quality, we don't know the relationship between the frequency of germ cell death and oocyte quality. Is it linear? Is the level of germ cell deaths in wildtype animals optimal during aging? To answer these questions, we are testing mutants with moderate effects on germ cell death. Mutations that increase the number of deaths include those in the transcription factor *pax-2*, which regulates the expression of *ced-9*; and in *csp-2*, which inhibits *ced-3* autoactivation. Mutations that decrease the number of germ cell deaths include weak alleles of *ced-3*. We found that *pax-2* mutants and *csp-2* mutants produce slightly worse oocytes than the wild type. Moreover, *ced-3* weak alleles also produce oocytes of poorer quality of than the wild type, but not as poor as those of strong *ced-3* mutants. Because either increasing or decreasing the number of germ cells can lower oocyte quality, the relationship between the level of germ cell deaths and oocyte quality is not linear.

Surprisingly, our studies of the DNA-damage-induced cell death pathway show that in some conditions, mutations in *egl-1* and *ced-13* improve oocyte quality in old animals. Since these *egl-1; ced-13* mutants have fewer germ cell deaths than the wild type, the number of germ cell death might not be optimal in older wildtype animals.

We also found that mutants that disrupt the Insulin/IGF signaling pathway improve oocyte quality in older females. However; this effect appears to be independent of apoptosis.

345A

Identifying natural genetic variation in stress and aging pathways in Caenorhabditis remanei populations. **John H. Willis**, Patrick Phillips. CEEB, Univ Oregon, Eugene, OR.

Numerous studies have implicated the insulin/IGF-1 signaling (IIS) cascade as playing a major role in both stress response and aging. In C.elegans, this cascade has been shown to negatively regulate various transcription factors including DAf-16, SKN-1 and HSF-1 in response to environmental conditions such as nutrients, heat and oxidative stress. To date, little is known regarding which, if any of these transcription factors, play a significant role in the observed phenotypic variation in both stress response and lifespan variation observed in natural populations of nematodes. To address these questions we have begun dissecting natural populations of C.remanei to identify the genetic networks that contribute to stress resistance and lifespan variation. First, we are determining the extent of genetic variation downstream of DAF-16 in C.remanei populations. We are utilizing commercially available antibodies against DAF-16 to perform ChIP-Seq experiments under environmental stress conditions and will correlate DAF-16 binding site patterns with total RNA changes as measured by RNA-Seq. In addition, we will are taking a traditional quantitative genetic approach by mapping lifespan variation and stress resistance in a set of recombinant inbreed lines (RIL's) that we are generating. To date, we have 55 lines which exhibit significant phenotypic variation in both lifespan and stress resistance. Progress on both fronts will be presented.

346B

A GATA transcriptional circuit guides aging in *C. elegans*. **Xiao Xu[1,2]**, Stuart Kim[1]. 1) Department of Developmental Biology, Stanford University School of Medicine, Stanford, CA; 2) Cancer Biology Program, Stanford University School of Medicine, Stanford, CA.

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 many of these genes are caused by three GATA transcription factors (*elt-3*, *elt-5* and *elt-6*)(Budovskaya *et al.*, *Cell* 2008). Here, we identify an additional GATA transcription factor, *egl-27*, that plays an important role during the aging process. We found that loss-of-function and gain-of-function alleles of *egl-27* have opposite effects on lifespan; specifically, overexpression of *egl-27* extends lifespan and knockdown of *egl-27* decreases *daf-2* lifespan without affecting wild-type lifespan. These results indicate that levels of *egl-27*(+) play a critical role in promoting longevity. To determine how *egl-27* promotes longevity, we performed ChIP-seq analysis to find EGL-27 targets. We found that EGL-27 targets are highly enriched for genes whose expressions decrease with age, suggesting that EGL-27 plays a direct role in regulating the aging process. Further, we found that slow aging *daf-2* mutants exhibit increased *egl-27* expression while fast aging *elt-3* mutants display decreased *egl-27* expression, suggesting that *egl-27* acts downstream of insulin signaling and GATA transcriptional networks. In addition to the GATA DNA binding domain, EGL-27 is 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 EGL-27 in old worms affect not only the GATA transcriptional network but also chromatin structure in general.

347C

Identification of molecular compensation system of *sod*-genes expression in nematode *C. elegans*. Koumei Yazaki[1], **Sumino Yanase[1,2]**, Taro Sakamoto[3], Yasuhito Nakagawa[3], Naoaki Ishii[2]. 1) Daito Bunka University School of Sports & Health Sci, Higashi-matsuyama, Saitama, Japan; 2) Tokai University School of Medicine, Bohseidai, Isehara, Kanagawa, Japan; 3) School of Pharmaceutical Sciences Kitasato University, Shirokane, Minato-ku, Tokyo, Japan.

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 previously found a functional compensation by the *sod-5* gene in the deletion mutants of *sod-1* gene encoding a Cu/Zn SOD in the nematode *Caenorhabditis elegans* (*C. elegans*). Here, we examined whether levels of *sod-1* gene expression alternatively are increased in the deletion mutant of *sod-5* gene also encoding other Cu/Zn SOD. As a result, not *sod-1* gene, but *sod-2* gene encoding an Mn SOD was induced several fold in the mutant. Likewise, we detected about induction of *sod-2* gene expression in a *sod-1;sod-5* double mutant. It is well known that the *sod-3* and *sod-5* genes are controlled via the insulin/insulin-like growth factor-1 (Ins/IGF-1) signaling pathway, which regulates longevity and stress resistance of *C. elegans*. The mammalian forkhead transcription factor FOXO ortholog, DAF-16, is located downstream on the Ins/IGF-1 signaling pathway. There are the DAF-16 consensus binding element (DBE) sequences, which bind the DAF-16 transcription factor, in each promoter region of *sod-2*, *sod-3* and *sod-5* genes on the DDBJ/GenBank/EMBL International Nucleotide Sequence Database. DBE in the promoter region of *sod-2* gene seemed to barely function using a *daf-16* gene null mutant, *daf-16(mgDf50)* strain, from a previous report. However, we propose that *sod-2* gene is also the target of DAF-16 transcription factor, and is associated with normal aging in *C. elegans* under intracellular stressful condition such as the *sod-1* and *sod-5* genes deletion mutant.

348A

A Mitochondrial Superoxide Signal Triggers Increased Longevity in *C. elegans*. **Wen Yang**, Siegfried Hekimi. Dept Biol, McGill Univ, Montreal, PQ, Canada.

The *nuo-6* and *isp-1* genes of *C. elegans* encode, respectively, subunits of complex I and III of the mitochondrial respiratory chain. Partial loss-of-function mutations in these genes decrease electron transport and greatly increase the longevity of *C. elegans* by a mechanism that is distinct from that induced by reducing their level of expression by RNAi. Electron transport is a major source of the superoxide anion, which in turn generates several types of toxic reactive oxygen species (ROS), and aging is accompanied by increased oxidative stress, which is an imbalance between the generation and detoxification of ROS. These observations have suggested that the longevity of such mitochondrial mutants might result from a reduction in ROS generation, which would be consistent with the mitochondrial oxidative stress theory of aging. It is difficult to measure ROS directly in living animals, and this has held back progress in determining their function in aging. Here we have adapted a technique of flow cytometry to directly measure ROS levels in isolated mitochondria to show that the generation of superoxide is elevated in the *nuo-6* and *isp-1* mitochondrial mutants, although overall ROS levels are not, and oxidative stress is low. Furthermore, we show that this elevation is necessary and sufficient to increase longevity, as it is abolished by the antioxidants NAC and vitamin C, and phenocopied by mild treatment with the prooxidant paraquat. Moreover, the results of treating a variety of short- and long-lived mutants with NAC and paraquat suggest that the pathway triggered by mitochondrial superoxide is distinct from previously studied mechanisms, including insulin signalling, dietary restriction, ubiquinone deficiency, the hypoxic response, and hormesis. These findings show that increased superoxide generation acts as a signal to trigger a distinct mechanism that prevents or attenuates the effects of subsequent aging. We propose that superoxide is generated as a protective signal in response to molecular damage sustained during wild-type aging as well. This model provides a new explanation for the well-documented correlation between ROS and the aged phenotype as a gradual increase of molecular damage during aging would trigger a gradually stronger ROS response.

349B

Nuclear Envelope Proteins: Regulation of Meiotic Recombination and Role in Reproductive Aging. **Alice Y. Ye**, Needhi Bhalla. Department of Molecular, Cell, and Developmental Biology, UC Santa Cruz, Santa Cruz, CA.

Deterioration of reproductive fitness with age is a common trait in many organisms, where aging results in both fewer progeny and an increased risk of defects. Although many of the mechanisms that underlie reproductive aging are not well understood, it is thought that errors in meiosis contribute. Meiosis is the specialized cell division that generates gametes for sexual reproduction, and is distinguished by its dependence on accurate meiotic recombination for proper chromosome segregation. A family of nuclear envelope proteins involved in tethering chromatin to the nuclear envelope - the LEM proteins - has been implicated in both meiotic recombination and somatic aging. There are three LEM family proteins in *C. elegans*: EMR-1, LEM-2, and LEM-3. Using cytological assays we have established that a subset of the LEM protein family regulates recombination. Loss of EMR-1 or LEM-2, but not LEM-3, activates a DNA damage checkpoint due to errors in meiotic recombination. We have also found that loss of either of these proteins delays the appearance of RAD-51, a protein involved in the repair of double-strand breaks, in meiotic nuclei. This raises the possibility that mutation of *emr-1* or *lem-2* affects double-strand break formation, RAD-51 recruitment to double-strand breaks, or both. In contrast to *emr-1* mutants, *lem-2* mutants are also delayed in the repair of double-strand breaks. This defect appears to result in reproductive defects: *lem-2* mutants have a reduced number and viability of progeny, and the severity of these defects worsens with age. Given the link between nuclear integrity and aspects of aging, this data suggests that the nuclear envelope regulates meiotic recombination and that this regulation might contribute to reproductive aging.

350C

Dissecting the interaction between insulin/IGF-1 and TOR signaling pathways. **Kelvin Yen[1]**, Heidi Tissenbaum[1,2]. 1) Program in Gene Function and Expression, University of Massachusetts Medical School, Boston, MA; 2) Program in Molecular Medicine, University of Massachusetts Medical School, Boston, MA.

Both the insulin/IGF-1 signaling and the TOR signaling pathways are known to regulate lifespan and feeding/fat in multiple species (1,2). Despite the fact that both pathways monitor energy levels and have been shown to have interconnected signaling cascades, lifespan analysis suggests that these pathways are independent of each other. We have analyzed single mutants and generated double mutant combinations of the insulin/IGF-1 pathway and the TOR pathway components. We have tested these strains for lifespan, lipid storage and protein phosphorylation status. We will present our data that suggest that there are multiple potential interaction points of the pathways. 1. Narasimhan, S., Yen, K., Tissenbaum, H. Converging Pathways in Lifespan Regulation. Current Biology 19, R657-R666 (2009). 2. Kapahi, P., et al. With TOR, Less Is More: A Key Role for the Conserved Nutrient-Sensing TOR Pathway in Aging. Cell Metabolism 11, 453-465 (2010).

351A

Deciphering mechanisms by which EGF signaling protects against the aging process. **Simon Yu**, Hiroaki Iwasa, Jian Xue, Monica Driscoll. Dept Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ.

We previously reported that EGF signaling confers a positive effect on multiple aging phenotypes, acting through the downstream branch of the EGF pathway involving PLCγand the IP3 receptor (Iwasa *et al*., 2010).To better understand operative mechanisms, we are analyzing mechanistic details of this healthspan pathway, looking at ligand requirements, testing other signaling components, and screening for downstream effectors. EGF ligand acts throughout development to influence healthy aging. *lin-3* encodes the *C. elegans* EGF ligand, which can be expressed as multiple isoforms due to alternative splicing (Dutt *et al*., 2004, Van Buskirk *et al*., 2007). Little attention has been given to adult expression pattern. We followed a *lin-3::gfp* fusion in adults and conclude that *lin-3* is expressed late into adult life in the pharynx.We tested for effects of over-expression of the intact gene and individual isoforms to address whether any one might promote locomotory healthspan extension and lifespan. We identify two of the individual LIN-3 isoforms that show anti-aging effects. Another question of interest is: when does LIN-3/EGF act to influence aging quality? We used RNAi approaches to show that LIN-3 is required both at larval stages and adult stages to influence maintained swimming performance and lifespan late in life. Implications of this finding are that: 1) EGF promotes maintenance throughout life, 2) effects of EGF signaling in development can have an impact later in life, and 3) EGF acts during adulthood to promote healthy aging. Another unresolved question is in what tissues does EGF signaling act to promote healthy aging. We expressed the *let-23(gf)* EGF receptor in specific tissues and asked whether age-associated mobility decline and lifespan are beneficially affected. We found ectopic expression of *let-23(gf)* in the intestine, neuron, and muscle can promote healthier aging phenotypes. Our previous data show that activated EGFR acts via increased IP3 activity through the IP3 receptor ER calcium release channel to confer healthspan benefits. We found that other known factors that modulate EGFR (*ark-1*) and control IP3 signaling and ER calcium release, including kinase (*lfe-2*) and phosphatase (*ipp-5*) can influence healthspan, consistent with the involvement of the core pathway in healthy aging. Searches for downstream factors that might mediate calcium signaling coming from EGF activation. Our data implicate appropriate/maintained calcium homeostasis in healthy aging. Though many factors could execute ER release and downstream calcium signaling, we identified one possible calcium-sensitive transcription factor downstream of IP3R in the EGF pathway that can regulate the effects on locomotory healthspan.

352B

The role of GATA transcription factors *elt-5* and *elt-6* in aging. **Stephanie M Zimmerman**, Yelena V Budovskaya, Stuart K Kim. Department of Genetics, Stanford University, Stanford, CA.

We previously found that three GATA transcription factors (*elt-3, elt-5, elt-6*) are responsible for much of the changes in gene expression in old age (Budovskaya et al 2008). The expression of *elt-3* declines with age due to increasing repression from *elt-5*. Resetting *elt-3* expression to high levels in old animals by *elt-5(RNAi)* extends lifespan by 15-20%. During development, *elt-3*, *elt-5*, and *elt-6* have important roles in the seam cells and hypodermis, suggesting that aging is caused by a drift of developmental pathways and not simply an accumulation of damage. To test this hypothesis, we are performing ChIP-seq and RNA-seq experiments on *elt-5* during aging, and will compare these results to *elt-5* developmental targets to determine whether the targets of *elt-5* in aging are equivalent to those during development. Single cell expression analysis of *elt-5* indicates that its expression in adults is restricted to the seam cells. In order to examine how different tissues interact during aging, I am examining whether the effects of *elt-5(RNAi)* in the seam cells can rejuvenate other tissues by examining the expression of aging biomarkers for the muscle (*unc-54:GFP*), the intestine (*sod-3:GFP*) and the pharynx (*pha-4:GFP*).

353C

Small molecule modulators of dauer signaling pathways and life span. **Ben Becker**, Adam Antebi. Molecular Genetics of Ageing, MPI for Biology of Ageing, Cologne, Germany.

C. elegans enter the stress-resistant long-lived dauer stage in response to harsh environmental conditions such as high temperature, food scarcity, or high population density. Multiple pathways control dauer entry during development and interestingly these pathways also modulate adult lifespan. In brief, the insulin/IGF-1-like signaling (IIS) pathway, together with TGF-β, induces a hormone biosynthetic pathway that converts cholesterol into bile-acid like steroids called the dafachronic acids (DA). DAs are endogenous ligands of the nuclear hormone receptor DAF-12, a key determinant of dauer formation, and promote reproductive development and short life. Conversely, when these pathways are downregulated the unliganded DAF-12 promotes dauer formation and long life. Little is known about other endogenous signaling molecules that modulate these well-characterized pathways. Small molecules involved in energy homeostasis such as sugars, amino acids, citric acid cycle intermediates, monounsaturated and polyunsaturated fatty acids are potential candidates to function as signaling molecules in this context, because their endogenous availability could reflect environmental conditions. C. elegans is auxotroph for cholesterol, the major precursor for sterol metabolism and therefore sterols are additional candidate modulators of the dauer decision. We are performing metabolite feeding experiments and use changes in dauer formation rates as a read out. The screen is done with *daf-2(e1368)* animals, carrying a mutation in the insulin receptor homologue DAF-2 that leads to increased dauer formation and longevity. In this sensitized background small changes in dauer signaling pathways will lead to a detectable alteration in dauer formation rates. As many dauer formation pathways also regulate lifespan, the candidate small molecules are analyzed in a secondary screen for changes in lifespan. To date, we have found several interesting sterols, which, at low concentrations, double the dauer fraction of *daf-2(e1368)* animals at 22.5 °C. Subsequent studies will determine the molecular mechanism and the physiological relevance for *C. elegans* health- and lifespan.

354A

CYP-35A5 and NHR-88 act together to coordinate brood size. **Alison Brooks**[1,2], Marc Van Gilst[1]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Molecular and Cellular Biology, University of Washington, Seattle, WA.

Optimizing fecundity in *Caenorhabditis elegans* necessitates tuning the allocation of nutrients to growth, longevity, and progeny production. Thus, it is often proposed that these traits are mutually exclusive under optimized conditions. Accordingly, crosstalk between the germline and soma in lifespan regulation has been extensively studied. In a screen for modifiers of NHR-49 activities, we identified two proteins that impact progeny production: the nuclear hormone receptor NHR-88 and the cytochrome P450 CYP-35A5. Initial mutant characterization showed that *nhr-88(tm1033)* does not affect progeny production, while the *cyp-35a5(ok1985)* allele decreases total brood. Analysis of the germline in *cyp-35a5* mutants revealed a reduction in the number of germ cells and a shortened mitotic region. Surprisingly, a strain carrying mutations in both *nhr-88* and *cyp-35a5* not only rescued the brood size defect of the *cyp-35a5* single mutant, but increased progeny production to 35% over that of wildtype. This dramatic effect on fecundity is seen despite incomplete rescue of germline phenotypes. Interestingly, the role of the *nhr-88* mutation in increasing brood size in a *cyp-35a5* background is temperature-sensitive, showing an increase only at moderate growth temperatures.

Consistent with the idea of an inverse correlation between fecundity and longevity, we see that the reduced germline in *cyp-35a5* mutants corresponds to a 17% increase in mean lifespan. However, the *nhr-88;cyp-35a5* double mutant shows a modest increase in lifespan while also increasing brood size, suggesting that the number of mitotic germ cells may have a stronger effect on longevity than the number of progeny produced. We propose that these genes act together to affect brood production by altering proliferation in the germline via a shift in the mitosis-to-meiosis transition zone. Additionally, *cyp-35a5* animals contain high levels of several polyunsaturated fatty acids, many of which have been shown to affect germ cell proliferation or brood size. Our data are consistent with a model in which CYP-35A5 negatively regulates NHR-88 to delay exit from mitosis, possibly by destroying an NHR-88 agonist. Further studies are underway to determine whether the NHR-88-dependent impact of CYP-35A5 on the germline is mediated by these fatty acid species.

355B

Role of G-protein coupled receptor signaling in C. elegans fat metabolism. **Claudio Comunian**, Tallie Noble, Supriya Srinivasan. Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

The sensory perception of the surrounding environment and the metabolic and behavioral adaptation to its changes are essential tasks for the survival of every organism. In C. elegans, environmental perception is mediated predominantly by sixty ciliated sensory neurons. Many signaling systems operate within these neurons to maintain energy balance, including GPCR-mediated pathways. The C. elegans genome encodes all of the components of canonical GPCR signaling pathways with significant homology to their mammalian counterparts, however their roles in energy balance and fat metabolism are largely unknown. We are systematically investigating the role of the different components of GPCR pathways with the goal of identifying signaling cascades that couple behavioral and metabolic adaptations to environmental conditions.

356C

Do Dauers differ ? - Comparative analysis of Dauer proteome and metabolome signatures in three nematode species. Zisong Chang[1], Guido Mastrobuoni[1], Hans-Joachim Knölker[2], Stefan Kempa[1], **Christoph Dieterich**[1]. 1) Berlin Insitute for Medical Systems Biology at the Max Delbrück Center for Molecular Medicine Berlin, Robert-Roessle-Str. 10, 13125 Berlin, Germany; 2) Department of Chemistry, Technical University Dresden, Bergstrasse 66, 01069 Dresden, Germany.

The life cycle of *Caenorhabditis elegans* proceeds through four larval stages. However, L2 larvae sense their environment and enter an alternative developmental stage, the Dauer stage, if adverse conditions such as high temperature, low food supply or high population densities prevail. Dauer larvae are non-feeding, stress resistant and long-lived. Dauer larvae morphologically resemble the infective larvae of parasitic nematodes. Intriguingly, a conserved endocrine mechanism controls the formation of Dauer as well as Infective larvae in nematodes. This conservation represents a fundamental link between Dauer and Infective larvae. In essence, we speculate that the Dauer stage could be a pre-adaptation for parasitism. If so, we hypothesize that Dauer larvae, Infective larvae and putative intermediate forms show similar molecular signatures.

We have established unlabeled quantitative methods to profile protein and metabolite abundance levels by shotgun LC-MS/MS and GC-MS techniques. Using these methods, we compared Dauer larvae to a mixture of non-Dauer stages in three different clade V species: *Caenorhabditis elegans*, *Caenorhabditis briggsae* and *Pristionchus pacificus*. Caenorhabditis and Pristionchus species diverged more than 250 million years ago and occupy different ecological niches. Especially, the genus Pristionchus shows species-specific associations with scarab beetles.

In general, we observe that Dauer larvae are depleted in proteins involved in RNA translation, Aminoacyl-tRNA biosynthesis and protein degradation. However, proteins involved in the central carbon metabolisms (e.g. Glycolysis / Gluconeogenesis, Citrate cycle and Oxidative phosphorylation) were highly abundant in Dauer larvae. Species-specific protein signatures will be presented as well. Metabolite levels show some surprising differences across species, which are in line with the phylogenetic tree. For example, Phosphoserine, a metabotropic glutamate receptor agonist, was found to be 10-100x fold enriched in Dauer larvae compared to Mixed Stage samples.

To our knowledge, we are the first to present an in-depth “shotgun” study on protein and metabolite abundance levels across nematode Dauer larvae. We will extend our efforts towards infective larvae from truly parasitic nematodes.

357A

Functions of Acyl-CoA Binding Proteins in *C. elegans*. **Ida C. Elle**[1], Pernille K. Birck[1], Thuc T. Le[2], Nils J. Faergeman[1]. 1) Dept. of Biochemistry and MolecularBiology, University of Southern Denmark, Odense M, Fyn, Denmark; 2) Nevada Cancer Insitute, 1800 West Charleston Boulevard Las Vegas, NV 89102 USA.

Acyl-CoA esters, the metabolically active form of fatty acids, are important intermediates in both anabolic and catabolic processes, but have also been identified as regulators of ion channels, enzymes, membrane fusion, and gene expression. Acyl-CoA binding protein (ACBP) is a small, primarily cytosolic protein, which binds acyl-CoA esters with high specificity and affinity. ACBP has been identified in all eukaryotic species, and the gene encoding the basal form displays all the hallmarks of a housekeeping gene, indicating that ACBP performs a basal cellular function. However, the existence of several ACBP paralogues in many eukaryotic species and differential tissue expression indicate that these proteins serve distinct functions. Studies in other model organisms have implicated ACBPs in triglyceride storage, lipid synthesis, autophagy, cold tolerance, and skin barrier function. C. elegans expresses seven functional ACBPs; four basal forms and three ACBP domain proteins. In the present study, we have obtained mutants with functional loss of each of six of the ACBPs and characterized their macroscopic and biochemical phenotypes. The seventh paralogue Membrane-Associated ACBP-1 has been shown to be involved in endosomal vesicle transport. We find that each of the six paralogues is capable of complementing growth of ACBP-deficient yeast cells, and that they exhibit distinct temporal and tissue expression patterns. All acbp single mutants display only subtle phenotypes, likely due to compensatory mechanisms and some extent of functional redundancy. ACBP-1 is shown to be involved in triglyceride storage and lipid droplet morphology. ACBP-2, containing an enoyl-CoA hydratase domain, is necessary for β-oxidation of unsaturated fatty acids, and ACBP-3 is needed for normal skin barrier function. We have generated a quadruple mutant, which we are currently investigating. This mutant is developmentally delayed compared to N2, and preliminary data suggest that it increases its

β-oxidation of exogenous fatty acids, suggesting that lack of all basal ACBPs elicits a starvation-like phenotype.

358B

Defining the role of ABC transporters in vitamin B12 metabolism using the model organism *Caenorhabditis elegans*. **Julie-Anne Fritz**[1], Megan McDonald[1], Padmaja Dharwadkar[1], Deborah Scheuchner[2], Floyd Snyder[2], Avalyn Lewis[3], Liang Li[3], Roy Gravel[1]. 1) Department of Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada; 2) Departments of Medical Genetics and Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada; 3) Chemistry Department, University of Alberta, Edmonton, AB, Canada.

Vitamin B12 (cobalamin; Cbl) is a micronutrient essential to human health. Genetic defects in eight complementation groups involved in the pathway for intracellular utilization of Cbl in humans have led to delineation of much of the subcellular pathway leading to Cbl cofactor synthesis. Nevertheless, the pathway remains incomplete, with genes governing mitochondrial transport of Cbl and possibly a mitochondrial reductase yet to be identified. Recently, the ABC transporter, MRP-1, was identified as a Cbl intestinal efflux transporter. Mutant mice lacking MRP-1 ($Mrp1^{(-/-)}$), however, display only diminished Cbl transport but no overt phenotype, suggesting multiple transport mechanisms involved in Cbl transport. We have chosen *C. elegans* as a model organism to define the role of ABC transporters in Cbl metabolism due to the organism's ease of use and wide availability of genetic tools. Importantly, the *C. elegans* genome contains 61 ABC transporters including an MRP-1 homolog and is ideal for modeling intestinal transport across the gut epithelium. We have screened 42 available ABC transporter mutant strains using a GC-MS based assay that detects the excretion of methylmalonic acid (MMA), which accumulates under Cbl deficiency. Null mutations in either MRP-1, MRP-2 or PGP-2 result in reduced excretion of MMA compared to wild-type worms. MRP-1 and MRP-2 are localized to the *C. elegans* intestine and may act redundantly as Cbl intestinal efflux transporters. PGP-2 is involved in intestinal granule biogenesis and may affect Cbl transport indirectly. In contrast, a null mutation in WHT-6 results in increased excretion of MMA compared to wild-type worms. WHT-6 is also localized to the intestine and may represent either a lysosomal or mitochondrial transporter. Further experiments, including the analysis of cytosolic homocysteine levels and the relevance to human Cbl transport, are underway. Overall, these studies suggest multiple ABC transporter involvement in Cbl transport and may lead to the identification of a mitochondrial Cbl transporter.

359C

Prostaglandin signaling in dauer formation and aging. **Makoto Horikawa**, Adam Antebi. Max Planck Institute for Biology of Ageing, Cologne, Germany.

Recent studies reveal a role of fatty acid metabolism in ageing. Oleic acid synthesis influences longevity in the gonadal pathway and polyunsaturated fatty acids (PUFA) have the potential to regulate insulin/IGF-1 signal (IIS). However, the function of fatty acids in ageing is poorly understood. Prostaglandins (PGs) are known as local hormones synthesized from PUFA, which are involved in inflammation and thermogenesis in mammals. Millller and colleagues recently showed that PGs contribute to sperm guidance in worms. We hypothesized that PGs can act as signaling molecules downstream of PUFA metabolism and influence IIS. Several homologs of PG synthase are present in worms: gst family, PGDS; R11A8.5, a PGES-2; ZC395.10, a p23/PGES-3; and the aldo-keto reductase family, PGFS. To begin a functional analysis, we first looked into dauer formation of mutants of the various homologs. We found that *p23(ok3052)* potently formed dauer at 27°C, whereas the other mutants did not. We then performed epistasis analysis to investigate how *p23* regulates dauer formation. We found that the Daf-c phenotype of *p23(ok3052)* was suppressed by Daf-d mutants in *daf-10*(sensory neuron), *daf-12*(steroid signal) and *daf-16*(IIS) mutations, but not *daf-5*(TGF-β). p23 is also known as a co-chaperone of HSP-90, which is encoded by the worm *daf-21* gene. It was previously shown that *daf-21(p673)* is Daf-c, and work at a similar step in dauer neurosensory processing as *daf-11*/guanylyl cyclase. It was revealed that dauer formation of both *daf-11(m47)* and *daf-21(p673)* is suppressed with *daf-10*, *daf-12* and *daf-16* mutations, and by cGMP treatment. We thus hypothesized that *p23* works in a complex with *daf-21*, to regulate dauer neurosensory processing either through its activity as a PGES-3 or as a chaperone complex. To test this hypothesis, we next analyzed interactions between *p23*, *daf-21* and cGMP signal. Surprisingly, cGMP did not rescue the Daf-c phenotypes of *p23(ok3052)*. We also performed the dauer formation assay with double mutants. We found that *p23(ok3052)* modestly enhanced dauer formation of *daf-21(p673)*. Unexpectedly, mutations of *pges-2* and *gst-4* enhanced *daf-21* dauer formation more strongly than *p23;daf-21*. These results suggest that *p23* regulates dauer but not via cGMP signal, and that *pges-2* and *gst-4* might have an unknown associated function with *daf-21* upstream of cGMP signal pathways. Finally, we analyzed longevity in these mutants. No significant changes were observed in any mutants at 20°C, whereas *p23* and *pges-2* mutants were significantly short lived at 15°C. We speculate that PGs signal has an unknown function in thermosensory neurons, and could be involved in ageing due to temperature adaptation mechanisms.

360A

GPCR signaling in free-living and parasitic nematode models: the cholecystokinin story. **T. Janssen**[1], L. Peeters[1], I. Beets[1], L. Temmerman[1], E. Meelkop[1], N. Suetens[1], W. Grant[2], L. Schoofs[1]. 1) Functional Genomics and Proteomics Unit, Department of Biology, KULeuven, Leuven, Belgium; 2) Nematode Functional Genomics lab, Department of Genetics, La Trobe University, Melbourne, Australia.

Members of the cholecystokinin/gastrin family of peptides, including the arthropod sulfakinins, and their cognate receptors, play an important role in the regulation of feeding behavior and energy homeostasis. The *C. elegans* genome contains one gene (two splice-isoforms) with strong homology to the mammalian cholecystokinin receptors and their invertebrate counterparts, the sulfakinin receptors. By using these potential C. elegans CCK receptors as bait, we have isolated and identified two CCK-like neuropeptides encoded by a peptide precursor protein called NLP-12, as the endogenous ligands of these receptors (Janssen *et al.* 2008, 2009, 2010). The NLP-12 peptides have a very limited neuronal expression pattern and seem to be very well conserved within four clades of nematodes. Both receptors and ligands share a high degree of structural similarity with their vertebrate and arthropod counterparts and also display similar biological activities with respect to digestive enzyme secretion and fat storage. Our recent microarray studies revealed that *C. elegans* CK signaling directly or indirectly affects the expression of several hundred genes, many of which are involved in sugar and fat metabolism and transport, reproduction and the response to starvation. These effects seem to be highly dependent on the animal's feeding status. The expression of the nematode CK system (receptor/ligand) itself is also reversibly upregulated under starvation, which is the opposite of the situation in mammals where feeding stimulates CCK expression and secretion. In addition, food-consumption tests revealed an inhibitory effect of nematode CK signaling on satiety, which is the complete opposite of their function in vertebrates and arthropods. It seems that some of the CCK functions have been conserved during evolution (i.e. related to fat storage, digestive enzyme secretion) whereas others have changed (i.e. opposite effects on satiety). Also L1 diapause and arrested dauer animals show an astonishing increase in CK receptor and ligand transcript levels. Many similarities exist between the arrested dauer larvae of free-living nematodes and the infective L3 stage of parasitic nematodes. This has led to suggestions that they are analogous lifecycle stages and has triggered us to investigate whether the *nlp-12/ckr-2* system is present and differentially expressed in infectious (iL3) or parasitic stage animals of the parasitic nematode *Parastrongyloides trichosuri*.

361B

ENPL-1/GRP94 interacts with ASNA-1 and promotes Insulin/IGF secretion in *Caenorhabditis elegans*. Balasubramanian Natarajan, Peter Naredi, **Gautam Kao**. Surgery, Molecular Medicine Center, Building 6M 4th Floor, Umea University, Umea, Sweden.

The HSP90 family member endoplasmin/GRP94 is a chaperone that is specialized for the maturation of secreted proteins. In mice endoplasmin is required for the secretion of IGFII. However there has so far not been a demonstration for a role of this protein in insulin signaling. We have analyzed the function of ENPL-1/endoplasmin in *C.elegans* for which two deletion mutants are available. *enpl-1* mutants are sterile, scrawny and starved in appearance. *enpl-1* mutants have a reduction in the strength of insulin signaling and this deficit may explain in part the starved appearance of the mutants. Analysis of insulin secretion in *enpl-1* mutants reveals that there is also a severe defect DAF-28:GFP secretion. We have previously studied the ASNA-1 gene and shown that it is a positive regulator of DAF-28 secretion. Since the constellation of phenotypes observed in *enpl-1* mutants are also seen in *asna-1* mutants we looked for a possible interaction between *asna-1* and *enpl-1*. Using yeast 2 hybrid analysis and GST pulldown techniques, we find that ENPL-1 binds to ASNA-1 and have defined the subdomain in ENPL-1 that is required for the binding. Western blot analysis shows that the ASNA-1 protein is destabilized in *enpl-1* mutants, but over-expression of ASNA-1 in *enpl-1* mutants is not sufficient to rescue the growth defects indicating that if they act together, ASNA-1 needs ENPL-1 for its activity. Further, *enpl-1;asna-1* double mutants display synthetic lethality. While both mutants have elevated ER stress levels, this aspect of the phenotype *per se* is likely not the cause of the DAF-28 secretion defect since *hsp-3* mutants, which have similarly elevated ER stress levels, secrete DAF-28 normally. ENPL-1 might act by regulating insulin levels because over expression of the insulins INS-4 and DAF-28 causes a partial rescue of the germ line phenotype of ENPL-1. Taken together we have identified a role for the chaperone ENPL-1/endoplasmin as a new positive regulator of insulin signaling that acts at the level of insulin availability and have shown that it acts in concert with ASNA-1. The findings suggest that ASNA-1 may act as a co-chaperone for ENPL-1 and that it needs the activity of ENPL-1 to promote insulin secretion.

362C

Data mining of global interactome studies yields new modulators of *C.elegans* insulin signaling. Ola Billing, Balasubramanian Natarajan, Ateequr Rahman Mohammed, Peter Naredi, **Gautam Kao**. Surgery, Molecular Medicine Center, Building 6M 4th Floor, Umea University, Umea, Sweden.

ASNA1 is a highly conserved ATPase involved in a seemingly wide range of functions including metal resistance, growth control, insulin secretion and the targeting of tail-anchored proteins to membranes. In *C. elegans*, depleting ASNA-1 results in reduced insulin/IGF signalling (IIS) and in a growth arrest at the L1 stage without any apparent feeding defects. We wished to identify novel interactors to ASNA-1 that may shed light on the various roles of ASNA-1. Genetic studies, global yeast two-hybrid studies and co-immunoprecipitation studies have led to the identification of proteins that interact with ASNA1 homologues in various model systems. We have undertaken a feeding RNAi screen on the homologues of these genes in *C. elegans* to identify genes that, like *asna-1*, cause a larval growth arrest in stage L1 with reduced IlS upon depletion by RNAi. Using bioinformatics we assembled a list of 143 possible ASNA-1-interactors, based on interaction maps from flies, worms and yeast. By means of feeding RNAi against 98 of these genes, we were able to identify six that upon inactivation caused *asna-1*-like L1 arrests: *rps-0* (ribosomal SA subunit B0393.1), T23B12.3 (mitochondrial ribosome S2 subunit) *iars-1* (iso-leucine aminoacyl tRNA synthetase), *ykt-6* (synaptobrevin), F41C3.4

(homolog of the human vesicle transport gene Got1), and *goa-1* (G protein alpha subunit Go). RNAi against two of those genes, *rps-0* and T23B12.3 led to decreased insulin signaling (using the DAF-16:GFP localization assay) in addition to the larval arrest. RNAi against T23B12.3 enhances synthetic semi-dauer formation in *daf-7(e1372)* mutants and enhances the L1 arrest phenotype of *asna-1* mutants in a sensitized background. *iars-1(rnai)* produces a strong L1 arrest without any feeding defects, but we are unable so far to find any evidence for an insulin signaling defect in the worms depleted for the gene activity. *ykt-6(rnai)* animals show defects in insulin signaling but also display moderate feeding defects as well. RNAi against *goa-1* and the GOT1 homolog led to severe feeding defects and were not considered further. Both *rps-0* and T23B12.3 are evolutionarily well conserved, raising the possibility that our screen may identify candidates that affect insulin signaling in vertebrates.

363A

Neuronal secretion of *C. elegans* DAF-28/insulin requires functional mitochondria. Ola Billing, Peter Naredi, **Gautam Kao**. Surgery, Umea University, Building 6M 4th Floor, Umea, Sweden.

The *C.elegans* genome encodes 40 insulin/IGF like peptides. They control diverse functions such as growth regulation in response to nutrient levels, exit from the dauer state, germ line proliferation, associative learning and aging. It is an open question whether some insulins function more like mammalian insulins - which regulate metabolic homeostasis, or like IGFs which control development. One feature which distinguishes insulins from IGFs is that the secretion of insulins, but not IGFs, is dependent on mitochondrial function since the critical first step in the secretion process is the closure of a ATP gated potassium channel which is dependent in turn on the proper mitochondrially generated ATP/ADP ratio. We asked whether the secretion of DAF-28/insulin, which is responsive to metabolic fluctuations, requires mitochondrial function. The *tomm-40* gene was targeted by RNAi and by a deletion mutation in order to create a loss-of-mitochondria phenotype. *tomm-40* encodes the pore through which all nucleus encoded proteins enter the mitochondrion. We find that animals with reduced levels of the mitochondrial outer membrane translocase homologue TOMM-40 arrest growth as larvae and have decreased insulin signaling. TOMM-40 acts as a mitochondrial translocase in *C. elegans* and in its absence animals fail to import a mitochondrial protein reporter across the mitochondrial membrane(s). Inactivation of *tomm-40* evokes the mitochondrial unfolded protein response and causes a collapse of the proton gradient across the inner mitochondrial membrane. Consequently these broadly dysfunctional mitochondria render an inability to couple food abundance to secretion of DAF-28/insulin. The secretion defect is not general in nature since two other tagged neuropeptides, ANF::GFP and INS-22::VENUS, are secreted normally. Further, coelomocytes in *tomm-40(rnai)* animals are competent to take up secreted proteins when the gene is inactivated at levels sufficient to compromise DAF-28:GFP secretion (but not produce the larval arrest phenotype). RNAi against two other putative members of the TOMM complex give similar phenotypes, implying that DAF-28 secretion is sensitive to mitochondrial dysfunction in general. Overexpression of DAF-28 in *tomm-40(rnai)* animals reverses their insulin signaling defect and inactivation of *tomm-40* function in daf-28 expressing neurons by transgene driven hairpin RNAi phenocopies the insulin signaling defect. We conclude that the insulin signaling defect seen in *tomm-40(rnai)* animals is due to a defect in DAF-28 secretion and that mitochondrial function is required for *C. elegans* to secrete DAF-28/insulin. Modulation of secretion likely represents an additional level of control over DAF-28 function.

364B

HRG-9 regulates systemic heme homeostasis in *C. elegans*. **T. Korolnek**, I. Hamza. University of Maryland, College Park, MD.

Hemes are metalloporphyrins that function as protein cofactors in almost every cellular process. Heme is also a significant source of bioavailable iron in the human diet. While the biosynthesis of heme has been well-characterized, the pathways for inter- and intracellular heme transport remain poorly understood. Because free heme is hydrophobic and toxic, we postulate that heme homeostasis is regulated by a coordinated network of heme transporters and chaperones. *C. elegans* does not synthesize heme but has a dietary requirement for environmental heme, thus making it an ideal animal model to identify heme uptake and trafficking pathways. Functional RNAi screens in our laboratory implicated *hrg-9* as a potential heme transporter in *C. elegans*. *hrg-9* encodes an ABC-type transporter that is expressed in most worm tissues and is transcriptionally regulated by heme. Worms lacking *hrg-9* were unable to lay viable eggs, a phenotype that was fully suppressed by heme supplementation. Depletion of *hrg-9* rescued worms from the lethality associated with ingesting gallium protoporphyrin IX, a toxic heme analog, indicating that *hrg-9* plays a role in transporting heme into the worm body. Furthermore, *hrg-9* RNAi worms accumulated the fluorescent heme analog, zinc mesoporphyrin, in intestinal cells, indicating a defect in heme export from the intestine. Functional assays in yeast support the hypothesis that HRG-9 is capable of exporting heme across cell membranes. Expression of HRG-9 in a yeast strain deficient for heme synthesis resulted in decreased growth in the presence of exogenous heme. Consistent with the reduced growth, HRG-9 expression caused lowered heme levels in the cytosol with a concomitant increase in heme levels in the secretory pathway, suggesting a role for HRG-9 in transporting heme from the cytosol into the secretory compartment. Altogether, our results implicate HRG-9 as a key regulator of systemic heme homeostasis in *C. elegans* that exports heme from the intestine to other tissues.

365C

Y47D7A.16: a riboflavin tranporter in *C. elegans* and homolog of human Riboflavin Transporter 2. **Craig W. Lamunyon**[1], Arundhati Biswas[2,3], Jason Rothman[1], Hamid Said[2,3]. 1) Dept Biological Sci, California State Polytech Univ, Pomona, CA; 2) Veterans Affairs Medical Center, Long Beach, CA; 3) Dept Medicine and Physiology, Univ of CA, Irvine, CA.

The *C. elegans* gene Y47D7A.16 is an ortholog of isoform 1 of the human Riboflavin Transporter 2 vitamin transporter. We became interested in this gene as the riboflavin transporter in *C. elegans*. Cloning of the cDNA revealed an exonic structure different from that predicted and an alternative splice in the first exon. Expression of the cDNA in human ARPE19 retinal cells showed enhanced riboflavin uptake, establishing the protein as a functional transporter of riboflavin (cRFT). Uptake by cRFT was pH dependent (uptake at buffer pH 5 > than uptake at pH 8), Na independent, and was inhibited by riboflavin structural analogues lumichrome and lumiflavin. Worms exposed to Y47D7A.16 RNAi laid defective eggs at a slowed pace and exhibited a slower defecation rate compared to controls. The protein is expressed primarily in the gut, with expression most pronounced in the distal gut, although expression also occurs in the excretory canal. We will discuss the effects of riboflavin supplementation on RNAi knockdown worms and on the expression of Y47D7A.16. [Supported by grants from the DVA and the NIH (DK-58057)].

366A

DHS-21, a dicarbonyl/L-xylulose dehydrogenase (DCXR) ortholog, regulates longevity and reproduction in *C. elegans*. **Son T. Le**[1], Tae-Woo Choi[1], Gunasekaran Singaravelu[2], Sun-Kyung Lee[1], Joohong Ahnn[1]. 1) Life Sci, College Natural Sci, Seoul, Korea; 2) Waksman institute, Rutgers University, Piscataway, NJ 08854, USA.

Dicarbonyl/L-xylulose dehydrogenase (DCXR) converts L-xylulose into xylitol, and reduces various α-dicarbonyl compounds, aromatic aldehydes, and aromatic ketones, thus performing a dual role in carbohydrate metabolism and detoxification. Various clinical and environmental conditions may induce DCXR expression in proportion to the metabolic stimulus. The enzymatic properties of DCXR in microbes and mammals, including humans, have been reported, as well as its pathological implications; however, the physiological functions of DCXR in the intact organism are not well understood. In this study, we identified DHS-21 as the only DCXR ortholog in *C. elegans*. The *dhs-21* is expressed in various tissues including the intestine, gonadal sheath cells, uterine seam (utse) cells and the spermathecal-uterus (sp-ut) valve. Recombinant DHS-21 was shown to covert L-xylulose to xylitol using NADPH as a cofactor. The *dhs-21* null mutants of *C. elegans* show defects in longevity, reproduction and egg-laying. Knock-down of *daf-16* and *elt-2* transcription factors affected *dhs-21* expression. These results suggest that DHS-21 is a bona fide DCXR of *C. elegans*, essential for normal life span and reproduction.

367B

Regulation of insulin and neuropeptide signaling by the Bardet-Biedl Syndrome complex. **Brian H. Lee**, Kaveh Ashrafi. Dept Physiology, Univ California, San Francisco, San Francisco, CA.

Bardet-Biedl syndrome, BBS, is a rare autosomal recessive disorder with clinical presentations including polydactyly, retinopathy, hyperphagia, obesity, short stature, cognitive impairment and developmental delay. Since disruptions of BBS proteins in a variety of organisms impair cilia formation and function, the multi-organ defects of BBS have been attributed to deficiencies in various cilia associated signaling pathways. In C. elegans, bbs genes are expressed exclusively in the sixty ciliated sensory neurons of these animals. We show that in contrast to many other cilia defective mutants, C. elegans bbs mutants increase release of dense-core vesicles. Consequently, bbs mutants exhibit phenotypes associated with increased insulin, neuropeptide and biogenic amine signaling including body size, feeding and metabolic abnormalities. Abrogation of enhanced secretions without concomitant correction of ciliary defects is sufficient to normalize these phenotypes in bbs mutants. Thus, our findings expand the role of BBS proteins to the regulation of dense-core-vesicle exocytosis and indicate that some features of Bardet-Biedl Syndrome may be cause by excessive neuroendocrine secretion.

368C

Dafadine Promotes Dauer-Formation and Lifespan-Extension of C. elegans by Inhibiting the Cytochrome P450 DAF-9. **Genna M. Luciani**[1,2], Lilia Magomedova[3], Rachel Puckrin[2], Malene L. Urbanus[2,4], Iain M. Wallace[2,4], Guri Giaever[1,2,3], Corey Nislow[1,2,4], Carolyn L. Cummins[3], Peter J. Roy[1,2]. 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, M5S 1A8, Canada; 2) The Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, M5S 3E1, Canada; 3) Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON, M5S 3M2, Canada; 4) Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, M5G 1L6, Canada.

The genetic analysis of dauer entry has provided fundamental insight into the insulin-like and TGF-beta signaling pathways that regulate entry into this diapause-like state. Despite the utility of dauer for understanding these conserved pathways, there is no known small molecule tool that robustly induces dauers. Such a reagent would facilitate further insight into the signaling cascades that regulate dauer entry. Through our on-going small molecule screens, we identified a compound that we call dafadine that robustly induces dauer formation in wild type worms grown in typical culture conditions. In addition, dafadine induces a penetrant distal tip cell (DTC) migration phenotype. Genetic analyses and cell-based assays show that dafadine antagonizes DAF-9. DAF-9 is a cytochrome P450 that functions downstream of insulin-like signaling to produce dafachronic acid, which is a hormone that that regulates dauer entry and DTC migration. Given that DAF-9 functions downstream of the insulin-like signaling pathway, which is well-characterized to regulate

longevity, we tested whether dafadine also affects the lifespan of worms. Dafadine-treated wild type adults had increased lifespan, while dafadine-treated daf-9 nulls did not. These data indicate that DAF-9 normally functions to restrict adult lifespan. We also found that dafadine can antagonize CYP27A1, which is DAF-9's functional ortholog in mammals. Our results suggest that dafadine is a novel tool with which to interrogate developmental control and longevity in C. elegans and perhaps other phyla as well.

369A

Local triglyceride synthesis by DGAT-2 promotes lipid droplet expansion. Ningyi Xu, Shaobing Zhang, Ronald Cole, Sean McKinney, Fengli Guo, **HoYi Mak**. Stowers Institute for Medical Research, Kansas City, MO.

Lipid droplets (LDs) are evolutionarily conserved organelles for cellular fat storage. We have previously shown that a loss of DAF-22 function, which attenuates peroxisomal fatty acid beta-oxidation, causes LD expansion and an increase in triglyceride levels. In order to identify genes that are required for LD expansion, we performed a genetic suppressor screen in the *daf-22* mutant background and recovered multiple alleles that fell into at least 5 complementation groups. We report here the molecular cloning of *dgat-2*, which encodes a conserved diacylglycerol acyltransferase, the terminal enzyme for triglyceride synthesis. Loss of *dgat-2* function inhibits LD expansion and reduced triglyceride levels of *daf-22* mutant animals while over-expression of DGAT-2 is sufficient to promote LD expansion in wild-type animals. Photo-bleaching demonstrates that DGAT-2 is a LD resident protein. Electron microscopy reveals that DGAT-2 clusters at discreet foci on the LD surface. We engineered a mutant DGAT-2 that was tethered to the ER. This mutant DGAT-2 failed to support LD expansion, indicating that LD localization of DGAT-2 is necessary for its proper function. Our results demonstrate that local synthesis and deposition of triglyceride is critical for LD expansion. We are currently searching for proteins that act in concert with DGAT-2 using genetic and proteomic approaches.

370B

Role of lipid signaling molecules in energy balance in C. elegans. **Thomas P. Mathews**, Tallie Noble, Benjamin F. Cravatt, Supriya Srinivasan. Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

Glycerol-based lipids are important energetic stores as well as important signaling molecules essential to animal physiology. A number of enzymes work to regulate these glycerol-lipid species through phosphorylation, hydrolysis or acylation. Diacylglycerol kinase type 1 (dgk-1) has been shown to be responsible for regulating diacylglycerol (DAG) concentrations in the C. elegans nervous system, regulating a host of behavioral phenotypes. In a recent RNAi screen, we found other enzymes responsible for regulating DAG species to have overlapping behavioral affects with dgk-1. We are currently investigating the role of DAG as a signaling molecule in energy balance in C. elegans.

371C

Histidine Protects Against Zinc and Nickel Toxicity in *C. elegans*. **John T. Murphy**[1], Janelle J. Bruinsma[1,5], Daniel L. Schneider[1], Sara Collier[1], James Guthrie[2], Asif Chinwalla[4], J. David Robertson[2,3], Elaine R. Mardis[4], Kerry Kornfeld[1]. 1) Developmental Biology, Washington Univ, St Louis, MO; 2) Research Reactor Center, University of Missouri, Columbia, MO 65211; 3) Department of Chemistry, University of Missouri, Columbia, MO 65211; 4) The Genome Center, Department of Genetics, Washington University School of Medicine, St. Louis, MO 63108; 5) Present Address: Exact Sciences, Madison, WI 53719.

Zinc is an essential trace element involved in a wide range of biological processes and human diseases. Zinc excess is deleterious, and animals require mechanisms to protect against zinc toxicity. To identify genes that modulate zinc tolerance, we performed a forward genetic screen for *C. elegans* mutants that were resistant to zinc toxicity. Here we demonstrate that mutations of the *C. elegans* histidine ammonia lyase (*haly-1*) gene promote zinc tolerance. *C. elegans haly-1* encodes a protein that is homologous to vertebrate HAL, an enzyme that converts histidine to urocanic acid. *haly-1* mutant animals displayed elevated levels of histidine, indicating that *C. elegans* HALY-1 protein is an enzyme involved in histidine catabolism. These results suggest the model that elevated histidine chelates zinc and thereby reduces zinc toxicity. Supporting this hypothesis, we demonstrated that dietary histidine promotes zinc tolerance. Nickel is another metal that binds histidine with high affinity. We demonstrated that *haly-1* mutant animals are resistant to nickel toxicity and dietary histidine promotes nickel tolerance in wild-type animals. These studies identify a novel role for *haly-1* and histidine in zinc metabolism and may be relevant for other animals.

372A

Starvation-signalling in *gpb-2*. **Robert H. Pollok**[1], Chanhee Kang[2], Leon Avery[1]. 1) Dept. of Molecular Biology, UTSouthwestern Med Ctr, Dallas, TX; 2) Dept. of Medicine, Brigham and Women's Hospital, Boston, MA.

During starvation, *C. elegans* has to adjust its behavior to survive. Using the starvation sensitive *gpb-2* loss-of-function mutant, we have identified components in a starvation-signaling pathway. The goals of my studies are to identify neurons that propagate a starvation signal, and to identify genes that regulate fat storage in the gut during starvation. Starvation induces MAP Kinase activation, which is lethal in *gpb-2* worms. MAP Kinase is also induced by arecoline, an acetylcholine receptor agonist, and inhibited by atropine, an acetylcholine receptor antagonist. This suggests cholinergic neurons send a starvation signal, and we believe the pharyngeal MC neurons are responsible. By ablating the MC neurons in newly hatched L1s, I hope to prevent starvation-induced lethality due to the *gpb-2* background. We have also identified several genes that act downstream of *gpb-2* in regulation of fat in the gut. Both *flp-20* loss of function and *mgl-2* loss of function rescue the starvation-induced lethality of *gpb-2,* while introduction of *gcy-28* loss of function restores lethality. When fat was assayed using oil-red-o, we found that GCY-28, a receptor-type guanylate cyclase, was necessary to maintain fat levels during starvation. GCY-28 is expressed in various head neurons and throughout the gut, and we believe that GCY-28 may be regulating how gut cells store fat. Using tissue specific rescue of GCY-28, we can test its role in gut fat storage. Ultimately I would like to tie both projects together into a model that describes a starvation-signaling pathway.

373B

Knockout of the folate tranporter *folt-1* induces sterility and metabolic defects that are alleviated by supplementation with other B vitamins. **Jason A. Rothman**, Craig LaMunyon. Dept Biol Sci, California State Polytech Univ, Pomona, 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. Our results show that *folt-1* knockout hermaphrodites have a substantially reduced germline, generate a small number of functional sperm, and only rarely produce a functional oocyte. We found no evidence of increased apoptosis in the germline of *folt-1* knockout mutants, suggesting that germline proliferation is defective. While *folt-1* knockout males are fertile, their rate of spermatogenesis was severely diminished, and the males were very poor maters. The mating defect is likely due to compromised metabolism and/or other somatic functions, as *folt-1* knockout hermaphrodites displayed a shortened lifespan and elongated defecation intervals. Folate supplementation did not rescue the *folt-1* knockout worms, but supplementation with other B vitamins did improve the knockout phenotype. Specifically, niacin, riboflavin, and thiamin all improved fertility and metabolic rate, as did oxaloacetate. Interestingly, *folt-1* knockout worms upregulate thiamine uptake, but we have not yet investigated uptake of other B vitamins. Alternatively, both *folt-1* knockout and N2 worms were harmed by methionine and homocysteine supplementation. Excess methionine should drive a buildup of homocysteine, which is recycled by the folate pathway. Because homocysteine is a known mitochondrial toxin, we conclude that much of the *folt-1* knockout phenotype is due to effects on mitochondrial function.

374C

The role of rsks-1 in regulation of fat metabolism. **Ming Sheng**[1], Josefin Nyström-Friberg[1], Staffan Lundstedt[2], Simon Tuck[1]. 1) Umea center of Molecular Medicine, Umeå University, Sweden; 2) Environmental chemistry Unit, Department of Chemistry, Umeå University, Sweden.

The storage of fat is crucial for the proper regulation of energy homeostasis. Work with mice has revealed that those lacking one of the two homologues of p70 S6 kinase have lower levels of fat and are resistant to high fat diet-induced diabetes. To understand how p70 S6 kinase regulates fat, we have undertaken a study of the function of rsks-1, the sole p70 S6 kinase homologue in C. elegans. In C. elegans, fat is primarily stored in granules present in the hypodermis and the intestine. Mutations that lead to an increase in fat accumulation cause a Darkened intestine (Din) phenotype resulting from increased refractility of the larger and more numerous granules. We found that C. elegans worms lacking the single p70 S6 kinase homologue in this organism, rsks-1, are Din and that the intestine is contains more and bigger granules than wild type. This phenotype was suppressed by multiple copies of wide-type rsks-1on a transgene. We quantified the fat accumulation in rsks-1 deficient worms by a combination of thin layer and gas chromatography and found that the mutants have 50% higher triglyceride levels compared to wild type. The GC analysis also revealed that the peaks corresponding to C16:1n9 and C18:1n9 were more prominent in the mutant. To help understand the reason for the increased fat content in the rsks-1 mutants, we performed a quantitative RT-PCR screen with (i) energy metabolism genes related to short-term food withdrawal (fasting), (ii) metabolic sensors, and (iii) genes that are known to regulate metabolic pathways in C. elegans coordinately. The results showed that RSKS-1 influenced the expression of the genes involved in β-oxidation, desaturation and elongation. We also tested these genes for their ability to reduce fat accumulation in rsks-1 mutants by RNAi and identified 10 genes that suppressed the dark intestine phenotype. Thus RSKS-1 is involved in fat metabolism in C. elegans; however, in contrast to the mouse model, it seems to act positively to promote fatty acid utilization.

375A

Determining the role of heterotrimeric G proteins in dauer entry. **Julien Shoenfeld**, Bryan Danzi, Edith Myers. Fairleigh Dickinson University, Madison, NJ.

C. elegans enter dauer under conditions of limited food, high temperature, and high concentrations of dauer pheromone. Signaling through heterotrimeric G proteins regulates sensitivity to food and dauer pheromone[1,2]. We are interested in whether the specific G proteins, EGL-30 (Gαq) and GOA-1 (Gαo) are important for sensing the environmental signals that regulate dauer entry because it has been suggested that GOA-1 may regulate dauer formation[3]. EGL-30 signaling antagonizes GOA-1 signaling, suggesting that EGL-30 may also somehow regulate dauer formation.

By separately controlling pheromone, temperature, and food levels we have begun to study the role of EGL-30 and GOA-1 in dauer formation and recovery. We assayed dauer formation in response to different environmental cues in *egl-30* and *goa-1* mutants. We found that EGL-30 and GOA-1 were not necessary for normal dauer formation at 25°C.

Because some proteins are only required for dauer entry at higher temperatures[4], we wanted to assay dauer formation in *egl-30* and *goa-1* mutants at 27°C. However, animals with *egl-30* and *goa-1* mutations were not viable at 27°C, therefore we could not determine whether these proteins are necessary for dauer formation in response to high temperatures. We determined that GOA-1 and EGL-30 were required for normal sensitivity to dauer-related ascarosides. Further studies are underway to determine the role of these specific heterotrimeric G proteins in relaying food related dauer signals.

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376B

Glycogen debranching enzyme AGL-1 regulates embryogenesis and lifespan through AMP Kinase dependent and independent pathways. **Jeffrey S. Simske**. Rammelkamp Ctr, Cleveland, OH.

Metabolic status generally affects embryogenesis and lifespan. *agl-1*, which encodes the single *C. elegans* glycogen debranching enzyme, is involved in embryogeneis and lifespan. The hypomorphic allele *jc11* and the more severe putative null allele *tm4809* are both partially penetrant, cold-sensitive, maternal-effect alleles that result in embryonic arrest at various stages. Hatching 'escaper' animals have shortened lifespans and suppress the longer lifespan of *daf-2* mutants at 20°C, and overexpression of AGL-1::GFP results in lifespan extension. AGL-1 affects embryogenesis through regulation of the energy sensor AMP-activated kinase (AMPK). AMPK is a heterotrimeric protein with a catalytic α subunit encoded by *aak-1* or *aak-2*, and regulatory subunits β (*aakb-1*, *aakb-2*) and γ (5 genes). The β subunit functions as a glycogen sensor and in one vertebrate system, the β1 subunit interacts directly with glycogen debranching enzyme. Strong binding of AMPK β1 to glycogen was shown to depend on the branch structure; binding in turn functions to inhibit AMPK activity. Loss of *agl-1* function is predicted to result in the formation of a 'limit dextrin' glycogen molecule with digestion stalled at branch points, and well-suited to bind to and inhibit AMPK activity. Supporting this model are the observations that AMPK activity is reduced in *agl-1* mutants, overexpression of *aak-2* or the *aakb-1* glycogen-binding domain suppresses *agl-1* phenotypes, and compounds thought to activate AMPK, such as metformin and resveratrol also suppress *agl-1* phenotypes. Finally, AGL-1 and the AAKB-1 co-immunoprecipitate from worm extracts, suggesting that AGL-1 and AAKB-1 physically interact to regulate energy metabolism and lifespan via glycogen. Dietary supplement with cornstarch (mixed linear and branched polysaccharides) bypasses *agl-1* phenotypes, whereas supplement with pure amylopectin (branched polysaccharides alone) not only fails to bypass, but enhances *agl-1* phenotypes. Supplement with various mono or di-saccharides does not suppress *agl-1* phenotypes. These findings support the hypothesis that branched carbohydrates generally inhibit AMPK activation. Interestingly, dietary supplement with glucose and glycerol dramatically enhance *agl-1* phenotypes, increasing penetrance of the most severe *agl-1* phenotypes to nearly 100%, even at permissive temperatures. Glycogen levels are higher in glucose supplemented animals, while AMPK inactivation is not further reduced and enhancement of *agl-1* by glucose is not suppressed by AMPK overexpression, suggesting there are AMPK-independent metabolic functions for *agl-1*. Genetic suppressors of glucose-enhanced *agl-1* phenotypes have been identified, and will be presented.

377C

S-adenosylmethionine synthetase SAMS-1 regulates brood size in *C. elegans*. **Hiroko Tamiya[1]**, Keiko Hirota[1], Yuta Takahashi[1], Hiroaki Daitoku[1], Naoaki Ishii[2], Akiyoshi Fukamizu[1]. 1) Life Science Center of TARA, University of Tsukuba, Tsukuba, Ibaraki, Japan; 2) Department of Molecular Life Science, Tokai University School of Medicine, Japan.

S-adenosylmethionine (SAM) is the main methyl donor and the common point between the three principal metabolic pathways: polyamine synthesis, transmethylation, and transsulfuration. The synthesis of SAM is catalyzed by SAM synthetase (SAMS), which transfers the adenosyl portion of ATP to methionine. Here, we investigate the physiological roles of the SAMS proteins in *C. elegans* by using loss-of-function mutants of *sams* genes, *sams-1*, *-3*, *-4* and *-5*. Among *sams* mutants, only *sams-1* mutant exhibited a marked reduction in brood size. To investigate the role of SAMS-1 in the regulation of brood size, we examined the expression pattern of *sams-1* using transgenic worms expressing GFP proteins under control of endogenous *sams-1* promoter. Unexpectedly, *sams-1* was expressed abundantly in the intestine and the body wall muscle, but not in the germline. Next, we examined whether SAM synthetic activity of *sams-1* is involved in the regulation of brood size. The reduction of brood size in *sams-1* mutant was efficiently rescued by over-expressing wild-type SAMS-1, but not an enzymatically inactive one. These results indicate that the brood size was modulated in SAM synthetic activity-dependent manner. Collectively, these data raise the possibility that SAM in the intestine and the body wall muscle might play an important role in maintaining the brood size in *C. elegans*.

378A

Genetic Regulation of Starvation Induced Adult Reproductive Diapause (ARD). **Bargavi Thyagarajan**, Giana Angelo, Marc Van Gilst. Basic Sci Division, Fred Hutchinson Cancer Res Ctr, Seattle, WA.

To survive food deprivation, *C. elegans* larvae often arrest development and adopt diapause states. Entry into dauer diapause and arrest at the L1 larval stage are examples of such response to the absence of food. When starved later in larval development, at the L4 larval stage, animals transition into adult reproductive diapause (ARD) and survive food deprivation, even up to 30 days. Adult worms in ARD are distinctive in appearance due to their small size and arrest as adults harboring one embryo per gonad arm. Remarkably, upon feeding, animals recover from ARD, produce progeny and live a normal adult life span. Thus, somatic and germline tissues are protected from the detrimental effects of ageing, maintained through extended periods of starvation and restored to near wild-type function when food is available. The molecular processes involved in successful ARD entry during starvation or recovery of tissues from prolonged periods of food deprivation remain largely unknown. We hypothesize that molecular pathways involved in exit from starvation induced ARD will include chemosensory pathways involved in sensing food, tissue maintenance/repair pathways and metabolic pathways that support the bio-energetic requirements of diapause exit. Entry into ARD upon food withdrawal requires a high population density of worms and the activity of the nuclear hormone receptor NHR-49 (the *C. elegans* ortholog of HNF4α). Furthermore, CED-3, a caspase essential for apoptosis, is critical for restoration of an organized germline following ARD exit. Lack of CED-3 activity, however, does not seem to affect the recovery of somatic tissues upon food restoration. To identify additional cellular mechanisms important for recovery of animals from ARD, we undertook a genome-wide RNAi screen. In particular, screening for genes, which when inactivated, prevent the recovery of somatic tissues during ARD exit. The RNAi phenotypes observed for worms in ARD (starved previously for 20 days or more) include failure to recover body size, movement defects, deformities in the hypoderm or vulva and lack of progeny. Thus far, a requirement for mRNA translation for recovery from ARD has been determined.

379B

Increasing *C. elegans* lifespan by inhibiting *E. coli* folate metabolism, but not *E. coli* proliferation. **Bhupinder Virk**, David Dixon, David Weinkove. School of Biological and Biomedical Sciences, Durham University, Durham, United Kingdom, DH1 3LE.

C. elegans provides an opportunity to understand how diet and microbial flora can affect lifespan. There is evidence, for example, showing that inhibition of bacterial proliferation by UV irradiation or antibiotic treatment increases *C. elegans* lifespan (Garigan *et al*, Genetics, v161, p1101 (2002)). Previously we found a spontaneous mutant of the RNAi bacteria HT115(DE3) that increases *C. elegans* longevity. The mutated gene *aroD* is involved in the shikimic acid pathway, which is responsible for producing many aromatic compounds, including the folic acid precursor para-aminobenzoic acid (PABA). Addition of PABA to *aroD* mutant bacteria eliminates this extended *C. elegans* lifespan completely, implicating folate synthesis as the cause of the lifespan increase. (Weinkove *et al*, unpublished) To test whether the hypothesis was also applicable in other *E. coli* strains including OP50 we used a sulphonamide drug, sulfamethoxazole (SMX), to inhibit bacterial folate metabolism. SMX treatment at various concentrations on OP50 bacteria increased *C. elegans* lifespan in a dose dependent manner. Interestingly, even though SMX is used as an antibiotic, the highest concentration used did not appear to decrease growth of the bacterial lawn substantially. This result was confirmed in liquid culture and by quantifying the cell content of bacterial lawns. Mass spectrometry was used to measure and compare folate levels in control and SMX treated OP50, and also in worms fed on these bacteria. The results show that levels of the most abundant folates in both worms and bacteria are reduced in the presence of SMX. Interestingly, we saw a greater reduction of folate in bacteria than in the worms themselves, suggesting that the worms can compensate for low folate levels in bacteria. Preliminary research suggests that SMX and kanamycin have similar effects on *C. elegans* lifespan, yet kanamycin kills bacteria and stops proliferation. Further work will determine whether inhibition of *E. coli* proliferation is necessary for the kanamycin effect. As SMX works at low concentrations and does not affect bacterial proliferation, it may be possible to use similar drugs to treat the mammalian gut flora without large changes in the microbial ecology.

380C

PNC-1 regulation NAD^+ synthesis but not absolute NAD^+ levels promotes muscle development and function in *C. elegans*. **Tracy L. Vrablik**, Wenqing Wang, Wendy Hanna-Rose. Dept Biochem & Mol Biol, Pennsylvania State Univ, University Park, PA.

Nicotinamide adenine dinucleotide (NAD^+) is an essential electron carrier in intermediary metabolism and a co-substrate for NAD^+ consumers such as sirtuins. Organisms must synthesize NAD^+ to both sustain metabolism and the activity of NAD^+ consumers. We previously reported that the nicotinamide (NAM) to NAD^+ salvage pathway enzyme *pnc-1* promotes *C. elegans* reproductive development. Here we investigate if our *C. elegans* model can provide insight into known roles for NAD^+ synthesis in vertebrates and employed a quantitative approach to evaluate how disrupting salvage affects the physiological levels of these metabolites. The NAM-NAD^+ salvage pathway enzyme Nampt is an important myogenic regulator in vertebrates, and we found a similar role for *pnc-1* in *C. elegans* muscle development and function. *pnc-1* males cannot mate due to a spicule muscle defect and multiple muscle types are impaired in the hermaphrodites. Nicotinamidase activity shuttles NAM into the NAD^+ salvage pathway and is therefore proposed to both promote NAD^+ synthesis and lower levels of the NAM. Pharmacological analysis revealed that PNC-1 promotes muscle function through both increasing NAD^+ synthesis and clearing of NAM. Interestingly we observed surprising muscle-cell-type specific sensitivity to perturbing NAD^+ production or NAM levels. Active NAD^+ biosynthesis during development is critical for function of the male spicule protractor muscles during adulthood, while vulval muscles are highly sensitive to elevated NAM levels. We sought to determine how NAD^+ salvage affects physiological metabolite levels and employed a selective reaction monitoring

LC/MS technique with isotopically labeled internal standards to quantify levels of NAM and NAD^+ in whole worm lysates. NAM levels are elevated in *pnc-1* mutants as predicted, however NAD^+ levels are surprisingly resistant to both genetic and pharmacological perturbations. Our results suggest that NAD^+ levels are maintained in response to metabolite availability through regulation of NAD^+ synthesis and/or utilization. To provide phylogenetic context, we investigated how these metabolite levels compare with other model systems such as flies, yeast, *E. coli*, mouse tissue and human cell culture. Samples that salvage NAM to NAD^+ through Nampt have NAM levels in excess of NAD^+, while organisms that use nicotinamidases all maintain NAD^+ in excess. Thus the relative level of NAM that an organism maintains may reflect the NAM-NAD^+ salvage enzyme it employs. *C. elegans* nicotinamidases have a much higher k_{cat} than is reported for Nampt, we propose the differences in NAM levels result from the catalytic rates of these NAD^+ salvage enzymes.

381A

Purification and characterization of *C. elegans* cytoplasmic malate dehydrogenase. Wei Gu, Penelope L. Lindsay, Justin W. Spengler, **Katherine M. Walstrom**. Div Natural Sciences, New College of Florida, Sarasota, FL.

Malate dehydrogenase (MDH) is the last enzyme in the citric acid cycle. MDH catalyzes the conversion of NAD^+ and malate to NADH and oxaloacetate. Since the forward reaction is energetically unfavorable, the reverse reaction is usually studied. Eukaryotes have two versions of this enzyme, one that is imported into the mitochondria and one that remains in the cytoplasm. The cytoplasmic MDH in *C. elegans* (F46E10.10) was originally mis-classified as lactate dehydrogenase. Hold and Riddle (Mech. Aging Dev. 124, 779, 2003) realized that the amino acids in the active site were compatible with malate binding rather than lactate. We named this enzyme MDH-1 and renamed the mitochondrial enzyme (F20H11.3) MDH-2 so that the naming convention corresponded to that used for other eukaryotes. We overexpressed MDH-1 in *E. coli* and removed the chitin binding domain tag used for the purification. In kinetic assays with the purified enzyme, MDH-1 had malate dehydrogenase activity that followed Michaelis-Menten kinetics. For the reverse reaction, we determined that the K_M for oxaloacetate was 37 μM, and the K_M for NADH was 70 μM. Our gel filtration results indicated that MDH-1 was active as a dimer, which is similar to the quaternary structure of most MDH enzymes (Minarik, P. et al., Gen. Physiol. Biophys. 21, 257, 2002). We are in the process of purifying endogenous MDH-1 from worms to compare its activity to our recombinant enzyme.

382B

Rationing of Energy Stores in C. elegans during Dauer Stage. **Meng Xie**[1], Richard Roy[2]. 1) W5/20 Biology, McGill University, Montreal, Quebec, Canada; 2) W5/17 Biology, McGill University, Montreal, Quebec, Canada.

The accumulation of excess body fat is associated with a number of major diseases including type II diabetes, coronary heart disease, hypertension, while it has also been correlated with certain forms of cancer. Caenorhabditis elegans provides an efficient model to address how fat is stored and hydrolyzed due to its amenability to genetic analysis. Using this model in the past we have shown that AMP-activated protein kinase (AMPK), a metabolic master switch of energy homeostasis that is conserved from yeast to humans, controls germ line stem cell quiescence, while AMPK mutant animals in the dauer stage expire prematurely due to hyperactive lipase activity. To identify further genes that may act as AMPK targets in energy regulation and to better understand how these genes might affect the fat related diseases, we conducted a global genome survey using an RNAi feeding strategy to knock down the expression of every predicted gene in C. elegans to identify genes that, when compromised, allow AMPK mutant dauer larvae to survive longer. 551 RNAi clones were identified in the survey. Among these we found that the knock down of a family of catalase genes (ctl-1 and ctl-2) was able to significantly increase the dauer life span of AMPK mutant worms. We provide evidence that the extension of dauer life span is due to an increased level of hydrogen peroxide, which results in a decreased rate of hydrolysis of the triglyceride stores, increased fatty acid de novo synthesis, and increased osmotic resistance. In addition, we found that expression of the catalase genes were regulated by AMPK via DAF-16. Further molecular characterization of the mechanism that connects the elevated hydrogen peroxide level and increased fatty acid de novo synthesis is likely to provide insight for the treatment of diseases associated with inappropriate lipid metabolism.

383C

Suppressors of *daf-18* L1 arrest phenotype and investigating a role for DAF-18 protein phosphatase activity. **Michael R. Zanetti**, Peter E. Hand, Ian D. Chin-Sang. Biology, Queen's University, Kingston, Ontario, Canada.

The *C. elegans* Eph receptor tyrosine kinase (RTK), VAB-1, has been identified as an important player in signaling pathways controlling lifespan, axon guidance and morphogenetic movements. As such, it is important to understand how the individual components of this signaling cascade are functioning. Mutations in one of the downstream targets, *daf-18(ok480)*, results in defective dauer formation and defective L1 arrest. DAF-18 is the homolog of the human tumor suppressor PTEN and is a negative regulator of an insulin/IGF-like pathway for longevity and dauer larva formation. In order to identify genes that function with *daf-18* in L1 arrest we used two approaches to look for suppressors of the *daf-18* L1 arrest phenotype. First, we used a candidate gene approach using RNAi knockdowns, which identified eleven suppressors. For suppressor genes that have mutants available, we will create double mutants with *daf-18(ok480)* to confirm suppression of the *daf-18* L1 arrest phenotype. Secondly, we used an unbiased EMS mutagenesis screen, which identified twelve potential suppressors. These suppressors are being mapped to chromosome locations and non-complementation tests will be carried out to determine if they are novel suppressors. Previous work in our lab showed that VAB-1 and DAF-18/PTEN are substrates for each other and mutually inhibit each other [1]. We also have shown vab-1 mutants can partially suppress *daf-18(ok480)* L1 arrest. Human EphA3, a VAB-1 homolog, is frequently mutated in lung cancers, in particular a missense mutation in the tyrosine kinase domain EphA3(K761N), and predicted to have oncogenic properties [2]. To test gain-of-function properties *in vivo* we created *C. elegans* transgenic lines with human EphA3(K761N) and the analogous mutation in VAB-1(K859N). VAB-1(K859N) transgenic worms have a phenotype similar to hyperactive VAB-1 gain-of-function mutations where PLM axons show premature termination [3]. Based on the antagonistic interaction between VAB-1 and DAF-18 we predict that DAF-18 will dephosphorylate VAB-1(K859N) and rescue the premature termination defects. We will also test whether this regulation is conserved in human EphA3 and PTEN. We will report our findings at the meeting. [1] Brisbin *et al.* 2009, Dev. Cell **17**, 459-469 [2] Ding *et al.* 2008, Nature **455**, 1069-1075 [3] Mohamed and Chin-Sang. 2006, Dev. Biol **290**, 164-176.

384A

Indolequinazoline Alkaloids Evodiamine and Rutaecarpine Suppress Multivulva of an Activating Ras Mutant. **Kosuke Amagasa**, Kahori Asano, Yusuke Morioka, Asuka Kambe, Hideshi Inoue. Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan.

C. elegans is a useful and cost effective model for initial drug screening. In mammmals, activation of the Ras/MAPK signaling cascade promotes cellular proliferation, and activating mutations of Ras are involved in tumorigenesis. This pathway is highly conserved and required for vulval development in C. elegans. Gain-of-function mutations of the Ras ortholog, let-60, lead to constitutive pathway signaling and cause a multivulva (Muv) phenotype. Using a gain-of-function mutant let-60(n1046), we screened 19 herbal medicines for inhibiting activity of the Ras/MAPK pathway signaling and found that an extract from Tetradium ruticarpum suppresses the Muv phenotype of the mutant. Indolequinazoline alkaloids evodiamine (Evo) and rutaecarpine (Rut) are known major bioactive components of T. ruticarpum. They have been reported to have various pharmacological activities including thermoregulation, vascular regulation, anti-allergic, anti-nociceptive and anti-inflammatory activities, and so on. We examined the effects of Evo and Rut on the let-60(n1046) mutant, and found that both the compounds of the concentration of 0.1 μM suppressed the Muv phenotype by 40%, which was comparable to effect of gliotoxin, an inhibitor of farnesyl transferase involved in activation of Ras. Transcription factors lin-1/ETS and lin-31/Forkhead are known downstream targets of the RAS/MAPK pathway. Neither Evo nor Rut, as well as gliotoxin, suppressed the Muv phenotype of lin-1(sy254), lin-1(e1777), and lin-31(n301). These results suggest that Evo and Rut suppress the Muv phenotype of let-60(n1046) by inhibiting Ras/MAPK pathway or farnesyl transferase.

385B

Role of FSHR-1 targets in regulating the *Caenorhabditis elegans* innate immune response. **Hannah L. Anthony**, Jonathan D. Hibshman, Jennifer R. Powell. Gettysburg College, Gettysburg, PA.

Innate immunity is crucial in the response and defense against pathogens for invertebrates and vertebrates alike. Prior research suggests that the G-protein coupled receptor (GPCR) FSHR-1 acts as a critical component in the innate recognition of intestinal infection in *Caenorhabditis elegans*. In order to characterize the function of FSHR-1 in the context of the *C. elegans* innate immune response, putative pathogen response genes that appear to be transcriptional targets of FSHR-1 are being evaluated. Six genes thought to be downstream of FSHR-1 were selected via a series of microarray analyses in worms with induced *Psuedomonas aeruginosa* infection. Quantitative infection-induced expression levels for the suspected pathogen response genes were determined by reverse transcription qPCR. RNAi was performed to assess differential survivorship in pathogen response gene knockouts and the relative contribution that each gene has to the innate defense against *P. aeruginosa* infection. Further, a transcriptional GFP reporter was constructed for one of the pathogen response genes [C17H12.6] that appears to be regulated by FSHR-1 based on qPCR experiments. Using these transgenic worms, we will examine the expression pattern of the gene under infectious conditions.

386C

O-GlcNAc cycling is a regulator of innate immunity in Caenorhabditis elegans. **Michelle R Bond**[1], Michael W Krause[2], John A Hanover[1]. 1) LCBB, NIDDK, National Institutes of Health, Bethesda, MD; 2) LMB, NIDDK, National Institutes of Health, Bethesda, MD 20892.

Playing critical roles in biological process including signaling and gene transcription, the O-linked N-acetylglucosamine (O-GlcNAc) post-translational modification of serine and threonine residues is dynamic. Modifying and/or regulating over 600 proteins, O-GlcNAc has been implicated in a variety of human conditions including Diabetes mellitus, cancer, and neurodegenerative diseases. O-GlcNAc cycling is governed by two proteins, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) that add and remove the modification, respectively. OGT interacts with evolutionarily conserved proteins that are key for the innate immune response: it interacts with components in the beta-catenin, p38 MAPK, and insulin signaling pathways. Given its role as a key signaling molecule and the pathways with which OGT interacts, we hypothesize that O-GlcNAc cycling plays a role in "fine-tuning" the innate immune response. Bacterial infections, especially those that are drug resistant, are of growing health concern in the USA. Although significant progress has been made in understanding organisms' responses to bacterial infections, the complexity of the mammalian system leaves in-depth characterization of immune pathways at the whole-organism level wanting. The bacteriovore C. elegans is an attractive candidate for studying the innate immune response: it is susceptible to many human pathogens, the animal's transparency allows real-time observation of bacterial accumulation and its short life span permits characterization of susceptibility by scoring longevity. Importantly, although the removal of OGT and OGA yields lethality in most organisms, null mutants of OGT and OGA are viable and fertile in C. elegans. The innate immune response was examined for wild type (WT), ogt-1 mutant, or oga-1 mutant worms to heat-killed S. aureus (HKSA). While the WT worms had similar longevity profiles regardless of the bacterial strain they were fed, ogt-1 and oga-1 mutant worms had an increased lifespan on HKSA in comparison to WT. These data suggest that defects in O-GlcNAc cycling may influence the animals' sensitivity to bacteria by changing the proteins modified by O-GlcNAc. In order to understand the implications of these data, we study the C. elegans innate immune response to live S. aureus and profile the proteins modified by O-GlcNAc in each case by immunoblot and mass spectrometry. The susceptibility of both O-GlcNAc cycling mutants and other innate immunity mutants is currently under investigation.

387A

Comparative Metabolite Profiling Identifies Toxic Phenazines Produced by *Pseudomonas aeruginosa*. **Brent Cezairliyan**[1,2], Nawaporn Vinayavekhin[3], Daniel Grenfell-Lee[1,2], Grace Yuen[2,4], Alan Saghatelian[3], Frederick Ausubel[1,2]. 1) Department of Genetics, Harvard Medical School, Boston, MA; 2) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 3) Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA; 4) Program in Immunology, Harvard Medical School, Boston, MA.

Bacterial pathogens employ a variety of methods to overcome host defenses. Using a novel approach combining genetics, biochemistry, and metabolite profiling, we identified two phenazine compounds produced by the Gram-negative human pathogen *Pseudomonas aeruginosa* that are toxic to the nematode *Caenorhabditis elegans*. Both 1-hydroxyphenazine and phenazine-1-carboxylic acid kill nematodes, even in the absence of *P. aeruginosa*. 1-hydroxyphenazine is toxic over a wide pH range, whereas the toxicity of PCA is strictly pH-dependent, demonstrating how diversity within a class of metabolites can be used to modulate bacterial toxicity in different environmental niches.

388B

Characterization of the pathogenicity of Enterohaemorrhagic *E. coli* in *C. elegans*. Ting-Chen Chou[1], Cheng-Ju Kuo[1], Hao-Chieh Chiu[2], Ching-Ming Wu[3], **Chang-Shi Chen**[1,4]. 1) Department of Biochemistry and Molecular Biology, National Cheng Kung University, Tainan, Taiwan, Taiwan; 2) Department of Clinical Laboratory Sciences and Medical Biotechnology, National Taiwan University, Taipei, Taiwan; 3) Department of Cell Biology and Anatomy, National Cheng Kung University, Tainan, Taiwan; 4) Institute of Basic Medical Sciences, National Cheng Kung University, Tainan, Taiwan.

Strains of Enterohaemorrhagic *Escherichia coli* (EHEC) that produce verocytotoxin, such as *E. coli* O157:H7, form an important group of zoonotic pathogens. *E. coli* O157:H7 has been implicated in large outbreaks as well as in sporadic cases of haemorrhagic colitis and the sometimes fatal haemolytic uremic syndrome. Here we applied the genetic tractable animal, *C. elegans*, as a model host to study the virulence of EHEC as well as the host innate immunity to this pathogen. Our preliminary data showed that EHEC intoxicated, induced severe Bag (bag-of-worms adults) phenotype, and eventually killed the worms. The transmission electron microscopy results indicated that EHEC induced attaching and effacing (A/E) lesions in the intestine. By further genetic analysis, we found that some important virulence factors of EHEC were required for its toxicity in *C. elegans*, and some conserved innate immune signal pathways were mediated in host susceptibility. Our results suggest that this EHEC-*C. elegans* model is suitable for future comprehensive genetic screens in both bacterial and host factors involved in the pathogenesis of EHEC.

389C

SIR-2.3 is involved in Cry5B pore-forming toxin defense in *C. elegans*. **Huan-Da Chen**[1], Cheng-Yuan Kao[2], Raffi V. Aroian[3], Chang-Shi Chen[1,4]. 1) Institute of Basic Medical Sciences,National Cheng Kung University, Tainan, Taiwan; 2) Center for Comparative Respiratory Biology and Medicine,University of California-Davis, Davis, California, United States of America; 3) Section of Cell and Developmental Biology,University of California-San Diego, La Jolla, California, United States of America; 4) Department of Biochemistry and Molecular Biology,National Cheng Kung University, Tainan, Taiwan.

Pore-Forming toxins(PFTs) are bacterial toxins that perforate holes at the plasma membrane of host cells and play essential roles in the pathogenesis of many human pathogens. Crystal(Cry) toxins are PFTs produced by *Bacillus thuringiensis*(Bt), and have been widely used as natural insecticides in agriculture for many decades. *C. elegans* has been developed as an *in vivo* model host to study the epithelial defenses against Cry toxins, including Cry5B in this study, and has proven instrumental in the identification of several molecular PFT-defense pathways. Here we showed that the transcription of the *sir-2.3* gene was significantly upregulated by Cry5B and *sir-2.3* was required for Cry5B defense in *C. elegans*. SIR-2.3 is a member of the SIR2 family proteins with NAD-dependent histone deacetylase activity. Our previous study has demonstrated that DAF-2 insulin-like signaling pathways were important for the intrinsic epithelial defense against PFTs. Interestingly, it has been reported that the mammalian SIR-2.3 homolog, SIRT4, can inhibit the insulin secretion in pancreatic β cells. Our current genetic epistasis data suggested that *sir-2.3* may act upstream of *daf-2* and function in the same signal pathway in response to Cry5B intoxication in *C. elegans*.

390A

Characterization of the Intestinal Autophagy Response Against *Vibrio cholerae* hemolysin. **Hediye N. Cinar**, Surasri N. Sahu, Oluwakemi Odusami. Div Virulence Assessment, Food & Drug Admin, Laurel, MD.

Autophagy is a tightly regulated process involving the degradation of the cellular components through the lysosomal machinery which helps cells to maintain a balance between production and degradation during development and growth, while acting as a survival pathway during starvation, stress, and bacterial infections (1). *Vibrio cholerae* infects and kills *C. elegans* via cholerae toxin and toxin-co-regulated pili independent manner. *V. cholerae* hemolysin (VCC), which is a pore forming toxin, is required for the lethality, growth retardation and intestinal cell vacuolation during the infection (2). VCC induced vacuoles found to be co-localized with LC3 (mammalian homolog of *lgg-1*) punctuate, a feature indicative of autophagosome formation, in cultured cells (3). To characterize the large intestinal vacuoles induced by the VCC in *C. elegans*, we have analyzed the autophagosome distribution in *C. elegans* intestine exposed to WT and *hlyA* deletion mutant strains of *V. cholerae*, using *lgg-1::GFP* marker. Exposure of *C. elegans* to

WT *V. cholerae* led to increased levels of *lgg-1::GFP* expression with increased number of dots in some of the intestinal cells, in comparison with intestines of worms exposed to *hlyA* deletion mutant strains. Accumulation of autophagosomes may reflect the induction of autophagy or reduced turnover of autophagosomes in blocked autophagy conditions. To differentiate between these two outcomes we genetically blocked the autophagy pathway using *unc-51/ atg-1* mutants. Inactivation of autophagy pathway using *unc-51(e369)* mutants led to a decrease in the formation of *lgg-1::GFP* punctuate, during *V. cholerae* infection, suggesting an induction rather than a blockage in intestinal autophagy (4). Intestinal autophagy activity was observed in a single cell or a group of intestinal cells while other parts of the intestine looked unaffected. We found that same intestinal cells showing induced autophagy upon *V.cholerae* infection also contain large vacuoles, and autophagosomes are located in close proximity of these vacuoles. To address the question of whether large intestinal vacuoles are autophagolysosome related, we used *lmp-1::GFP* (lysosome-associated membrane protein-1) marker, and found that *lmp-1::GFP* is expressed in the membranes of the large vacuoles. Altogether our data suggest that *V. cholerae* induces autophagy response in *C. elegans* intestine via VCC and large intestinal vacuoles observed during infection are originated from autophagolysosomes. 1) Jia K. et al. PNAS, 106 (2009) 14564-14569 2) Cinar et al. PLoS ONE, 2010, 5(7): e11558. doi:10.1371/journal.pone.0011558 3) Gutierrez MG. et al. PNAS, 104 (2007) 1829-1834 4) Cinar HN. et al. Ag. meta. pathogenesis in *C. elegans* Meeting 2010.

391B

Testing the "glycine zipper" model for the formation of toxic β-amyloid oligomers. **Vishantie Dostal**, Gin Fonte, Christine M. Roberts, Patrick Gonzales, Christopher D. Linlk. Institute for Behavioral Genetics, University of Colorado, Boulder, CO.

Accumulation of aggregating β-amyloid peptide (Aβ) has been implicated in Alzheimer's disease pathology (1). However, neither the toxic species of Aβ nor the mechanism of neurotoxicity has been clearly resolved. Previous studies have shown that the ability of synthetic Aβ to form transmembrane pores in vitro depends upon a glycine zipper motif located in the hydrophobic C-terminal region: Gly-XXX-Gly-XXX-Gly (2,3). We have engineered several transgenic C. elegans lines to express wild type Aβ42 and Aβ42 variants (G37L, N27G, I31G, M35G, G37F) in order to test the glycine zipper motif hypothesis. Substitutions in the glycine zipper region, particularly the G37L variant, were found to be significantly less toxic than wildtype Aβ. We then engineered Aβ variants with second site substitutions (N27G G37L, I31G G37L, or M35G G37L) that we predicted might restore toxicity based on structural models. Our results indicated that these second site substitutions did indeed restore Aβ toxicity. We have previously shown that activation of the *skn-1*/Nrf2 oxidative stress response pathway by exposure to 10 percent coffee extract strongly protects worms expressing wild type Aβ (4). Interestingly, we found that coffee exposure did not further reduce toxicity in the Aβ variants with glycine zipper substitutions, suggesting there may be some convergence between the protective mechanisms resulting from Aβ sequence changes or activation of *skn-1* . We have also assayed the toxicity of the Aβ variant peptides in Neuro 2a cells, primary hippocampal cultures, and rat cortical neurons. In all of the neuronal models G37L was less toxic when compared to wildtype Aβ42. (1.) Tiraboschi, P., Hansen, L.A., Thal, L.J., Corey-Bloom, J, 2004 The importance of neuritic plaques and tangles to the development and evolution of AD. Neurology 62 (11): 1984-1989. (2.) Kim, S., Jeon, T.J., Oberia, A., Yang, D., Schmidt, J.J., Bowie, J.U, 2005 Transmembrane glycine zippers: Physiological and pathological roles in membrane proteins. Proc Natl Acad Sci (102): 14278-14283. (3.) Arispe, N., Pollared, H.B., Rojas, E,1995 Zn2+ interaction with Alzheimer amyloid β protein calcium channels. Proc Natl Acad Sci (93): 1710-1715. (4.) Dostal, V., Roberts, C.M., Link, C.D., 2010 Genetic mechanism of coffee extract protection in a Caenorhabditis elegans model of β-amyloid peptide toxicity. Genetics 186(3):857-66.

392C

Characterization of Viruses that infect Caenorhabditis Nematodes. **Carl J Franz**[1], Yanfang Jiang[1], Guang Wu[1], Tony Belicard[2], Marie-Anne Felix[2], David Wang[1]. 1) Washington University, Saint Louis, MO. United States; 2) Jacques Monod Institute, CNRS-Univ. Paris, France.

We recently described the first natural viral infections of Caenorhabditis nematodes (PLoS Biol. 2011 Jan 25: 9(1)). Initially, two positive strand RNA viruses, Orsay virus and Santeuil virus, were discovered that were demonstrated to be capable of infecting nematodes in the laboratory. We have now identified a third related virus, Le Blanc virus. The complete genomes of all three viruses have been sequenced and have been determined to be most closely related to known nodaviruses. Nodaviruses possess bipartite positive sense RNA genomes and infect fish and insects. All three viruses share a similar genome organization that differs from that of the known nodaviruses as follows: (1) both RNA genome segments of each virus are larger than those of the canonical nodaviruses; (2) the RNA2 segments encode an extra ~ 1 kb open reading frame (protein δ) of unknown function that is not present in any of the known nodaviruses; (3) none of the viruses encodes an obvious ortholog of the nodavirus B2 protein, which is a known inhibitor of RNA interference. Under laboratory infection conditions, these viruses exhibit species specificity, where Orsay virus only infects C. elegans and Santeuil and LeBlanc viruses only infect C. briggsae. We are working now to develop essential reagents to take advantage of this novel experimental infection system in order to probe host-virus interactions. These efforts include generating virus specific antibodies and transgenic animals capable of expressing recombinant viruses. With these reagents we are broadly interested in addressing questions such as "What is the cellular tropism of the virus?", "Can infected animals clear viral infection?", "How are viruses transmitted from animal to animal?", "What is the function of the δ protein?" and "What host factors restrict viral infection?".

393A

Muscle degeneration: a protein misfolding problem? April R. REEDY, Angela REA-BOUTRIOS, Maité CARRE-PIERRAT, Laurent SEGALAT, **Kathrin GIESELER**. CGphiMC, CNRS - Université Claude Bernard Lyon1, UMR5534, Villeurbanne, France.

Duchenne Muscular Dystrophy (DMD) is a lethal X-linked muscle disease, characterized by progressive muscle necrosis, myofiber atrophy, and endomysial fibrosis. DMD is caused by the loss of the Dystrophin protein, a large multidomain protein, which bridges the actin cytoskeleton to the transmembrane Dystrophin Associated Protein Complex (DAPC) and thereby to the extracellular matrix. The pathology of DMD resembles diseases in which the homeostasis of the proteome has been compromised. The phenotypic hallmarks of such diseases include age dependent onset, mitochondrial damage/ROS production, calcium mishandling and variable pathology. Our transcriptomic analyses of a C.elegans DMD model showed that many genes involved in metabolism, gene expression, ion homeostasis, autophagy, and protein synthesis and protein degradation are differentially expressed in comparison to wild type. Many of these cellular mechanisms have been shown to modulate the toxicity of conformational diseases. This was further supported by a large scale RNAi screen for suppressors of muscle degeneration in which we pulled out genes necessary for protein synthesis, and regulation of protein degradation and stress signaling. Based upon this, we have introduced mutations and transgenic constructs, which have been implicated in the stabilization of proteostasis, into the C. elegans DMD model and shown that these gene products can dually suppress muscle degeneration. It has been recently shown in the mdx mouse, that the loss of Dystrophin, does not actually decrease transmembrane DAPC protein expression, but rather stability: many of the transmembrane DAPC proteins were shown to form oligomers or SDS insoluable aggregates, rendering them inaccessible to classical immunohistochemistry and soluablizing treatments {Daval, 2010}{Cluchague, 2004}. This initial perturbation may provide a context for compromised proteostasis. We are currently introducing destabilizing protein polymorphisms into Dystrophin mutants so as to test for genetic enhancement.

394B

Mitochondrial Fission Protects *C. elegans* from Amyloid-Beta Toxicity. **Patrick K Gonzales**[1,2], Christopher D Link[1,2]. 1) Institute for Behavioral Genetics, Boulder, CO; 2) University of Colorado, Boulder, CO.

Alzheimers disease (AD) is a late-onset age-dependent, neurodegenerative disorder affecting 5.3 million Americans. Current evidence points to amyloid beta (Aβ), a peptide derived from the amyloid precursor protein (APP), as playing a central role in AD pathogenesis, but mechanisms of Aβ toxicity remain unclear. One possible mechanism of Aβ toxicity is mitochondrial dysfunction. Amyloid beta induces mitochondrial dysfunction in many AD model systems. Evidence of abnormal mitochondria morphology is present in AD post mortem brain tissue, APP over-expressing neuroblastoma cells, and our Aβ expressing transgenic worm. Mitochondria morphology is controlled by a number of fission and fusion genes. Fission and fusion genes are abnormally expressed upon induction of Aβ in neuronal cell lines. In this study, we explored the effect of fission and fusion genes on Aβ toxicity in a transgenic Aβ (1-42) producing worm. We found increased mitochondrial fragmentation in response to Aβ induction. We also report that RNAi knockdown of fission genes *drp-1* and *fis-1* result in increased toxicity, while RNAi of fusion gene *eat-3* protects against Aβ toxicity. Our results suggest that mitochondrial fission is a protective mechanism against Aβ toxicity. Currently, we are exploring autophagy as a potential mechanism for mitochondrial fissions protection against Aβ toxicity.

395C

Molecular characterization and suppression of *srf-2*, a DUF23 surface-antigenicity gene that affects bacterial infection. **Maria J. Gravato-Nobre**, Simon Spiro, Tom Baker, Dave Stroud, Jonathan Hodgkin. Dept Biochemistry, Univ Oxford, Oxford, United Kingdom.

The gene *srf-2* was initially identified in screens for mutants with altered surface antigenicity (S. Politz et al., 1990). Subsequently it has been shown to affect susceptibility to several bacterial pathogens. Srf-2 mutants are resistant to the formation of Yersinia biofilms on the head (the Bah, or Biofilm Absent on Head phenotype) and to tail-swelling induced by Microbacterium nematophilum (the Bus, or Bacterially Un-Swollen phenotype). Conversely Srf-2 mutants are hypersensitive to Leucobacter Verde1.

Molecular cloning of *srf-2* reveals that it encodes a predicted secreted protein with a DUF23 domain, belonging to a family of 61 related genes in C. elegans. Most of these DUF23 genes have no currently known biological function, but one of them is the gene *bah-1*, which affects adhesion of Yersinia (K. Drace et al., 2009). Bah-1 mutants, however, are resistant only to Yersinia, unlike Srf-2 mutants which are resistant to multiple pathogens. Double mutants (*srf-2 bah-1*) resemble *srf-2* single mutants. The DUF23 domain is currently of uncertain biochemical function, but may be associated with glycosylation. The sequence alterations in 9 *srf-2* mutations (both EMS and mutator-induced) have been determined. These include transposon insertions and lesions affecting a splice site and most exons of the coding region. All are likely to result in severe or complete loss-of-function, and have similar phenotypes.

Transgenic reporter constructs indicate that the major site of expression for *srf-2* is in the seam cells, like many other surface-affecting genes including *bah-1*. Effects on the cuticular surface have been examined by scanning EM. This reveals differences in several aspects of cuticle morphology in Srf-2 mutants, including the annuli, furrows and alae, which appear

less defined than in wildtype. Movement of Srf-2 mutants is also slightly abnormal, with a weak Skiddy phenotype.

Srf-2 mutants are unable to grow on bacterial lawns containing the surface-adherent bacterial pathogen Leucobacter Verde1, probably as a result of lethal cuticle damage. Suppressors or revertants of *srf-2* can therefore be efficiently selected by mutagenizing *srf-2* populations and exposing them to Verde1 lawns. By this means, several suppressed lines have been obtained. Further analysis of these suppressors is in progress.

396A

Host seeking and the evolution of olfactory behavior in parasitic nematodes. **Elissa A. Hallem[1]**, Adler R. Dillman[2], Manon L. Guillermin[1], Paul W. Sternberg[2]. 1) MIMG Department, University of California, Los Angeles, Los Angeles, CA; 2) Biology Division, California Institute of Technology, Pasadena, CA.

Many parasitic nematodes rely on olfactory cues to locate their hosts, yet little is known about how olfactory preferences contribute to the specificity of host-parasite interactions. We are investigating this question using the insect-parasitic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*; the mammalian-parasitic nematodes *Nippostrongylus brasiliensis* and *Strongyloides ratti*; and the free-living nematode *C. elegans* as a comparative model. We examined the chemotaxis behavior of parasitic infective juveniles (IJs) and *C. elegans* dauers in response to a large and diverse panel of ecologically-relevant odorants, including host odorants that we identified by gas chromatography-mass spectrometry of live hosts. We found that the insect-parasitic nematodes have different odor response profiles despite their overlapping host ranges. However, the odor response profiles of the insect-parasitic nematodes are more similar to each other than to *C. elegans* despite their phylogenetic distance, likely reflecting a key role for olfaction in their convergently evolved parasitic lifestyles. We also examined the responses of insect-parasitic nematodes to host versus non-host insects, and found that these parasites are capable of discriminating between different insect species using olfactory cues. We are now extending these studies to mammalian-parasitic nematodes to gain insight into the niche-specific olfactory adaptations of different parasitic species as well as the evolution of olfactory behavior. We are also examining the relative contributions of the universal cue carbon dioxide (CO_2) versus host-specific odorants in mediating host-seeking behavior. Our results indicate that parasites rely on both CO_2 and host-specific odorants for host location, but the relative importance of CO_2 versus host-specific odorants varies for different parasites and different hosts. Finally, we are investigating the neural basis of host-seeking behavior by identifying the sensory neurons and downstream neural circuits that are required for successful host location.

397B

Manganese-transporters have an important roles in innate immune system in Caenorhabditis elegans. **Hwan-Jin Kim**, Ju-Yeon Lee, Joeng-Hoon Cho. Dept of Biology Education, Chosun University, Gwangju, Korea.

Nramp1 is a functionally conserved manganese transporter in macrophages. Manganese (Mn), a superoxide scavenger, is required in trace amounts and functions as a cofactor for most antioxidants. Three Nramp homologs, smf-1, smf-2, and smf-3, have been identified thus far in the nematode Caenorhabditis elegans. A GFP promoter assay revealed largely intestinal expression of the smf genes from early embryonic through adult stages. In addition, smf deletion mutants showed increased sensitivity to excess Mn and mild sensitivity to EDTA. Interestingly, these smf deletion mutants demonstrated hypersensitivity to the pathogen Staphylococcus aureus, an effect that was rescued by Mn feeding or knockdown of the Golgi calcium/manganese ATPase, pmr-1, indicating that Mn uptake is essential for the innate immune system. This reversal of pathogen sensitivity by Mn feeding suggests a protective and therapeutic role of Mn in pathogen evasion systems. We propose that the C. elegans intestinal lumen may mimic the mammalian macrophage phagosome and thus could be a simple model for studying Mn-mediated innate immunity.

398C

A novel, high-throughput, liquid killing assay of *P. aeruginosa* infection of *C. elegans* provides insight about pathogenesis and host defense. **Natalia V. Kirienko[1]**, Jonah Larkins-Ford[1], Carolina Wählby[2], Frederick M. Ausubel[1]. 1) Dept Molecular Biol, Mass General Hospital, Boston, MA; 2) Imaging Platform, Broad Institute of Harvard & MIT, Cambridge, MA.

The opportunistic pathogen *Pseudomonas aeruginosa* is a serious threat to patients with severe burns, cystic fibrosis, or compromised immune systems. The same virulence mechanisms are often used in both mammalian and *C. elegans* infections by this pathogen. We are using a streamlined *P. aeruginosa* - *C. elegans* infection model to gain insight into the pathology of a *P. aeruginosa* infection and for development of treatment options. Two different modes of killing have been described in infections of *C. elegans* with *P. aeruginosa* strain PA14. “Fast killing” is toxin-dependent and kills worms within several hours of exposure. “Slow killing” takes place over the course of days and does not appear to involve low molecular weight toxins, but the precise mechanism of action is currently unknown. Here we describe a third mode, which we call “liquid killing”. We developed a reliable assay for measuring liquid killing in both 96- and 384-well plate formats. With this method, we can identify biochemical and genetic pathways of the bacterium as well as virulence factors that are involved in the infectious process, and the host defense pathways. Liquid killing exhibits overlapping and independent aspects when compared with slow killing. For example, mutations in a *C. elegans* MAPK pathway increase the susceptibility of worms to both. In contrast, liquid killing shows characteristic differences in the degree of intestinal colonization by PA14, the nature of the cytopathology, and the specific bacterial virulence factors involved. As this assay is amenable to high-throughput methodologies, we are currently using it for screening a transposon insertion library of *P. aeruginosa* to identify genes involved in pathogenesis. In addition, we are also using this assay in a high-throughput chemical genetics screen to discover novel antimicrobials and immunostimulatory compounds. A preliminary screen of approximately 10,000 compounds has already yielded several primary hits.

399A

Screening of natural products as anti-infectives towards *Staphylococcus aureus* using an *in vivo Caenorhabditis elegans* infection model. **Cin Kong[1]**, Man-Wah Tan[2], Sheila Nathan[1,3]. 1) Universiti Kebangsaan Malaysia, Bangi, Malaysia; 2) Department of Genetics, Stanford School of Medicine, Stanford University, USA; 3) Malaysia Genome Institute, Bangi Lama, Selangor, Malaysia.

A growing threat of antibiotic-resistant *Staphylococcus aureus* continues to persist in both nosocomial and community settings. The failure of existing antibiotics to control *S. aureus* infections has led to calls for novel therapeutic approaches to combat this human pathogen. To discover potential new anti-infective agents, we utilized the nematode *Caenorhabditis elegans* to perform an *in vivo* screen of a number of natural product extracts and synthetic compounds for anti-infective properties. To enable a rapid and cost-effective screen, the assay was carried out in liquid medium in 24-well plates. Worms fed on *S. aureus* in liquid medium were killed over the course of several days in an infection-like process. The screen was performed on the premise that the addition of extracts that are able to rescue *C. elegans* from ensuing death upon *S. aureus* infection are deemed to exhibit anti-infective properties. Screening of 60 compounds revealed 14 natural extracts and 12 synthetic compounds that prolonged the survival of *S. aureus*-infected nematodes. All positive hits contributed to more than 50% *C. elegans* survival following infection compared to almost 90% death of untreated *S. aureus* infected worms. The 26 compounds identified most probably act through several distinct mechanisms. When tested *in vitro* for inhibition of bacterial growth, a number of these extracts failed to affect bacterial growth. This demonstrates the utility of the screen to detect hits that may be overlooked in a conventional *in vitro* cell culture based screen. Two natural extracts identified in the screen did not inhibit the growth of *S. aureus* but appeared to act on the bacterial virulence *in vivo*. The extract-treated *S. aureus* killed the worms at a significantly slower rate compared to untreated *S. aureus* ($P<0.0001$). These findings indicate that the whole-animal *C. elegans in vivo* screening model is able to identify compounds that target bacterial virulence, a largely unexplored class of anti-infectives.

400B

Genetic analysis of *Caenorhabditis elegans* responses to grassland soil bacteria reveals both specific and general functions. **Vinod Kurumathurmadam Mony**, Michael Herman. Division of Biology, Kansas State University, Manhattan KS, USA.

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 interested in studying the genetic responses of native grassland nematodes to the environment focusing on their interactions with bacterial communities. Since native nematodes do not have well developed genetic resources, we are employing *Caenorhabditis elegans* to model their bacterial interactions in laboratory. We identified 204 *C. elegans* genes differentially expressed in response to growth on four different bacteria: *Bacillus megaterium*, *Pseudomonas sp.*, *Micrococcus luteus* and *Escherichia coli*. To assess whether these genes were functionally relevant in this interaction, we used available *C. elegans* loss-of-function mutants in 21 of these genes to perform fitness and lifespan (as an indicator of detrimental bacterial effects on nematodes) assays. For many of the genes the degree of differential gene expression between two bacterial environments predicted the magnitude of the effect of the loss of gene function on life-history traits in those environments (Coolon et al., 2009). We are now focusing on those differentially expressed genes that appear to be involved in defense and metabolism functions. We further classified these genes based on putative molecular functions, and found those encoding lysozymes, lectins and proteases were predominant. Functional analysis of mutations in defense or metabolism genes, including five *lys*, five *lec* and four protease genes, yielded several significant genotype-by-environment interactions. Among *lys* gene mutants, *lys-4* showed differential effects on all bacteria tested, *lys-1* showed reduced lifespan on *E. coli* and B. *megaterium*, but *lys-10* showed reduced lifespan only in response to *E. coli*. However, among *lec* gene mutants, *clec-50* showed lowered lifespan in response to *E.coli*, *B. megaterium* and *S. maltophilia* whereas *lec-9* displayed a longer lifespan in *E. coli*, *B. megaterium* and *Pseudomonas sp*. Thus, in response to *E. coli*, *C. elegans* requires different lysosomal components which might play an important part in defense against or metabolism of *E. coli*, whereas it employs same *lec* genes as a general response in different bacterial environments. We have also found the involvement of proteases (*cpr-5* and *cpi-1*) in regulating worm lifespan in different bacterial environments, also suggesting a general response. Thus it appears that *C. elegans* employs different combinations of lysozymes, but the same lectins and proteases to defend against diverse soil bacteria.

401C

Identifying host factors required for Sindbis virus replication using *C. elegans*. **Michael Laha[1,2]**, Hugo Decker[1,2], Ellen Bradley[1,2], Charles Rice[1], Margaret MacDonald[1], Shai Shaham[2]. 1) Laboratory of Virology and Infectious Disease, The Rockefeller University, NY; 2) Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

Members of the *Alphavirus* genus infect equine and human hosts and cause symptoms

such as rash, arthritis, fever, encephalitis and in some cases death. Of the *Alphaviruses*, Sindbis virus (SIN) is the most extensively studied and viral factors required for SIN genome replication have been characterized. While proteomic approaches have uncovered host factors that bind viral proteins, the absence of a genetic model has hindered progress in uncovering the functional roles of such factors.

Although a natural *C. elegans* virus has not been described, a number of viruses have been reported to replicate in *C. elegans*, suggesting that this animal may provide a suitable setting to study host factors required for SIN replication. To study SIN replication, we generated transgenic *C. elegans* harboring a SIN replicon in which the structural proteins open reading frame was replaced with GFP, and whose expression is controlled by the heat-shock promoter (HSP). GFP expression from similar SIN replicons in other systems is dependent on viral replication and expression of a subgenomic viral RNA. We found that upon heat shock, transgenic animals expressed low and variable levels of GFP. Introducing the SIN transgene into *rde-4* RNAi-defective mutants greatly enhanced expression as assessed by GFP and by Northern analysis, suggesting that the double-stranded RNA SIN replication intermediate is likely inactivated by the RNAi machinery. Although HIP::mCherry co-injected with the HIP::SIN transgene was expressed in all cells upon heat shock, GFP expression was primarily detected in *C. elegans* muscle cells. This result suggests that SIN preferentially replicates in this cell type. Similar muscle-cell replication tropism has been described for SIN in murine models, and suggests that *C. elegans* may indeed be an appropriate setting for uncovering host factors required for SIN replication.

To uncover such host factors, we performed EMS mutagenesis and isolated 12 mutants with reduced GFP expression. We are using SNP mapping, whole genome sequencing and fosmid rescue experiments to identify the relevant genes. Once identified, genes with obvious mammalian homologs will be studied for roles in established cell culture systems. Our studies may provide insight into the complicated host-*Alphavirus* interplay that leads to infection and disease.

402A

Innate immune response to microsporidian infection. **Amy T. Ma**, Emily R. Troemel. Cell and Developmental Biology, University of California, San Diego, La Jolla, CA.

Nematocida parisii is a natural intracellular pathogen of *C. elegans* and was originally isolated from a wild-caught worm near Paris. *N. parisii* is an obligate intracellular parasite in the Microsporidia phylum, which is made up of over 1200 species of fungal-related pathogens that infect a wide range of hosts (Troemel, 2011). Microsporidia infect their hosts by injecting infectious sporoplasm directly into host cells via a specialized infection apparatus contained within spores, called a polar tube. After infection, *N. parisii* reproduces within the intestinal cell as a replicative meront and is in direct contact with the host cell cytostol. Subsequently, spore formation occurs and spores exit host cells to establish new infections. During later stages of infection, intestinal cells are loaded with *N. parisii* spores and eventually *C. elegans* die prematurely. We are interested in identifying novel components of the *C. elegans* innate immune that are elicited in response to *N. parisii* infection. First, to see if previously identified pathogen defense pathways are involved in host response to *N. parisii* infection, we are knocking down select pathways and evaluating changes in *N. parisii* infection. Previously, we have shown that *pmk-1* and *daf-2* have little effect on pathogen development (Troemel, et al., 2008), indicating that response to this natural intracellular pathogen may be distinct from response to predominantly extracellular pathogens. To identify novel components of innate immune response, we are undertaking an unbiased RNAi screen to identify host genes that affect the rate of *N. parisii* development and *C. elegans* survival. This work will contribute to the overall understanding of *C. elegans* defense against pathogen infection and could identify novel mechanisms for *C. elegans* to contain intracellular pathogens. References. Troemel, E. (2011). New Models of Microsporidiosis: Infections in Zebrafish, C. elegans, and Honey Bee. PLoS Pathogens, 7 (2), e1001243. Troemel, E., Félix, M.-A., Whiteman, N., Barrière, A., & Ausubel, F. (2008). Microsporidia are natural intracellular parasites of the nematode Caenorhabditis elegans. PLoS biology, 6 (12), 2736-52.

403B

Identifying *P. aeruginosa* factors that activate *C. elegans* immunity. **Deborah L. McEwan**, Frederick M. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

Intestinal epithelial cells must quickly determine whether a microbe is a commensal or a pathogen. Failures in this decision process can result in serious infections and have been associated with many human disorders including metabolic syndrome, Crohn's disease, and irritable bowel syndrome. However, the mechanisms underlying pathogen recognition by epithelial cells is poorly understood, especially since virulent bacteria often contain the same conserved microbial features as related but non-virulent strains that fail to activate immune pathways. To understand how hosts distinguish pathogens from non-pathogens at epithelial barriers, we are studying how the virulent *P. aeruginosa* strain PA14 elicits an immune response in *C. elegans* intestinal cells. *P. aeruginosa* virulence factors likely act redundantly to establish infection and analysis of PA14 avirulent mutants has yet to identify a *P. aeruginosa* factor, such as an exotoxin, that is necessary for *C. elegans* immune activation. Because of this, we are screening for individual *P. aeruginosa* genes that can trigger the expression of *C. elegans* antimicrobial effectors. Specifically, we are feeding worms candidate PA14 genes expressed in a normally non-pathogenic *E. coli* strain. Previous experiments have found that heat-killed PA14 fails to activate *C. elegans* immunity, and so we are focusing on factors known to be secreted only by viable bacteria. Using this approach, we have identified a preliminary *P. aeruginosa* candidate that upregulates *C. elegans* immunity. Further characterization of this candidate and progress towards discovering additional *P. aeruginosa* immune triggers will be discussed.

404C

Protective role of *dnj-27*, a thioredoxin family member, on *C. elegans* models of Alzheimer's and Parkinson's Diseases. **F Munoz-Lobato**[1], CD Link[2], S Hamamichi[3], KA Caldwell[3], GA Caldwell[3], A Miranda-Vizuete[1]. 1) Centro Andaluz de Biologia del Desarrollo. Seville, Spain; 2) Institute for Behavioral Genetics. University of Colorado. Boulder, USA; 3) Department of Biological Sciences. University of Alabama. Tuscaloosa, USA.

Common cellular mechanisms underlie many aging-associated neurodegenerative diseases (ND). For instance, protein aggregation and oxidative stress have been shown to lie beneath the initiation and progression of many ND such as Alzheimer's (AD) and Parkinson's (PD) diseases. The thioredoxin (TRX) system is one of the most important antioxidant defenses and among its functions are not only a cytoprotective effect against oxidative stress but also chaperone function. One of the main interests in our lab is to study the implication of the TRX system in the development of ND. *Caenorhabditis elegans* is an attractive model system to study the TRX system since available mutants of all TRX family members are viable. To ascertain the role of the thioredoxin system in ND, we performed an RNAi screen of all known genes of the TRX family in transgenic *C. elegans* models of AD (by overexpression of the human beta-amyloid (Aβ) peptide) and PD (by overexpression of a fusion protein composed of human alpha-synuclein (α-syn) and GFP). In these models, Aβ and α-syn::GFP peptides aggregate in the cytoplasm leading to pathological phenotypes that can be easily monitored. We found that, out of all the thioredoxin genes screened, only Y47H9C.5/*dnj-27* appears to have a protective effect in both AD and PD models. dnj-27 is the orthologue of the mammalian endoplasmic reticulum (ER) resident protein ERdj5, a protein required as a disulfide reductase for degradation of misfolded proteins by the ER-associated degradation (ERAD) pathway. Using a *Pdnj-27::GFP* transgene we show that *dnj-27* expression is induced by both human Aβ and α-syn in *C. elegans* body wall muscle cells. We also found that *dnj-27* expression in worms is induced by ER stress upon tunicamycin treatment via IRE-1/XBP-1 pathway and by ero-1 RNAi, an ER-resident sulfhydryl oxidase required for disulphide bond formation in protein folding, suggesting an evolutionary conserved role of ERdj5/dnj-27 in ERAD. Phsp-4::GFP expression, a reliable indicator of the level of unfolded proteins and of UPR activation, was used to determine whether UPR activation occurs upon Aβ and α-syn overexpression. Surprisingly, we found UPR activation in the AD model but not in the PD model. Furthermore, overexpression of *dnj-27* attenuated Aβ-induced Phsp-4::GFP expression. We hypothesize that Aβ and α-syn aggregation in the cytoplasm might affect ER homeostasis and that DNJ-27 could be involved in the maintenance of this ER homeostasis.

405A

Genetic architecture of natural variation in *Caenorhabditis elegans* pathogen avoidance. **R. Nakad**[1], B. Snoek[2], S. Ellendt[1], T.G. Mohr[1], K. Dierking[1], J. E. Kammenga[2], H. Schulenburg[1]. 1) Evolutionary Ecology and Genetic, Christian-Albrechts University, Kiel, Germany; 2) Laboratory of Nematology, Wageningen University,Wageningen, The Netherlands.

The nematode *Caenorhabditis elegans* feeds on microbes in its natural environment. Some of these microbes are pathogenic and thus harmful to *C. elegans*. To minimize resulting fitness reductions, *C. elegans* has evolved various defence mechanisms including behavioral responses (e.g. avoidance behavior) that reduce contact with the infectious microbes. In this study, we characterized the genetic architecture of natural variation in *C. elegans* avoidance behavior against the infectious stages of the Gram-positive bacterium *Bacillus thuringiensis*. We performed an analysis of quantitative trait loci (QTL) using recombinant inbred lines (RILs) and near isogenic lines (NILs) generated from a cross of two genetically as well as phenotypically distinct natural isolates. The results identified at least three QTLs that underlie variation in pathogen escape behavior. One of the candidates is the *npr-1* gene which encodes a homolog of the mammalian neuropeptide receptor and which was previously indicated to contribute to behavioural defense. Interestingly, in our case, *npr-1* alleles appear to influence avoidance behavior in exactly the opposite way than the previous studies. Our findings highlight the central role of *npr-1* in fine-tuning nematode behavior in an ecological context.

406B

Morphological Study of Muscle Degeneration in *C.elegans*. **Nicolas Brouilly**, Claire Lecroisey, Laura Pierson, Kathrin Gieseler. CGPhiMC, Lyon, France.

Duchenne Muscular Dystrophy (DMD) is a severe myopathy caused by the absence of functional dystrophin in cardiac and skeletal muscles. Dystrophin is a large protein which bridges the muscle cytoskeleton to the extra-cellular matrix. In the absence of dystrophin, the structural anchorage of the contractile apparatus and the integrity of the plasma membrane are impaired and the muscle degenerate progressively. Despite more than 20 years of research, the physio-pathology of DMD is still poorly understood.

C.elegans has a dystrophin homolog, DYS-1, which exhibits conserved function with the human dystrophin protein. In a sensitized genetic background, the loss of DYS-1 leads to a progressive muscle degeneration.

We have undertaken a morphological characterization of dystrophin-dependent muscle degeneration in *C.elegans* by electron microscopy. Using high-pressure freezing and low temperature substitution, we were able to observe intact tissues at high resolution. We analyzed the progression of muscle degeneration throughout *C.elegans* life cycle. In depth

observations and statistical analysis on electron-micrographs, as well as 3D reconstructions of wild-type and degenerating muscle cells allowed us to investigate the pathogenesis of muscle degeneration.

During the muscle degeneration process, we observed that the *C.elegans* DMD worms display progressive decomposition of muscle cell structures including organelles and cytoskeleton elements. In addition, we noted the appearance of a variety of single and multi-membranous vesicles. These vesicles are reminiscent of pathologies linked to dysfunctionning autophagy and/or deficient endosomal clearance. Interestingly, our previous studies suggested an implication of endocytosis regulators during muscle degeneration. How and why this mecanism is implicated in the dystrophin-dependent muscle degeneration process is yet unknown.

407C

Mutations in *bus-10*, encoding a putative membrane protein, alter both susceptibility and resistance to different bacterial infections. **Delia M. O'Rourke**[1], Rebecca Price[1], Nicholas Lillios[2], Maria Gravato-Nobre[1], Jonathan Hodgkin[1]. 1) Dept of Biochemistry, University of Oxford, Oxford, UK; 2) University of Wisconsin-Madison, Madison, USA.

C. elegans is used extensively to study host-pathogen interactions. We have conducted genetic screens to identify mutations that cause resistance to infection by the nematode-specific Gram-positive bacterial pathogen, *Microbacterium nematophilum*. This bacterium attaches to the rectal and post-anal cuticle and causes slow growth and a defensive swelling of the rectal epithelial cells. Many of the mutations (representing at least 20 different genes) isolated in our screens alter the cuticle of the worm and prevent attachment of bacteria and the establishment of an infection.

We used whole genome sequencing to identify one of these mutants, *bus-10*, as ZK596.3. This gene encodes a 322 amino acid nematode-specific protein, predicted to be integral to the membrane. We have characterised several large deletions of the gene as well as multiple transposon insertions derived from a *mut-7* induced mutagenesis screen, in which *bus-10* was found to be a conspicuous hot-spot for transposon-induced mutation (>30 *bus-10* alleles recovered). Two EMS-chemically induced alleles were identified as stop mutations in exons 2 and 3. Using a bicistronic construct with the *bus-10* promoter and gene linked to the *gpd2/3* sequence and TagRFPT, we demonstrate that a transgenic worm strain expressing ZK596.3 can rescue the Bus-10 phenotype. We find that the *bus-10* promoter is active in many tissues, most notably in the seam cells, rectal gland cell, rectal valve and the excretory gland.

In addition to preventing attachment of *M. nematophilum*, *bus-10* mutants are also strikingly altered in the response to two other pathogens, Leucobacter strains Verde1 and Verde2. Whereas wild type worms survive infection by Verde 1, but are killed by infections with Verde2, *bus-10* worms, in contrast, are killed by infection with Verde1, but survive infection with Verde2. We have use the differential susceptibility of *bus-10* infection by Verde1 to conduct a suppressor screen for mutagenised *bus-10* worms that can survive on Verde1. This screen identified 8 suppressors, and initial mapping followed by complementation assays suggest that we have identified 8 alleles of one extragenic suppressor gene. We will present further data on the identification and properties of this suppressor gene and propose a model to explain how the interactions of *bus-10* and its suppressor result in the striking differential responses of these worms to infection by two different Leucobacter strains.

408A

Regulation of PMK-1 p38 MAPK-dependent immunity by the transcription factor ATF-7 in *C. elegans*. **Daniel J. Pagano**, Dennis H. Kim. Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

Innate immunity in *C. elegans* requires a conserved PMK-1 p38 mitogen-activated protein kinase (MAPK) pathway that regulates the basal and pathogen-induced expression of immune effectors. We identified ATF-7, a conserved basic-region leucine zipper (bZIP) transcription factor, as a downstream target of PMK-1 p38 MAPK and a regulator of immune effector gene expression. Loss of *atf-7* function can partially suppress the enhanced susceptibility to pathogen phenotype conferred by loss of *pmk-1* function by restoring the basal expression of PMK-1-regulated genes. In contrast, the pathogen-induced expression of these genes is not restored by loss of *atf-7* function in a *pmk-1* mutant. This suggests a model in which, when PMK-1 p38 MAPK is inactive, ATF-7 functions as a transcriptional repressor of PMK-1-regulated genes that, during infection, undergoes a switch to a transcriptional activator upon phosphorylation by activated PMK-1 p38 MAPK. To identify other components that regulate the PMK-1 p38 MAPK-dependent immune response, we performed a *pmk-1* suppressor screen. We screened 35,000 haploid genomes and identified 51 mutants that partially suppress the enhanced susceptibility to pathogen phenotype conferred by *pmk-1* loss-of-function. We anticipate that our genetic screen will identify genes that function with *atf-7* and provide insight into the mechanism of the ATF-7-mediated transcriptional control of PMK-1 p38 MAPK-dependent immunity.

409B

Genomic Analysis of Immune Response Against *Vibrio cholerae* Hemolysin in *Caenorhabditis elegans* . **Surasri Sahu**[1], Jada Lewis[1], Isha Patel[1], Serdar Bozdag[2], Joseph LeClerc[1], Hediye Cinar[1]. 1) Divn. of Virulence assessment, Food & Drug Administration, Laurel, MD; 2) Neuro-Oncology Branch, Natioal Cancer Institute, NIH, Bethesda, MD.

Vibrio cholerae, a natural inhabitant of aquatic ecosystem, causes acute severe gastroenteritis in large populations through epidemic and pandemic dissemination. The major virulence factor responsible for massive fluid loss via watery diarrhea is cholerae toxin (CT). *V. cholerae* strains, which lack CT, and *V. cholerae* vaccine strains with deleted CT locus are also capable of causing diarrhea, soft tissue infections, sepsis, inflammatory enterocholitis, in humans in a sporadic fashion through mechanisms that are currently unclear. *V. cholerae* cytolysin (VCC) is among the accessory *V. cholerae* virulence factors that may contribute to sporadic disease pathogenesis. VCC, encoded by *hlyA* gene, belongs to the most common class of bacterial toxins, pore-forming toxins (PFTs), which are important virulence factors. *Vibrio cholerae* infects and kills *C. elegans* via cholerae toxin independent manner. VCC is required for the lethality, growth retardation and intestinal cell vacuolation during the infection (1). Little is known, however about the gene expression responses against VCC. To address this question we performed a microarray study in *C. elegans* which was exposed to *V. choleare* strains with intact and deleted *hlyA* genes, for 18 hours. 2611 differentially expressed genes were identified when we compared expression in *C. elegans* exposed to wild type E7946 versus E7946 Δhly, and 758 differentially expressed genes when we compared expression in *C. elegans* exposed to nearly isogenic *V. cholerae* vaccine strains CVD109 (*hlyA*+) versus CVD110 (*hlyA*-) [considering fold change (+/-) 1.2, FDR=0.5 and P<0.01]. We found significant overlap between these two comparisons, such that 582 genes are common between the two. Many of these genes have been previously reported as mediators of innate immune response against other bacteria in *C. elegans*. Among the differentially expressed genes are: C-type lectins, such as *clec-45, clec-174, clec-209, clec-17, clec-47, abu* (activated in blocked unfolded protein response) genes, which contain Prion-like (Q/N-rich)-domain; *pqn-5, abu-6, abu-7, abu-8*, lipase related *lips-6*, tollish gene *toh-1*, genes regulated by *daf-16; dod-22* and *dod-24*. Protective function of the subset of the differentially expressed genes against *V. cholerae* infection was confirmed using RNAi. Our results suggest that VCC is a major virulence factor, which induces wide variety of immune response related genes during *V. cholerae* infection in *C. elegans*. (1) Cinar et al. PLoS ONE, 2010, 5(7): e11558. doi:10.1371/journal.pone.0011558.

410C

A Feeding RNAi Screen to identify novel genes in anti-viral immunity in C. elegans. **Shruthi Satish**[1], Zhihuan Gao[1], Stephanie Coffman[1], YuanYuan Guo[1], Michael Cantrell[2,1], Gina Broitman-Maduro[1], Morris Maduro[1], Shou-wei Ding[1]. 1) University of California Riverside, Riverside, CA; 2) University of Idaho.

Viruses with an RNA genome replicate, interact with hosts and evolve via mechanisms distinct from DNA genomes and are important human, animal and plant pathogens. We have been using C. elegans to examine immune mechanisms that defend against replication of the Flock House Virus (FHV), one of the best characterized positive-strand RNA viruses. We have previously shown that FHV replication launched from an inducible transgene integrated in the C. elegans genome triggers production of virus-derived siRNAs, which guide viral RNA clearance by RNA silencing or RNA interference (RNAi). The FHV genome includes two RNAs, one of which encodes an RNA-dependent RNA polymerase (RdRP) and B2, a protein that blocks host RNAi. Using a heat shock-driven transgene to express RNA1 in which the B2 coding region has been replaced with GFP, we can visualize replication of viral RNA by observing GFP expression. We have used this transgene in an rrf-3(pk426) background to perform an RNAi screen for genes that when knocked down lead to greatly increased appearance of GFP, and hence replication of RNA1. We have used the ORFeome library to knock down ~55% of C. elegans genes. Approximately 250 genes showed reproducible ability to enhance viral genome replication. These genes fall into several different pathways, the main ones being those involved in protein synthesis and post translational modification, development, stress response, autophagy and apoptosis, as well as genes with no known function. Several genes were identified that function in RNAi, consistent with results we have previously obtained using chromosomal mutants, suggesting that these new genes will be relevant to antiviral mechanisms. We will report on our progress in further characterization of the genes identified. Although FHV is not a known C. elegans pathogen, we expect that some of these genes may be important for immunity to natural pathogens, such as the recently identified Orsay Virus in C. elegans, which also is a member of the Nodaviridae as is FHV (Felix et al,. 2010).

411A

Characterization of pathogenesis of S. aureus and involvement of checkpoint response in C. elegans. **Lone V. Scholer**[1], Steffen Noerregaard[1], Frederik D Hansen[2], Kurt Fuursted[3], Anders Olsen[1]. 1) Dept Molec Biol, Aarhus Univ, Aarhus C, Denmark; 2) Dept Med Microbiology and Immunology, Aarhus Univ, Aarhus C, Denmark; 3) Dept Clinical Microbiology, Aarhus University hospital Skejby, Denmark.

Staphylococcus aureus (S. aureus) is a frequent colonizer of humans contributing to normal bacterial flora in nose, skin and mucosa. In addition, it is an important human pathogen causing various diseases ranging from superficial skin infections to life-threatening infections. Methicillin resistant S. aureus (MRSA) strains are rapidly spreading in the community (CA-MRSA) and is consequently becoming a more serious health threat. The capacity of S. aureus to cause this spectrum of human diseases reflects an ability to adapt to distinct microenvironments in the human body and suggests that the pathogenesis of S. aureus infection is a complex process involving a diverse array of virulence determinants that are coordinately expressed at different stages of infection. Studies have shown that virulence factors are very differently distributed among strains and are not always regulated in the same way, reflecting the ability of S. aureus to adapt and survive in different environmental niches and resist antibiotic treatment. C. elegans has previously been established as a model host for the study of S. aureus pathogenesis and CA-MRSA strains are capable of killing C. elegans[1]. We have screened thirty clinical MRSA isolates and found that in terms of pathogenicity they can be divided into separate classes. Using these classes we wish to identify new virulence markers and novel immunity pathways activated in response to S. aureus infection. We are particularly interested in evaluate

virulence determinants during colonization versus infection and establishing their clinical relevance in humans. Inactivation of some S-M checkpoint proteins increase stress resistance and lifespan of C. elegans[2]. Since there may exist a potential correlation between stress response and immune function we are studying the role of checkpoint proteins to S. aureus exposure. We have identified several novel genes with potential checkpoint function which increase lifespan and thermotolerance. We find that that mutation of one of these, the transmembrane protein NDG-4, also confers resistance towards S. aureus infection. Epistasis analysis reveals that increased tolerance towards S. aureus infection is partially dependent on insulin signaling but that another unidentified component is involved as well. 1. Sifri, C.D., et al. 2003. 71(4): p. 2208-17. 2. Olsen, A., M.C. Vantipalli, and G.J. Lithgow. Science, 2006. 312(5778): p. 1381-5.

412B

Towards a SNP map for *Heterorhabditis bacteriophora*. **Hillel Schwartz**, Paul Sternberg. Division of Biology and HHMI, California Institute of Technology, Pasadena, CA.

Heterorhabditis bacteriophora is a species of insect-parasitic nematode that lives in mutually beneficial symbiosis with pathogenic *Photorhabdus* bacteria. *Photorhabdus* bacteria are lethal to insects and to other nematodes, including the soil nematode *Caenorhabditis elegans*, but are required for *H. bacteriophora* growth. The symbiosis between *Heterorhabditis* nematodes and *Photorhabdus* bacteria therefore offers the potential to study the molecular genetic basis of their cooperative relationship. We are interested in developing tools to make such studies more feasible; in particular, we have obtained and inbred independent isolates of *H. bacteriophora* and identified polymorphisms using high-throughput sequencing. We are in the process of creating a SNP map; comparison of genomic sequences of the reference *H. bacteriophora* strain M31e and an inbred derivative of a Moldovan isolate identified approximately one SNP for every 3.5 kbp; these polymorphisms are being used to score recombinant inbred lines to place supercontigs in a genetic map. We are also developing techniques for the use of *H. bacteriophora* in the laboratory, and are investigating other *Heterorhabditis* nematodes. We anticipate that these resources will enable us and the wider insect-parasitic nematode community to identify induced mutations and natural variations affecting the interactions involved n the symbiosis between *Heterorhabditis* nematodes and pathogenic *Photorhabdus* bacteria.

413C

LIN-7 PDZ Modulates DAF-2-mediated Innate Immunity Signaling and Infection Sensitivity in Caenorhabditis elegans. **Xiao-Hui Sem[1,3]**, Jason Kreisberg[1], Mikael Rhen[3], Patrick Tan[1,2]. 1) Genome Institute of Singapore, Agency for Science, Technology and Research; 2) Duke-NUS Graduate Medical School Singapore; 3) Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden.

BACKGROUND: We have previously utilized the soil nematode Caenorhabditis elegans as a model host to characterize the mechanisms by which pathogenic bacteria mount an in vivo infection. To further define these host-pathogen interactions, we have carried out a reverse genetic analysis in the worm to identify genes that modulate host responses to pathogenic bacteria.

RESULTS: We have identified a new role for the C. elegans intestinal cell junction protein, LIN-7, whose expression regulated the nematode's sensitivity to multiple pathogens. We discovered that genetic inhibition of *lin-7* significantly enhanced C. elegans survival after infection by Burkholderia spp. Similarly, *lin-2* and *lin-10* mutants also resisted infection. Several lines of evidence indicate that *lin-7* likely acts to regulate the DAF-2/DAF-16 insulin-like innate immunity pathway. The DAF-2 insulin-like receptor C-terminus is predicted to bind LIN-2, and the pathogen resistance of *lin-7* mutants is dependent genetically on *daf-2/daf-16* signaling. *lin-7* mutants exhibited transcriptional upregulation of DAF-16-regulated innate immunity genes even in the absence of infection, and both *daf-2* and *lin-7* mutants exhibited similar patterns of intestinal nuclear DAF-16 subcellular localization following heat stress. *lin-7* mutants also resisted infection by Pseudomonas and Salmonella spp., further supporting a role for *lin-7* in general innate immunity.

CONCLUSION: Our results have demonstrated that LIN-7 functionally interacted with LIN-2 and LIN-10 during bacterial infection, and that this complex may regulate the localization of the insulin-like receptor, DAF-2, affecting the evolutionarily conserved DAF-2/DAF-16-mediated signaling pathway of the worm innate immunity. These data collectively suggests that in polarized epithelial cells lining the C. elegans gastrointestinal tract, the precise subcellular localization of innate immunity signaling components, mediated by factors such as LIN-7, may play an important role in controlling host responses to in vivo bacterial infections.

414A

Key residues of Cry5B structure and function: mutagenesis by alanine screening. **Jillian Sesar**, Yan Hu, Raffi V Aroian. Division of Cell Biology, University of California, San Diego, San Diego, CA.

Soil-transmitted helminthes (hookworms, whipworms, and *Ascaris*) infect more than 1 billion people worldwide. Only one drug (albendazole) is able to show a high enough efficacy against parasitic worms under conditions for mass drug administration. However, recent studies have shown an increase in resistance to this drug, signifying that a new treatment option is quickly needed. Crystal (Cry) proteins produced from the soil bacterium *Bacillus thruingiensis* have been used for decades as a means to control insects that destroy crops and transmit human diseases and are known to be safe to humans. Our lab is currently investigating several of these crystal proteins and their potential to be safe and effective anthelmintics. Cry proteins intoxicate invertebrates by acting as pore-forming toxins. Several defined steps in their mechanism of action have been suggested from insect studies, and there is still yet great uncertainty as to the importance of these various steps. There is also another school of thought that, in addition to acting as pore-forming toxins, Cry proteins also intoxicate cells via signal transduction events independent of pore formation. We believe the *C. elegans* - Cry5B system has great potential to unlock mysteries surrounding Cry proteins. Here, we are investigating structure-function relationships of Cry proteins via alanine scanning. We are in the process of mutating every residue of Cry5B with alanine to determine which amino acids are important for Cry5B. Here we will present our latest data from an initial screen in domain III of the protein in which we have already found mutations of interest that show both increased and decreased toxicity towards *C. elegans* as compared to the wild type Cry5B. Our ultimate goal will be to correlate these changes in activity with specific changes in protein functionality (eg, receptor biding). This study provides a way to systematically identify the important residues in Cry5B, which will provide more information on the protein function, as well as to identify improved protein variant candidates for treating parasitic worms.

415B

Caenorhabidits elegans commensals provide protection from *P. aeruginosa* infection. Sirena Montalvo-Katz[1], Oliver Huang[1], Susannah Tringe[2], **Michael Shapira[1]**. 1) Integrative Biol, UC Berkeley, CA; 2) DOE Joint Genome Institute, Wallnut Creek, CA.

Caenorhabditis elegans is used extensively as a model to study infection and host-pathogen interactions. However, very little is known about the natural context in which such interactions occur and which is responsible for the evolution of the participating mechanisms, both in the worm and in its pathogens. To fill this gap we set out to characterize the bacteria with which *C. elegans* interacts in its soil environment. To this end, initially sterile wild-type N2 worms were grown to adulthood in rich soil. The Microbial communities associated with these worms were characterized by pyrosequencing of 16S rDNA and were found to consist mostly of proteobacteria and bacteriodetes, with a small representation of firmicutes. From worms grown on soil we were also able to isolate and culture thirteen different resident species, among them several *Bacillus* and *Pseudomonas* species, including *P. aeruginosa*. Additional analyses assessed the survival of worms when grown on pure cultures of three of these isolates. All three supported worm lifespan comparable to that of worms grown on *E. coli*, suggesting that they are commensals. When used as food source to support worm growth from egg to L4, two of the three tested were found to increase resistance to a subsequent infection with the pathogenic PA14 *P. aeruginosa* strain. Additional experiments suggest that this protection is provided by distinct mechanisms. In summary, our data support previous assumptions of long-standing interactions between *C. elegans* and *P. aeruginosa*, and extend those to include commensalism. They further suggest that certain soil-derived species can colonize the worm and may be involved in the ability of *C. elegans* to resist infection in its natural habitat.

416C

Fat Burning in Sleeping Worms: Regulation of Multiple Signaling Pathways by Kruppel Like Factors. **Shahid S. Siddiqui[1]**, Sarwar Hashmi[2], Jun Zhang[2], Chen Yang[2], Immanuel Dhansingh[1], David Gozal[3]. 1) Dept Med, Univ Chicago, Chicago, IL; 2) New York Blood Center, New York, NY; 3) Dept of Pediatrics , University of Chicago, IL.

Sleep is an important behavior across diverse species, regulated by conserved signaling pathways. OSAS (obstructive sleep apnea syndrome) is characterized by episodic hypoxia, affecting about 5% of adult American population and may lead to hypertension, if left untreated. In *C. elegans* post-embryonic development is accompanied by four larval stages [Sulston et al., 1983] that are interrupted by periods of inactivity called lethargus, and has been suggested to represent 'sleep' [Raizen et al., 2008]. The cAMP-CREB pathway has been shown to affect sleep-wake cycle in *Drosophila, C. elegans* and mammalian systems [Zimmerman et al., 2008], and cGMP dependent protein kinase expression is regulated by Rho and KLF4 [Zeng et al., 2006]. Genetic analysis has suggested the role of LIN-3 and LET-23 (the EGF like-ligand and EGF receptor, respectively) in sleep like behavior in *C. elegans* [Van Buskirk and Sternberg, 2007]. Overexpression of LIN-3 results in quiescent behavior during periods of normal activity, through the activity of ALA neuron that is regulated by paired (a family of Kruppel like transcription factors) and LIM class of transcription factors CEH17, CEH-14/Chx10, and CEH17/Phox2 through a feedback regulatory pathway [Van Buskirk and Sternberg, 2010]. We have shown the role of KLF-3 in beta oxidation of fatty acids in *C. elegans* that results in accumulation of fat in intestinal cells in *klf-3(ok1975)* homozygous loss of function mutants, arrested development and poor fecundity [Hashmi et al., 2008; Zhang et al. 2011 submitted], and may also affect sleep cycle by affecting the lethargus timings during larval molts. Interestingly, KLF4 in mammals not only mediates adipogenesis but also regulates kallistatin expression [Shen et al., 2009] that regulates kidney protein expression during episodic hypoxia (EH), by inhibiting vasodilation mediated by kallikrein-Kallistatin signaling pathway [Thonhboonkered et al., 2002]. The sleep regulating *egl-4* gain of function mutation in cyclic GMP also results in the accumulation of fat in the intestine [Raizen et al., 2006]. Thus accumulating results suggest that *klf-3* signaling pathway analysis in *C. elegans* may elucidate fat utilization (see the abstract by Hashmi et al., this meeting), sleep, and reproductive behavior.

417A

Microarray analysis of pathogen response in *C. elegans* and *P. pacificus* reveals differences in molecular effectors of innate immunity. **Amit Sinha**, Igor Iatsenko, Robbie Rae, Ralf J. Sommer. Dept. of Evolutionary Biology, Max Planck Institute for Developmental Biology,

Tuebingen, Germany.

Free-living nematodes such as *C. elegans* and *P. pacificus* encounter a variety of bacteria in their natural environment, some of which are utilized as food source while others are pathogenic and need to be defended against. Studies on innate immunity in *C. elegans* have uncovered various signaling pathways and their downstream targets involved in response to various bacterial pathogens. Unlike the bacteriovore *C. elegans*, *P. pacificus* is omnivorous and found in necromenic association with scarab beetles. Because of the different ecology, genes and pathways involved in its innate immunity might differ from *C. elegans*. Indeed, both nematodes show different susceptibilities to pathogenic bacteria (Rae et al 2010). Study of genetic basis of these differences can shed light on how innate immunity evolves with different ecologies. *P. pacificus* is a well established model for studies in evolution, development and ecology, and genetic and transgenic tools and a sequenced genome are available. We have now added a microarray based gene expression profiling platform to the toolkit for studying *P. pacificus* biology. Here, we use these microarrays to identify and compare the genes induced /repressed upon exposure of *C. elegans* and *P. pacificus* to different pathogens. Since *P. pacificus* arrays were custom manufactured by Agilent Technologies, we used the commercial *C. elegans* arrays also from Agilent, to avoid platform specific effects on expression profiles. Gram-positive bacteria *Bacillus cereus* (strain DB27) and *Staphylococcus aureus*, and gram-negative bacteria *Serratia marcescens* and *Xenorhabdus nematophilium* were tested. While all 4 were lethal to *C. elegans*, *P. pacificus* survived on *Bacillus* DB27 and *S. aureus*. We compared expression profiles of the two nematode species after 4-hour exposure to each of these pathogens. Age-matched worms fed on *E. coli* (OP50) were used as controls. We observe induction of many genes typically associated with immune response, e.g. C-type lectins, lysozymes, genes involved in detoxification, stress response, osmoregulation and starvation response. Many of these genes are targets of p38-MAPK *pmk-1*, while others are targets of RNAi pathway members *dcr-1*, *rde-4* etc. However, the overlap between the expression profiles of the two species is minimal, suggesting that they activate different sets of immune effectors in response to pathogens. This might explain their different susceptibilities to the same pathogen. We are currently investigating the upstream factors regulating these effectors, using computational methods, and forward and reverse genetics.

418B

Expression patterns of an FSHR-1 transcriptional target gene upon infection of *C. elegans* by *P. aeruginosa*. **Jordann A. Smak**, Jennifer R. Powell. Department of Biology, Gettysburg College, Gettysburg, PA.

The innate immune system is evolutionarily ancient and is responsible for the initial response to an invading pathogen. This innate response requires specific receptors to recognize diverse pathogens or the damage caused by these pathogenic microorganisms. The G-protein coupled receptor FSHR-1 has been previously identified as a potential receptor involved in the innate immune system of *C. elegans*. FSHR-1 is required for the response to intestinal infection by a variety of pathogens, and it regulates the expression of several pathogen-response genes. We have begun to characterize the expression pattern of the gene F01D5.5, which has been shown by qPCR to depend on FSHR-1 for its induction in *C. elegans* upon infection by *P. aeruginosa*. To analyze the tissue specificity and basal expression of this putative FSHR-1 target gene, worms carrying an F01D5.5::GFP transcriptional reporter will be examined. We will perform a time course analysis of F01D5.5::GFP induction during exposure to *P. aeruginosa* to better understand the expression of this gene under pathogenic conditions.

419C

Responses of *C. elegans* to Pathogenic Challenge. **Gregory Stupp**[1], Steven L. Robinette[1], Ramadan Ajredini[1], Paul A. Gulig[2], Arthur S. Edison[1,3]. 1) Department of Biochemistry & Molecular Biology; 2) Molecular Genetics & Microbiology; 3) National High Magnetic Field Laboratory, University of Florida.

C. elegans releases and responds to many different chemicals necessary for regulating important behaviors such as mating attraction, dauer formation, aggregation, and recognition and differentiation of food and pathogens. The nematode can sense bacterial populations through small-molecule messengers such as acyl-homoserine lactones, and are able to interfere with certain quorum sensing systems. Additionally, *C. elegans* has a complex olfactory system which allows the nematode to avoid detrimental conditions such as areas of high population density or osmolarity and areas containing pathogenic bacteria. Moreover, this system, which is affected by released pheromones, exhibits behavioral plasticity that allows the nematode to learn and adapt, for example, to avoid an odorant associated with harmful conditions. This study seeks to determine changes in *C. elegans* behavior and exometabolome, the set of small molecules released into the environment by the worms, in the presence of a pathogen. We collected exudates from a synchronous population of *C. elegans* under standard conditions and in the presence of synthetic compounds produced by *Pseudomonas aeruginosa*, for the purpose of identifying worm responses to pathogenic conditions. We acquired 2D NMR spectra of the exudates, and used a novel method developed in our lab for 2D NMR alignment and pattern recognition (Robinette et al., 2011) to identify NMR peaks correlated with worm responses. We also quantified behavioral responses to these pathogen-challenged exudates using custom software that tracks individual nematodes, quantifies reversal frequency and calculates average speed for a set of nematodes on an agar plate in order to identify potential alarm responses. Additionally, we are working on a high-throughput version of the bioassay apparatus that consists of six webcams mounted on a custom lighting unit which will enable recording and analysis of six bioassays simultaneously. The availability of libraries of mutants for both *C. elegans* and *P. aeruginosa* makes this an exciting project to probe mechanisms of interspecies chemical interactions. 1. Edison AS. *Current opinion in neurobiology.* 2009;19(4):378-88. 2. Beale E, et al. *Applied and environmental microbiology.* 2006;72(7):5135-7. 3. Kaplan F, et al. *Journal of chemical ecology.* 2009;35(8):878-92. 4. Schulenburg H, Ewbank JJ. *Molecular microbiology.* 2007;66(3):563-70. 5. Yamada K, et al. *Science.* 2010;329(5999):1647-1650. 6. Robinette SL, et al. In Press, *Analytical Chemistry* (2011).

420A

Molecular characterization of *Coprinopsis cinerea* lectins-mediated toxicity against *C. elegans*. **Katrin Stutz**[1], Alex Butschi[1], Silvia Bleuler-Martinez[2], Mario Schubert[3], Markus Aebi[2], Markus Künzler[2], Michael O. Hengartner[1]. 1) Institute of Molecular Life Sciences, Zurich, Switzerland; 2) Institute of Microbiology, ETH Zurich, Switzerland; 3) Institute of Molecular Biology and Biophysics, ETH Zurich, Switzerland.

Lectins are non-immunoglobulin, carbohydrate-binding proteins without catalytic activity towards the recognized carbohydrate. They are widely distributed among eukaryotes such as plants, fungi and mammals as well as prokaryotes. Several lectins of *Coprinopsis cinerea* and of other fungi display toxicity towards *Caenorhabditis elegans* and other organisms such as *Aedes aegypti*, *Acanthamoeba castellani* and HeLa cells and thus may be part of a lectin-mediated defense system of higher fungi against predators and parasites.

Our goal is to unravel the glycotargets that are bound by the *C. cinerea* lectins CGL2 and CCL2 in *C. elegans* as well as the underlying toxicity mechanisms. The results may reveal an Achilles heel in this organism and might pave the way to new approaches in fighting animal parasitic nematodes.

Toxicity of lectins against *C. elegans* is assessed by feeding *E. Coli* overexpressing lectins. This results in inhibition of development and reproduction and eventually leads to premature death. As a proof of principle, we could show for the galectin CGL2 that its toxicity depends on its ability to bind to a galactose-containing glycoconjugate. We used Mos1-transposon mutagenesis to isolate mutants resistant to CGL2. The molecular analysis of the resistance genes allowed us to predict the structure of the glycoepitope bound by CGL2, and also revealed a novel, evolutionary conserved glycosyltransferase (GALT-1).

Interestingly, toxicity assays of a second *C. cinerea* lectin, CCL2, showed toxicity exclusively towards *C. elegans* and *Drosophila melanogaster*; unlike CGL2 that is toxic to a broad range of species. Glycan array analysis of recombinant CCL2 revealed a pronounced carbohydrate-specificity for Fucα1,3GlcNAc-containing glycans. Resistance of *C. elegans* mutants (*bre-1*, *ger-1*, *fut-1*) defective in the biosynthesis of the α1,3-core fucoside confirmed this glycoepitope. Feeding *C. elegans* with a dTomato::CCL2 fusion protein showed that CCL2 binds, like CGL2, to the surface of the intestinal epithelium. By EMS mutagenesis and RNAi screens as well as biochemical purification of the glycotarget, we want to learn more about the structure of the CCL2 target. In addition, we will perform transmission electron microscopy and metabolomics to elucidate toxicity mechanisms.

421B

A *C. elegans* model for cell autonomous, non-autonomous, and delayed hypoxic injury. **Chun-Ling Sun**[1], C. Michael Crowder[1,2]. 1) Department of Anesthesiology; 2) Department of Developmental Biology, Washington University School of Medicine, St. Louis, Missouri.

Despite accounting for one third of US mortality, there is still no approved treatment for hypoxic cell death. In order to understand better the biology of hypoxic death and identify determinants of survival, we have performed a variety of forward genetic screens in *C. elegans* for genes whose mutant phenotype is resistance to hypoxic death. These screens have all used as their primary endpoint survival of the organism after a severe hypoxic incubation. A complexity of this model to study hypoxic cell death is cell non-autonomous contributions to the hypoxic sensitivity of a specific cell type. For example, germline-deficient *C. elegans* have increased survival of neurons and myocytes and the entire organism following hypoxic incubation. To focus on cell death as opposed to organismal death, we built a strain that expresses GFP and wild type RARS-1 only in pharyngeal myocytes in the background of *rars-1(gc47 rf),* which renders the entire organism resistant to prolonged hypoxia (*Science* 323:630-3, 2009). Consistent with our goal, hypoxia of the pharyngeal rescued strain did not result in significant organismal death after a 24 hour recovery but pharyngeal pumping rate was drastically reduced (193 $\pm$ 9 to 26 $\pm$ 5 pumps/min). We also observed pharyngeal pathology visible by Normarski. In addition to these pharyngeal phenotypes, we observed a marked reduction in the locomotion speed of these animals after recovery from hypoxia (0.18 $\pm$ 0.01 to 0.03 $\pm$ 0.01 mm/sec) whereas the locomotion of *rars-1(gc47)* fully recovers to pre-hypoxia speeds. We presume this is a cell non-autonomous phenotype and is likely due to killing of neighboring nerve ring neurons. We are examining this hypothesis by scoring nerve ring neuronal survival directly. Finally, we have observed a delayed organismal death in this strain where the animal survival drops from about 85% after a 24 hour recovery to 16% after 96 more hours . Thus, this strain offers the ability to study cell autonomous, cell non-autonomous, and delayed hypoxic cell death.

422C

G-protein coupled receptor OCTR-1 controls innate immunity by regulating non-canonical unfolded protein response genes. **Jingru Sun**, Varsha Singh, Rie Kajino-Sakamoto, Alejandro Aballay. Duke University, NC.

The endoplasmic reticulum (ER) has developed specific signaling pathways called the unfolded protein response (UPR) to cope with ER stress and restore ER homeostasis. Recent studies indicate that the increased demand on protein folding in the ER, that may occur during bacterial infections, must be successfully alleviated by canonical and non-canonical UPR pathways for a complete immune response to be mounted. We found that the

nervous system controls the activity of a non-canonical UPR pathway required for innate immunity in *Caenorhabditis elegans*. OCTR-1, a G-protein coupled receptor, functions in ASH and ASI neurons to actively suppress innate immune responses by down-regulating the expression of non-canonical UPR genes *pqn/abu* in non-neuronal tissues. *Pseudomonas aeruginosa* exposure up-regulates *pqn/abu* genes to levels comparable to those induced by treatment with the ER stressor tunicamycin. Our studies highlight pathways that may play important roles at dealing with pathogens directly and with the damages caused by the infection.

423A

A Calpain based muscle repair/remodelling system in *C. elegans*. Beth Oczypok[1,2], Tim Etheridge[1], Brandon Fields[2], Lew Jacobson[2], **Nate Szewczyk[1]**. 1) University of Nottingham, School of Graduate Entry Medicine and Health, Royal Derby Hospital, Derby, DE22 3DT, UK; 2) University of Pittsburgh, Department of Biological Sciences, Pittsburgh, PA, 15260, USA.

Integrin containing attachment complexes transmit force between the inside and outside of muscle cells and signal changes in muscle size in response to force and, perhaps, disuse atrophy. Two members of an integrin containing muscle attachment complex, UNC-97/PINCH and UNC-112/MIG-2/Kindlin-2, have decreased expression in response to spaceflight, a condition thought to induce disuse atrophy. We find that acute disruption (by RNAi or temperature sensitive mutations) of integrin containing muscle attachments induces muscle protein degradation, myofibre and mitochondrial dystrophies, and a movement defect in adult *C. elegans*. Degradation in response to attachment disruption is blocked in *dim-1* mutants and by RNAi or drugs targeting calpain genes or gene products. These results suggest that sustained disruption of integrin based attachment leads to bulk degradation of muscle cytosol by calpains. While we have shown that calpain activation is sufficient to yield bulk degradation of muscle cytosol, we are unconvinced that this activation is likely to be physiologically, as opposed to pathologically, relevant. Certainly individuals with mutations in genes such as integrin would suffer sustained disruption of attachment and congenital myopathies do exist as a result. However, we doubt that sustained disruption of attachment complexes occurs under physiological conditions given that they are thought to be mechanosensitive. Rather, we speculate that these complexes are subject to constant physical stress and short term disruption of attachment may occur. Clearly transient activation of a protease such as a calpain might be beneficial to clear physically disrupted proteins that cannot be refolded appropriately and such a situation has been postulated for mammalian muscle (involving Calpain-3). Thus, we examined RNAi against calpain gene(s) and found such treatment(s) results in myofibre, mitochondrial, and integrin muscle attachment disruptions. We therefore propose a calpain based repair/remodelling mechanism exists for dealing with attachment complex disruption, for example contraction/exercise, and that inability to repair, for example genetic defects or possibly aging, results in calpain based degradation of bulk muscle protein. This work is funded by the UK MRC (G0801271) and US NIH NIAMS (AR054342).

424B

Understanding the effects of the intracellular pathogen *Nematocida parisii* infection of the *C. elegans* intestine on intracellular trafficking and cytoskeleton components. **Suzy Szumowski**, Emily Troemel. Biological Sciences, University California San Diego, San Diego, CA.

Uncovering the interactions between a pathogen and its host is of critical importance to understanding pathogenesis. The morphological similarities between worm and human intestinal cells make *C. elegans* an excellent system for probing host defenses against pathogenic microorganisms infecting the gut. One such pathogenic organism is the microsporidian *Nematocida parisii*, which is an obligate intracellular pathogen that infects worms. The Microsporidia phylum is composed of over 1200 species, including those that may play a role in honeybee colony collapse disorder as well as those that contribute to mortality in AIDS patients. Microsporidia have highly reduced genomes and are dependent on nutrients obtained from their host in order to reproduce and develop within host cells. By working with a species of microsporidia that infects worms, we are able to investigate host-pathogen interactions in the context of intact, differentiated epithelial cells.

N. parisii was isolated from nematodes residing in a compost heap in Paris and is thus a natural pathogen of *C. elegans*. Because these two species have likely coevolved, we may uncover fundamental aspects of *C. elegans* intestinal biology by studying their interaction. In particular, we have been focusing on the process of endocytosis and how this may be exploited or perturbed by *N. parisii* infection. To investigate this interaction, we are examining fluorescently tagged *C. elegans* proteins during the course of infection. We have been investigating both cytoskeletal components, as well as components involved in vesicle trafficking. Additionally, we are examining the functional impact of *N. parisii* infection on intestinal endocytosis by monitoring the rate at which fluorescently labeled BSA travels through the endocytic pathway in infected and uninfected worms. Our preliminary results indicate that endocytosis plays an important role in response to infection by this intracellular pathogen. We are also using these assays to examine the nature of spore-filled vesicles that appear later in infection, which may result from intracellular trafficking events. Our studies will provide insight into the mechanisms by which a natural intracellular pathogen is able to co-opt host intestinal pathways in order to survive and replicate. These studies may provide insights into the mechanisms by which microsporidia cause disease in other hosts as well.

425C

C. elegans clhm-1 encodes a novel voltage-gated, Ca^{2+} permeable ion channel that regulates cell excitability. **Jessica E. Tanis**, Zhongming Ma, Predrag Krajacic, Liping He, J. Kevin Foskett, Todd Lamitina. Department of Physiology, University of Pennsylvania, Philadelphia, PA.

Calhm1 is a recently described integral membrane protein that modulates cellular Ca^{2+} homeostasis. A polymorphism in Calhm1 has been associated with certain forms of late-onset Alzheimer's disease, suggesting a potential role for mutant Calhm1 in disease. However, the physiological functions of Calhm family members are unclear. While humans express six Calhm genes, *C. elegans* expresses a single Calhm gene, *clhm-1*, which is 16% identical / 28% similar to human Calhm1. CLHM-1 is expressed in excitable cells, including sensory neurons and the body-wall muscles. Quantitative biomechanical analysis showed that *clhm-1* mutants exhibit an uncoordinated swim gait. Over-expression of *clhm-1* was highly toxic, causing completely penetrant embryonic/larval lethality when expressed in the body-wall muscles and neurodegeneration when expressed in touch neurons. Together, these data suggest that CLHM-1 plays important roles in excitable cell function. Heterologously expressed human Calhm1 exhibits properties consistent with it being an ion channel. To determine if *C. elegans* CLHM-1 also encodes an ion channel, we measured CLHM-1-dependent currents in Xenopus oocytes using two electrode voltage clamp experiments. Although CLHM-1 lacks a canonical voltage sensor domain, expression of CLHM-1 produced a voltage-sensitive current that was outwardly rectifying. Removal of extracellular Ca^{2+} shifted the voltage-dependent activation towards hyperpolarized voltages, suggesting that extracellular Ca^{2+} normally inhibits CLHM-1 function. CLHM-1 is permeable to both cations and anions, with some preference for Ca^{2+} ($P_{Ca2+} : P_{Na+} \approx 4$). Ca^{2+} and Cl^- permeability was altered by mutation of a charged residue in a potential pore-forming domain, suggesting that CLHM-1 is a bona fide ion channel. CLHM-1 currents were inhibited by gadolinium, ruthenium red and Zn^{2+}, while specific blockers of voltage-gated Ca^{2+} channels and NMDA receptors had no effect. Our analyses demonstrate that *C. elegans* CLHM-1 exhibits biophysical properties similar to human Calhm1, indicating that the function of this novel class ion channels is evolutionarily conserved from *C. elegans* to humans. Further study of *C. elegans* CLHM-1 will be used to characterize the physiological functions of members of this protein family and the biological / biophysical mechanism of CLHM-1-dependent neurotoxicity.

426A

Non-lytic, actin-based exit of intracellular parasites from *C. elegans* intestinal cells. Kathleen A. Estes, **Emily R. Troemel**. Dept Biol, Univ California, San Diego, La Jolla, CA.

The intestine is a common site for invasion by intracellular pathogens, but little is known about how pathogens restructure and exit intestinal cells, particularly *in vivo*. Recently, we identified a natural intracellular pathogen that infects intestinal cells of the nematode *C. elegans* and found that it is a new species of microsporidia. Microsporidia are fungal-related obligate intracellular parasites that infect a wide range of hosts, including humans: infections of AIDS patients can cause lethal diarrhea and microsporidia have been deemed priority pathogens by the NIH. We named the *C. elegans*-infecting microsporidian species *Nematocida parisii*, or nematode-killer from Paris. *N. parisii* invades *C. elegans* intestinal cells, undergoes its life cycle, and then exits cells in the transmissible spore form. Here we show that *N. parisii* causes rearrangements of intestinal actin, ACT-5, as part of a novel parasite exit strategy. First, we find that removal of actin from the apical side of intestinal cells creates gaps in the terminal web, a cytoskeletal barrier to escape. Second, we show that gap formation is a regulated process, and that all contagious animals exhibit terminal web gaps, consistent with gaps being part of an exit strategy. Third, we find that actin is not required for infection progression or spore production, but actin is required for spore exit. And finally, we show that despite large numbers of spores exiting host cells, this process does not cause cell lysis. These results provide insight into parasite restructuring of the cytoskeleton and non-lytic escape from intestinal cells *in vivo*.

427B

Development of C. *elegans* models for Amyotrophic Lateral Sclerosis. **Alexandra Vaccaro[1,2,3]**, Arnaud Tauffenberger[1,2,3], Alex Parker[1,2,3]. 1) Department of Pathology and Cell Biology, Universite de Montreal, Montreal, Quebec, Canada; 2) CRCHUM, Montreal, Canada; 3) Center of Excellence in Neurosciences of Universite de Montreal, Montreal, Canada.

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by a progressive and selective loss of motor neurons in the brain stem, the motor cortex and the spinal cord. The progression of ALS is marked by fatal paralysis and respiratory failure leading to death within 2 to 5 years. Unfortunately, to date there is no effective treatment known to slow or halt disease progression. Two recently discovered causative genes for ALS, TDP-43 (TAR DNA Binding Protein 43) and FUS/TLS (Fused in Sarcoma/Translocated in Liposarcoma), are under further investigation regarding their biological roles in neuropathies. Since TDP-43 and FUS are evolutionarily conserved we turned to the model organism C. *elegans* to learn more about their biological functions. The following objectives are being carried out: 1. The worm orthologues of TDP-43 and FUS are *tdp-1* and *fust-1* and we have obtained deletion mutants for each gene. These mutants are being characterized for their contribution to cellular stress resistance and longevity. We observed that *tdp-1* and *fust-1* have roles in the oxidative and osmotic stress and that *tdp-1* may act to specify stress response signaling. 2. We have taken a transgenic approach to study the *in vivo* consequences of TDP-43 and FUS mutations. We engineered strains to express wild type and mutant human TDP-43 or FUS in worm motor neurons. The expression of mutant TDP-43 or FUS produces robust, adult onset, age-dependent motility defects ultimately leading to paralysis. These phenotypes are distinct from animals expressing wild type TDP-43 or FUS alleles. These phenotypes are useful for genetic and

pharmacological suppressor screening. We have conducted a genetic screen and isolated a number of suppressors of mutant TDP-43 toxicity. An update of our findings will be presented.

428C

The interaction between *C. elegans* and the emerging nosocomial pathogen *Stenotrophomonas maltophilia*. **Corin White**, Vinod Mony, Brian Darby, Michael Herman. Ecological Genomics Institute, Division of Biology, Kansas State University, Manhattan, KS, USA.

Stenotrophomonas maltophilia is a ubiquitous aerobic gram-negative bacterium that can cause nosocomial and community-acquired infections. In the course of our studies on the ecological genomics of nematode-bacterial interactions, we discovered a pathogenic interaction between *Caenorhabditis elegans* and a local *S. maltophilia* environmental isolate, JCMS. To characterize this interaction, we developed a GFP-labeled *S. maltophilia* JCMS strain to determine the fate of the bacteria upon ingestion by *C. elegans*. The accumulation of bacteria in the gut, an indicator of bacterial pathogenesis, was recorded for six days of feeding on GFP-labeled JCMS or *E. coli* OP50. The degree of intestinal distension caused by JCMS was significantly different from that caused by OP50 on each day of observation ($p < 0.0001$), suggesting that *S. maltophilia* induces a pathogenic response in *C. elegans*. We have also found that JCMS exposed to UV light kills *C. elegans* less effectively than live bacteria. This suggests that the accumulation of living bacteria contributes significantly to the lethality of *C. elegans* after prolonged exposure to JCMS. Pathogenic bacteria such as *P. aeruginosa* PA14 have exhibited faster killing on rich media such as brain heart infusion (BHI) or peptone-glucose-sorbitol (PGS). However, the pathogenicity of JCMS was not affected by growth on BHI or PGS. Thus, the pathogenic mechanism employed by PA14 appears to be different from JCMS. To determine the contributions of major defense pathways to the *C. elegans* response to JCMS, we measured the lifespan of representative innate immune response mutants grown on JCMS and OP50. In the *DAF-2/DAF-16* pathway, *DAF-2* negatively regulates the transcription factor *DAF-16*. As a result, *daf-2* mutants are long-lived on most pathogenic bacteria, including human pathogens. However, we have discovered that *daf-2* mutants have reduced lifespan on *S. maltophilia* JCMS, suggesting that the *DAF-2/16* pathway is not involved. We also observed that *pmk-1*, *sek-1* and *dbl-1* mutants were hypersensitive to *S. maltophilia* JCMS, suggesting minor involvement of these pathways. However, the observation that each strain, including the wild type, displayed a shorter lifespan on *S. maltophilia* JCMS suggests the involvement of other undiscovered pathways. We are currently using RNA-seq to identify these genes and pathways. Genes that are differentially regulated in worms exposed to *S. maltophilia* JCMS as compared to OP50 are potential candidate immune response genes. Our combined studies will provide insight on the co-evolution between pathogen and host.

429A

Enterococcus infection of *Caenorhabditis elegans* as a model of innate immunity. **Grace J. Yuen**, Read Pukkila-Worley, Frederick M. Ausubel. Department of Molecular Biology, Massachusetts General Hospital, Boston, MA.

Enterococcus is a Gram-positive commensal that is found in the gastrointestinal and biliary tracts of all healthy humans. It is also an important opportunistic pathogen, causing nosocomial urinary tract and wound infections that are often complicated by antibiotic drug resistance. Most human enterococcal infections are caused by either *E. faecalis* or *E. faecium*. Our laboratory has modeled *Enterococcus* infection in *C. elegans* using both strains. We have previously shown that infection with either strain leads to gut distention, but only *E. faecalis* is able to establish a persistent infection and kill the nematode. We now provide evidence that at least two canonical *C. elegans* immune signaling pathways are important for survival during infection with both *E. faecalis* and *E. faecium*. While the lifespan of wild-type worms is unaffected by an *E. faecium* infection, mutations in the PMK-1 and FSHR-1 immune signaling pathways lead to an immunocompromised phenotype, where *pmk-1* and *fshr-1* mutants die rapidly upon *E. faecium* feeding. This new finding suggests that an active immune response is required to keep *E. faecium* infection "in check" in the worm intestine, and that *E. faecium* is indeed pathogenic to the nematode. We are now using microscopy and genetic studies to understand the biology of the *Enterococcus* infection in *C. elegans* and to identify novel *Enterococcus*-activated immune signaling pathways.

430B

Dissecting the molecular pathway underlying IFT modeling. **Qing Zhang**, Yuxia Zhang, Qing Wei, Jinghua Hu. Mayo Clinic. 200 First Street S.W. Rochester, MN 55905.

Cilia act as motile or sensory devices on the surfaces of most eukaryotic cells, and cilia dysfunction results in a variety of severe human pathologies, collectively termed ciliopathies. Phylogenetically conserved intraflagellar transport (IFT) machinery, which is composed of IFT-A and IFT-B subcomplexes, mediates the bidirectional movement of IFT cargos that are required for the biogenesis, maintenance, and signaling of cilia. However, an understanding of how IFT particles are assembled at the ciliary base and turned around at the ciliary tip remains elusive. Our previous data suggested that IFT-B component DYF-2 is the key factor involved in regulating IFT modeling at both the ciliary base and ciliary tip. From a mutagenesis screen for C. elegans mutants with defective IFT modeling at the ciliary tip, we identified jhu616, which encodes a mutant DYF-2 with a G361R alteration in the conserved WD40 domain. Further studies indicated that DYF-2 and BBS (Bardet-Biedl syndrome) proteins coordinate IFT assembly/reassembly at the ciliary base/tip (See Wei Q. et al's abstract from the Hu lab). To further dissecting the molecular pathway underlying IFT modeling, we performed a suppressor screen of the jhu616 allele. Dye-filling (Dyf) assay was used to assess cilia integrity. jhu616 animals are 100% Dyf. In ~300,000 haploids screened, we retrieved 7 independent strains that could rescue the Dyf phenotype of jhu616 allele. Sequencing results showed that no new mutations were introduced into dyf-2 locus, indicating that the suppressors contain mutations in other players involved in IFT modeling. We are mapping the suppressors and looking forward to fully dissecting the molecular pathway underlying IFT modeling and ciliogenesis.

431C

The Role of the Argonaute Protein, ALG-1, in *C. elegans* Neural Development. **Cristina Aguirre-Chen**, Christopher M. Hammell. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

MicroRNAs (miRNAs) are small (22 nucleotides), non-coding RNAs that post-transcriptionally regulate the expression of target genes, and have been implicated in playing a critical role in a variety of biological processes such as developmental timing and cell fate determination. *C. elegans* miRNAs exert their regulatory functions through the Argonaute protein, ALG-1, a key component of the miRNA-induced silencing complex (miRISC) which acts to physically couple the mature miRNA and target mRNA and is required for miRNA activity. Aside from the finding that the *lsy-6* miRNA is involved in the determination of left/right asymmetry of the *C. elegans* ASE neurons, roles for miRNAs in *C. elegans* neural development or maintenance are largely undefined. Our current studies aim to determine whether miRNA function is required for the proper patterning of the *C. elegans* nervous system. To this end, we are currently analyzing a novel, antimorphic allele of *alg-1*, *alg-1(ma192)*, in a variety of neuron-specific transcriptional reporter backgrounds, including *juIs76* (DD/VD motor neurons), *zdIs13* (HSN and NSM neurons), *oyIs14* (PVQ neurons), *mgIs18* (AIY neurons), and *otIs138* (PVD neurons). Our strategy of analyzing the axonal and dendritic architecture of animals mutant for *alg-1*, rather than animals harboring mutations in individual miRNAs, probes the global requirement of miRNAs in the development of the *C. elegans* nervous system.

432A

The atypical Cdc-42-like GTPases CHW-1 and CRP-1 redundantly regulate axon pathfinding and cell migration. **Jamie K. Alan**, Erik A. Lundquist. Molecular Biosciences, University of Kansas, Lawrence, KS.

Rho family small GTPases are Ras-related small GTPases that regulate cytoskeletal organization, motility, trafficking, and cell migration. Numerous studies have shown that Rho GTPase signaling pathways contribute to proper neuronal axon pathfinding and migration in *C. elegans*. There are 7 Rho GTPases in C. elegans: RHO-1 (RhoA), CDC-42 (Cdc-42), CED-10 (Rac1), RAC-2 (Rac1), MIG-2 (RhoG), and the atypical Cdc-42-like molecules CHW-1 (Chp/Wrch-1), and CRP-1 (putative TC10, TCL). Despite extensive knowledge of Rho/Cdc-42/Rac GTPases, little is known about the contribution of the *C. elegans* atypical Cdc-42-like family members CHW-1 and CRP-1 to axon pathfinding and neuronal migration. CHW-1 is similar to both Chp (Wrch-2, RhoV) and Wrch-1 (RhoU), while CRP-1 is similar to both TC10 (RhoQ) and TCL (RhoJ). CHW-1 and CRP-1 are predicted to, or have been shown to display altered GDP/GTP cycling characteristics (Jenna et al., 2005). CHW-1 contains an alanine instead of the conserved glycine at the 12 position (using Ras numbering), which is predicted to be a partially activating mutation. CRP-1 resembles TC10 and TCL, and like these GTPases, CRP-1 also shows altered GDP/GTP binding characteristics (Neudauer et al., 1998; Vignal et al., 2000; Jenna et al., 2005). To test the roles of CHW-1 and CRP-1 in axon pathfinding and cell migration, we assayed the PDE axons and the migrations of the Q descendants AQR and PQR in *chw-1(ok697)* and *crp-1(ok685)* mutants. We found that loss of chw-1 or crp-1 alone resulted in only modest pathfinding defects in PDE neurons, similar to *cdc-42(gk388)*. However loss of both *cdc-42* and *chw-1* or *chw-1* and *crp-1* resulted in synergistic increases in PDE axon pathfinding defects. These results suggest that, similar to the Rac-like GTPases CED-10 and MIG-2 (Lundquist et al., 2001), the Cdc-42-family GTPases CDC-42, CHW-1, and CRP-1 act redundantly in axon pathfinding. Preliminary studies indicate that loss of either *chw-1* or *crp-1* result in modest defects in AQR migration, suggesting that they might redundantly control cell migration as well. As a complementary approach, we plan to drive the expression of mutant versions of CHW-1 and CRP-1 in the PDE neurons and AQR/PQR to determine the effects of overactivity, similar to studies on the Rac GTPases (Struckhoff and Lundquist, 2003). Preliminary results indicate that expression of a mutant CHW-1 transgene predicted to have increased GTPase activity disrupted neuronal morphology. Taken together, these data provide preliminary evidence for a role of the atypical Cdc-42-like GTPases CHW-1 and CRP-1 in axon pathfinding and neuronal migration.

433B

Dissections of *lin-11* neuronal enhancers in *C. elegans* and *C. briggsae*. **Siavash Amon**, Bhagwati Gupta. Biology, McMaster University, Hamilton, Ontario, Canada.

The LIM homeodomain transcription factor *lin-11* plays an important role in the morphogenesis of the egg-laying system and differentiation of several neurons. We are dissecting the enhancer regions of *lin-11* to understand its mechanism of transcriptional regulation. Our lab had earlier identified conserved vulva and uterine modules that are located in the 5' UTR (Marri and Gupta, 2009). The uterine module was shown to be regulated by f*os-1/fos* and two LIN-12/Notch pathway targets *lag-1/Su(H)* and *egl-43/evi1*. To identify the neuronal enhancers of *lin-11* we are now focusing on its intronic regions. We have found that two of the *lin-11* introns (intron 3 and intron 7) activate reporter gene expression in a subset of olfactory and chemosensory neurons. This suggests that the neuronal expression of *lin-11* utilizes regulatory inputs distinct from those in the vulva and uterus. The corresponding introns in *C. briggsae* show similar expression profiles suggesting that the mechanism of *lin-11* regulation is likely to be evolutionarily conserved. We did four-way sequence comparison of the two introns using *C. briggsae, C. remanei, C. brenneri* lin-11 orthologs and found limited conservation in some regions. It is likely that these regions are involved in regulating *lin-11* expression. We are using bioinformatics to identify putative transcription factor binding sites, especially within the conserved sequences. The dissection of *lin-11* introns will help us understand the signaling network of *lin-11* and how it regulates neuronal differentiation. In parallel, we are also generating chimeric LIN-11 protein by swapping LIM and Homeodomain regions from other LIM-HOX family members to understand domain specificity in different cell types. These experiments will provide a better understanding of *lin-11* function in nematodes and how it regulates developmental processes in multiple tissues.

434C

Rapid and reversible arborization of IL2 neurons during dauer development. Nathan E Schroeder, **Rebecca J Androwski**, Maureen M Barr. Dept. of Genetics, Rutgers Univ, Piscataway, NJ. 08854.

Dendrite morphology and plasticity profoundly affect neuronal signaling and behavioral outputs (1). However, little is known regarding the molecular signals governing morphogenesis of dendrite structure. During non-dauer stages of *C. elegans* development the inner labial (IL2) neurons, a set of six ciliated putative chemosensory neurons, display a bipolar morphology with an unbranched dendrite and axon. Under adverse environmental conditions, *C. elegans* can develop into a dauer larva, an alternative juvenile stage with altered morphology and behavior. We found that during the dauer stage, the IL2 neurons exhibit hierarchical dendritic branching and a switch from a bipolar to multipolar morphology.

During dauer formation the ventral and dorsal IL2 primary dendrites establish branching and extend *de novo* processes from the cell bodies which undergo additional branching. The lateral IL2 neurons branch exclusively at the distal dendrites, forming a circular "crown" extending around the circumference of the head. Using time-lapse imaging, we found that plasticity in the IL2s begins with the onset of the dauer molt. Following the cessation of pharyngeal pumping puncta begin forming and resorbing in a dynamic fashion along the primary dendrite for several hours. Rapid and dynamic branch formation with periodic pruning events occur during a 3-4 hour period preceding radial shrinkage. Following recovery from dauer, the branches are incompletely resorbed, leaving behind occasional remnant secondary branches.

Using a forward genetics screen, we isolated 28 candidate mutants with branching defects. Variations in defects include ectopic branches, disorganized branching or an incomplete crown. Several mutants were backcrossed and are being identified using traditional mapping and whole genome sequencing. We are currently using laser ablation to examine tiling and possible roles of surrounding tissue on IL2 branching. Additionally, we are testing candidate genes that may play a role in the IL2 dauer branching phenotype. Various developmental disorders are associated with defects in dendrite structure (1). IL2 branching in dauers may serve as a new and rapid model to understand the molecular basis of arborization and dendritic pruning that underlie these disorders.

1. Jan and Jan. 2010. Nat. Rev. Neurosci. 11:316-328.

435A

To branch or not to branch? Role of *png-1* in limiting axon branching in *C.elegans*. **Nasrin Babadi**, Claudia Arauz, Anna Su, Antonio Colavita. Cellular and Molecular Medicine, University of Ottawa, Ottawa, ON, Canada.

Formation of neural networks is complex process that not only requires axon growth and guidance, but also depends on the ability of the axons to generate branches and connect with multiple targets. We use the worm's egg laying organ and the neuronal networks that control this process to study the process of axon branching. VC4, VC5 and the HSNs are the neurons that innervate the vulva muscles and regulate egg expulsion. VC4 and VC5 motor neurons project their axons laterally around the vulva, where they overlap and form branches. Our lab has showed that mutations in *png-1*, the sole worm orthologue of peptide N-glycanase (PNGase), cause excessive and complex ectopic branching defects in VC4 and VC5. Additionally, we also found that these *png-1* branching defects are strongly enhanced when combined with mutations in *sax-1/NDR kinase* and *sax-2/Furry*. To better understand how VC neurons develop in wild-type and mutant backgrounds we are currently peforming a time-lapse analysis using an *unc-4p::GFP* reporter to visualize VC4 and VC5 from neuritogenesis to target innervation. We will present our findings.

436B

Cholinergic motor neurons are required for the positioning of GABA receptor clusters at the neuromuscular junction. **Belinda Barbagallo**, Michael Francis. Neurobiology Department, University of Massachusetts Medical School. Worcester, MA.

Chemical synapses are the primary means of communication between neurons, with post-synaptic neurons often receiving inputs from multiple presynaptic partners. The proper organization of diverse receptor classes within the post-synaptic membrane is a key requirement for the appropriate integration of diverse inputs. The *C. elegans* neuromuscular junction (NMJ) provides a genetic model for studying the molecular mechanisms that govern the organization of polyinnervated synapses. At the *C. elegans* NMJ, body wall muscles receive innervation from both cholinergic (excitatory) and GABAergic (inhibitory) motor neurons at *en passant* synapses along the nerve cord. We are interested in defining the role of motor neurons in establishing the organization of post-synaptic receptors on body wall muscle cells and sculpting the post-synaptic receptor field. In previous work, we have shown that expression of the pore-modified acetylcholine receptor subunit ACR-2(L/S) leads to cell-autonomous death of the A and B cholinergic motor neuron classes shortly after hatch. Despite death of the cholinergic motor neurons, gross GABA motor neuron morphology, including projection patterns and pre-synaptic release sites, appear normal in these animals. We now show that ACR-2(L/S)-induced loss of cholinergic motor neurons leads to the disruption of both cholinergic and GABAergic postsynaptic receptor fields at the NMJ. We found that GFP-tagged subunits of homomeric ACR-16 receptors and heteromeric levamisole receptors show altered localization patterns, including aggregation

in muscle arms. The altered distribution of acetylcholine receptors presumably arises as a direct consequence of the loss of cholinergic presynaptic release sites. Interestingly however, despite the presence of normal presynaptic specializations in GABA motor neurons, we also observe large regions of the nerve cord that lack appropriately localized clusters of postsynaptic $GABA_A$ (*unc-49*) receptors. We are currently evaluating whether these defects in the GABA postsynaptic receptor field are due to altered muscle arm coverage of the nerve cord or reflect mislocalization of receptors clusters within individual muscle arms. Our data suggest that the cholinergic motor neurons play an essential role in the development and/or maintenance of both excitatory and inhibitory synapses at the *C. elegans* NMJ. Ultimately, we hope to identify motor neuron-derived signals that dictate the organization of receptor clusters within the post-synaptic receptor field and our progress in these studies will be presented.

437C

An Investigation into the Affect of Neuronal Activity on Proper Neural Connectivity in C. elegans. **Ben Barsi-Rhyne**, Kristine Miller, Joori Park, Emma Holdrich, Miri VanHoven. Department of Biological Sciences, San Jose State University, San Jose, CA 95192.

Neuronal activity has been implicated in the establishment and maintenance of appropriate synaptic connections in vertebrate and invertebrate systems. However, the molecular mechanisms by which activity affects connectivity is poorly understood. Our hypothesis is that correct sensory and synaptic activity during development is necessary for proper neural connections in Caenorhabditis elegans. Activity may affect axon outgrowth, axon guidance, synaptogenesis, or the maintenance of neural connections. To test these hypotheses we have looked at strains of C. elegans with mutations affecting activity pathways. We have obtained a number of strains with mutations in genes required for sensory activity and synaptic transmission and have crossed them with a transgenic fluorescent synaptic partner recognition marker that allows us to visualize specific neurons and their synapses. This synaptic partner recognition marker includes NLG-1 GFP Reconstitution Across Synaptic Partners, which labels synapses between PHB sensory neurons and AVA interneurons, as well as an mCherry fluorophore that allows us to observe defects in axon outgrowth, axon guidance, and contact between pre- and postsynaptic neurites in vivo. Intriguingly, our preliminary results indicate different roles for different types of neuronal activity in neural development. A loss-of-function mutation in odr-3, which encodes a G-alpha subunit required for sensory activity, results in reduced synapses between PHB and AVA neurons. These results indicate that sensory activity is likely necessary for correct synaptic partner recognition. Surprisingly, we also found that loss-of-function mutations in unc-7, a gap junction component, result in reduced contact between PHB and AVA neurites without affecting synapses between these neurons. Interestingly, this indicates that electrical synapses are likely required for correct neurite contact between pre- and postsynaptic neurites within a complex nerve bundle. Our future goal is to further characterize the roles of these genes to understand their roles in neural development.

438A

Investigation of the UNC-6/Netrin and UNC-40/DCC-Mediated Synaptic Partner Recognition Pathway in C. elegans. **Kelli L Benedetti**, Aruna Varshney, Akshi Goyal, Dianicha Santana-Nuñez, Pooja Prasad, Joori Park, Miri VanHoven. Department of Biological Sciences, San José State University, San José, California, 95192.

The nervous system consists of neurons organized into circuits through synaptic connections, allowing information to be transferred to and processed in the brain. To form circuits, neurons must identify their correct synaptic partners among the many neurites in a target region and proceed to form synapses. Elucidation of the molecular mechanisms neurons employ to identify the correct synaptic partners may help us gain a better understanding of neurological diseases in humans, including autism and schizophrenia. Our research focuses on the synapses formed between the PHB sensory neuron and the AVA interneuron in the model organism Caenorhabditis elegans. Previous research in our laboratory indicates that the UNC-6/Netrin ligand and the UNC-40/DCC transmembrane receptor regulate synaptic partner recognition between the PHB and AVA neurons in C. elegans. The goal of this study is to determine if genes that function with unc-6/Netrin and unc-40/DCC in earlier developmental pathways also play a role in the UNC-6/Netrin and UNC-40/DCC synaptic partner recognition pathway. We hypothesized that genes that function with UNC-6/Netrin and UNC-40/DCC in axon guidance and cell migration, including sdn-1/SynDecaN , gpn-1/GlyPicaN , vab-1/EPH, unc-115/abLIM, unc-5/UNC5, and mig-10/Lamellipodin may be part of conserved modules that additionally play a role in synaptic partner recognition. To test this hypothesis, we introduced a transgenic synaptic marker called Neuroligin 1-mediated GFP Reconstitution Across Synaptic Partners (NLG-1 GRASP) into gpn-1/GlyPicaN , sdn-1/SynDecaN , vab-1/EPH, unc-115/abLIM, unc-5/UNC5, and mig-10/Lamellipodin mutants using standard genetic crosses to fluorescently label synapses between the PHB and AVA neurons. However, our preliminary analysis suggests that there are no severe defects in synaptic partner recognition in these mutants. Interestingly, this suggests that although both pathways are mediated by UNC-40/DCC, the downstream signaling pathways specifying synaptic partner recognition and axon guidance may be largely distinct. Our results also suggest that a forward genetic screen will be a productive way to identify new pathway members.

439B

A Family of Eight Small 2-Ig Domain Proteins Functions to Protect Neuronal Architecture. **Cassandra Blanchette**, Claire Bénard. Department of Neurobiology, UMass Medical School, Worcester, MA.

A critical but poorly understood aspect of neurobiology is how the integrity and function of an established nervous system is protected throughout life, despite post-embryonic body growth, movement and the incorporation of new neurons to existing neural circuits. Research using *C. elegans* has implicated five molecules in neuronal maintenance: the secreted two-Ig domain proteins ZIG-4 and ZIG-3, the FGF receptor EGL-15(5A), the L1-like SAX-7 cell-adhesion protein, and the giant DIG-1 protein. Besides *zig-3* and *zig-4*, the *C. elegans* genome encodes 6 other 2-Ig domain proteins: the transmembrane bound ZIG-1 and 5 other secreted ZIGs. To decipher how the ZIG proteins mediate neuronal maintenance, we have constructed multiple mutant combinations of the *zig* genes, including triple, quadruple, quintuple, sextuple and octuple mutants.

We have found that *zig-5* and *zig-8* mediate the maintenance of proper neuronal position and that the defects of the double mutant are similar to those of *sax-7* null. *sax-7* encodes an L1-like cell adhesion protein that functions in neurons to maintain their position in the worm. SAX-7 exists as two distinct forms, which differ in the number of extracellular Ig domains and adhesiveness. The long form appears to be auto-inhibited by the folding of the two external Ig domains, which occlude the two critical adhesive Ig domains. Our genetic analysis suggests that the adhesiveness generated by SAX-7L depends on ZIG-5 and ZIG-8, as the function of ZIG-5 and ZIG-8 is required only if SAX-7L is present. Also, a mutated form of SAX-7L that is in the open conformation is active in maintenance, even in *zig-5 zig-8* double mutants. We hypothesize that ZIG-5 and ZIG-8 interact with the two external Ig domains of the long isoform of SAX-7/L1, thus opening the SAX-7L molecule so that its critical central Ig domains are free to mediate adhesion and thus neuronal maintenance.

The *zig*-less octuple mutant that we generated displays specific neuronal maintenance and behavioral defects, while being otherwise wild type. Progress will be reported on the characterization of the octuple mutant.

440C

A *prkl-1*suppressor screen to identify new planar cell polarity components involved in neuronal polarity. **D. Carr**[1,2], J. Visanuvimol[1,2], L. Sanchez-Alvarez[1,2], A. Colavita[1,2]. 1) Department of Neuroscience, University of Ottawa, Ottawa, Ontario, Canada; 2) Neuroscience Research Institute, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada.

The planar cell polarity (PCP) pathway is responsible for organizing cell polarity in the plane of the epithelium. We have recently shown that the core PCP pathway components Van Gogh (*vang-1*), Prickle (*prkl-1*), and Dishevelled (*dsh-1*) are required in a subset of peripheral motor neurons (VC neurons) to restrict neurite emergence to a specific tissue axis. In loss-of-function mutants, VC4 and VC5 neurons display ectopic neurites that extend abnormally along the anteroposterior (AP) body axis. The *unc-4* promoter is expressed in the VA, DA and VC motor neurons. *unc-4* driven over-expression of PRKL-1 in the VC neurons is sufficient to suppress neurite growth. These transgenic animals also displayed backwards locomotion impairment; a movement controlled by the VA and DA neurons. This inability to move backwards is suppressed by *vang-1* null mutants, which are known to interact with *prkl-1* in the PCP pathway. We used these observations as the basis of a genetic screen to identify other suppressors that can restore backwards locomotion. Our preliminary findings include the identification of several suppressor mutants including at least 8 new alleles of *vang-1* and at least one allele of *fntb-1*, the beta subunit of farnesyltransferase. Farnesyltransferases post translationally modifies proteins by attaching a 15-carbon isoprenoid lipid, farnesyl, to the cysteine residue of a C-terminal CAAX motif. The addition of a farnesyl group facilitates membrane association of the protein due to the hydrophobic nature of the lipid. We believe that FNTB-1 prenylates PRKL-1 at its C-terminal CAAX motif to promote membrane association and interaction in the PCP pathway. We will be describing the characterisation of *fntb-1* and genetic interactions with *prkl-1* as well as further findings from the genetic screen.

441A

Loss of the soluble guanylate kinase *guk-1* results in multiple developmental defects. **Margaret R Casazza**, Douglas Portman. Biomedical Genetics, University of Rochester, Rochester, NY.

The guanylate kinase family of proteins can be divided into two main groups—soluble guanylate kinases and membrane-associated guanylate kinases. The latter, known as MAGUKs, are enzymatically inactive, localize to synapses and tight junctions and have been implicated in cell signaling, ion channel trafficking and cell-specification. The soluble GKs localize to the cytosol and catalyze the phosphorylation of GMP to produce GDP. They are involved in guanine nucleotide synthesis and cyclic nucleotide recycling. While much is known about MAGUKs, especially their roles in development and signaling, little is understood about the role that soluble GKs might play in development and physiology. We show here that a soluble guanylate kinase has a role not only in sensory neuron differentiation in the male tail, but in a variety of other developmental processes as well.

An EMS screen in the lab for male tail defects identified a new mutation in the soluble guanylate kinase *guk-1*. Interestingly, both the novel allele *fs5*, and a deletion allele, *tm2620*, have pleiotropic phenotypes. Homozygous mutants display a variety of defects including larval arrest and sterility in both sexes, protruding vulva and defects in ray neuron differentiation. Though *guk-1* appears to be the sole soluble GK in the C. elegans genome, it appears to have a surprisingly restricted expression pattern as determined by recombinereed fosmids. Expression is limited to a small number of cells including several head neurons, AFD, AQR and URX, as well as PQR, all cells in which cGMP signaling is known to play an important role. Expression is also observed in the somatic gonad, although this expression is brighter in males than hermaphrodites. We are currently working to further characterize where and when *guk-1* functions and to elucidate how it acts to promote normal development in *C. elegans*.

442B

Integrin Cooperates with UNC-40 to Guide Muscle Arm Extension. **Kevin K Chan**, Peter J Roy. Department of Molecular Genetics, The Donnelly Centre for Cellular and Bimolecular Research. 160 College St., University of Toronto, Toronto, ON, M5S 3E1, Canada.

Body muscles of nematodes extend specialized plasma membranes, called muscle arms, to the dorsal and ventral nerve cords to form neuromuscular junctions. The extension of *C. elegans* muscle arms is stereotypical and likely guided by a chemoattractant that is secreted from motor axon targets. We recently found that an UNC-40/DCC pathway, which includes the UNC-40 receptor, the MADD-2 adaptor protein, and the UNC-73 Rho-GEF, functions cell-autonomously in *C. elegans* to direct muscle arm extension towards the motor axons (Alexander et al., 2009, 2010). Two lines of evidence suggest that this signalling pathway may be a non-canonical one. First, UNC-6/Netrin, which is the canonical ligand of UNC-40, is dispensable for muscle arm extension to motor axons. Second, the extra-cellular domains of UNC-40 are not required for UNC-40's role in directing muscle arm extension. These data suggest that a second receptor complex may co-operate with UNC-40 to guide muscle arms. To identify other plasma membrane-associated proteins that might cooperate with UNC-40, we screened candidate receptors for their ability to facilitate UNC-40-mediated muscle arm extension. We previously reported that PAT-2/α-integrin, but not INA-1/α-integrin, is required for muscle arm extension to the motor axons (Dixon et al., 2006). Here, we present evidence that the PAT-2/PAT-3 integrin heterodimer is required for UNC-40-dependent muscle arm extension. We are currently investigating the mechanism by which integrin facilitates UNC-40's role in directing muscle arm extension.

443C

UNC-119 acts to maintain neuronal differentiation. **Brittney Chilton**, Andrew Gorzalski, Fern Bosada, Scott Clark. Biology Department, University of Nevada, Reno, NV.

During differentiation, neurons extend axons to their targets, form synaptic connections and begin to exhibit attributes of mature neurons. Neurons must retain their appropriate differentiated state and express the correct complement of 'terminal differentiation' genes throughout the life of the organism to maintain a functional nervous system. *sra-6::gfp* is expressed exclusively and continuously in PVQ, ASH and ASI soon after the cells are born during embryogenesis and serves as a terminal differentiation marker for PVQ. Mutations in *unc-119* and five other genes that disrupt axon growth were found to also eliminate expression of *sra-6::gfp* in PVQ soon after hatching. Expression of *sra-6::gfp* was high in embryos and newly hatched L1 larvae, yet the GFP signal was greatly reduced within 2-3 hours and was typically not detectable after 5-6 hours. Mutation of all six genes caused a similar time course in the extinction of the GFP signal, supporting the idea that these genes act in a common pathway. Expression in ASH and ASI was not altered and unrelated axon outgrowth mutations did not affect *sra-6::gfp* expression in PVQ, indicating that axon outgrowth defects *per se* do not perturb *sra-6::gfp* expression. We examined three additional genes expressed in PVQ and found that *egl-47::gfp* and *gpa-14::gfp* expression was reduced after hatching but that *gpa-9::gfp* expression was not affected by mutation of these six genes. These observations indicate that these six axon outgrowth genes act in a process that maintains the correct expression profile or differentiated state of PVQ after hatching.

To understand further how these genes affect PVQ differentiation, we undertook a genetic screen to isolate mutations that restore expression of *sra-6::gfp* in PVQ in *unc-119* mutants. Eight suppressors that define two genes were isolated. *unc-119* animals harboring a suppresser mutation still exhibit a strong Dpy and Unc *unc-119* phenotype and PVQ still has axon outgrowth defects. As such, these suppressors are specific to the restoration of *sra-6::gfp* expression in PVQ and do not appear to rescue other *unc-119* functions. We are testing whether these suppressors can restore *egl-47::gfp* and *gpa-14::gfp* expression in PVQ as well as whether they can suppress the *sra-6::gfp* expression defects caused by mutation of the other five axon growth genes. We are addressing whether *unc-119* acts autonomously in PVQ and whether it is needed continuously or transiently during development. We are also investigating whether the *unc-119* process affects *zag-1* and *pag-3*, which are needed for PVQ differentiation and block expression of *sra-6::gfp* in PVQ when mutated.

444A

An F-box protein FSN-1 Regulates Retrograde Insulin Signalling. Wesley Hung, Christine Hwang, Edward Liao, **Jyothsna Chitturi**, Ying Wang, Hang Li, Mei Zhen. Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada.

The formation of a functional synapse requires the co-ordinated growth of both pre-synaptic and post-synaptic termini. Communication between the pre- and post-synaptic termini involves many proteins and factors. The pre-synaptic F-box protein FSN-1 is an evolutionarily conserved protein that plays an important role in axon growth and synapse development. FSN-1 is a part of an SCF-like E3 ubiquitin ligase complex that comprises of the RING finger domain protein RPM-1, Cullin and Skp (1). FSN-1/RPM-1 complex negatively regulates a MAPK kinase cascade comprised of DLK-1/MKK-4/PMK-3 during synapse formation (2).

We report here that FSN-1 also regulates synapse development, in part, through the regulation of a retrograde insulin/IGF-signalling pathway in the post-synaptic terminal. Mutation in *fsn-1* causes over-development of some synapses along the dorsal nerve cord while other areas of the dorsal cord have little or no synapses. In *fsn-1* mutants there is an increased activity of the insulin/IGF signalling pathway in the post-synaptic muscle cells. Accordingly, loss-of-function mutations in the insulin/IGF pathway components suppressed the synaptic morphology defects of *fsn-1* mutants. We identified several neuronal insulin-like ligands that regulate the postsynaptic insulin/IGF signal during synapse development; all require a proprotein convertase, EGL-3 (3, 4), for maturation. We further demonstrate that, in vivo, EGL-3::GFP's level is increased in *fsn-1* mutants. in vitro, FSN-1 interacts with, and ubiquitinates EGL-3. We propose that FSN-1 modulates the activity of the post-synaptic insulin/IGF pathway via its regulation of EGL-3.

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445B

Sex pheromone perception in *C. elegans* is under the regulation of TGFβ and IGF signals acting in the nervous system. KC Fan, **KL Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, ----, Hong Kong.

In dioecious species, effective mate searching is critical for reproduction success. The use of a sexual-attractant, the sex pheromone, is a common strategy found in diverse species. Our previous studies on the sexual behavior of the 4 species within the ELEGANS group had revealed the existence of sex pheromone in nematodes, with the females of dioceous species producing attractants to recruit males. Our genetic analyses and cell-specific ablation experiments had also defined the requirement of three neuronal types: chemo-sensory AWAs, interneuron AIZs and the male-specific CEMs. *C. elegans* males with any of these neurons defective failed to respond to the sex pheromone. We find that this sex pheromone-mediated behavior is regulated by TGFβ and IGF signals. Here we showed by genetic manipulation that TGFβ/IGF ligands, receptor components and signal transduction molecules are required in pheromone response. We then asked for the cellular targets of TGF-beta and IGF signals in the sex pheromone behavior. Studies on TGFB and IGF signals in *C. elegans* showed that they were coordinating multiple biological processes through their functions in the nervous system, for example the dauer development and food searching behavior. In this study, we were able to demonstrate that the pheromone-nonresponsive phenotype displayed by TGFβ type-I receptor *daf-1* and IGF PI3 dependent kinase *age-1* mutants could be rescued by ectopical expression of corresponding cDNA in the nervous system, implying that the nervous system is the target of these paracrine signals in sex pheromone perception. Subsequently, the impact of specific IGF or TGFβ component elimination on the gross-morphology of the sex pheromone perceiving neurons were also examined by confocal microscopy. The interplay of paracrine factors and how they are used to modulate rapid chemosensory function will be discussed. (This study is funded by the Research Grants Council, Hong Kong.).

446C

DAF-18/PTEN regulates neurodevelopmental axon outgrowth through isoform-specific DAF-16/FOXO activity. **R. Christensen**[1], A. Byrne[2], M. Hammarlund[2], D. A. Colón-Ramos[1]. 1) Cell Biology, Yale University, New Haven, CT; 2) Genetics, Yale University, New Haven, CT.

The phosphatidylinositol 3-kinase (PI3K) signaling pathway is a conserved signal transduction cascade that is fundamental for the correct development of the nervous system. The major negative regulator of PI3K signaling is the lipid phosphatase DAF-18/PTEN, which can modulate PI3K pathway activity during neurodevelopment. We have identified a novel role for DAF-18/PTEN in promoting axon outgrowth during development in C. elegans. We find that DAF-18/PTEN modulates the PI3K signaling pathway to activate DAF-16/FOXO and promote developmental axon outgrowth. This activity of DAF-16/FOXO in promoting outgrowth is isoform-specific, effected by the *daf-16b* isoform but not the *daf-16a* or *daf-16f* isoforms. This outgrowth-promoting role is also specific for development, as DAF-16 and DAF-18 mutants do not show decreases in injury-induced outgrowth following laser axotomy. This data provides a novel mechanism by which the conserved PI3K signaling pathway can regulate developmental axon outgrowth.

447A

A screen for mutants with defective hypodermal attachment of mechanosensory neurons. **Brian Coblitz**, Martin Chalfie. Biological Sci, Columbia Univ, New York, NY.

The touch receptor neurons (TRNs) project processes anteriorly along the body adjacent to the hypodermis. Hemidesmosomal-like structures on the hypodermis link it to TRN extracellular proteins. In newly hatched larvae the TRN processes lie next to the body wall muscle, but as the animals mature intervention by the hypodermis separates the TRN processes from the muscle. Mutations affecting the extracellular proteins MEC-1, MEC-5 (a collagen), and HIM-4 (hemicentin) disrupt this separation. To identify other components needed for the separation, we mutagenized animals in which the body wall muscles were labeled with $_{Pmyo-3}$*mCherry* and the TRNs were labeled with *mec-18::GFP*. Attachment mutants had ALM processes adjacent to the muscle. In addition to identifying new alleles for *mec-1*, *mec-5*, and *him-4*, we also found alleles in at least 7 other genes. Two of these new genes cause the mechanosensory abnormal (Mec) phenotype, suggesting novel roles of *mec* genes in attachment. Efforts are underway to map and sequence the new mutations.

448B

Mechanisms of BAG sensory cilia specification. **Astrid Cornils**, Piali Sengupta. Biology Department, Brandeis University, Waltham, MA.

The nervous system relies on specialized sensory neurons to sense environmental conditions and initiate appropriate physiological and behavioral responses. Functions of many sensory neurons are dependent on the presence of morphologically specialized cilia which are microtubule-based organelles acting as cellular 'antennae'. A mechanism called intraflagellar transport (IFT) is required to build cilia and transport cargo and ciliary precursors into the ciliary compartment. However, little is known about how diverse, specialized sensory cilia are shaped. *Caenorhabditis elegans* is an established model for the study of cilia generation and maintenance. Worms possess 60 ciliated neurons, each of which is specialized to sense specific cues. The BAGL/R sensory neurons, so-called due to the bag-like appearance of their cilia, sense carbon dioxide and decreases in oxygen levels. Little is known about the mechanisms by which BAG cilia are formed, although interestingly, the essential IFT component *osm-6*/IFT52 has been reported not to be expressed in the BAG neurons. To investigate the formation of BAG cilia, I am first analyzing BAG cilia structure in known ciliary mutant backgrounds to determine the extent to which BAG ciliogenesis depends on known ciliary genes. I am analyzing *in vivo* IFT as well as the ultrastructure of BAG cilia in wild-type animals, and plan to carry out a forward genetic screen to identify new genes required for BAG ciliogenesis. Comparing the pathways for BAG cilia specialization to those of other specialized *C. elegans* neuronal cilia will allow me to define new mechanisms for the generation of ciliary structure diversity.

449C

The LAMMER kinase MADD-3 Functions Cell-Autonomously to Direct Muscle Arm Extension. **Serena A D'Souza**, Louis Barbier, Rachel Puckrin, Peter Roy. The Donnelly Centre, Department of Molecular Genetics, University of Toronto, Toronto, Canada.

The body wall muscles of nematodes project plasma membrane extensions, called muscle arms, to the motor axons to establish neuromuscular junctions. We have previously shown that an UNC-40 pathway, consisting of the UNC-40 receptor, the MADD-2 adaptor protein, and the UNC-73 Rho-GEF, functions cell-autonomously to direct muscle arm extension to the motor axon targets. Through screens for additional muscle arm development defective (Madd) mutants, we discovered three new genes that we call madd-3 X, madd-4 I, and madd-5 V. Here we present our characterization of madd-3, which encodes a LAMMER kinase. The LAMMER kinases are a well-conserved family of dual specificity kinases found ubiquitously throughout eukaryotes. The most extensively-studied family member is Drosophila Doa, which encodes multiple isoforms of the kinase. The 55 kDa Doa isoform is nuclear localized and plays an essential role in sex determination via the phosphorylation of SR proteins, which in turn regulates the sex-specific splicing of doublesex pre-mRNA. SR protein substrates are a conserved target of LAMMER kinases. The 105 kDa isoform of Drosophila Doa is localized exclusively to the cytoplasm, suggesting that it may have a role beyond regulating mRNA splicing. Like Doa, madd-3 encodes multiple isoforms. The madd-3 allele that was isolated in our screen (tr186) creates an early non-sense codon within the madd-3a-specific exon, indicating that MADD-3A is necessary for muscle arm extension. Muscle specific-expression of MADD-3A can rescue the muscle arm extension defects of madd-3 mutants, demonstrating that MADD-3A functions cell-autonomously. We therefore focused our efforts on characterizing MADD-3A in detail. Functional reporters show that MADD-3A's expression is restricted to non-pharyngeal muscles. Similar to the localization of the 105 kDa Doa isoform, we found that a functional MADD-3A::YFP fusion protein is localized to the cytoplasm of muscle cells and cannot be readily detected in nuclei. MADD-3A contains an N-terminal region with no defined domains and a C-terminal kinase domain. A structure-function analysis indicates that the kinase domain is necessary for MADD-3A function, and that the N-terminal region keeps MADD-3A from accumulating in the nucleus, but is unnecessary for function. Genetic analyses indicate that MADD-3A functions in an UNC-40 pathway. RT-PCR results, however, indicate that MADD-3A does not regulate the splicing of several components of the UNC-40 pathway, including unc-40, madd-2, and unc-73. Ongoing experiments to understand MADD-3A`s role in muscle arm extension may uncover new roles for the cytoplasmic isoforms of this conserved family of kinases.

450A

Regulation of axon extension and termination by RPM-1. **Maria Fernanda daSilva**, Scott Clark. Biology Department, University of Nevada, Reno, NV.

During development, neurons extend axons along stereotypic pathways to their targets where they form synaptic connections with their appropriate partners. The trajectory of an individual axon is determined by its growth cone responding to various guidance cues along its path via cell surface receptors, such as SAX-3/Robo, UNC-5 and UNC-40/DCC. The connectivity established during embryogenesis is maintained and refined as an animal grows in part by further extension of axons to retain their relative organization. The neuron AVG is located in the retrovesicular ganglion and extends two processes during embryogenesis: a short anteriorly directed process and a long posteriorly directed process that pioneers the ventral nerve cord and extends to the tail region. To identify genes controlling axonal development, we screened for mutants with AVG axonal growth or guidance defects. We recovered five mutations that cause the posteriorly directed AVG process to extend past its normal termination site. Four are alleles of *rpm-1*, which encodes a conserved E3 ubiquitin ligase that regulates presynaptic differentiation of GABAergic neurons and growth termination of ALM and PLM axons. Previous studies indicate that RPM-1 plays a key role in controlling the transition from axon extension to synapse formation.

For ALM/PLM axon termination, RPM-1 is known to act via two parallel pathways: a Rab GTPase GLO-1 and Rab GEF GLO-4 pathway and a FSN-1 and DLK-1 MAP kinase cascade pathway. We found that *fsn-1* mutants had AVG axon defects like *rpm-1* mutants, yet *glo-1* and *glo-4* mutants did not. The penetrance of *fsn-1* mutants was much lower than that of *rpm-1* mutants, indicating that a non-GLO-1/GLO-4 pathway acts in parallel to FSN-1 downstream of RPM-1 in AVG. Furthermore, while RPM-1 regulates SAX-3 and UNC-5 activity to affect ALM/PLM axon growth, we determined that RPM-1 does not act by affecting SAX-3, UNC-5 or UNC-40 activity in AVG.

We examined *rpm-1, fsn-1* and *zd203* (the non-*rpm-1* allele recovered in our screen) animals at different stages and found that L4 and younger animals exhibited a wild-type AVG axon extension pattern. However, in young adults, the AVG axon was overgrown, indicating that these genes act to stop axon extension once an animal has reached adulthood. Together, our studies reveal that RPM-1 acts via the FSN-1 pathway and an unknown third pathway to prevent overgrowth of the AVG axon at later stages of development.

451B

Identification of novel loci interacting with the Kallmann Syndrome gene *kal-1*. **Carlos A. Diaz-Balzac**[1], Nathali Gomez[1], Hannes Buelow[1,2]. 1) Department of Genetics, Albert Einstein College of Medicine, Bronx, NY; 2) Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Kallmann syndrome (KS) is characterized by two major deficits, anosmia and hypogonadism. These deficits are the likely result of a neuronal targeting defect of the olfactory axons and a failure of GnRH-secreting neurons to migrate to the hypothalamus. To date, five genes associated with this syndrome have been identified, namely, *KAL1*, *FGFR1*, *FGF8*, *PROKR2*, and *PROK2*; though these only account for approximately 30% of all KS cases. The first gene identified was KAL1, which encodes a secreted cell adhesion protein named anosmin-1 and is responsible for the X-linked form of the KS. Misexpressing the homolog of anosmin-1/KAL1 in *Caenorhabditis elegans* causes a highly penetrant axonal branching phenotype. Expanding a small pilot modifier screen of the *kal-1* gain of function phenotype we have to date isolated a total of 43 mutations in both screens, including 41 suppressor mutations and 2 enhancer mutations. With complementation tests still in progress, we know already that the mutants comprise a minimum of eleven complementation groups. The genes represented by three of these complementation groups have previously been described, including the Heparan sulfate (HS) 6O-sulfotransferase *hst-6*, the HS epimerase *hse-5* and the PAPS (phosphoadenosyl-phosphosulfate) transporter 1 *pst-1*. Of the remaining eight novel complementation groups we have identified the molecular lesions in two complementation groups as alleles of the HS 3O-sulfotransferase *hst-3.2* (see also poster by Tecle et al.) and the large extracellular matrix protein *dig-1*. We will report on the progress of characterizing both the newly identified mutations and complementation groups. Characterization of these genes should afford us a deeper understanding of how *kal-1* acts during the development of the nervous system and the role of the extracellular matrix in this process. Additionally, these genes may represent candidate genes to cause Kallmann Syndrome in humans.

452C

Syndecan modulates growth cone migration and morphology during axon regeneration. **Tyson Edwards**, Marc Hammarlund. Department of Genetics and Program in Cellular Neuroscience, Neurodegeneration, and Repair, Yale University School of Medicine, New Haven, CT.

Restoring neuronal function after an injury requires the successful migration of the regenerating axon back to its target cells. Cell surface heparan sulfate proteoglycans (HSPGs) mediate important interactions with extracellular signals and have been shown to change expression after neuronal injury (Murakami et al. *Neuroscience*. 2006), suggesting that they may mediate some aspects of axon regeneration. In order to elucidate a potential role for HSPGs in regeneration, we severed fluorescently labeled GABAergic motorneurons in HSPG mutants using a pulsed dye laser, and assessed subsequent regeneration. A novel regeneration phenotype was observed in mutants for the transmembrane HSPG syndecan (*sdn-1*), but not for the GPI-anchored glypicans. Although axons in *sdn-1* mutants initiate regrowth as frequently as wild type, average neurite lengths are decreased and many *sdn-1* mutants axons are characterized by branch-like structures and dysmorphic growth cones. Further, very few regenerating axons in *sdn-1* mutants reach their target on the dorsal nerve cord. These data demonstrate that syndecan affects the migration step of axon regeneration, possibly through an underlying defect in growth cone morphology.

Syndecan is post-translationally modified by the addition of heparan sugar chains, which are themselves modified by sulfation, epimerization, and deacetylation (Bernfield et al. *Annu Rev Biochem*. 1999). The HSPG modifying enzymes are essential for axon guidance and cell migration during *C. elegans* development (Bülow and Hobert. *Neuron*. 2004), and *sdn-1* mutant worms recapitulate many of these neuronal defects (Rhiner et al. *Development*. 2005). Interestingly, mutants for the modifying enzymes *hse-5*, *hst-2*, *hst-6*, *hst-3.1*, *hst-3.2*, and *sul-1* showed no obvious regeneration phenotypes, and the HSPG synthesizing enzyme *rib-2* mutant did not phenocopy the *sdn-1* regeneration defects. These results indicate that syndecan may function independently of heparan sulfate sugars to affect growth cone migration and morphology during regeneration, and may represent a mechanistically distinct function from its known role in axon guidance. Our preliminary data suggest that syndecan functions in the hypodermis to affect axon guidance, while we are currently testing the hypothesis that syndecan functions cell-intrinsically in neurons to regulate the cytoskeleton. Thus, our data suggest that syndecan may have two separable functions: a canonical function in axon guidance, and a novel function in growth cone morphology and migration during regeneration.

453A

COEL-1, a metazoan specific protein, is required for proper function of microtubules in Touch Receptor Neurons. **M.Y. Frederic**[1], V.F. Lundin[2], M.D. Whiteside[1], D.K. Tu[1], D.L. Baillie[1], J-M. Bellanger[3], H. Hutter[1], F.S. Brinkman[1], M.R. Leroux[1]. 1) Simon Fraser University, Burnaby, BC, Canada; 2) Stanford University, Stanford, CA, USA; 3) CRBM-CNRS, Montpellier, France.

The emergence of the ancestral metazoan from a Choanoflagellate-like unicellular organism likely necessitated a swift acquisition of novel proteins required to support novel cytological features needed for multicellularity, and specialised cells such as neurons. Using a comparative genomics approach, we identified a set of genes that encode proteins strictly found in and widely conserved across metazoans. Many have unknown functions or are poorly characterized. To experimentally validate the metazoan-specific character of a representative protein, we chose to study *coel-1*, the *C. elegans* ortholog of cofactor E-like, a poorly characterised protein implicated in tubulin degradation in mammalian cells. Expression studies show that *coel-1* is broadly expressed in embryos but becomes largely restricted to neurons in larvae and adults. Disrupting COEL-1 function in the worm is tolerated but results in hypersensitivity to the microtubule (MT)-stabilizing drug paclitaxel (taxol). Overexpressing COEL-1 causes embryonic lethality, and escapers have an egg-laying defect and a reduced response to gentle body touch. This latter behavior is mediated by the Touch Receptor Neurons (TRN) that express *coel-1* throughout development. We analyzed the morphology of these neurons using a *mec-4*::GFP reporter and observed process length and branching defects when *coel-1* is disrupted or overexpressed. Consistent with a role for COEL-1 in MT function, transmission electron microscopy analyses reveal defects in the structure of MTs found along TRN processes. In *C. elegans*, these neurons are the main cells where MTs are acetylated. We therefore tested for a possible link between *coel-1* and tubulin acetylation. Using a genetic approach we discovered that HDAC-6, the evolutionarily-conserved tubulin deacetylase, or the recently discovered tubulin acetylases, ELPC-3 and MEC-17, functionally interact with COEL-1. Together these results suggest that COEL-1 regulates microtubule stability and structure, working together with components of the tubulin acetylation/deacetylation pathways to maintain proper TRN morphogenesis and function. Cofactor E-like therefore has the characteristics of a protein that is specifically required in a metazoan, providing evidence for the importance of our metazoan-specific proteins dataset.

454B

Characterization of intracellular calcium signaling within damage *C. elegans* neurons . Lin Sun, Samuel Chung, Fay Gao, Kevin Roodhouse, **Christopher V. Gabel**. Dept. of Physiology and Biophysics, Boston University School of Medicine, Boston, MA.

Traumatic neuronal damage triggers large intracellular calcium transients that are associated both with subsequent neuronal degeneration and cell death, and alternatively with regenerative repair and outgrowth [1, 2]. Combining femtosecond laser ablation with the use of genetically encoded calcium sensitive fluorophores, we can optically measure intracellular calcium signaling within a specific target neuron of an intact adult *C. elegans* in response to precision laser damage [3-5]. Here we characterize the damage induced calcium signal across a variety of laser ablation experiments. This includes variations in the proximity of the damage point to the cell soma, targeting of distinct morphological structures and different neuronal types, modulation of laser power, different animal ages, and multiple surgeries to the same neuron. Results are revealing complex subcellular calcium dynamics that are precisely tuned to control cell fate. We find that in general large, extended calcium transients correlate with cell degeneration, while particularly small transients are associated with reduced regenerative outgrowth. This suggests an optimum window in which elevation of cytoplasmic calcium successfully facilitates neuronal repair and outgrowth without initiating cell death. Our results are helping to pinpoint the critical aspects of this signaling pathway that dictate neuronal survival and regeneration following traumatic damage.

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455C

An EMS screen in *C. elegans* for genes affecting RID axon guidance. **Q. Ge**[1,2], X. Li[1], X. Huang[1]. 1) Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China; 2) Graduate School of Chinese Academy of Sciences, Beijing 100049, China.

During the development of the nervous system, neurons extend axons over long distances in order to reach their targets. Axons could respond to both dorsal-ventral and anterior-posterior guidance cues and translate them into correct cytoskeletal changes. How to correctly decode these spatial cues is a daunting task in establishing neural connection. RID motoneuron requires dorsal-ventral guidance in the nerve ring and anterior-posterior guidance on the dorsal midline. To identify more regulators in the axon guidance, we performed an EMS screen for mutants with RID axon guidance defects. The mutants we got can be classified into two major categories according to their phenotypes: (1) RID overgrowth in the tail and (2) RID dorsal guidance defects. In addition, we found some mutants with two RID neurons.

456A

Development and Function of RIS, a *Caenorhabditis elegans* GABAergic interneuron. **Marie Gendrel**, Diana Cai, George Baison, Darym Alden, Oliver Hobert. Howard Hughes Medical Institute, Columbia University, Department of Biochemistry and Molecular Biophysics, New York, NY, USA.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate brain and dysfunction of GABAergic neurons can have profound pathological implications. In *C. elegans*, 26 neurons express conserved GABAergic terminal differentiation markers, such as the enzyme producing GABA (GAD/UNC-25), the GABA-specific vesicular transporter (VGAT/UNC-47) and the protein targeting VGAT to the synaptic membrane (a LAMP-like protein/UNC-46). 25 of these are motoneurons and only one neuron, named RIS, is an interneuron which most closely resembles the dominant type of GABA neurons in vertebrates. Preliminary evidence indicates that LIM-6 - a LIM homeobox transcription factor - is partly involved in RIS development, and loss of LIM-6 suggests that RIS might be involved in the control of metabolism and life span. To identify cis-regulatory elements necessary for the expression of known RIS terminal differentiation markers, we used mutational analysis of the gfp reporter genes. Initial results of the *unc-47* promoter analysis define a 25bp area that is necessary for *unc-47* expression in RIS. Further analysis of this region combine with the study of the other promoters should allow us to define cis regulatory motifs and therefore potential binding sites for transcription factors that control RIS differentiation. In parallel, in order to identify these factors we undertook EMS screens to identify trans-acting factors necessary for the expression of RIS terminal differentiation markers. Using the worm sorter, we isolated one temperature-sensitive mutant, in which *unc-47* expression disappears at the adult stage. The whole-genome deep sequencing technique combine with the SNP mapping strategy should allow us to quickly clone the mutant. Altogether we should learn whether the RIS terminal differentiation markers are co-regulated through common cis-regulatory elements and trans-acting factors, with the LIM homeobox gene *lim-6* being one but not the only component. In the end, we will use transgenic animals in which RIS is genetically eliminated to determine RIS function. Together, these results should give us a detailed picture of GABA neuron development and function.

457B

Mechanisms of UNC-86-mediated gene regulation in the BDU neurons. **Patricia Gordon**, Oliver Hobert. Department of Biochemistry and Molecular Biophysics, Columbia University, New York, NY.

UNC-86 is a POU domain transcription factor that is expressed in 57 neurons in the adult hermaphrodite. The role of UNC-86 has been thoroughly characterized in six of those cells: the mechanonsensory neurons ALML/R, PLML/R, AVM, and PVM. In these neurons, unc-86 and its cofactor mec-3 are known to be a terminal selectors: transcription factors that not only determine cell fate, but also directly regulate the battery of genes that distinguish a terminally differentiated cell. The mechanisms by which UNC-86 regulates cell fate in the other 51 neurons are less well understood. Here we examine the role of unc-86 in the BDUL/R interneurons, the sister cells of the ALML/R mechanosensory neurons. Using GFP reporters for the terminally expressed BDU genes ser-2, flp-10, zig-3, and ceh-14, we have shown that unc-86 fulfills the criteria to also be a terminal selector gene in BDU. We have systematically dissected the promoters of each reporter gene to determine the consensus UNC-86 binding site in the BDU cells. In addition, we have examined the interactions of unc-86 with a putative BDU cofactor, pag-3. Mutations in the pag-3 gene cause the BDU neurons to convert to ALM neurons; here we show that mec-3 mutations similarly cause a conversion of ALM neurons to BDU neurons. This cell-fate conversion phenotype is also seen in unc-86 mutants whose mec-3 binding site has been disrupted. We propose a model by which interactions between unc-86, mec-3, and pag-3 allow correct differentiation of both the ALM and the BDU neurons.

458C

EGL-13, a SOX transcription factor, regulates neuronal cell fate determination of the BAG neurons. **Jakob Gramstrup Petersen**, Roger Pocock. Biotech Research and Innovation Centre - BRIC Ole Maaloes Vej 5, University of Copenhagen, 2200 Denmark.

Responses to changes in the oxygen environment are crucial for maintaining homeostasis and survival. In C. elegans these responses are orchestrated by soluble guanylate cyclases acting primarily in the two sets of sensory neurons - BAG and URX. The molecular factors that determine BAG cell fate are unknown. Therefore, we have conducted an EMS mutagenesis screen to isolate mutants that have lost BAG cell fate. From the screen we isolated three mutants, *rp13*, *rp14* and *rp15*. *rp14* has a strong Egl phenotype due to defective connection of the gonad (Cog phenotype). Due to the phenotype we hypothesized that *rp14* may be an allele of *egl-13*. We find that *egl-13::GFP* is expressed in the BAG neurons and that the *egl-13(ku194)* allele causes 100% loss of BAG expression using the *pflp-19::GFP* reporter strain. *egl-13* is a member of the SOX transcription factor family and has previously been described to play an essential role for the proper development of the utse[1]. Vertebrate homologs of EGL-13 are SOX5 and SOX6 which have been shown to be involved in chondrogenesis and cell cycle progression of neural progenitors in the chick spinal cord[2,3]. The discovery that *egl-13* plays a role in neuronal cell fate specification in the

worm might reveal that SOX5 and SOX6 also plays specific roles in the development of the human nervous system. 1Cinar, H. N., Richards, K. L., Oommen, K. S. & Newman, A. P. The EGL-13 SOX domain transcription factor affects the uterine pi cell lineages in Caenorhabditis elegans. Genetics 165, 1623-1628 (2003). 2Smits, P. et al. The transcription factors L-Sox5 and Sox6 are essential for cartilage formation. Dev Cell 1, 277-290, doi:S1534-5807(01)00003-X [pii] (2001). 3Martinez-Morales, P. L., Quiroga, A. C., Barbas, J. A. & Morales, A. V. SOX5 controls cell cycle progression in neural progenitors by interfering with the WNT-beta-catenin pathway. EMBO Rep 11, 466-472, doi:embor201061 [pii] 10.1038/embor.2010.61 (2010).

459A

RAE-1 a novel PHR binding protein regulates axon termination and synapse formation. **B. Grill**[1], L. Chen[2], E.D. Tulgren[1], S.T. Baker[1], M. Anderson[3], W. Bienvenut[4], Y. Jin[2], C.C. Garner[3]. 1) Department of Pharmacology, University of Minnesota, Minneapolis, MN; 2) Department of Neurosciences and HHMI, University of California San Diego; 3) Department of Psychiatry and Behavioral Sciences, Stanford University; 4) Protein Analysis Facility, University of Lausanne.

Pam/Highwire/RPM-1 (PHR) proteins are conserved from C. elegans to mammals, and play a critical role in synapse formation, axon guidance and axon termination. In C. elegans, the Regulator of Presynaptic Morphology (RPM)-1 functions with FSN-1 to negatively regulate the DLK-1 MAP kinase pathway. RPM-1 also positively regulates a Rab GTPase pathway that includes: GLO-4 and GLO-1. To further understand the mechanism of how RPM-1 functions, we biochemically purified RPM-1, and used mass spectrometry to identify RPM-1 binding proteins. Here we report the identification of RNA Export protein (RAE)-1 (also called Nuclear Pore Protein (NPP)-17) as a novel RPM-1 binding protein. The biochemical interaction between RPM-1 and RAE-1 is evolutionarily conserved as it also occurs between Pam (the human PHR protein), and rat Rae1. A conserved domain in the PHR proteins is sufficient for binding to RAE-1, and point mutations were identified that reduce binding to RAE-1.

Previous studies in yeast and mammals have shown that Rae1 functions as a cell cycle regulator, as a regulator of chromosome segregation, and as an mRNA export factor. While mammalian Rae1 and C. elegans RAE-1 are expressed in neurons, their postmitotic function remains unknown. To address the functional significance of RAE-1 binding to RPM-1, we performed a series of genetic experiments. Previous studies have shown that *rpm-1* loss of function (lf) mutants have defects in axon termination in the mechanosensory neurons, and have defects in synapse formation in the motor neurons. *rae-1(tm2784)* mutants have similar axon termination defects to *rpm-1* (lf) mutants, although they occur with lower penetrance. Double mutants of *rae-1* and other RPM-1 binding proteins, such as *fsn-1* and *glo-4*, have enhanced defects in axon termination. *rae-1;fsn-1* and *rae-1;glo-4* double mutants also have enhanced defects in synapse formation in the GABAergic motor neurons. *rae-1;rpm-1* double mutants are not enhanced demonstrating that *rae-1* functions in the same pathway as *rpm-1*. Transgenic expression of RPM-1 that is point mutated to reduce binding to RAE-1 does not rescue axon termination defects in *rpm-1* (lf) mutants with the same efficacy as expression of wild-type RPM-1. These observations highlight the importance of RAE-1 and its interaction with RPM-1 for axon and synapse development.

460B

The role of Eph signaling in amphid commissure guidance. **Emily N. Grossman**, Andrew D. Chisholm. Univ California: San Diego, La Jolla, CA.

Eph receptor tyrosine kinases and their ephrin ligands function in cell contact mediated signaling in many developmental and physiological processes. C. elegans encodes a single Eph receptor (VAB-1) and four GPI-linked ephrin (EFN) ligands. Eph signaling plays a number of roles in C. elegans neural development, including regulation of collective neuroblast movements (George et al., 1998) and ventral guidance of the amphid commissure (AC) (Zallen et al., 1999). Amphid commissures are bundles of ~20 axons that emerge from lateral amphid cell bodies, extend to the ventral midline, then turn anteriorly into the nerve ring. Ventral guidance of the AC requires at least three partly redundant pathways: UNC-6 netrin, SAX-3 Robo and VAB-1/Eph. Previous work suggested AC guidance requires both kinase-dependent and kinase-independent Eph signaling (Zallen et al.,1999). Kinase-independent Eph signaling may provide a model for reverse signaling via GPI-linked ephrins. We find that EFN-1 is the dominant ligand involved in AC guidance whereas EFN-2 and EFN 3 have minor roles. Although in morphogenesis the efn-1,2,3 triple mutant recapitulates the phenotype of the vab-1 receptor null, in AC guidance the ephrin triple knockout is significantly less severe than vab-1, suggesting involvement of additional ligands. We are testing orthologs of known Eph signaling genes for their potential roles in this ephrin signaling pathway. We find that the Src family kinase ABL-1 is required for AC ventral guidance and may act in the VAB 1 kinase-independent pathway. Abl is involved in axon guidance in other organisms but has not previously been linked to C. elegans axon guidance or Eph signaling. Conversely, gain of function in the EGL-19 calcium channel suppresses Eph guidance defects and leads to a synthetic dendrite extension defect, suggesting crosstalk between Eph and calcium signals. We are examining where VAB-1 and EFN-1 are expressed and required for AC guidance. Preliminary data indicate VAB-1 may be required in the amphid neurons themselves for normal guidance. The AC trajectories are largely bilaterally symmetrical in the wild type. Unexpectedly, Eph signaling mutants display a consistent left-right bias in the penetrance of AC guidance defects, in that left-hand axons are more frequently defective than right-hand axons. We find this asymmetry for several AC neuron types in Eph mutants, but not in other ventral guidance mutants. These observations suggest an unanticipated left-right asymmetry in the expression or requirement for Eph guidance cues. We are exploring whether known left-right asymmetry pathways are involved in this aspect of AC guidance.

461C

Axon guidance defects displayed by *zfp-1* and *lin-35* mutants depend on the presence of the transgene *oxIs12*. **Stephan Gysi**[1,2], Michael Hengartner[1,2]. 1) Institute of Molecular Life Sciences, University of Zurich, Zurich, Switzerland; 2) Neuroscience Center Zurich, University of Zurich, Zurich, Switzerland.

Heparan sulfate proteoglycans (HSPGs) are known in different organisms to influence a wide variety of signaling pathways. We previously showed that the HSPG core protein Syndecan (SDN-1) plays an important role during nervous system development. With the aim to find new components in HSPG related signaling processes influencing guidance of commissural axon of the D-type motor neurons we designed a forward genetic screen. One of the candidates of this screen, *op481*, mapped to a region not containing any previously characterized axon guidance gene. Molecular analysis revealed that *op481* is a premature stop codon mutation in the gene *zfp-1*. Surprisingly we observed that the axon guidance defects of the *sdn-1(zh20); zfp-1(ok554)* double mutant were only visible if the D-type motor axons were labeled with the *oxIs12[unc-47::gfp; lin-15+]* transgene. Combined with one of the two other transgenes (*oxIs268[unc-47::gfp]* or *juIs76[unc-25::gfp; lin-15+]*) labeling the same neurons, the *sdn-1(zh20); zfp-1(ok554)* double mutant failed to show increased axon guidance defects. Interestingly we observed the very same phenomenon in animals carrying a mutation in *lin-35*: animals with the genotype *lin-35(n745); sdn-1(zh20) oxIs12* showed axon guidance defects while *lin-35(n745); sdn-1(zh20)* double mutants failed to show defects in combination with *oxIs268* or *juIs76*. Our efforts to shed light on this effect indicated that the site of integration of *oxIs12* is the relevant difference. Using data from a whole genome sequencing approach of animals carrying *oxIs12*, we were able to localize the precise place of integration of *oxIs12* at the very end of the 3'UTR of the gene *grd-1* on the X chromosome. Furthermore, from the number of sequencing reads covering the transgene sequence we were able to roughly estimate the size of *oxIs12* to about 3.6Mb. We are currently trying to find a satisfying answer to why *oxIs12* is influencing axon guidance in *zfp-1* and *lin-35* mutants.

462A

A new class of mutants alters dendrite length in URX oxygen-sensing neurons. Ian G. McLachlan[1], Valeri J. Thomson[2], Shai Shaham[3], **Maxwell G. Heiman**[4]. 1) Program in Neuroscience, Harvard Medical School, Boston, MA; 2) Bard High School Early College II, Elmhurst, NY; 3) Laboratory of Developmental Genetics, The Rockefeller University, New York NY; 4) Department of Genetics, Harvard Medical School and Children's Hospital Boston, Boston, MA.

Neurons develop specialized and characteristic morphologies to carry out their functional roles in the nervous system. Fine aspects of neuronal shape, such as dendrite length, can be studied in *C. elegans* due to the highly stereotyped anatomy of the organism. Previous work has shown that neurons of the amphid sensory organ anchor their dendritic tips at the nose while the cell body migrates away, thus establishing the length of the mature dendrite. The identified anchors, DEX-1 and DYF-7, resemble zonadhesin (a mammalian sperm protein) and zona pellucida proteins (of the mammalian egg), respectively. It is presently unknown whether sensory neurons outside the amphid use similar anchoring mechanisms to specify their dendrite lengths. To address this, a visual genetic screen was conducted to identify factors required for appropriate dendrite morphology in the URX oxygen-sensing neurons. Like amphid neurons, URX neurons extend dendrites to the nose but, unlike amphid neurons, their dendrites reside in a separate fascicle, project to a distinct target site, and are not ensheathed by glia. Two of the mutants isolated from this screen, *ns302* and *ns303*, fell into one complementation group. These mutations shorten URX dendrites to half-length and do not affect the amphid, in contrast to *dex-1* and *dyf-7* mutations which shorten amphid dendrites severely and do not affect URX. Through SNP mapping between CB4856 (Hawaiian) and N2 strains, *ns303* was mapped to a ~130 kb interval on chromosome V containing 18 putative protein-coding genes. One gene within this interval is predicted to encode a protein containing a cysteine-rich domain with sequence similarity to DEX-1 and mammalian zonadhesin. Other genes in the interval also share features with previously identified anchors of amphid sensory neuron dendrite tips. This raises the possibility that variants in a shared family of adhesion molecules may mediate dendrite morphogenesis in multiple classes of sensory neurons.

463B

Wnt signals and Frizzled receptors regulate dendrite formation in *C. elegans* . Leonie Kirszenblat, Divya Pattabiraman, Brent Neumann, **Massimo A. Hilliard**. Queensland Brain Inst, Univ Queensland, Brisbane, QLD, 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 ligands and receptors that regulate dendrite development. To understand how dendrites develop in *C. elegans* we focused on the PQR oxygen sensory neuron. PQR has its cell body positioned in the left lumbar ganglion on the posterior-lateral side of the body. A single dendrite extends posterior with sensory cilia at its tip, while the axon extends anterior along the ventral nerve cord. PQR is born post-embryonically allowing easy visualization of dendrite development using the *gcy- 36::GFP* transgene. In a genetic screen for dendrite defective mutants we isolated a previously uncharacterized mutation in *lin-17*, a *C. elegans* Frizzled receptor gene. We found that in *lin17(vd002)*, the PQR dendrite was absent, shortened or misrouted anterior. Similar dendrite defects were also observed in other known alleles of *lin-17*. Cell-

specific expression of wild-type LIN-17 in PQR indicated a cell-autonomous role of this molecule in regulating dendrite development. LIN-44 is a Wnt ligand known to bind the Frizzled receptor LIN-17 and is expressed by four hypodermal cells in the tip of the tail. We found that *lin-44* mutants presented PQR dendrite defects similar to those observed in *lin-17* mutants. We expressed LIN-44 ectopically from more anterior regions of the body and found that it worsened the PQR dendrite defects of *lin-44* mutants, indicating LIN-44 functions as an instructive cue. Furthermore, we induced LIN-44 expression at different stages of development and found that expression of this molecule is necessary and sufficient prior to PQR dendrite formation. Analysis of the *lin-17 lin-44* double mutant indicated a genetic interaction between these molecules. Our studies provide the first direct evidence that specific Wnt signals and Frizzled receptors regulate dendrite formation *in vivo*. We propose a model in which LIN-17, present on the cell surface of PQR, interacts with LIN-44 through an attractive mechanism to mediate dendrite formation.

464C

Regulation of expression of the Netrin axon guidance system. **Kelly L. Howell**, Oliver Hobert. Columbia University, New York, NY.

The Netrin system of extracellular ligands and receptors is a highly conserved system of molecules that control the axon guidance of many neurons throughout development. Depending on the Netrin receptor expressed, neurons will either be attracted or repelled toward or away from Netrin ligand cues in order to reach their synaptic targets. Given the sometimes long and complicated paths axons must travel, expression of the Netrin family of molecules must be precisely regulated in order for axons to be guided properly. Unfortunately, however, little is known about how the expression of these guidance genes is controlled. To better understand this regulation, we have made fosmid-based reporters for the Netrin ligand, UNC-6, and its two receptors, UNC-5 and UNC-40, to analyze their expression. These genes are expressed in several neuronal types whose differentiation has been shown to be controlled by a terminal selector gene, a transcription factor that controls the terminal identify of a neuron type by directly regulating the expression of terminal differentiation genes. We are currently determining if expression of UNC-6, UNC-5, and UNC-40 is dependent on specific terminal selector genes in a given neuron type, thus suggesting axon path-finding genes are part of the terminal gene battery. In addition, we are using promoter bashing to identify cis-regulatory elements responsible for expression of these genes in order to help identify transcription factors required for their regulation. These experiments will provide new insight into how expression of the Netrin family of proteins is controlled for proper axon guidance during development.

465A

The *C. elegans* CDK8 Mediator module regulates specific axon guidance decisions in the ventral cord and suppresses SAX-3/ROBO activity during dorsal axon navigation. Andreas Steimel[1], Jinkyo Suh[1], Angela Hussainkhel[1], Samineh Deheshi[1], Jennifer Grants[2], Richard Zapf[3], Donald Moerman[3], Stefan Taubert[2], **Harald Hutter[1]**. 1) 1Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada; 2) Centre for Molecular Medicine and Therapeutics, University of British Columbia, Vancouver, BC, Canada; 3) Department of Zoology, University of British Columbia, Vancouver, BC, Canada.

Navigational cues, used by outgrowing axons, are detected by receptors on the growth cone. The pathway choices available to an axon are in part defined by the set of guidance receptors present on the growth cone. Regulated expression of receptors and genes controlling the localization and activity of receptors ensures that axons respond only to guidance cues relevant for reaching their targets. In genetic screens for axon guidance mutants, we isolated an allele of *let-19/mdt-13*, a component of the Mediator, a large ~30 subunit protein complex that processes the regulatory information of transcription factors. LET-19/MDT-13 is part of the CDK8 module of the Mediator. By testing other Mediator components, we found that all subunits of the CDK8 module as well as other Mediator components are required for specific axon navigation decisions in a subset of neurons. The CDK8 module components *cdk-8* and *cic-1* are expressed in most if not all cells throughout development, similar to the other components of the CDK8 module. Comparative transcriptome analysis of embryonic interneurons using SAGE revealed that the Mediator regulates the expression of a large number of genes in those neurons. In a subset of motoneurons the CDK8 module appears to specifically repress SAX-3/ROBO activity to ensure proper commissure navigation.

466B

New methods to study nerve bundle organization. **Richard Ikegami**, Gian Garriga. Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Recent findings have shown that mammalian nerve bundles are organized and that this organization facilitates topographic targeting[1,2]. John White and colleagues observed that nerve bundles in C. elegans are also organized[3], suggesting to us that the C. elegans ventral nerve cord (VNC) could be a useful model for understanding how organization within bundles develop. Sets of neurons have axons run adjacent to one another over long distances in C. elegans nerve bundles. Neighbors of a particular axon presumably reflect the path that guided the neuron's growth cone along the nerve bundle during development.

We developed approaches to study the organization of the HSN motor neurons in the VNC. These are the last neurons to extend axons along an otherwise, fully developed VNC. Until now, only EM analysis of serially sectioned worms provided the resolution to monitor axon positions within nerve bundles[3]. We adapted the membrane-localized split-GFP (GRASP)[4] to label the interface between the HSN axons and those of the PVP and PVQ neurons that guide HSN fasciculation in the VNC[5]. The labeled interfaces reproduce the fasciculation pattern predicted from EM analysis. Earlier in development, the PVP axon provides a track for the PVQ growth cone[6]. PVP/PVQ GRASP along the VNC is continuous, which differs from the EM analysis. We suspect that GRASP mediated adhesion maintains interactions between axons that would normally be disrupted during postembryonic development. This discrepancy has important implications for the use of GRASP and how the VNC develops.

In order to further investigate how the VNC develops, we have developed several additional techniques. These include a heterologous cell adhesion system to drive changes in HSN fasciculation, an RNAi system to target HSN fasciculation before the HSN axons extend but after the VNC has developed and strains that are missing one of the redundant cells involved in HSN fasciculation. These techniques will be used to re-evaluate the roles of molecules implicated in HSN fasciculation as well as identify new molecules. Furthermore, these techniques are easily adapted for use with other neurons.

[1]Imai T. et al. (2009) Science 325(5940):585-90. [2]Plas D.T. et al. (2005) J Comp Neurol. 491(4):305-19. [3]White J.G. et al. (1986) Phil. Trans. R. Soc. Lond. B. 314(1165):1-340. [4]Feinberg E.H. et al. (2008) Neuron 57(3):353-63. [5]Garriga G. et al. (1993) Development 117(3):1071-87. [6]Durbin R. (1987) Ph.D. Dissertation, Cambridge University, England.

467C

Searching for factors required for BAG cell fate determination. **Vaida Juozaityte**, Konstantinos Kagias, Roger Pocock. Biotech Research and Innovation centre, , Ole Malløs vej 5, Copenhagen, Denmark,.

The BAG neurons enable *C. elegans* to generate rapid behavioral responses to oxygen and carbon dioxide. However, the molecular factors that determine BAG cell fate are not known. The aim of this project is to identify the molecular factors required for the differentiation of BAG neurons. First, we aim to identify elements in the promoters of genes expressed in the BAG neurons that are required for BAG expression. We are systematically dissecting the promoters of genes expressed in the BAG neurons - *flp-11, flp-13, flp-17, flp-19, gcy-31* and *gcy-33*. Such deletion analysis will enable the identification of minimal elements within these promoters that are required for BAG expression. Second, we will search for putative transcription factor binding sites within these minimal elements that are required for BAG expression. We will subsequently use BAG::GFP reporter strains to examine BAG cell fate in relevant transcription factor mutant strains. The results of the project will enable us to better understand how the specification of BAG neurons is regulated in *C. elegans* and potentially identify crucial factors required for oxygen and carbon dioxide sensing in higher organisms.

468A

Sexual specification of ventral cord neurons. **Andrea K. Kalis**, Djem Kissiov, Breanna Tetreault, Jennifer Ross Wolff. Biology, Carleton College, Northfield, MN.

C. elegans males and hermaphrodites crawl into postembryonic life with identical complements of ventral cord neurons (VCNs). By the end of L1, VCN lineages begin to diverge to take on sex-specific roles that support reproduction. In hermaphrodites, P3.aap-P8.aap become VC neurons that regulate egg laying. In males, P3.aap-P11.aap divide to generate CA and CP neurons (Pn.aapa/p), which innervate targets including male-specific neurons, muscles, and gonad[1]. How does Pn.aap differentiate into VCs in hermaphrodites, but divide to produce CAs and CPs in males? Pn.aap fates in both sexes are dependent on the activity of the Hox transcription factor LIN-39[2,3]. Not surprisingly, the sex determination pathway is also instrumental. Our experiments using a *tra-2(ts)* allele reveal that active TRA-1 is postembryonically required to prevent male fate in the Pn.aap lineage. XX *tra-2(ts)* worms raised at the restrictive temperature after hatching display the male-specific pattern of serotonin expression in VCNs. While it is evident that Hox proteins converge with sexual regulators to instigate sexually dimorphic neurogenesis, the details of this interaction remain unclear.

To identify new regulators of sex-specific VCN fates, we are screening for mutations that alter VCN expression of the normally CP-specific marker *tph-1::gfp*. Preliminary analysis reveals mutants in three phenotypic classes: 1) Mutants with sexual transformation in multiple tissues including VCNs. These mutants confirm a role for sex determination in determining VCN fate. 2) Mutants with reduced expression of *tph-1::gfp* in CPs. Some mutants in this class are Unc, suggesting a disruption of general VCN neurogenesis. More interesting are mutants with CP-specific reduction of *tph-1::gfp* expression and normal locomotion. We have mapped one such mutation, *ccc1*, to a small region of the X chromosome (X:12.6 to X:13.95) containing about 50 candidate genes. 3) Mutants with increased VCN expression of *tph-1::gfp*. This class includes alleles of *pag-3* and *unc-3*, both of which cause supernumerary *tph-1::gfp(+)* VCNs in males. This is consistent with previous studies demonstrating a similar role for these genes in hermaphrodite Pn.aap[4]. Surprisingly, we find that *unc-3*, but not *pag-3*, hermaphrodites occasionally have *tph-1::gfp(+)* CP-like neurons, suggesting a link between *unc-3* and sex determination in VCNs. Further analysis of these mutants will reveal mechanistic links between sex determination and neurogenesis. [1]Male Wiring Project (worms.aecom.yu.edu); [2]Salser SJ, et al., Genes & Dev 7:1714-1724; [3]Clark SG, et al. Cell 74: 43-55; [4]Prasad BJ, et al. Dev Biol 323: 207-15.

469B

Roles of 2-Ig domain proteins ZIG-1, ZIG-5, ZIG-8 and their interactions with SAX-7 in neuronal maintenance. **Anagha Kulkarni**, Claire Bénard. Department of Neurobiology, UMass Medical School, Worcester, MA.

Neuronal circuitries established during development must persist throughout life. This is a challenge to the integrity of a nervous system given postnatal body size increase, body movements, addition of new neurons, and aging. Research in *C. elegans* reveals that molecular systems ensure that the overall organization of neuronal ensembles is

appropriately maintained throughout life. Five molecules have been identified as mediators of neuronal maintenance: the secreted 2-Ig domain proteins ZIG-4 and ZIG-3, the FGF receptor EGL-15(5A), the L1-like SAX-7 cell-adhesion protein, and the giant DIG-1 protein. The mechanisms by which these molecules maintain the neuronal architecture remain unknown.

The *C. elegans* genome contains 8 *zig* genes that encode 2-Ig domain proteins. Numerous related genes are expressed in adult brains in flies and mammals, but their function remains unknown. We seek to understand the roles of the *zig* genes in neuronal maintenance and their interplay with other neuronal maintenance factors. Our analysis of multiple mutant combinations of the *zig* genes reveals that each of these genes contributes to neuronal maintenance and that they function in redundant and antagonistic ways. Notably, while the single mutants *zig-5* and *zig-8* are wild type, the double mutant *zig-5 zig-8* displays progressive displacement of head chemosensory neurons in late larval life and adulthood. This phenotype is similar to that of *sax-7* null mutants. The gene *sax-7* encodes two isoforms, SAX-7S and SAX-7L, that have 4 and 6 Ig domains respectively, and differ functionally. SAX-7L might form an autoinhibitory horseshoe structure wherein the 2 external Ig domains occlude the two central critical Ig domains. We have found that *zig-5 zig-8* double mutant defects can be fully suppressed by the loss of *sax-7L*, indicating that ZIG-5 and ZIG-8 are required only when SAX-7L is present. Together with other genetic evidence, we hypothesize that ZIG-5 and ZIG-8 regulate SAX-7L by directly interacting with the external Ig domains of SAX-7L, leading to an open conformation that exposes the central domains required for maintenance.

zig-5 and *zig-8* also interact with *zig-1*, as loss of *zig-1* fully suppresses *zig-5 zig-8* double mutant defects. *zig-1* encodes the only transmembrane ZIG protein. We find that *zig-1* functions in muscles and the transmembrane domain is essential for maintenance. We speculate that *zig-1* antagonizes the function of *zig-5* and *zig-8*. We are testing the interactions of ZIG-5, ZIG-8, SAX-7 and ZIG-1 by cell aggregation assays.

470C
Cell fate determination of oxygen sensing neurons in *C. elegans*. **Kasper Langebeck-Jensen**, Teresa Rojo, Roger Pocock. BRIC - Biotech Research & Innovation Centre, Copenhagen Biocenter, Ole Maaløes Vej 5, 4th floor, Copenhagen, Denmark.

In the environment, *C. elegans* has to respond to a variety of attractive and repulsive cues such water-soluble chemicals, odors and gases. The URX neurons in*C. elegans* are responsible for the detection of up-shifts in environmental oxygen, enabling the worm to generate rapid behavioral responses. However, the molecular factors critical for determining URX cell fate are not fully understood. Using forward EMS mutagenesis screening approaches we have isolated 10 mutants that fail to express *flp-8::GFP* in the URX neurons. Through the cloning and phenotypic characterization of these mutants we have revealed part of the underlying pathway required for the differentiation of the URX neurons and a potential temperature-dependent circuit, where *flp-8::GFP* is only induced in URX at high temperatures. Currently, we are working on identifying the molecular lesions of mutants isolated from the genetic screening, in addition to elucidating why neuropeptide expression is required in these neurons under stress.

471A
Genome-wide RNAi screening for *vhp-1* suppressors in *C. elegans*. **Chun Li**[1], Naoki Hisamoto[1], Paola Nix[2], Shuka Kanao[1], Tomoaki Mizuno[1], Michael Bastiani[2], Kunihiro Matsumoto[1]. 1) Div. of Biol. Sci., Grad. School of Sci., Nagoya Univ., Japan; 2) Div. of Biol., Utah Univ., USA.

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 *Caenorhabditis elegans*, the stress response is controlled by p38 and JNK MAPK pathways, consisting of DLK-1 (MAPKKK)/MKK-4 (MAPKK)/PMK-3 (MAPK) and MLK-1(MAPKKK)/MEK-1(MAPKK)/KGB-1 (MAPK), respectively. The *vhp-1* gene encodes an MAPK phosphatase that negatively regulates both MAPK pathways. Animals with the *vhp-1* deletion are arrested during larval development due to hyperactivation of the MAPK pathways. To identify components involved in MAPK-mediated signaling, we performed a genome-wide RNA interference (RNAi) screening for suppressors of the *vhp-1* lethality. With this RNAi screening, we isolated *dlk-1*, *pmk-3*, *cebp-1*, *mlk-1*, *mek-1* and *kgb-1* as *vhp-1* suppressors. Furthermore, we identified 10 novel *svh* (suppressor of *vhp-1*) genes. They encode protein kinases, transcription factors, mediator complexes, a receptor-like transmembrane protein and an RNA-binding protein. Here, we present results showing that a novel receptor-like transmembrane protein is required for neuronal regeneration in *C. elegans*.

472B
Neuron developmental defects caused by ethanol exposure. **Conny H C Lin**, Sasha Sa, Ken Ke-Chih Huang, Catharine Rankin. Brain Res Ctr, Univ British Columbia, Vancouver, BC, Canada.

Fetal Alcohol Spectrum Disorder (FASD) caused by maternal alcohol consumption affects more than 1% of all live births. Affected individuals exhibit a wide range of brain damage in areas such as cerebellum, corpus callosum, hippocampus and optic nerves. These nervous system abnormalities were shown to underlie the behavioral and cognitive disabilities such as problems in executive functioning, verbal learning, and bimanual coordination found in individuals with FASD. Ethanol is a promiscuous ligand that can affect a wide variety of proteins. As a result, we are still far from comprehending the full spectrum of molecular mechanisms involved in ethanol neuro-teratology. By modeling FASD using C. elegans, a powerful genetic model, we can hasten the progress in understanding the mechanisms of ethanol damage to the developing nervous system. We are using C.elegans as a model system to investigate the effects of ethanol on the developing nervous system. We have found similar neuronal developmental defects in C. elegans during larval development as those found in mammalian models of FASD. C. elegans exposed to 0.5mM ethanol (~0.12% blood alcohol concentration) from hatch to L4 exhibit signs of cell death, defective migration and axon pathfinding. In ethanol exposed worms, mechanosensory neurons labeled with pmec-4::GFP or pmec-7::GFP sometimes have AVM and PVM missing or dislocated. The expression of pmec-4::GFP in AVM/PVM was consistently fainter compared to ALM/PLM, but this differential expression level did not occur in the pmec-7::GFP strain. In addition, PLM neurites in 70% of the exposed worms are grossly abnormal (overextension, premature termination, excess branching and/or dorsal-ventral wandering). Vulval motor neurons VC4 and VC5 labeled with CyIs4[pcat-1::GFP] were also abnormal in ethanol exposed worms (strain obtained from the Colavita lab, University of Ottawa, Canada). In addition, the vulva slit was found to be posteriorly dislocated in exposed worms. The two GFP-positive vulval neurons innervating the dislocated vulva were both posterior to the vulva slit instead of positioning on opposite side of the vulva; their neurite innervations were grossly abnormal, and the expression of pcat-1::GFP was consistently fainter in the neuron more anterior of the two compared to the one posterior. These findings suggest that larval ethanol exposure in C. elegans 1) selectively affects gene expression, 2) causes neurite misguidance, and 3) produces migration defects in neurons as well as vulva cells. Future studies will focus on further characterization of ethanol neuro-teratology on other neurons such as HSN and DD/VD and the ethanol's effect on axon guidance.

473C
DM-domain genes *dmd-3* and *mab-23* specify critical cell fate characteristics in the male *C. elegans* ray sensorimotor circuit. M. Siehr[1], A. Sherlekar[1], P. Koo[1], X. Bian[1], D. Portman[2], **R. Lints**[1]. 1) Dept Biol, Texas A & M Univ, College Station, TX; 2) University of Rochester, Center for Aging and Developmental Biology, Rochester, NY.

DM-domain (DSX-MAB-3) transcription factors promote gender-specific development in widely divergent organisms. However, precisely how their activity sex-specifically shapes developmental patterning is known for only a few cases. Two DM-domain genes, *dmd-3* (1) and *mab-23* (2), are expressed in cells of the ray sensorimotor circuit. This circuit induces apposition of the male tail against the hermaphrodite during mating (see Koo et al abstract). There are nine bilateral pairs of ray sensilla on the male tail (numbered 1 to 9) and each contain the sensory endings of two neurons, type A and B. The A-neurons are critical for circuit function and release dopamine and acetylcholine to effect tail posture. We find that *dmd-3* and *mab-23* are expressed in these neurons and that their loss of function dramatically alters the balance of dopaminergic (DA) and cholinergic (ACh) fate in this population. In wild type males DA fate is confined to three of the nine A-neuron pairs (those of rays 5, 7, and 9). However, in *dmd-3* and *mab-23* mutants virtually all A-neurons express DA fate; ACh fate (normally expressed by the A-neurons of rays 1 to 4 and 6) is rarely observed. ETS-domain transcription factor gene *ast-1* (3) and the *C. elegans distal-less* homolog *dopy-2/ceh-43* (4) establish expression of dopamine biosynthesis genes in all DA neurons in the worm during development. We find that *ast-1* and *ceh-43* are expressed in all A-neurons and that the ectopic DA fates in the DM-domain gene mutants depend on *ast-1/ceh-43* function. The results of genetic analyses suggest a model in which DA fate corresponds to the ground state of A-neurons and is conferred by *ast-1/ceh-43*. However, in most A-neurons *dmd-3*/*mab-23* suppress *ast-1/ceh-43* function and instead promote ACh fate in a *ceh-43*-dependent manner. The A-neurons of rays 5, 7 and 9 are able to adopt DA fate because DM-domain gene function is blocked by the action of a TGF-beta (DBL-1) signaling pathway, which is specifically activated in the cells of these rays. Mating behavior-, optogenetic- and pharmacological analyses of the DM-domain mutants reveal that these animals are severely defective in mate apposition behavior. These defects can be attributed to mis-specification of A-neuron fates and to defects in the sex-specific patterning of core body wall muscles, which also express *dmd-3* and *mab-23* during development. 1. Mason et al. (2008) Development.135: 2373-82. 2. Lints & Emmons (2002) Genes Dev. 16: 2390-402. 3. Flames & Hobert (2009) Nature. 16: 885-9. 4. Doitsidou et al. (2008) Nat Methods. 5:869-72.

474A
Sensory Nerve Ending Regeneration during Development of the C. elegans Dopaminergic PDE Neurons. **Xuemin Lu**[2], Tori Hatch[1,2], Rita Droste[1,2], Robert Horvitz[1,2]. 1) HHMI; 2) Department of Biology, MIT, Cambridge, MA 02139 USA.

Neural regeneration is a fundamental issue in neuroscience. Successful regrowth or repair of neural tissues could play a vital role in patient recovery after nervous system injury. Despite our current knowledge about axon regeneration, much is still unknown about the regeneration of sensory endings, as in retinal regeneration in damaged retinas and hair cell regeneration after hearing loss. To investigate molecular mechanisms and factors involved in neural regeneration, especially sensory nerve ending regeneration, we are using C. elegans to identify new molecules and pathways involved in this process. We are focused on a simple sensory structure in the posterior part of the worm, the postdeirid. Each of the two postdeirids encompasses three cells, the PDE dopaminergic neuron and two associated glial cells, the PDE sheath and PDE socket cells. The simple structure and relative isolation of the postdeirid makes it an excellent system for imaging in live animals and for genetic screens. During development, the sensory ending of the PDE neuron in the postdeirid is shed with the cuticle during at least the L4 molt and regenerates in the adult. By combining fluorescent live-cell imaging techniques using a GFP reporter with electron microscopic techniques, we are investigating the structural and morphological changes of the PDE sensory ending during the last molt. We are also performing both RNAi screens using a

sensitized genetic background (sid-1 overexpression in neurons) and EMS screens in an effort to identify genes that function in PDE sensory ending regeneration. Genes indentified in either screen will be further characterized. We hope that our findings will reveal mechanisms of neural regeneration.

475B

Identifying Genes that turn Skin Cells into Neurons. **G. Minevich**[1], M. Doitsidou[1], S. Gowtham[1], H. Korswagen[2], O. Hobert[1]. 1) Department of Biochemistry & Biophysics, Columbia University, New York City, NY; 2) Hubrecht Institute, Utrecht, The Netherlands.

Under specific circumstances, skin cells can change their differentiated state from a skin to a neuronal state. This is observed in several systems, from worms to flies to vertebrates. For example, in the L2 stage of C. elegans, the differentiated skin cell V5 generates a neuroblast that then forms two mechanoreceptor neurons, PVD and PDE. Emmons and co-workers previously described that a loss of the conserved bHLH transcription factor *lin- 32* (Atoh1 in mouse) results in a failure of this skin-to-neuron transformation (Zhao, et al., 1995); instead, the neuroblast converts into a skin cell, like all other V cells do.

Using forward genetic screens, we have identified additional genes required in this skin-to-neuron transformation. Our mutant collection contains a subset of conserved transcription factors that may have similar roles in vertebrates. As proof of principle, we have isolated several *lin-32* alleles: one of which is a regulatory mutation in a region that is completely conserved between 5 species of nematode and contains a C2H2-type zinc finger binding site. In another mutant, *dopy-1*, *lin-32::gfp* expression fails to be induced in the V5 lineage, suggesting that we isolated a regulator of *lin-32* expression. *dopy-1* is a C2H2 zinc finger transcription factor and while it may control *lin-32* directly, it is broadly expressed, suggesting that other activators of *lin-32* remain to be identified. Another gene we have implicated in the skin-to-neuron transition is *hlh-2*, a gene encoding a highly conserved protein known to heterodimerize with LIN-32 and play a role in male ray neurogenesis (Portman, et al., 2000). The allele of *hlh-2* that we retrieved also appears to be a regulatory allele that deletes upstream promoter elements. Using a parallel candidate gene approach, we identified *vab-15* as another mutant where the skin-to-neuron transformation fails to occur. *vab-15* was previously found to encode the C. elegans homolog of the vertebrate Msx homeobox genes (Du, et al., 2001). We find that *vab-15* also controls *lin-32* expression.

From a collection of over 30 mutant alleles in which neurons in the postdeirid lineage fail to be induced, we expect to find more genes that act to induce *lin-32* expression and/or cooperate with *lin-32* to control induction of the postdeirid lineage. We will present our analysis of these additional mutant loci.

476C

UNC-40 is required for neuronal morphogenesis and synapse distribution in the serotonergic NSM neuron in C. elegans. **Jessica C Nelson**[1], Daniel Colón-Ramos[2]. 1) Interdepartmental Neuroscience Program, Yale University, New Haven, CT; 2) Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University, New Haven, CT.

Serotonin is a conserved neurotransmitter that modulates a diverse array of behaviors and physiological processes such as reproduction, respiration, sleep, and affect. Despite their far-reaching effects, serotonin neurons are relatively few in number within the CNS. These neurons achieve their vast influence through two neurodevelopmental strategies: elaborate axonal arborization and the formation of neurosecretory serotonin synapses. Although the appropriate morphological development of serotonin neurons is crucial for their function, the molecular and genetic underpinnings of these processes are not yet known.

Serotonin axon arborization and synaptogenesis occur within precise spatial coordinates and with stereotyped developmental timing. For example, serotonin neurons in the rat are born and extend simple, unbranched neurites in the embryo. The neurons remain unbranched until the post-natal period, when they extend synapse-containing axonal arbors within specified target fields (Lidov et al).

The precisely timed and spatially regulated extension of axonal arbors is conserved in the nematode C. elegans. Here, the main serotonergic neuron (called NSM) extends a single, unbranched neurite in the embryo. This neurite remains unbranched until just prior to the L4 stage, when synaptic axonal branches extend in a precise neuroanatomical coordinate overlying the nerve ring (Axang et al).

I have conducted a candidate screen for genes required for axonal arborization in the C. elegans NSM neuron. I have identified a novel role for the canonical axon guidance molecule UNC-6 (Netrin) and its receptor UNC-40 (DCC) in localization and morphology of axon arbors. In NSM, mutants lacking the genes for these axon guidance cues display wild-type guidance of the main ventral axons, but do not correctly execute the distinct process of axon arborization. I have determined that UNC-40 is sufficient cell-specifically for the designation of axonal arbor position and morphology. Interestingly, I have observed that UNC-40 localizes transiently to nascent axonal arbors in an UNC-6-dependent manner. Finally, I have preliminary evidence that UNC-40 also plays a role in synapse distribution along the main NSM axon. These data suggest that the canonical axon guidance cue UNC-40 is required for neuronal morphogenesis and synapse distribution in the serotonergic NSM neuron in C. elegans.

477A

C. elegans as model system to study Nephrin/Neph protein functions. Nicola Wanner, Tobias B. Huber, **Elke Neumann-Haefelin**. Nephrology, University Hospital Freiburg, Freiburg, Germany.

The Neph and Nephrin cell recognition module is essential for multiple morphogenetic processes including neuronal synaptogenesis in *C. elegans* (Shen & Bargmann 2003, Shen et al. 2004) and formation of the kidney filtration barrier in vertebrates. In *C. elegans* SYG-1 and SYG-2, the homologs of human Neph and Nephrin, act on HSN neurons and guidepost cells of the vulva to drive synapses from motor neurons onto adjacent target neurons and muscles. We demonstrate now that the Neph/Nephrin family proteins can form cell-cell adhesion modules across species: *C. elegans* SYG-1 directly binds to human Nephrin and SYG-2 binds to Neph1-3. Based on these findings, we established a *C. elegans* model system to study functional and structural conservation of Nephrin and Neph proteins in vivo. Expression of mammalian Nephrin in *C. elegans* syg-2 mutants can relocate SYG-1 to appropriate sites and restore defective synaptic connectivity, indicating functional compatibility of Nephrin and SYG-2 proteins. Furthermore, we investigated the ability of SYG-1 and SYG-2 respectively to engage homophilic interactions in trans between the HSN and vulva epithelial cells. Strikingly, synapse assembly can be induced by homophilic SYG-1 but not SYG-2 binding indicating a critical role of SYG-1 intracellular signalling for morphogenetic events. In summary, our findings corroborate that *C. elegans* synapse formation is an excellent tool for investigating fundamental Nephrin/Neph protein functions, cell recognition and intracellular signalling events.

478B

UNC-73/Trio, the Rac GTPases, UNC-33/CRMP and UNC-44/Ankyrin are required for limiting the extent of growth cone filopodia protrusion and ectopic axon branching. **Adam Norris**, Erik Lundquist. University of Kansas, Lawrence, KS.

The growth cone consists of actin-based lamellipodial and filopodial protrusions, which provide both propulsive force and the sensation of extracellular guidance cues. While many molecules involved in axon pathfinding have been identified, little is known about their role in the developing growth cone. Here we show that UNC-73/Trio, the Rac GTPases, UNC-33/CRMP and UNC-44/Ankyrin, which are all known axon guidance genes, were required for the inhibition of excessive axon branching and the inhibition of filopodia protrusion in developing growth cones. The *unc-73* mutant phenotype was rescued by an activated *mig-2* construct, indicating that MIG-2 likely acts downstream of UNC-73. The ectopic axon defects seen in each of these mutants appeared to be a direct consequence of the filopodia protrusive defects. The genes studied here may be downstream of a repulsive UNC-40/UNC-5 heterodimer, as they suppressed an ectopically-expressed UNC-5-dependent activated UNC-40 phenotype in the VD growth cones. Thus we have described a developmental role for a series of genes known to be important for proper axon guidance, arriving at a developmental explanation for the defects seen in the endpoint analysis of the nervous system.

479C

A copine like protein CPNA-2 regulates neural localization of UNC-6/Netrin in *Caenorhabditis elegans*. Taro Asakura, Kumiko Fujita, Yoshio Goshima, **Ken-ichi Ogura**. Dept Pharmacology, Yokohama City Univ, Yokohama, Japan.

UNC-6/Netrin is an evolutionarily conserved secretory protein required for axon guidance, cell migration, cell polarity and synapse formation. In *C. elegans*, UNC-6/Netrin is expressed by ventral glia, neurons, muscles and vulval precursor cells. The proper localization/secretion of UNC-6/Netrin is important for exhibiting the positional information. However, little is known about the localization/secretion mechanism.

In order to reveal the localization mechanism of UNC-6/Netrin, we screened mutants that exhibited abnormal localization of Venus::UNC-6 (Asakura et al., Genetics, 185:573-585, 2010). We found that, in mutants of *unc-51*(autophagy related kinase), *unc-14* (RUN domain protein) and *unc-104* (kinesin motor protein), Venus::UNC-6 was abnormally accumulated in the cell bodies in ventral neurons, resulting in little Venus::UNC-6 in the axons. Aberrant accumulation of Venus::UNC-6 in ventral muscles was seen in mutants of *unc-18* (SM protein) and *unc-68* (ryanodine receptor). In addition, we isolated *gh23* that exhibited abnormal accumulation of Venus::UNC-6 in neural cell bodies. The *gh23* phenotype is very similar to that of the *unc-104* mutant.

We cloned this gene and found that the responsible gene of *gh23* was a *cpna-2* gene that encoded a copine like protein. As copine is a Ca2+-dependant phospholipid-binding protein, we think that CPNA-2 may be an adaptor protein of UNC-104 for trafficking of the secretory vesicles including UNC-6/Netrin. We will discuss possible mechanism on the localization of UNC-6/Netrin.

480A

Wnt, Frizzled, CAM-1/ROR and LIN-18/RYK signaling establish anteroposterior neuronal polarity. **Daniel Oliver**, Scott Clark. Biology Department, University of Nevada, Reno, NV.

Wnts are conserved signaling molecules that control a diversity of developmental processes, including asymmetric cell division, cell fate specification and axon guidance. Wnts can act via several cell surface receptors, including seven-transmembrane Frizzleds, CAM-1/ROR tyrosine kinases and LIN-18/RYK atypical tyrosine kinases. We and others found that Wnt and Frizzled signaling regulate the anteroposterior polarity of the mechanosensory neurons ALM and PLM. When Wnt or Frizzled signaling is disrupted, the polarity of ALM and PLM is inverted: the anterior process adopts the length, branching pattern and synaptic properties of the posterior process and vice versa. These studies indicated that different but overlapping sets of Wnts control neuronal polarity in different body regions: CWN-1, CWN-2 and EGL-20 orient ALM, while CWN-1, EGL-20 and LIN-44 polarize PLM. The role of the Wnt MOM-2 in ALM/PLM polarity had not been fully addressed because *mom-2* mutations cause maternal effect embryonic lethality. Using a temperature-sensitive *mom-2* allele, we found that the loss of MOM-2 activity by itself failed to perturb ALM or PLM polarity. However, we discovered that *mom-2* in combination with other Wnt mutations affected both ALM and PLM polarity. Our further analysis of various mutant combinations revealed that all five Wnts regulate ALM and PLM

polarity.

The five Wnts required to polarize ALM and PLM do not have simple redundant or additive functions. Different Wnts have different affects on ALM and PLM and they act in a hierarchical and sometimes antagonistic fashion. For example, we found that CWN-1 and CWN-2 act as permissive signals and EGL-20 functions as an instructive cue to polarize ALM. However, CWN-1 became an instructive signal in the absence of EGL-20. The sequential removal of individual Wnt activities could enhance or reduce ALM and PLM polarity defects. For example, most ALMs exhibited a reversed polarity when three specific Wnts were mutated, yet ALM often adopted a symmetric polarity when these and a fourth Wnt were eliminated.

Previous studies indicated that the Frizzled LIN-17 acts in PLM to control its polarity. Using a variety of approaches, we examined the role of CAM-1/ROR, LIN-18/RYK and all four Frizzleds in ALM and PLM polarity. We found that CAM-1, LIN-18 and the Frizzleds MOM-5 and CFZ-2 affected ALM polarity, while at least LIN-17 and LIN-18 influenced PLM polarity. In summary, our results show that signaling by five Wnts and several types of Wnt receptors are needed to establish ALM and PLM polarity.

481B

microRNA regulation of neuronal guidance in *C. elegans*. **Mikael Egebjerg Pedersen**, Goda Snieckute, Roger Pocock. BRIC, University of Copenhagen, Ole Maaløesvej 5, DK-2200 Copenhagen N, Denmark. mikael.pedersen@bric.ku.dk.

Background

Neurons must extend axons in the correct direction and at the proper time during development. Important regulators of developmental timing are microRNAs. In fact, the first microRNAs to be identified, lin-4 and let-7, were discovered based on their heterochronic phenotypes. The Caenorhabditis elegans hermaphrodite specific motor neurons (HSNs) present a convenient system for further investigating the temporal control of axon guidance and cell migration. The HSNs extend a single axon ventrally, which then migrates anteriorly during the L4 larval stage. Previous studies have identified a molecular pathway involving the highly conserved microRNA lin-4 to be required for correct neuronal guidance of the HSN neurons. Here, we show yet another conserved microRNA family, the mir-79 family, homologues of the highly conserved miR-9, to be involved in neuronal guidance of the HSN neurons in C. elegans.

Results

To study the role of the mir-79 family in neuronal guidance we obtained knockout strains for both members of the mir-79 family, mir-75 and mir-79. In our preliminary analysis, we found that deletion of both microRNAs causes severe defects in HSN cell migration and axon guidance. Defects include anterior misplacement of HSN cell bodies and posteriorly directed axons. We will present further work regarding the analysis of the specific roles of the mir-79 family during neuronal guidance.

482C

A FORWARD GENETIC SCREEN TO IDENTIFY NEW SYNAPTIC PARTNER RECOGNITION GENES. Shanté O'Hanlon, Anabel Ortiz, **Minh Pham**, Mekala Raman, Miri VanHoven. Biological Sciences, San José State University, San José, CA.

Proper formation of synapses between neurons is essential for the function of the nervous system. This process is required for correct messages to be sent, allowing functions that are vital for survival including perception, thought, and behavior. The purpose of this work is to understand the molecular mechanisms required for neurons to identify and form synapses with their correct partners. We use the model organism C. elegans to address this question because they have a simple, well-characterized nervous system with a complete synaptic map. We study PHB sensory neurons and AVA interneurons, which contact and form synapses in the posterior ventral nerve cord of the animal. The transgenic fluorescent marker Neuroligin-1 GFP Reconstitution Across Synaptic Partners, is used to label PHB-AVA synapses and cytosolic mCherry is used to visualize the neurites. Our group has previously identified two genes required for PHB-AVA synaptic partner recogntion: unc-6/Netrin, which encodes a secreted ligand expressed in AVA, and unc-40/DCC, which encodes a trans-membrane receptor expressed in PHB. However, it is not known how the synaptic partner recognition signal is transduced downstream of this receptor and ligand. Therefore, we are using an unbiased forward genetic screen to identify new genes that mediate synaptic partner recognition. We have conducted a semi-clonal F2 screen for mutants with defects in neurite contact and synaptogenesis. 1854 haploid genomes have been assayed and 11 synaptic partner choice mutants have been isolated and characterized. Our goal is to genetically characterize and map these mutants.

483A

CEH-28 regulates neuroendocrine function of the M4 pharyngeal neuron by activating *dbl-1* expression. **Kalpana Ramakrishnan**, Paramita Ray, Peter Okkema. Laboratory for Molecular Biology and the Department of Biological Sciences, University of Illinois at Chicago.

The homeodomain transcription factor CEH-28 is specifically expressed in the M4 pharyngeal motor neuron, where it regulates synaptic assembly and neuronal polarity. Here we describe a second role for CEH-28 in regulating a neuroendocrine function of M4 that is mediated by the BMP/TGFβ family factor DBL-1. In wild-type animals, *dbl-1::gfp* is strongly expressed in M4 and a subset of other pharyngeal and non-pharyngeal neurons, while, in *ceh-28* mutants, *dbl-1::gfp* expression is specifically lost in M4. The *dbl-1* promoter contains several potential CEH-28 binding sites, and we have evidence that *dbl-1* is directly activated by CEH-28. Using a series of 5'-deletions of the *dbl-1* promoter, we have identified sequences necessary for *dbl-1::gfp* expression outside of M4, and we find that deletion of potential CEH-28 binding sites progressively reduces *dbl-1::gfp* expression in M4. Within the *dbl-1* promoter, we have identified a 633 bp enhancer containing two potential CEH-28 binding sites that activates a *Δpes-10::gfp* reporter specifically in M4. Activity of this enhancer is lost in *ceh-28* mutants, while mutation of the potential CEH-28 binding sites reduces the frequency and level of M4 GFP expression in wild-type animals. DBL-1 is a ligand for the Sma/Mab signaling pathway that regulates body size and male tail patterning, but its function in the pharynx is unknown. The pharyngeal g1 gland cells are located near M4, and we find similar morphological defects the g1 gland cells in *ceh-28* and *dbl-1* mutants, as well as some other mutants affecting Sma/Mab pathway signaling. We can partially rescue the gland cell defects in *dbl-1* mutants by expressing *dbl-1* only in M4. Based on these findings, we hypothesize that CEH-28 is a direct activator of *dbl-1* expression in M4, and that DBL-1 secreted from M4 regulates gland cell activity or morphology.

484B

HLH-3 expression is necessary during embryogenesis for proper HSN differentiation and function. **Saleel V Raut**, Aixa Alfonso. University Of Illinois at Chicago, Chicago, IL.

HLH-3 is a basic helix-loop-helix (bHLH) transcription factor of the Achaete-Scute family that plays a role in the differentiation of the serotonergic hermaphrodite specific motor neurons (HSNs) in C. elegans. Hermaphrodites harboring molecular null alleles of *hlh-3 (bc248 and tm1688)* are egg-laying defective, their HSNs have defects in axon pathfinding and 65% have no immunodetectable serotonin (Doonan et al., 2008). In order to understand how *hlh-3* expression is regulated, we have begun characterizing the *cis*-acting sequences required for normal expression. We had previously shown that UNC-86 regulates hlh-3 expression in the HSNs. Our data showed that 75% of the HSNs did not express a HLH-3:: GFP translational fusion construct in an *unc-86(e1416)* mutant background. We hypothesized that three potential UNC-86 binding sites in the *hlh-3* promoter and intron are required for expression of *hlh-3*. To test whether these potential binding sites were sufficient for *hlh-3* expression, we generated a *hlh-3* promoter - intron construct fused with *gfp* and injected it in wild type animals. We find that this truncated construct is expressed during embryogenesis but there is no expression in the L1 stage or further. To pursue whether sequences in the 3' untranslated region were necessary for hlh-3 expression in postembryonic stages, we characterized endogenous expression using fosmid WRM0625aA01 (kindly provided by the Hobert laboratory) where ~ 34 Kb of genomic DNA is included and the *hlh-3* coding sequence is fused to YFP at the carboxyl terminus. We find that *hlh-3::yfp* expression is only detectable during embryogenesis. Since the fosmid rescues the Egl phenotype of *tm1688* and the HSNs do not acquire their final differentiated state before the L4 stage, we conclude that *hlh-3* function in the embryo is necessary for proper HSN differentiation and function. To determine what sequences in the *hlh-3* promoter are necessary for expression we are currently looking at a deletion allele, *bc277*, which is missing 664 bp of the promoter.

485C

A genetic analysis of the axon guidance of the C. elegans pharyngeal neuron M1. **Osama M. Refai**, Evvi Rollins, Patricia Rohs, Jeb Gaudet. Dept Biochemistry and molecular biology, University of Calgary, Calgary, AB, Canada.

The guidance of axons to their correct targets within an organ is a critical step in development. We are interested in understanding how individual neurons find their targets in the context of a developing organ. We are using the pharyngeal neuron M1 as a model because it extends a process from the posterior pharyngeal bulb to the anterior end of the pharynx. Furthermore, M1 uses the g1P gland as a substrate to guide its axon. Electron micrographs show that the M1 axon bundles with the g1P gland projection through the anterior pharynx but not in the more posterior part of the pharynx. Ablation of glands results in defects of the M1 trajectory, suggesting that g1P is necessary for M1 guidance. Growth cone defective mutants (e.g. unc-51 and unc-119) showed severe defects of the M1 trajectory at the procorpus, where it contacts g1P, but no defects in the posterior portion of M1, where there is no contact with g1P. These results suggest that the M1 axon develops through two phases: a growth cone-independent phase in the posterior pharynx (as observed for other pharyngeal neurons; Morck et al., 2003) and a growth cone-dependent phase in which the axon is guided by the g1P cell. Tests of mutations of the major guidance pathways (e.g. unc-6, sax-3, vab-1, smp-1 and smp-2), show little or no defect in M1, suggesting the involvement of novel molecules that act via the g1P gland. To identify additional genes involved in M1 guidance, we performed a forward genetic screen to identify mutants that affect M1 axon migration. We isolated 12 mutants with defective M1 trajectories, including mutants that overextend their anterior process, mutants that underextend the anterior process and a single mutant that appears to be missing M1. We are currently in the process of cloning the relevant mutations and will present our findings. Overall, the results are consistent with a model in which growth of the M1 axon involves two phases: a growth cone-independent phase in the posterior pharynx, which likely occurs passively due to pharyngeal elongation; and a growth cone-dependent phase in the anterior pharynx, that establishes the rest of the M1 trajectory and is at least partially dependent on the gland cell extension.

486A

PLR-1 regulates Wnt signaling by reducing cell surface levels of Frizzled and CAM-1/ROR. **Ryan Robinson**, Scott Clark. Biology Department, University of Nevada, Reno, NV.

Wnts are secreted signaling proteins that regulate a variety of developmental processes by interacting with different cell surface receptors, including seven-transmembrane Frizzleds

and the receptor tyrosine kinase CAM-1/ROR. During formation of the C. elegans nervous system, Wnt-Frizzled signaling controls cell migration, neuronal polarity and axon guidance along the anteroposterior body axis. We identified a transmembrane RING finger protein, PLR-1, that governs the response to Wnts by reducing the cell surface levels of Frizzled (L. Moffat, R.R., A. Bakoulis & S.C., unpublished results). Loss of PLR-1 in the neuron AVG caused its anteroposterior polarity to be symmetric or reversed because signaling by the Wnt CWN-1 was inappropriately activated; while ectopic expression of PLR-1 blocked Wnt signaling. Frizzleds are enriched at the plasma membrane; however, when PLR-1 and Frizzled were coexpressed, Frizzled was no longer detected at the cell surface but instead was colocalized with PLR-1 in endosomes and Golgi. Our results indicate that PLR-1 coordinates the spatial and temporal response to Wnts during neuronal development by regulating Frizzled trafficking.

Using various fluorescently tagged transmembrane protein chimeras, we found that the extracellular domain of PLR-1 was both necessary and sufficient for interaction with the extracellular cysteine rich domain (CRD) of Frizzled and that the Frizzled CRD was both necessary and sufficient for interaction with the extracellular domain of PLR-1. These experiments and others support the model that PLR-1 and Frizzled interact via their respective extracellular/luminal domains to form a complex that is targeted to endoscopes via the activity of the PLR-1 RING finger. The CRD is a conserved feature of Frizzleds and is also present in CAM-1/ROR proteins. As such, we examined whether PLR-1 affected CAM-1/ROR trafficking. Indeed, we found that PLR-1 also reduced the cell surface levels CAM-1/ROR, showing that PLR-1 regulates the trafficking of two distinct families of Wnt receptors. We are currently investigating whether PLR-1 escorts Frizzled and CAM-1/ROR to the endosome directly from the Golgi or whether PLR-1 recruits them to the endosome from the cell surface via endocytosis.

487B

MIG-10/Lamellipodin and Abelson-interactor-1(ABI-1) act together to mediate directed migration and outgrowth in *C. elegans*. M. McShea[1], S. Zhang[1], M. Kuhlwein[1], S. Hashmi[1], M. Dubuke[1], C. Grant[1], K.L. Schmidt[2], E.G. Stringham[2], **E.F. Ryder[1]**. 1) Dept Biol & Biotechnology, Worcester Polytechnic Inst, Worcester, MA; 2) Dept Biology, Trinity Western University, Langley, BC, Canada.

Neuronal and axonal migration are vital to the establishment of proper neuronal connections during development. The process of directed migration is dependent on reorganization of the actin cytoskeleton in response to external guidance cues. The MRL signaling proteins are thought to transmit positional information from surface guidance cues to the actin polymerization machinery, and thus to promote polarized outgrowth of axons during nervous system development (Quinn and Wadsworth, 2008). In *C. elegans*, mutations in the MRL family member *mig-10* result in animals that have defects in axon guidance, neuronal migration, and the outgrowth of the processes or 'canals' of the excretory cell, which is required for osmoregulation in the worm. To determine more of the molecular partners of MIG-10, we conducted a yeast two hybrid screen using MIG-10 as bait. ABI-1, a downstream target of ABL non-receptor tyrosine kinase, was independently isolated 6 times as a strong MIG-10 interactor. ABI-1 is known to be part of the WAVE complex involved in the initiation of actin polymerization. Single mutants for *mig-10* and *abi-1* displayed similar phenotypes of incomplete migration of the ALM neurons and truncation of the excretory canal. Furthermore, abi-1(RNAi) enhanced the excretory canal truncation observed in *mig-10* mutants. Using both the yeast two-hybrid and a co-immunoprecipitation system, we are identifying domains within MIG-10 and ABI-1 that are necessary for their interaction. We have demonstrated that cell autonomous expression of MIG-10 in the excretory cell rescues the canal truncation phenotype, consistent with MIG-10 and ABI-1 functioning together in this cell. Our results suggest that MIG-10 and ABI-1 act together to link cell surface signaling with polarized actin polymerization.

488C

MADD-2 Regulates Guidance to the Midline through an UNC-40/DCC pathway in *C. elegans*. **Ashwin Seetharaman**, Mariam Alexander, Guillermo Selman, Peter Roy. University of Toronto, Department of Molecular Genetics, The Donnelly Centre for Cellular and Bimolecular Research. 160 College St.,Toronto, ON, M5S 3E1, Canada.

The body wall muscles of C. elegans extend plasma membrane projections, called muscle arms, to the dorsal and ventral midlines to establish neuromuscular junctions with the motor axons. We previously reported that the UNC-40/DCC netrin receptor functions cell-autonomously to direct muscle arm extension through the UNC-73 Rho-GEF. Through a screen for muscle arm development defective (Madd) mutants, we identified a gene that we call madd-2 that functions cell-autonomously in muscle arm extension. In addition, madd-2 is also required for numerous other midline-oriented guidance events, including HSN, AVM, and PVM axon guidance, and the extension of other muscle cells to the ventral midline. The madd-2 locus was independently characterized by other groups and found to be involved in ADL axon branching (by the Bargmann and Tessier-Lavigne groups), male tail ray axon guidance (by the Emmons and Huang groups), and anchor cell invasion (by the Hajnal group). MADD-2 belongs to the C1 subfamily of tripartite motif (TRIM) proteins, which are characterized by an N-terminal ring finger (RING) domain followed by two B box (BB) domains, a coiled-coil domain, a COS domain, a fibronectin-type III (FNIII) domain, and a C-terminal B30.2 (SPRY) domain. MADD-2 is homologous to human MID1, the most extensively characterized C1-TRIM protein in mammals. Mutations in MID1 result in Opitz syndrome, a disease characterized by numerous defects along the ventral midline. Although it is known that MID1 can polyubiquitinate PP2Ac via its RING domain, how MID1 governs midline development is unclear. We have shown that MADD-2 functions in the UNC-40 pathway to guide muscle arm and axon extensions to the midline. MADD-2 may potentiate signalling through the UNC-40 pathway by facilitating a physical interaction between UNC-40 and UNC-73. We have also shown that eliminating or mutating the RING domain disables MADD-2 function. However, the subcellular localization or the abundance of transgenically-expressed UNC-40 or UNC-73 is not altered in madd-2 null mutants, suggesting that MADD-2 does not ubiquitinate its known binding partners in the worm. Whether MADD-2 regulates the abundance of C. elegans PP2Ac (i.e. LET-92) is currently unclear due to conflicting lines of evidence. We are currently working to better understand the role of MADD-2's RING domain and whether MADD-2 has additional roles within the UNC-40 pathway.

489A

The glutamatergic nervous system of *C.elegans*. **E. Serrano**, O. Hobert. Dept Biochem, Columbia Univ, New York, NY.

Glutamate is an essential neurotransmitter in the nervous system whose dysfunction can lead to a range of neurodegenerative diseases. Previous molecular studies have characterized some of the components of glutamatergic transmission in *C.elegans* although this information is not complete yet. The developmental mechanisms that result in terminal differentiation of glutamatergic neurons are incompletely understood both at the level of cis-regulatory elements and trans-acting factors. Extensive work, at the functional and molecular level, has been done to show that eat-4 is a vesicular glutamate transporter homologue to the mammalian VGluT. Still the expression pattern information is incomplete. We have addressed this gap by using two types of reporters, a fosmid based one and a PCR product containing the whole genomic locus (10 kb aprox). We created a fusion cassette in two pieces such as only when they recombine *in vivo* the mCherry gene will be transcribed. In both cases we saw a large number of cells expressing eat-4, either in the head of the worm and in the tail compared to the previous reporter (lee et al, 1999). We have identified most of these cells. Two other genes related to VGluT by sequence homology are K10G9.1 (vglu-2) and T07A5.3 (vglu-3). 2 kb promoter fusions of vglu-2 and vglu-3 genes showed patterns of expression more restricted than eat-4 but that may account for "orphan" neurons not yet associated with specific neurotransmitters. Fosmid-based reporters for these genes are being analyzed. On a functional level, we find that vglu-3 mutants (tm3990) are defective in motor tasks and impaired in a reversal assay. More functional assays will confirm that these two genes are indeed glutamate transporters. The existence of more than one vesicular glutamate transporter correlates with the three VGluTs described in mammals. Eat-4 expression is regulated in a piece-meal fashion. By dissecting the eat-4 promoter into individual pieces we have been able to identify independent modules of expression. For instance, in the first 300 bp upstream to the start codon resides the minimal information for eat-4 expression in the mechanosensory neurons and some head neurons (AUA, ADA and ASK). Moreover eat-4 is lost in the mechanosensory neurons in mec-3 mutants and in unc-86 mutants, indicating that the mechanosensory terminal selector genes, mec-3/unc-86, might be regulating the glutamatergic fate of this neuronal type. Further studies are being done in order to address the regulatory logic underlying the expression of eat-4 in this neuronal type. Other components of the glutamate metabolism are being explored as well as its potential regulation by the mechanosensory terminal selector genes.

490B

Doublets' well kept secret: the sister singlets. **Malan Silva[1]**, Robert O'Hagan[1], Ken Nguyen[2], David H. Hall[2], Maureen Barr[1]. 1) Genetics, Rutgers University, Piscataway, NJ; 2) Center for *C. elegans* Anatomy, Albert Einstein College of Medicine, 1410 Pelham Parkway, Bronx NY10461.

Cilia are cellular antennae; in humans, defects in ciliary form or function give rise to ciliopathies. *C. elegans* sensory organs, or sensilla, consist of morphologically and functionally diverse arrays of cilia that mediate sensation. The length and shape of cilia depends on axonemal microtubule (MT) architecture. In the amphid channel cilia, the ciliary axoneme is anchored to the membrane by a region known as the transition zone (TZ). In the TZ, MT doublets are made of a 13 protofilament A-tubule and an attached 11 protofilament B-tubule. MT doublets are anchored to the membrane with "Y" links of unknown composition. Beyond the TZ, amphid channel cilia display two distinct zones classified using MT architecture. The middle segment consists of nine MT doublets that lack 'Y' links. The distal segment is composed entirely of MT singlets.

Not all *C. elegans* cilia share this axonemal structure. For example, we have shown that cilia of the male cephalic neurons (CEMs) are composed of singlet MTs *(1)*. We used high pressure freeze fixation and electron tomography to observe CEM cilia ultrastructure. By comparing cephalic and amphid channel cilia, we hope to understand at the molecular level how axonemes are specialized in form and function. We focused on transition from MT doublets to singlets and traced individual A- and B-tubules at the doublet-to-singlet transition. We found that the A- and B-tubules in middle segment doublets in CEM cilia separate into two sister singlets in the distal segment. These sister singlets are continuous to the distal segment of the cilium and spatially diverge from each other. Additionally we found that as compared to TZs of amphid neurons, CEM TZs span a longer distance. We are interested in determining how this spatial and structural disposition of the CEM MTs occurs, and how it affects ciliary motors. We are currently testing the hypothesis that tubulin post-translational modifications contribute to MT arrangement and stability in *C. elegans* sensory cilia (see Abstract by O'Hagan et al).

1. A. R. Jauregui, K. C. Q. Nguyen, D. H. Hall, M. M. Barr, The JCB 180, 973-988 (2008).

491C

Netrin instructs presynaptic assembly through a synaptic MIG-10 isoform and the actin cytoskeleton. **Andrea K Stavoe**, Jessica C Nelson, Daniel A Colón-Ramos. Department of Cell Biology and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University, New Haven, CT.

The development of the nervous system is orchestrated with a limited number of molecular cues. For example, molecules originally identified for their roles in axon guidance are now known to play important roles during synapse formation. While examples of guidance molecules controlling synaptogenesis abound, we understand less about how these molecules instruct different neurodevelopmental outcomes in a cell-specific manner. We have previously demonstrated that Netrin, originally identified as guidance molecule, instructs presynaptic assembly in the nerve ring interneuron AIY. The Netrin receptor UNC-40 is cell-autonomously necessary for presynaptic assembly in AIY. Here we identify the mechanisms by which the axon guidance receptor UNC-40 instructs presynaptic assembly in vivo. Using genetic analysis and cell biological experiments, we find that UNC-40 is required at presynaptic sites for organizing the actin cytoskeleton through the Rac GTPase pathway, specifically CED-5/DOCK180 and CED-10/Rac1. Null or strong loss-of-function mutants in these pathway components exhibit similar presynaptic vesicle clustering phenotypes as UNC-40 in the interneuron AIY. Downstream of CED-10, we find that MIG-10/Lamellipodin is cell-autonomously required for proper presynaptic vesicle clustering in AIY. We also identify a unique MIG-10 isoform, MIG-10B, which is specifically required to regulate vesicle clustering in response to Netrin. This isoform localizes to AIY synaptic regions, as determined by MIG-10B:GFP fusion proteins. MIG-10B is required for organization of the actin cytoskeleton at these sites, as determined by protein-GFP fusions and recombineering fosmid rescue experiments. Other MIG-10 isoforms do not localize to synaptic regions, nor do they rescue the presynaptic assembly phenotype in mig-10 null mutants. Thus, our data provide a novel mechanism for presynaptic targeting in vivo. Our data also indicate that signaling modules that organize the actin cytoskeleton during guidance can be co-opted at a cell biological level to organize the actin cytoskeleton in presynaptic assembly.

492A

Regulatory logic of pan-neuronal gene expression in *C. elegans*. **Nikolaos Stefanakis**[1], Inés Carrera[2], Oliver Hobert[2,3]. 1) Department of Biological Sciences, Columbia University, New York, NY; 2) Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY; 3) Howard Hughes Medical Institute.

The adult *C. elegans* nervous system is remarkable diverse consisting of 302 neurons grouped in 118 anatomical classes. The morphological and functional diversity of mature differentiated neurons of each class is reflected in their different molecular composition. The expression of different sets of genes gives each neuron its "neuron-type specific" identity. However, all neurons in a nervous system have common morphological characteristics namely cellular projections and synapses. The molecular correlates to those common features are encoded by pan-neuronal genes. While a lot of information is available about how specific neuronal types are generated, the regulatory programs that govern pan-neuronal gene expression are poorly understood.

In this study our main goal is to dissect the *cis*-regulatory logic of pan-neuronal gene expression and to identify the *trans*-acting factors responsible for its execution. To set the basis of our analysis we have first constructed a reporter for the pan-neuronal synaptic gene *rab-3*. We have extensively studied the expression pattern of this transgene, which appears to be expressed in almost all the neurons of an adult hermaphrodite worm. We then defined a pan-neuronal gene battery consisting of synaptic proteins and neuronal-specialized cytoskeleton components that are broadly expressed in the nervous system. We study the spatial and temporal expression pattern of each of these genes by tagging their locus with a nuclear localized YFP using fosmid recombineering, and comparing the overlap of expression between the *rab-3* reporter and these fosmid reporters. We are currently dissecting the *cis*-regulatory architecture of each member of our list by generating NLS-TagRFP deletion constructs of their promoter regions.

Although we are verifying that most of the genes under study are expressed throughout the nervous system of the adult worm in a similar spatial and temporal pattern, deletion analysis of their promoters shows that there might not be a common regulatory logic among them. While for many genes a piece-meal manner of regulation is suggested, there are other genes where only a small region of the promoter is still sufficient to drive expression in most neurons. Progress on this analysis will be presented.

493B

The Arp2/3 complex and UNC-115/abLIM act downstream of CDC-42 in neuronal lamellipodia and filopodia formation. **Eric C. Struckhoff**, Erik A. Lundquist. Molecular Biosciences, University of Kansas, Lawrence, KS.

Rho-family GTPases (Rho, Rac, and Cdc-42) regulate cell morphology by influencing the structure and dynamics of the actin cytoskeleton. Actin responses controlled by Rho GTPases include induction of focal adhesion complexes, assembly of actin stress fibers, and formation of filopodia and lamellipodia at the leading edge of axon growth cones in neurons. Cdc-42 is an important regulator of actin polymerization, particularly of filopodia formation and nucleation. Filopodia are a key feature of growth cones of developing axons. In order to elucidate the role of Cdc-42 and associated components of actin regulation in axon development, we constructed a transgene composed of a constitutively active form of CDC-42 (the G12V mutation) under the control of the osm-6 promoter. CDC-42(G12V) caused defects in PDE neuron morphology, consisting of ectopic extensions from the PDE axon and cell body, including fan-like lamellipodial extensions and thin filopodial extensions. Axon polarity and guidance errors were also observed. These results indicate that CDC-42 might normally be involved in lamellipodia and filopodia formation, possibly in the growth cone. We next set out to determine the downstream effectors of CDC-42(G12V) in ectopic formation of lamellipodia and filopodia. Cdc-42 has been implicated in controlling actin-based cell morphology via the WASP pathway and the Arp2/3 complex. We examined whether loss-of-function alleles of putative downstream effectors were able to suppress the defects caused by CDC-42(G12V). Mutations in wsp-1, wve-1, gex-2, and gex-3 (activators of the Arp2/3 complex), toca-1 and toca-2 (upstream regulators of WASP), arx-4 (a component of the Arp2/3 complex), and in unc-115 (an actin binding protein) were tested. wsp-1(gm324) and arx-4(ok1093) completely suppressed, consistent with known roles of these molecules downstream of Cdc-42. WAVE is thought be downstream of Rac GTPases, and wve-1(ne350) did not fully suppress. However, gex-2 and gex-3, which are thought to act with WAVE, did suppress, suggesting that they might also act with WASP. toca-1, but not toca-2, also suppressed. Surprisingly, unc-115, thought by previous studies to act downstream of Rac GTPases, also suppressed, suggesting that UNC-115 might also act downstream of CDC-42. Thus, a pathway consisting of TOCA-1/WASP-1/GEX-2/GEX-3/ARX-4 mediates ectopic lamellipodia and filopodia induced CDC-42(G12V), and UNC-115/abLIM might act in this or in a parallel pathway to the actin cytoskeleton.

494C

UNC-40/DCC, PTP-3/LAR, and MIG-21 regulate anterior-posterior polarization and migration of the Q neuroblasts. **Lakshmi Sundararajan**, Erik Lundquist. Molec Biosci, Univ Kansas, Lawrence, KS.

Polarization and migration of neurons is essential during nervous system development. We use Q neuroblasts as a system to study neuronal cell migration. The QR and QL neuroblasts, born in the posterior lateral region of the worm, undergo initial polarizations at about one hour after hatching in the anterior and posterior directions respectively. They then migrate in the direction of protrusion and divide to produce three neurons of which AQR (from QR) migrates anteriorly to near the anterior deirid, and PQR (from QL) posteriorly to near the phasmid ganglia. Consistent with previous studies (Honigberg and Kenyon, 2000; Williams et al., 2003), we show that mutations affecting the transmembrane receptors UNC-40/DCC and PTP-3, a LAR type receptor tyrosine phosphatase, cause defects in direction of QR and QL polarization and migration. We found that UNC-40 and PTP-3 might act redundantly in directing posterior migration of QL, as defects were significantly more severe for QL in *ptp-3(mu256); unc-40(RNAi)* than the single mutants alone. Furthermore, we show that loss of MIG-21, a small transmembrane protein with thrombospondin repeats (Williams 2003), caused near randomization of QL and QR protrusion and migration as previously observed. In an attempt to understand how these genes might be acting together in controlling Q cell migrations, we constructed double mutants of *mig-21* with *unc-40* and *ptp-3*. Alone, *mig-21* mutants displayed many defects in QR anterior migration (QR often migrated posteriorly). Surprisingly, in *mig-21; unc-40* double mutants, the defects in QR anterior migration were suppressed (QR migrated to the posterior), suggesting that posterior migration of QR in *mig-21* mutants required functional UNC-40. In contrast, *mig-21; ptp-3* resembled *mig-21* alone for both QR and QL, suggesting that MIG-21 and PTP-3 might be working in the same pathway or in an independent pathway. Taken together, our data suggest that UNC-40 and PTP-3 might redundantly control posterior migration of QL. Furthermore, MIG-21 might normally inhibit UNC-40 in QR, such that in a *mig-21* mutant, UNC-40 drives posterior migration of QR. Current studies are directed at determining the focus of action of these genes. *unc-40* and *mig-21* have been reported to be expressed in QL and QR (Chan et al., 1996; Williams et al. 2003), and we have found that the *ptp-3B* promoter is active in cells that are consistent with QL and QR, suggesting cell autonomous function. Furthermore, *unc-40(RNAi)* driven in QL, QR, and the seam cells induces Q cell defects. We are using mosaic analysis to pinpoint where these genes are required.

495A

The Role of 3-O Sulfation of Heparan Sulfate in Neuronal Development in *C. elegans*. **Eillen Tecle**, Hannes Buelow. Department of Genetics. Albert Einstein Col Med, 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 of modifications, including sulfation, 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 the patterning of the nervous system. However, the role of HS 3-O sulfation, the most rare HS modification has not been established. Vertebrate genomes code for at least seven members of the HS 3O-sulfotranferase gene family and are grouped into two distinct classes. We have identified one gene coding for a predicted HS 3O-sulfotranferase of each class in the C. elegans genome which we have named *hst-3.1* and *hst-3.2*, respectively. Reporter analyses of *hst-3.1* and *hst-3.2* reveal discrete, largely complementary expression patterns. The *hst-3.2* reporter is primarily expressed in ectodermal tissues (hypodermis and neurons) whereas the *hst-3.1* reporter shows expression in body wall muscle and a few select neurons. Defects in synaptic branch formation and axon termination in select neurons are observed in *hst-3.2* mutants; indicating that *hst-3.2* is required for the proper development of subset of neurons. In addition, perturbations in behaviors regulated by the affected neurons are observed in the *hst-3.2* mutants. Some of the phenotypes identified in the *hst-3.2* mutants are shared by mutations in *rpm-1*, a conserved regulator of pre-synaptic maturation (Schaefer et al., 2000; Zhen et al., 2000). Double and triple mutant analyses indicate that *rpm-1* and *hst-3.2* act in parallel genetic pathways. Our data suggests that HS 3-O sulfation, introduced by *hst-3.2*,

may be a crucial determinant of and/or be part of a novel pathway in the establishment of neuronal connectivity.

496B

HBL-1 patterns synaptic remodeling in *C. elegans*. **Katherine L. Thompson-Peer**[1,2], Jihong Bai[1,2], Joshua Kaplan[1,2]. 1) Dept of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept of Neurobiology, Harvard Medical School, Boston, MA.

Synapses are added and removed extensively during the development of the nervous system, and to a lesser extent throughout the lifetime of an animal. Although synaptic refinement plays a pivotal role in establishing circuit and likely cognitive function in many if not most systems, little is known about the genetic pathways governing this process. In *C. elegans*, the GABAergic DD motor neurons undergo developmentally programmed remodeling, whereby synapses onto ventral body muscles are eliminated and replaced with dorsal synapses. We show that DD remodeling is extensively patterned and we identify the transcription factor HBL-1 as a central regulator of this process. Remodeling is restricted to the DD neurons by UNC-55, which represses *hbl-1* expression in the GABAergic VD neurons, explaining the cell-specificity of remodeling. Mutants lacking HBL-1 have delayed DD remodeling, whereas precocious remodeling is observed in mutants lacking the microRNA *mir-84*, which inhibits translation of the *hbl-1* mRNA. Finally, we observe that DD remodeling is activity-dependent. Moreover, bidirectional changes in network activity alter the transcription of *hbl-1*, and adjust the timing of remodeling in an HBL-1-dependent manner. Thus microRNA, network activity, and transcriptional regulatory pathways converge on *hbl-1* to pattern DD plasticity.

497C

The Messiness of Combinatorial Control. **Irini Topalidou**, Martin Chalfie. Columbia Univ, NY, NY.

Because of combinatorial expression, transcription factors can determine the development of more than one type of cell. For example, the transcription factor complex of MEC-3 and UNC-86 is required in embryos for the development of both the touch receptor neurons (TRNs) and the FLP neurons. Despite sharing these transcription factors, the TRNs, but not the FLP neurons, express several proteins needed for gentle touch, e.g., MEC-2, MEC-4, and MEC-18. Both the embryonic TRNs and the FLP neurons upregulate mRNAs for approximately 300 genes. Twenty three percent of the genes are upregulated in both cells. We were surprised to find that some of the commonly upregulated genes are MEC-3-regulated genes that had previously been identified as being specific to the TRNs. Although the mRNAs for these genes are detected in the FLP cells in low amounts, these mRNAs do not appear to be translated to detectable levels. These results suggest that transcriptional control is relatively inexact, but these apparent transcription errors are well tolerated and do not alter cell fate. Either a threshold amount of transcription is required for translation or these mRNAs are subjected to some sort of translational control. The FLP cells are, nonetheless, poised to make TRN-specific proteins. Expression of the transcription factor ALR-1, a downstream target of MEC-3 that ensures, but does not direct, TRN differentiation caused FLP neurons to make higher levels of the mRNAs for these genes and their proteins. This ectopic expression also prevented the FLP neurons from forming the fine branches that characterize the adult cells. Thus, *alr-1* appears to change the fate of these cells. Our experiments suggest that combinatorial control, although potentially able to produce many different types of cells, can be messy. Such messiness may need to be controlled, but it also could be advantageous, allowing cells to take on additional characteristics under different conditions.

498A

The prolyl 4-hydroxylase dpy-18 is required for specific axon guidance events. **Nanna Torpe**, Roger Pocock. Biotech Research & Innovation Center, University of Copenhagen, Copenhagen, Denmark.

In the developing nervous system of both vertebrates and invertebrates the guidance of axonal growth cones is regulated by several signaling mechanisms and interactions with the extracellular matrix (ECM). We are interested in the identification of novel ECM modifiers that regulate axonal guidance. Through a screen of enzymes that modify the ECM we identified the collagen prolyl 4 -hydroxylase (P4H) α-subunit, DPY-18, as a new protein required for neuronal development in C. elegans. P4Hs are enzymes known to be involved in the synthesis of collagen and the dpy-18 mutant is characterized by a short and fat (dumpy) phenotype. In preliminary studies, we have found that dpy-18 is also specifically required for axonal guidance of the PVQ and HSN neurons in C. elegans. We are in the process of identifying the tissues in which dpy-18 is expressed. Tissue specific rescue is also currently being performed to identify in which tissues dpy-18 is required. We hypothesize that the DPY-18 protein interacts and modifies other proteins to regulate neuronal development. We propose to characterize the role(s) of DPY-18 at the molecular and cellular level and to identify molecular targets of DPY-18 through candidate gene approaches and genetic screening. This will enable us to better understand how prolyl 4-hydroxylation regulates neuronal development.

499B

PPM-1, a PP2Cα/β phosphatase, regulates axon termination and synapse formation in C. elegans. **E. Tulgren**, S. Baker, L. Rapp, A. Gurney, B. Grill. University of Minnesota, Minneapolis, MN., 55455.

Previous studies have shown that the Regulator of Presynaptic Morphology (RPM-1) regulates synapse formation, axon guidance, and axon termination. RPM-1 functions as an ubiquitin ligase to negatively regulate a MAPK pathway that includes DLK-1, MKK-4 and the p38 MAP kinase, PMK-3. The ubiquitin ligase activity of RPM-1 requires the F-box protein, FSN-1. RPM-1 is also a positive regulator of a Rab GTPase pathway that includes *glo-4* and *glo-1*. While RPM-1 is the only known negative regulator of the DLK-1 pathway, studies in yeast and mammals have shown that Protein Phosphatase 2C (PP2C) α and β isoforms can negatively regulate MAPK pathways. C. elegans has a single homolog of mammalian PP2Cα and β that we call Protein Phosphatase Magnesium/manganese-dependent (PPM)-1 (previously called TAG-93). Here we test the role of *ppm-1* in axon termination and synapse formation using two loss-of-function (lf) alleles (*ok578* and *tm653*).

ok578 and *tm653* are molecular null alleles as they both delete large amounts of the *ppm-1* gene including sequence encoding conserved residues that are essential for PP2C phosphatase activity. *ppm-1* (lf) mutants have defects in axon termination similar to mutants of molecules in the *rpm-1* pathway, including *fsn-1*, *glo-4* and *glo-1*. In *ppm-1;fsn-1* double mutants axon termination defects are significantly enhanced demonstrating that *ppm-1* and *fsn-1* function in parallel pathways.

The presynaptic terminals of GABAergic motor neurons can be visualized using SNB-1::GFP, which localizes to evenly distributed puncta along the dorsal cord in wild-type animals. While the organization of SNB-1::GFP puncta is normal in *ppm-1* (lf) mutants, *ppm-1;fsn-1* double mutants have significantly enhanced defects in organization and numbers of puncta. In contrast, *ppm-1,rpm-1* double mutants are not enhanced. These results indicate that *ppm-1* functions in a parallel pathway to *fsn-1*, and in the same pathway as *rpm-1*.

We have found that *ppm-1* functions through its phosphatase activity downstream of *rpm-1*, which suggests that PPM-1 may dephosphorylate and inactivate a kinase in the DLK-1 pathway. To test this hypothesis, we generated double mutants of *ppm-1* with *dlk-1*, *mkk-4*, or *pmk-3*. Only *ppm-1;pmk-3* double mutants were suppressed when compared to *ppm-1* (lf) mutants. Further, defects caused by transgenic overexpression of DLK-1 were rescued by coexpression of PPM-1. Overall, our results show that PPM-1 regulates axon termination and synapse formation by negatively regulating the DLK-1 pathway at the level of PMK-3.

500C

Sox genes in early nervous system development. **Berta Vidal Iglesias**[2], Oliver Hobert[1,2]. 1) Howard Hughes Medical Institute; 2) Department of Biochemistry & Molecular Biophysics, Columbia University Medical Center, New York, NY.

One of the first steps in nervous system development is the generation of neural progenitor cells that will subsequently give rise to the vast diversity of neurons present in the adult. How neuronal progenitor specification is achieved is a fundamental question that we are still far away from completely understanding. The Sox proteins are a highly conserved group of transcription factors and the expression of some of them (group B) correlates with the commitment of cells to a neural fate. The evolutionary conserved expression of sox genes in neuronal progenitors and the fact that they are regulated by signaling molecules involved in neuronal specification suggests a role for sox genes in establishing neural fate. However, the relevance of Sox proteins in vertebrate neuronal development has been difficult to prove genetically mainly because of presumed redundant functions. C. elegans has only 5 sox genes (sem-2, sox-2, sox-3, sox-4 and egl-13) and we have investigated their potential role in early neurogenesis. First, we have analyzed the expression pattern of all sox genes during embryogenesis by using fosmid-based reporter genes. sem-2 and sox-2 are the sox genes expressed earliest and in a more broad manner during embryogenesis, being expressed in neuron progenitors, although not exclusively neither in all of them. Their expression pattern would be be compatible with a role in early neuronal specification. On the other hand, sox-3, sox-4 and egl-13 are expressed in a few cells during late embryogenesis, when most neurons are already born. Hence, we have mainly focused our efforts in further characterizing sem-2 and sox-2 as potential regulators of early neuronal specification events. For that purpose we have generated a new sox-2 deletion allele taking advantage of the MosDel tecnique. Both sem-2 and sox-2 null mutants are embryonic or early larval lethal and show different degrees of morphogenesis defects. When we crossed sem-2 or sox-2 mutants with a panneuronal reporter we did not observe any obvious neuronal loss and the expression of neuron-type specific reporters was not generaly affected either. These results strongly suggest that, contrary to our initial hypothesis, sox genes are not required for correct neuronal specification. The possibility of sem-2 and sox-2 acting redundantly is unlikely, at least in a broad manner, since their co-expression during embryogenesis in rather limited. Potential redundancy or compensatory mechanisms between different sox genes are currently being investigated.

501A

Identifying new players involved in dorsal-ventral axon guidance in *C.elegans*. **Jinbo Wang**[1,2], Xia Li[1], Mei Zhen[3], Xun Huang[1]. 1) Key Laboratory of Molecular and Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, China; 2) Graduate School, Chinese Academy of Sciences, Beijing, China; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada.

Neurons in nervous system have specific identity, which is achieved by accurate control of neural differentiation and proper communication with environmental factors during development. One important step in neuron maturation is the navigation of neuronal processes. Different neurons send their processes along putative pathway to specific targets. Incorrect navigation may leads to severe locomotion defects. Several signaling pathways including *unc-6*/*unc-40*/*unc-5*, *slt-1*/*sax-3*, *ephrin*, *semaphorin*, *wnt*/*Frz*, and *unc-129* are involved in axon guidance regulation. In order to find more players involved in axon

guidance, we performed genetic screen using RID neuronal marker in *C.elegans*. The cell body of RID neuron locates in the dorsal ganglion. The process of RID runs down to the ventral ganglion, then runs back to the dorsal cord and, at last grows along the dorsal cord. Several mutants were isolated. One of them is *xd86*. *xd86* mutant shows 100% dorsal-ventral guidance defect. The RID process in *xd86* mutant runs down to the ventral ganglion then runs along the ventral cord. Besides the dorsal-ventral guidance defect, about 70% *xd86* animals show duplicated RID neurons. Further analysis is in process.

502B
Ciliogenesis in *Caenorhabditis elegans* requires genetic interactions between *nphp-2* and transition zone associated genes. **Simon RF Warburton-Pitt**[1], Andrew R Juaregui[1], Chunmei Li[2], Michel R Leroux[2], Maureen M Barr[1]. 1) Dept. of Genetics, Rutgers, The State University of NJ, Piscataway, NJ; 2) Dept. of Mol. Biol. and Biochem., Simon Fraser University, Burnaby, BC.

The cystic kidney diseases Nephronophthisis (NPHP), Meckel Syndrome (MKS), and Joubert Syndrome (JBTS) share an underlying etiology of dysfunctional cilia. INVS, mutated in NPHP type II, encodes inversin, a cilia localizing protein. Here we show that the *C. elegans* inversin homolog, *nphp-2*, localizes to the transition zone (TZ) and middle segments of sensory cilia, and is partially redundant with the NPHP4/nephrocystin-4 homolog *nphp-4* for the proper biogenesis of cilia, namely positioning and axoneme formation. *nphp-2* is not required for correct localization of other TZ proteins. *nphp-2* genetically interacts with other ciliopathy gene homologs in a cell-dependent manner to control cilia formation. We conclude that *nphp-2* plays an important role in *C. elegans* cilia, and that this gene defines a third cell-type specific pathway that acts in conjunction with previously described NPHP and MKS pathways in cilia formation and development.

503C
Understanding the molecular mechanisms that mediate axon outgrowth termination in *C. elegans*. **William Wung**, Johann Zaroli, Kelli Benedetti, Phil Knezevich, Joori Park, Nathan Cook, Miri VanHoven. Biological Sciences, San Jose State University, San Jose, CA.

The nervous system is made up of neurons that communicate through synapses formed between cellular extensions called dendrites and axons. Critical processes such as perception, movement, and thought rely on correct communication between neurons. Dendrites acquire information from the environment or other neurons, while axons relay information to other neurons. Injury to axons is difficult to treat because most axons in the central nervous system extend only during development. It is thought that molecular stop signals instruct axons when to stop extending during development and may maintain this state afterwards. Identifying these stop signals and their receptors may be an important step in designing therapies for nervous system injuries. The simple, well-characterized nervous system of *C. elegans*, make it an ideal model organism for such studies. We have labeled PHB sensory neurons with a cytosolic mCherry fluorophore to assay axon length. Analysis of loss-of-function mutants indicates that the transmembrane receptor *sax-3/Robo*, previously isolated for its role in directing axons and cell bodies away from certain body regions, is also required for correct termination of axon outgrowth. Our project aims to investigate the role of *sax-3/Robo* in axon outgrowth termination and to elucidate the pathway by which it transduces the termination signal. Timecourse experiments implicated *sax-3/Robo* in terminating initial axon outgrowth rather than in the maintenance of PHB axon length. Furthermore, preliminary genetic analysis indicates that *slt-1*, a *sax-3/Robo* ligand, has an antagonistic effect on the pathway. We have also identified three cytosolic genes that may function downstream of *sax-3/Robo*: *unc-73*, which encodes guanine nucleotide exchange factor (GNEF) similar to the Trio protein, *unc-34*, which encodes an EVH1 domain-containing protein orthologous to Enabled/VASP, and *unc-69*, which encodes a coiled-coil protein orthologous to human SCOC. We are currently testing a series of candidate genes to identify new pathways members, and conducting genetic analysis to order these genes in a pathway.

504A
The RFX Transcription Factor DAF-19 Couples Toll-interleukin 1 Receptor Domain Protein TIR-1 Signaling to Regulation of Neuronal Activities in C. elegans. **Y. Xie**, M. Moussaif, S. Choi, J. Sze. Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

Prior studies from our laboratory and others have demonstrated two layers of transcriptional regulation of the serotonin-biosynthesis enzyme tryptophan hydroxylase tph-1 in the ciliated chemosensory neurons ADF. The OCR-2/OSM-9 TRPV channel regulates the basal tph-1 expression under optimal growth conditions (Zhang et al., 2004). Stress conditions or mutations of the sensory cilia further upregulate tph-1 expression in ADFs (Zhang et al., 2005; Chang et al., 2006; Moussaif and Sze, 2009; Shivers et al., 2009). To identify new genes involved in the regulation of tph-1 expression in the ADF neurons, two genetic screens were carried out. We identified that yz58, a gain-of-function allele of the Toll-interleukin 1 receptor domain protein TIR-1, restored tph-1 expression in the ocr-2 mutant background. Characterization of mutations that suppress tph-1 upregulation by tir-1 (yz58) led to the identification of two daf-19 alleles. Interestingly, both mutations are located in the DAF-19 dimerization domain. Mutations in DAF-19 do not affect the expression of CAT-1/VMAT, indicating that the mutations do not transform the ADF serotonergic cell fate. Further analysis suggests that DAF-19 also interacts with TIR-1 signaling in other cellular and physiological functions. References Chang AJ, Chronis N, Karow DS, Marletta MA, Bargmann CI. 2006. A distributed chemosensory circuit for oxygen preference in C. elegans. PLoS Biol 4:e274. Moussaif M, Sze JY. 2009. Intraflagellar transport/Hedgehog-related signaling components couple sensory cilium morphology and serotonin biosynthesis in Caenorhabditis elegans. J Neurosci 29:4065-75. Shivers RP, Kooistra T, Chu SW, Pagano DJ, Kim DH. 2009. Tissue-specific activities of an immune signaling module regulate physiological responses to pathogenic and nutritional bacteria in C. elegans. Cell Host Microbe 6:321-30. Zhang S, Sokolchik I, Blanco G, Sze JY. 2004. Caenorhabditis elegans TRPV ion channel regulates 5HT biosynthesis in chemosensory neurons. Development 131:1629-38. Zhang Y, Lu H, Bargmann CI. 2005. Pathogenic bacteria induce aversive olfactory learning in Caenorhabditis elegans. Nature 438:179-84.

505B
Tectonic contributes to transition zone complexes required for proper ciliogenesis. **Laura Yee**[1], Rachel Bowie[2], Oliver Blacque[2], Kaveh Ashrafi[1], Jeremy Reiter[1]. 1) University of California San Francisco, San Francisco, CA; 2) University College Dublin, Dublin, Ireland.

Primary cilia are microtubule based projections on the cell surface that mediate processes such as signal transduction and sensory reception. Disruption of cilia results in human disorders with a spectrum of symptoms, collectively known as ciliopathies. Several ciliopathy genes such as those underlying Nephronophthisis (*nph*) and Meckel Syndrome (*mks*) encode proteins that localize to the transition zone, a mysterious structure at the base of the ciliary axoneme. Previous studies in *C. elegans* indicate that NPH and MKS proteins act in two functionally redundant complexes at the transition zone. We are studying the transition zone through another protein, Tectonic (TCTN-1), which in mammals is required for ciliogenesis in a tissue-specific manner, localizes to the transition zone and binds other transition zone proteins. Through double mutant analysis, we have found that in *C. elegans*, *tctn-1* is redundant with *nph* genes but not *mks* genes, thereby placing *tctn-1* within the MKS complex. In addition, *tctn-1; nph-4* animals have disrupted transition zones and display a subset of phenotypes associated with ciliary dysfunction. We are continuing to investigate the role of TCTN-1 in the transition zone by determining if ciliary protein localization is altered in *tctn-1; nph-4* mutants.

506C
Characterization of a L1 axon guidance phenotype in the mechanosensory neurons and the role of lethal genes in axon guidance. **Fabian P.S. Yu**, Ahmed M. Mohamed, Ian D. Chin-Sang. Biology, Queen's University, kingston, Ontario, Canada.

Our lab has been investigating the function of VAB-1, an Eph receptor tyrosine kinase (RTK), in neuronal development. *vab-1* mutations result in abnormalities in epidermal morphogenesis, neuroblast movement, and also axon guidance [1-3]. We use the mechanosensory neurons with particular focus on the PLM to study the axon guidance roles of *vab-1* and related genes. Work on axon guidance has mainly utilized the L4 or young adult stage of worms to characterize defects in axon development. Recent work in our lab has shown that the L1 stage provides a greater degree of sensitivity. At the L1 stage *vab-1* null mutants display a 22% versus a 15% overgrowth defect, and for the hyperactive *vab-1* tyrosine kinase strain L1 worms achieved 97% versus a reported 85% axonal undergrowth defect [3]. With this improved PLM scoring phenotype we aim to focus on 1) studying the role of lethal genes in axon guidance , and 2) studying genes known to be synthetic lethal with *vab-1* null mutations. To overcome the issue of lethality we have used a tissue specific RNAi technique that is resistant to the spreading effects of RNAi [4]. The knockdown assay utilizes the *rde-1* mutant background to inhibit RNAi activity in the worm. These worms are then injected with a touch neuron *rde-1* specific rescuing construct via the *mec-4* promoter along with an RNAi construct consisting of a dual *mec-4* promoter flanking our gene of interest. We have created a number of constructs for lethal genes to study their role in axon guidance and will test whether they function in the *vab-1* signalling pathway. Our preliminary study focused on *arx-1* and *arx-2* which code for the Arp 2/3 complex for actin nucleation. Both *arx-1* and *arx-2* display penetrant embryonic lethality using standard RNAi feeding or injection methods. In contrast, our neuronal specific RNAi approach bypassed the embryonic lethality and *arx-1* or *arx-2* RNAi displayed ~80% PLM undergrowth, suggesting that *arx-1* and *arx-2* are required in PLM for axon outgrowth. Thus this technique allows us to study the function of essential genes or *vab-1* synthetic lethal genes in axon guidance. 1. Boulin, T., Pocock, R., and Hobert, O. (2006). Current Biology 16, 1871-1883. 2. George, S.E et al., (1998). Cell 92, 633-644. 3. Mohamed, A.M., and Chin-Sang, I.D. (2006). Dev. Biol. 290, 164-176. 4. Qadota, H et al., (2007). Gene 400, 166-173.

507A
Searching for Negative Regulators of Neurite Outgrowth in *Caenorhabditis elegans*. **Bo Zhang**[1,2], Song Song[1,2], Xun Huang[1], Mei Ding[1]. 1) Key Laboratory of Molecular and Developmental Biology, IGDB, CAS, Beijing 100101, China; 2) Graduate School of Chinese Academy of Sciences, Beijing 100049, China.

How do individual nerve fibers find their way along specific paths in a complex environment such as the developing central nervous system? A principal mechanism in axon guidance is binding of a receptor protein on the axon surface to a guidance molecule. However, it remains a mystery exactly how a limited number of guidance molecules can pilot the growth of billions of neurons.

In our previous work, we identified a conserved Wnt pathway composed of Wnt ligand CWN-2, Frizzled receptors CFZ-2 and MIG-1, co-receptor CAM-1/Ror and downstream component DSH-1, which is crucial for RME neurite anterior-posterior (A-P) outgrowth in *C. elegans*. To address how axon outgrowth is terminated and how the termination signal interacts with Wnt pathway, we further searched mutants with longer RME processes. From an unbiased screen, we recovered an *xd49* mutant, which displays a longer RME process

phenotype. Furthermore, we also identified two more genes that could negatively regulate RME neurite outgrowth. Currently, we are taking genetics and biochemistry approaches to address how Wnt pathway interacts with this newly identified axon termination signal and how Wnt activity could be attenuated on time to fine tune the proper length of the axon. Detailed analysis will be reported in this meeting.

508B
The C2H2 Zn finger transcription factor LSY-27 is involved in controlling a lateralized neuronal differentiation program in *C.elegans*. **Feifan Zhang**, Maggie O'Meara, Oliver Hobert. Department of Biochemistry and Molecular Biophysics, Howard Hughes Medical Institute, Columbia University Medical Center, New York, NY 10032.

Functional lateralization is a common feature of many nervous systems. The genetic programs that control left/right neuronal asymmetry in the nervous system are, however, poorly understood. The ASE neurons are a pair of chemosensory neurons morphologically symmetric yet functionally asymmetric. A class of putative chemoreceptors of the GCY family is expressed in a left/right asymmetric manner. Genetic mutant screens have revealed several classes of genes (called "*lsy* genes" for Lateral SYmmetry defective) that control the left/right asymmetric expression of the *gcy* genes. We describe here the molecular characterization of two distinct mutant *C.elegans* strains in which the left/right asymmetry of ASEL and ASER is disrupted and the differentiation program of the ASER neuron now becomes derepressed in the ASEL. We show that in one mutant strain the LIM homeobox gene *lim-6* is defective while in another strain a broadly expressed, novel member of a *C.elegans*-specific, fast-evolving family of C2H2 Zn finger transcription factors, *lsy-27* is mutated. Based on phenotypic similarities between the two strains we propose that they cooperate to repress ASER fate in ASEL.

509C
Identification of the sex pheromone receptors in AWA neuron. **Yuan ZHOU**, King-Lau CHOW. Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Chemosensation is an important process used by animals to perceive changes in the environment and to coordinate appropriate responses. The chemosensory system detects a diverse range of chemical cues, including sex pheromone, via different chemosensory receptors. We have previously shown that *C. remanei* females produce a sex pheromone with long-range attractiveness for both *C. remanei* and *C. elegans* males. Our data also implicate the involvement of G protein-coupled receptors (GPCRs) acting in AWA neurons in this perception process.
In order to identify the receptors acting within AWA, an *in vivo* cell-specific pull-down experiment was conducted, followed by a microarray analysis. Over 150 GPCR-transcripts were identified from the AWA-specific transcriptome, the validity of their involvement demands further experimental verification.
Some mutants with defective candidate GPCRs have been tested with the standard pheromone-attractant assays. As a result, 21 candidate GPCRs were eliminated from functioning in the sex pheromone perception process, while a male-specific candidate was shown to be involved. Tagged with a *gfp* reporter, this gene showed expression in cilia of the ASIs of both sexes and in male AWAs. Preliminary experiments were conducted to evaluate AWA-specific expression of this gene for function rescue in the null mutant, while AWA-specific knock-down of this same gene will be tested in transgenic animals. In addition, the relationship between its function and its cellular targets will be ascertained by ASI-expression rescue and the results will be presented.
In parallel, transcriptional reporter expression study was conducted to validate the other candidates. Nine GPCRs were confirmed to express in AWA from the top 50 candidates. Behavioral tests will be conducted using the corresponding mutants or knock-down animals in order to explore their functions in AWA-mediated chemosensation.
In addition, this subset of AWA-specific genes opens the door for the definition of the potential AWA-specific *cis*-element, which might help uncover the regulatory architecture of AWA cell fate specification. (This study is supported by Research Grants Council, Hong Kong.).

510A

Interaction of an alpha-catulin homologue, CTN-1, with dystrobrevin (DYB-1) determines the localization of BK channels in *Caenorhabditis elegans* muscles. **Linu S. Abraham**, Hyun J. Oh, Hongkyun Kim. Cell Biology & Anatomy, Rosalind Franklin University of Medicine and Science, North Chicago, IL.

Muscular dystrophy is a family of neuromuscular disorders that is characterized by progressive muscle weakness and wasting. A number of muscular dystrophies are caused by mutations in components of the dystrophin associated protein complex (DAPC). The DAPC plays several different roles including linking the actin cytoskeleton to the extracellular matrix, acting as a membrane organizer for signaling molecules, and regulating calcium homeostasis. Recent evidence indicates that the DAPC is essential for the localization of the calcium-activated BK potassium channel, SLO-1, near calcium channels in the *C. elegans* muscle. Previously, in a forward genetic screen to identify additional genes responsible for SLO-1 localization and function, we identified *ctn-1*, an alpha-catulin homolog with sequence similarity to α-catenin and vinculin. CTN-1 exhibits a punctate expression pattern that closely resembles the pattern of muscle dense bodies. *ctn-1* mutation affects the integrity of the DAPC, since the expression pattern of SGCA-1, a component of the DAPC, is perturbed in *ctn-1* mutants. Consequently, SLO-1 localization was greatly diminished in muscles of ctn-1 mutants. Thus, CTN-1 uses the DAPC to localize SLO-1 to specific regions of the muscle membrane. In order to further understand the nature of interaction between CTN-1 and the DAPC, we performed yeast two-hybrid interaction studies. Our results indicate that CTN-1 interacts with dystrobrevin (DYB-1), a component of the DAPC that is also known to bind other DAPC components such as dystrophin and syntrophins. Through deletion mapping, we found that the C-terminal region of DYB-1 binds to the N-terminal domain of CTN-1. To further dissect the *in vivo* role of different domains of CTN-1, we examined expression patterns of GFP-tagged deletion constructs of CTN-1. The characteristic punctate pattern shown by GFP::CTN-1 was not abolished when either the coiled-coil domain, or the N terminal domain of CTN-1 were deleted. However, C-terminal domain deletion resulted in alterations in the punctate pattern. Based on these results, we propose that CTN-1 interacts with the DAPC by binding to DYB-1, thus helping to anchor the DAPC near dense bodies. The DAPC in turn ensures that SLO-1 is localized to regions of the membrane that are rich in calcium channels. It can thus be concluded that α-catulin is an important gene that regulates neuromuscular function and may play a role in the pathogenesis of certain forms of muscular dystrophy.

511B

Sink or Swim: A Screen for Dopamine-Dependent Modulation of Swimming Behavior. **Sarah R Baas**[1], J Andrew Hardaway[2], Shannon L Hardie[1], Sarah Whitaker[1], Bing Zhang[3], Randy D Blakely[1,2,3,4]. 1) Pharmacology, Vanderbilt University, Nashville, TN; 2) Neuroscience Graduate Program, Vanderbilt University, Nashville, TN; 3) Sylvio O. Conte Neuroscience Research Center, Vanderbilt University, Nashville, TN; 4) Psychiatry, Vanderbilt University, Nashville, TN.

The monoamine neurotransmitter dopamine (DA) modulates brain circuitry relevant to cognition, reward, motor control, and arousal. Defects in DA signaling have been implicated in risk for addiction, attention-deficit hyperactivity disorder (ADHD), schizophrenia, and Parkinson's disease. The presynaptic dopamine transporter (DAT) is a major control point for DA signaling and a major target for psychostimulants, including cocaine and amphetamine. In the nematode Caenorhabditis elegans, DA signaling modulates egg-laying, locomotion, touch response, and defecation. The C. elegans DAT, DAT-1, modulates these behaviors by reuptake of DA from the synapse, limiting receptor availability and synaptic spillover of DA and enabling DA re-release following DA recycling to the presynaptic terminal. Our work has shown that DAT-1 activity is particularly important to ensure normal swimming behavior. Whereas wild-type worms thrash in water for up to 20 minutes at a relatively constant rate (~1 Hz), dat-1 mutants paralyze in a few minutes, a phenotype known as swimming-induced paralysis (swip). Swip can be rescued by pharmacological inhibition of monoamine packaging with reserpine as well as by genetic ablation of the D2-like DA receptor, DOP-3. To determine novel presynaptic regulators of DA signaling, we undertook a chemical mutagenesis screen to identify reserpine-sensitive swip animals. From ~10,000 haploid genomes screened to date, we obtained 12 mutants that exhibit robust reserpine-reversible swip but that show no overt motor phenotype on solid media. Two of these lines carry mutations in dat-1, verifying the specificity of our screen. The other mutants are currently being mapped, and although they phenocopy loss of dat-1, are not deficient in dat-1 and thus may yield novel regulators of DAT-1 activity, function, or trafficking. One of these mutants, swip14, maps to the negative end of the X chromosome, exhibits normal responsiveness to exogenous DA on plates, and does not appear to influence the gross morphology or projections of DA neurons. We are currently pursuing genes within the mapped region of swip14 that harbor mutations identified by whole genome sequencing. The identification of the molecular lesion associated with swip14, as well as that of our other swip mutants, may yield important and conserved clues to the presynaptic regulation of DA signaling. Supported by NIH awards MH065215 to S.B., MH093102 to A.H., and DA027739 to R.D.B.

512C

The Stomatins UNC-1 and STO-6 Function in the *C. elegans* Motor Circuit. **Louis Barbier**[1,2], Michelle Po[1,2], Taizo Kawano[1], Mei Zhen[1,2]. 1) Samuel Lunenfeld Research Institute, Toronto, Canada; 2) Department of Molecular Genetics, University of Toronto, Toronto, Canada.

The knowledge of the wiring diagram and functional tests (i.e. ablation studies) of the *C. elegans* nervous system has led to the identification of the core components of the motor circuit, which contains five pairs of command interneurons signaling to at least six classes of motoneurons to generate patterns of locomotion. The control of *C. elegans* locomotion, however, is not fully understood at the molecular and cellular level. At the molecular level, mutations in many neural genes lead to specific, quantifiable movement phenotypes, but the underlying circuit defects remain poorly understood. We have used a combined molecular genetic and calcium imaging approach to dissect the function of innexin genes in regulating different layers of this motor circuit. To this end, we have shown that UNC-7 and UNC-9 innexins form gap junctions that control the output balance between the forward and backward components of the motor circuit (Kawano *et al.* submitted; see also Po *et al.*, 2010 C. elegans Neuro meeting).

How these gap junctions are regulated at the molecular level is unknown. In a screen for suppressors of the *unc-7(e5)* innexin kinker mutant, we isolated gain-of-function alleles of two stomatin genes, *unc-1* and *sto-6*, that allow *unc-7(e5)* animals to move forwards. Stomatins are a conserved family of membrane-associated proteins that regulate ion channels[1]. Stomatins contain a stomatin domain, oligomerization domain, and occasionally other functional motifs. Like innexin *unc-7* and *unc-9* loss of function mutants, *unc-1(lf)* results in a kinker phenotype, whereas a *sto-6* frameshift deletion mutant does not display an overt locomotion phenotype. *unc-1(gf)* and *sto-6(gf)* mutants, however, display unique phenotypes. We will present studies that indicate a differential role of these two classes of stomatins in regulating *C. elegans* motor circuit function.

1) Salzer U, Mairhofer M and Prohaska R. *Dynamic Cell Biology*, 2007.

513A

Degeneracy and neuromodulation in the thermosensory circuit regulates robustness in thermosensory behaviors in *C. elegans*. **Matthew Beverly**, Sriram Anbil, Harold Bell, Piali Sengupta. Department of Biology and National Center for Behavioral Genomics, Brandeis University, Waltham, MA 02454.

Animals must exhibit physiologically important behaviors under a wide range of conditions. The mechanisms by which neural circuits generate robust behaviors are not well understood. *C. elegans* maintains optimal body temperature by regulating locomotory behavior. When exposed to temperatures greater than their cultivation temperature (T_c), *C. elegans* moves towards colder temperatures in a behavior called negative thermotaxis. The AFD and AWC sensory neurons are thermosensory and are required for negative thermotaxis under the limited set of conditions that have been examined to date. However, whether these neurons contribute similarly to negative thermotaxis under all conditions, or whether additional circuit components are recruited under specific conditions, are unknown. By analyzing the requirement of different sensory neurons in mediating negative thermotaxis under conditions of varying T_c and gradient temperature ranges, we have identified the ASI chemosensory neurons as a third thermosensory neuron type. Different combinations of the AFD, AWC and ASI thermosensory neuron types are necessary and sufficient for negative thermotaxis under different conditions. We find that the ASI neurons respond to changes in temperature within an operating range whose bounds are defined by T_c. Interestingly, this operating range of ASI is altered in mutants affecting neuropeptidergic signaling suggesting that neuromodulation, possibly from AFD, coordinates thermosensory neuron response ranges to ensure a coherent behavioral output. In preliminary work, we have identified neuropeptide receptor and ligand genes, mutations in which alter the temperature response range of ASI, and are currently characterizing their behavioral phenotypes and sites of action. Our work suggests that multiple circuit configurations can act degenerately to generate the same behavior under different conditions, and emphasize the importance of defining context when describing neuronal contributions to a behavior.

514B

Genetic and Neural Pathways Underlying Light-induced Inhibition of Pharyngeal Pumping by *C. elegans*. **Nikhil Bhatla**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA.

C. elegans moves away from ultraviolet and blue light, and this avoidance is dependent on LITE-1, a putative light sensor, as well as the uncloned *lite-2* and *lite-3* (Edwards..Miller 2008). We found that worms also stop pharyngeal pumping in response to light. For example, worms immediately stop pumping when exposed to violet light (the "acute response") and maintain reduced pumping after light is removed (the "sustained response").

Mutations in *lite-1*, *lite-2* or *lite-3* have little effect on the acute response but lead to a completely defective sustained response, such that pumping quickly recovers to pre-light levels after light is removed. After testing neurotransmission mutants we found that *eat-4*, a vesicular glutamate transporter, is required for the acute but not the sustained response to light. *unc-13*, a regulator of neurotransmitter release, is required for both responses. Since *eat-4; lite-1* mutants lack both the acute and sustained responses, *eat-4* and *lite-1* likely act in parallel, indicating that an additional light sensor upstream of *eat-4* might be present in the worm.

The *C. elegans* nervous system consists of 2 anatomical networks connected by gap junctions between the main network's RIP neurons and the pharyngeal network's I1 neurons. Consistent with the hypothesis that the main network is involved in the pumping response to light, laser ablation of both I1s yielded worms that lacked the acute but not the sustained light response, similar to *eat-4* mutants. To investigate whether *eat-4* acts downstream of the I1s, we ablated individual classes of *eat-4*-expressing pharyngeal neurons (M3s, NSMs, and I5). We found that these ablations had no effect on the worm's light response, indicating that *eat-4* likely acts outside the pharynx to control the acute response. We also tested available mutants of other glutamate transporters and glutamate receptors, but all responded normally to light.

We plan to identify the sites-of-action of *lite-1* and *eat-4* via cell ablation and cell-specific rescue experiments. Additionally, we plan to image calcium to measure the physiological

sequence in which these neurons are activated. We also plan to do a mutagenesis screen for additional mutants defective in the pumping response to light to seek the light sensor upstream of *eat-4*, the glutamate receptor downstream of *eat-4*, and the signaling molecules downstream of *lite-1*. We hope that such analysis will elucidate important principles about neural communication that are relevant more generally across species.

515C

Genetic, pharmacological and calcium imaging analysis of mutants affecting HSN activity. **Robyn Branicky**, William R. Schafer. Dept Cell Biol, MRC LMB, Cambridge, United Kingdom.

We are using the egg-laying system as a model to investigate how neural circuits generate behavioural outputs. The egg-laying behaviour is regulated by a simple motor circuit, made up of only a few neurons (the HSNs and the VCs) and muscles (the VMs). Despite the anatomic simplicity of the egg-laying system, genetic, pharmacological, and more recently, calcium imaging studies have revealed several layers of complexity including the involvement of multiple neurotransmitters and their receptors as well as neuropeptides. We have used calcium imaging to monitor the activity of the HSNs and VMs in a collection of characterised and uncharacterised egg-laying defective (Egl) mutants with presumed defects in HSN activity. We have identified mutants acting both upstream and downstream of calcium entry into the HSN. We will present our phenotypic and molecular characterisation of these mutants and propose models for how they could be affecting HSN activity and ultimately the activity of the egg-laying circuit.

516A

The receptor tyrosine phosphatase *clr-1* is required for axon regeneration in *C. elegans*. **Rebecca Brown**[1], Marc Hammarlund[2], Stephen Strittmatter[1]. 1) Neurology, Yale University, New Haven, CT; 2) Genetics, Yale University, New Haven, CT.

Axons in the central nervous system display limited regeneration after injury. Recent expression studies in rodents have identified dozens of genes that are upregulated after CNS injury, however, confirming each gene's role in regeneration in a mouse model is cumbersome and labor intensive. *C. elegans* is a useful model for studying the genetics of axon regeneration. Individual GFP-labeled axons can be lesioned by laser microsurgery, and their regrowth can be monitored *in vivo*. *C. elegans* motor neurons display robust regeneration and can reestablish functional connections after laser axotomy. Genes identified as differentially expressed in microarray or RT-PCR analyses of mammalian CNS or PNS regeneration were compiled from the literature (Benowitz and Yin, 2007) and from preliminary studies in our lab. This list was refined to include about 30 genes with *C. elegans* homologues and further to include only genes for which loss of function alleles were viable and available. Using these genes, we conducted a candidate screen for genes that contribute to regeneration in the GABA motor neurons. We identified *clr-1*, which encodes a receptor tyrosine phosphatase related to the LAR family, as a potential mediator of regeneration. *clr-1* loss of function mutants are capable of regeneration per se, as the percentage of fibers that regenerate after axotomy in *clr-1* mutants is similar to that seen in wild-type worms. However, *clr-1* mutant regenerating axons display extensive lateral branching and rarely cross the lesion site to reach their targets in the dorsal cord.

517B

A screen for suppressors of RHO-1 neuronal signaling identifies the dopamine reuptake transporter DAT-1. **Kimberley Bryon**[1], Andrew Porter[1], Rachel McMullan[2], Stephen Nurrish[1]. 1) MRC LMCB, University College London, London, United Kingdom; 2) Imperial College London, London, United Kingdom.

$G\alpha_q$ (EGL-30) is central to neuronal communication, regulating the production and destruction of the 2nd messenger diacylglycerol (DAG). A major target of $G\alpha_q$ is the small GTPase RHO-1. RHO-1 inhibits diacylglycerol kinase (DGK-1) leading to increased levels of DAG at the synaptic membrane, in turn increasing the amount of the neurotransmitter, acetylcholine (ACh) released. Animals over-expressing constitutively active RHO-1 (RHO-1*) in cholinergic motor-neurons have a loopy locomotion and an increased rate of ACh release shown by faster paralysis on 1mM aldicarb effects. RHO-1 still modulates neurotransmission in *dgk-1* mutants identifying that there are other DGK-1 independent pathways. To identify other effectors of RHO-1, we conducted an EMS-mutagenesis screen. 2000 haploid genomes were screened and worms that suppressed the neuronal phenotype of loopy locomotion associated with RHO-1* were identified. A secondary screen was then performed to look for suppression of RHO-1* expressed from the heat shock promoter of a separate transgene, which causes additional non-neuronal phenotypes. From these two screens 12 suppressors were identified that suppressed the neuronal effect of RHO-1* (loopy locomotion) but not the non-neuronal effects. Intriguingly, locomotion and absolute levels of ACh release don't appear to be as strongly correlated as previously thought. Many of our suppressors of neuronal RHO-1* loopy locomotion do not suppress the hypersensitivity to aldicarb, suggesting that levels of ACh release still remain high. Whole genome sequencing of one of these suppressors, nz99, has identified a premature stop codon in T23G5.5, the gene encoding a dopamine transporter DAT-1. Expressing RHO-1* in the cholinergic motor neurons of a *dat-1* deletion mutant also gives rise to worms with more wildtype locomotion suggesting that DAT-1 is the suppressor identified by the screen. Experiments addressing the role of DAT-1 in RHO-1 signaling will be presented. As dopamine is known to be important in co-ordinating locomotion this raises interesting questions about how DAT-1 and RHO-1 interact.

518C

Comparative connectomics reveals the neurobiological basis for predatory feeding behavior in *Pristionchus pacificus*. **Daniel J. Bumbarger**, Metta Riebesell, Ralf J Sommer. Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany.

A fundamental tenet of neuroscience is that nervous system function emerges from synaptic connectivity. How evolutionary modifications in behavior manifest themselves in the nervous system is poorly understood due to a paucity of system-level connectivity data. Predatory feeding in the nematode *Pristionchus pacificus* is an evolutionary novelty that increases the complexity of its behavioral palette. In the first comparison of its kind, we use thin-section TEM to compare synaptic connectivity in the pharyngeal nervous systems of *P. pacificus* with *Caenorhabditis elegans* to understand how the connectome evolves. We reveal homology between the nervous systems of *P. pacificus* and *C. elegans*, which are composed of identical sets of identified neurons that can generate very different behavioral output. Synaptic connectivity was found to be surprisingly divergent given the anatomical similarity. We identified the I1 and I2 interneurons as candidates for enabling differential regulation of pharyngeal muscle cells during predatory vs. bacterial feeding in *P. pacificus*. Changes in connectivity of the M5 motorneuron, which in *C. elegans* is presynaptic to the grinder muscle cells (pm7) are a neurobiological correlate to the loss of a grinder in *P. pacificus*. A system-level comparison in network topology reveals substantial shifts in nervous system architecture. In *P. pacificus*, the I4 interneuron is shown to be the only neuron in the pharynx that is not presynaptic to muscle cells, compared to 9 neurons in *C. elegans*. This suggests information flow is substantially different in the two species. Interestingly, I4 has much higher betweenness centrality in *P. pacificus* than in *C. elegans*. Various measures show pm4 (median bulb muscle cell), pm5 (isthmus muscle cell) and g1D (dorsal gland cell) to be the primary outputs of the system in both species. Using centrality measures and a force-directed graph analysis, we show that the *C. elegans* network focuses primarily on the isthmus, whereas the *P. pacificus* network focuses primarily on the median bulb. Previous work has shown that there are more classes of neurons expressing serotonin in *P. pacificus* than in *C. elegans* (Rivard et al., 2010). Consistent with these observations, eigenvector and PageRank centrality measures show a reduced role for NSM as an output of the system in P. pacificus. Our comparison has revealed the network architecture associated with evolutionary changes in behavior and yields testable hypotheses of network function in both *P. pacificus* and *C. elegans.* .

519A

Dopamine-mediated effects of phenylethylamine in living worms and isolated embryonic neurons. Bryan Safratowich, Rochelle Wickramasekara, **Lucia Carvelli**. Pharmacology, Physiology and Therapeutics, University of North Dakota, Grand Forks, ND.

In brain catecholaminergic terminals a single decarboxylation step affected by aromatic amino-acid decarboxylase converts phenylalanine to phenylethylamine (PEA) at a rate comparable to that of the central synthesis of dopamine (DA). Subnormal PEA levels have been linked to disorders such as attention deficit and depression, while excess has been invoked particularly in paranoid schizophrenia, in which it is thought to act as an endogenous AMPH and, therefore, would be antagonized by neuroleptics. Though PEA and AMPH show similar effects, both increase extracellular DA concentration, questions still remain in regards to the true function of PEA in the central nervous system. In order to further examine the molecular mechanisms of PEA actions, we have chosen the nematode Caenorhabditis elegans (C. elegans) as our experimental model system. In C. elegans the catecholaminergic neurotransmission includes mostly the dopaminergic system. DA is synthesized in eight sensory neurons (4 CEP, 2 ADE and 2 PDE) and modulates locomotion, learning and egg laying activity. All the known dopaminergic components involved in DA synthesis, vesicle storage, release and reuptake are highly conserved between the worm and mammals. Previously, we showed that AMPH induces a complete lack of motor function in nematodes placed in water. We named this behavior swimming-induced paralysis (SWIP). Evidences suggested that AMPH-induced SWIP behavior is mediated by DA efflux through the dopamine transporter (DAT-1). This causes an excess of extracellular DA concentrations which over-stimulate the DA receptors and ultimately generates SWIP. Here we show that, although PEA and AMPH have very similar molecular structures, PEA is more potent than AMPH in generating SWIP behavior. Our data show that PEA significantly induces SWIP behavior in wild-type animals at a stronger rate when compared to AMPH. To further examine the mode of action of PEA-induced SWIP, we treated DAT-1, DOP-2 and DOP-3 receptor knockout animals (dat-1, dop-2 and dop-3) with different concentration of PEA. dat-1 and dop-3 animals displayed less susceptibility to PEA-induced SWIP than wild-type animals suggesting that these two proteins partially mediated PEA-induced phenotype. On the contrary, in dop-2 animals, PEA-induced SWIP occurred at levels similar to wild-type animals. Ongoing biochemistry and amperometric studies will establish the molecular mechanism underling PEA effects in cultured neurons isolated from C. elegans embryos. Importantly, these findings demonstrate the utility of the nematode model for the dissection of molecular determinants of PEA-modulated behavior.

520B

CALM-1 is a Calcium Dependent Regulator of Synaptic Adhesion During Neuronal Development. **Raymond Caylor**, Brian Ackley. University of Kansas, Lawrence, KS.

Synaptic connections rely upon specialized machinery to ensure proper morphology and neuronal transmission. We describe the identification of a putative calcium signaling cascade and its role in regulating synaptic morphology. This pathway is comprised of the voltage gated calcium channel (VGCC) subunits *unc-2* and *unc-36* along with a previously uncharacterized gene, *F30A10.1*, predicted to encode an EF-hand containing protein that we

are naming the homolog of calmyrin-1 (*calm-1*).

unc-2, *unc-36* and *calm-1* were discovered to regulate synapse morphology by suppressing the defects observed in nidogen mutants. Nidogen is a conserved ECM molecule, and when absent results in irregularly shaped, elongated synaptic puncta. Removing *unc-2*, *unc-36* or *calm-1* in a *nid-1* background returns synapses to wild type size. These results indicate the abnormal synaptic areas of *nid-1* mutants require functional calcium signaling.

unc-2, *unc-36* and *calm-1* single mutants display enlarged, regularly shaped synaptic areas and an overall reduction in synaptic puncta. Double mutant analysis between VGCC;*calm-1* reveals these genes work in a linear pathway. Synaptic areas in a gain of function allele of *unc-2* resemble the diffuse, elongated nidogen synapses, and *calm-1*, through epistatic analysis, was found to operate downstream of the VGCCs. This suggests CALM-1 is responsive to calcium influx from activated calcium channels and is subsequently activated to regulate synaptic morphology.If this is true, CALM-1 would be expected to interact with any number of target proteins after calcium binding and activation. We found that CALM-1 binds RACK-1 in a calcium dependent manner. RACK-1 is an intracellular scaffold protein that interacts with signaling and adhesion molecules. *rack-1* mutants phenocopy nidogen synaptic defects, and these defects can be suppressed by *calm-1*.

Synapses can be added dynamically during organismal growth and based on our synaptic phenotype data, we believe to have uncovered one possible mechanism. Nidogen may serve as an adhesive force providing synaptic stability as indicated by mutant synaptic spreading. Conversely, calcium signal component (*unc-2*, *unc-36* and *calm-1*) mutations result in slightly enlarged, punctate synapses and suppress the spreading of nidogen mutants. A proposed mechanism may be a calcium signaling pathway that, in response to growth signals, mediates synaptic adhesion through the nidogen complex. Modulation of synaptic adhesiveness may allow for addition of new synapses during development.

521C

Muscarinic signaling in motor neurons facilitates neurotransmission and is dependent on Ca++ and sphingosine kinase, *sphk-1*. **Jason P. Chan**, Derek S. Sieburth. Zilkha Neurogenetic Inst, Univ So California, Los Angeles, CA.

Acetylcholine can act as a potent neuromodulator in the brain by activating intracellular G-protein signaling pathways that drive neurotransmission. In *C. elegans*, agonists for muscarinic AChRs (mAChRs) promote synaptic transmission at the NMJ by activating the heterotrimeric G-protein EGL-30/Gαq. However, the identity of the GPCR activated by acetylcholine and the signaling pathways downstream of muscarinic signaling are not clear. Here, we show that arecoline acts through *gar-3*, a mAChR of the M1/M3 subclass, and two EGL-30 effector proteins, EGL-8/PLC and UNC-73/Trio. Furthermore, we identify two novel downstream components activated by this pathway: 1) *sphk-1*/sphingosine kinase, an enzyme that converts sphingosine to sphingosine-1-phosphate, and 2) *unc-2*, a VGCC required for neurotransmission. We provide evidence that *sphk-1* and *unc-2* are required for arecoline-induced hypersensitivity to aldicarb. Previously, we have shown that SPHK-1 facilitates neurotransmitter secretion by regulating the synaptic vesicle cycle, indicating that muscarinic signaling may utilize SPHK-1 to facilitate ACh release. Indeed, acute treatment of arecoline recruits a GFP-tagged SPHK-1 fusion protein to presynaptic release sites. In contrast, synaptic SPHK-1 levels are decreased in *egl-30* and *unc-73* loss of function mutants, suggesting that SPHK-1 may be activated by this pathway. Arecoline and UNC-73 mediated recruitment of SPHK-1 depends on *unc-2*; both *unc-73* and *unc-2* mutants decrease SPHK-1 abundance but *unc-73;unc-2* double mutants do not have additive effects. Furthermore, the ability of arecoline to recruit SPHK-1 to synapses is completely dependent on *gar-3*, which acts cell autonomously in motor neurons. Experiments to identify the source of endogenous ACh are under way, but preliminary data suggests that ACh released from motor neurons themselves is not involved. For example, aldicarb treatment (to promote spillover) or mutations that alter ACh secretion from the NMJ do not have detectable effects on SPHK-1 abundance. Together, our results indicate that acetylcholine facilitates neurotransmitter release at the NMJ by activating GAR-3 on motor neurons. This, in turn, activates an EGL-30 - UNC-73 pathway that leads to Ca++ dependent recruitment of SPHK-1 to sites of neurotransmitter release.

522A

How larvae wiggle: functional analysis of the L1 larva locomotory circuit. **Sway P. Chen**[1,2], Anji Tang[2,3], Quan Wen[1,2], Aravinthan D. T. Samuel[1,2]. 1) Department of Physics, Harvard University, Cambridge, MA; 2) Center for Brain Science, Harvard University, Cambridge, MA; 3) Department of Neurobiology, Harvard University, Cambridge, MA.

At all developmental stages, *C. elegans* navigates its environment by generating and propagating sinusoidal bending waves along its body. The neural circuit controlling the locomotory behavior, however, changes significantly at the late L1 larval stage [1]. Neuroanatomy shows that almost no cholinergic motor neurons innervate the ventral body wall muscles in the L1 larva, raising the question of how the larva is capable of normal locomotion. Here we hypothesize that ventral body wall muscles in the L1 larva contract by default. During the dorsal muscle contracting phase, DB cholinergic motor neurons activate DD GABAergic motor neurons and cause periodic relaxation of the ventral muscles. This hypothesis is supported by two experimental observations. First, we examined the swimming behavior of GABA-deficient mutant *unc-25* in the L1 stage and found that the tail consistently curved to one side. Second, we found that wild-type worms expressing GFP in VNC neurons consistently curved to the ventral side after being paralyzed by ivermectin, a drug that silences the motor circuit but has no effect on muscles. Further optogenetic and calcium imaging experiments will be carried out to quantify and relate motor neuron and muscle activity in the free-moving larva. Our approach will build toward a detailed mechanistic model of L1 locomotion. Hopefully, comparison of L1 and adult worm locomotion will shed light on conserved principles of rhythmic motion in general.

1.White, J.G., et al., The structure of the ventral nerve cord of Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci, 1976. 275(938): p. 327-48.

523B

Sensory inputs regulate excitatory synaptic transmission at neuromuscular junctions. **S. Choi**[1,2], Z. Hu[1,2], J.M. Kaplan[1,2]. 1) Dept of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept of Neurobiology, Harvard Medical School, Boston, MA.

How are sensory inputs transformed into motor outputs? Several *C. elegans* sensory behaviors have been extensively analyzed. These studies have typically focused on detection and discrimination between sensory cues. Much less is known about the circuit mechanisms and signaling molecules that engender the motor responses to sensory inputs. The NPR-1(Neuropeptide Receptor) inhibits the activity of a subset of sensory neurons (1). Thus, *npr-1* mutants have increased sensory responsiveness in these neurons, which causes abnormal locomotive behaviors such as social feeding and increased locomotion rate. We found that *npr-1* mutants also have an increased rate of endogenous excitatory post-synaptic currents (EPSCs) at neuromuscular junctions (NMJs), suggesting that altered sensory responsiveness leads to increased synaptic transmission at NMJs and consequently to faster locomotion rates. We show two lines of evidences supporting this idea. First, expression of *npr-1* in the sensory circuit completely rescued the EPSC rate defect of *npr-1* mutants. Second, mutations that block sensory transduction, for example, *ocr-2* (TRPV channel) and *tax-4* (cGMP-gated ion channel), blocked the EPSC rate increase of *npr-1* mutants. These mutations also block the increase in locomotion rate of *npr-1* mutants (2, 3). Conversely, chronic activation of ASH (with capsaicin treatment of ASH::VR1 transgenic animals) induced hypersensitivity to aldicarb (inhibitor of acetylcholine esterase), suggesting that increased sensory activities are not only necessary but also sufficient for the increase in excitatory synaptic transmission at NMJs. Analysis of double mutants suggests that neuropeptides and fast synaptic transmission are both required for the increased EPSC rate of *npr-1* mutants. Analysis of *npr-1* mutants should allow us to begin describing the circuit mechanisms linking sensory responses to changes in locomotion. (1) Macosko et al. (2009) Nature 458(7242):1171-5 (2) de Bono et al. (2002) Nature 419(6910):899-903 (3) Coates and de Bono. (2002) Nature 419(6910):925-9.

524C

C. elegans: A Neurological Model to Characterize a Small Conductance Calcium-Activated K+ Channel. **CK Chotoo**[1], DC Devor[1], CJ Luke[2]. 1) Department of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, Pennsylvania 15261; 2) Department of Pediatrics and Cell Biology & Physiology, University of Pittsburgh, Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania 15224.

Small conductance calcium-activated potassium (SK) channels function to regulate neuronal firing frequency through the generation of a component of the medium afterhyperpolarization that follows action potentials. In humans, irregular action potential frequency underlies diseases such as ataxia, epilepsy, schizophrenia and Parkinson's disease. An SK channel homologue, kcnl2, is encoded for by the F08A10.1 gene in C. elegans. Kcnl2 shares 38% and 34% homology with the human SK2 and SK3 channels, respectively, and shows a great degree of conservation in the six transmembrane domains, the pore motif and the calmodulin binding domain. These structural motifs have been shown to be important for the channel's biogenesis and pharmacology in humans and mice. The F08A10.1 gene was amplified from the WRM063DE08 fosmid and was fused to GFP at the two proposed stop codons. Microinjection of these DNAs indiscriminately yields overexpression of all splice variants. Transgenic lines show that the kcnl2 channel was localized to neurons of the head, the ventral nerve cord, the dorsal cord and the phasmids of the tail. Phenotypic analyses of both knockout and overexpressing transgenic lines identify a potential role of kcnl2 in development and egg laying. We propose that C. elegans will serve as an elegant and simplistic neurological model to study the physiological functions of SK channels that are otherwise masked in complex mammalian systems.

525A

A computational model of associative learning and chemotaxis in the nematode worm C. elegans. **Netta Cohen**[1], Peter Appleby[2]. 1) School of Computing and Institute of Membrane and Systems Biology, University of Leeds, Leeds, United Kingdom; 2) Department of Computer Science, University of Sheffield, United Kingdom.

The challenge of understanding how the effectively hard wired circuitry in *C. elegans* generates complex behaviors is particularly interesting in the context of circuits that serve multiple functions and that undergo plasticity or learning. One such circuit is the relatively well characterized chemotaxis circuit in the head. In chemotaxis *C. elegans* will move up or down a chemical gradient dependent on whether the chemical acts as an attractant or repellent. It does this by a combination of two navigational strategies: (i) gradually steering left or right until the worm points up or down the gradient and (ii) modulating the probability of pirouettes and choosing the final orientation of the worm after the pirouette has finished. A large body of work has shown that the chemotaxis response is dynamic and that the degree of influence a particular chemical has on navigation can be changed, or even reversed depending on experience. Changes are reversible, specific to the chemical in question, and can be generated by classical conditioning experiments. All of these are hallmarks of associative learning, a sophisticated process that requires integration of multiple signals to produce a coordinated change in a behavioral response.

We focus on the worm's chemotaxis and its ability to learn associations between salt

(NaCl) concentrations and food. We draw upon existing experimental data from a variety of sources including electrophysiological and anatomical data to construct a simplified NaCl chemotaxis circuit in *C. elegans*. The circuit includes the two key NaCl sensory neurons ASEL and ASER, a simplified integration unit and a motor interface. We also define a set of experimentally observed behaviors we wish to reproduce including gentle turning, modulation of reversals and pirouette frequency, control of final orientation following a pirouette, and associative learning. In particular, we are interested in the alteration in behavioral response to NaCl that arises due to the pairing of high concentrations of NaCl with food or starvation. We present a computational model of NaCl chemotaxis and learning and show that model worms, placed in a simulated environment, exhibit qualitatively realistic chemotaxis behavior and adaptation. We further demonstrate that our model is robust and tolerant to noise. Our proposed chemotaxis circuit leads to a number of distinct predictions that could be used to test the model experimentally, including specific consequences from our postulated locus of associative learning and predicted consequences from ablations of ASEL and ASER.

526B

The *C. elegans* ERG (Ether-a-Go-Go Related Gene) K^+ channel is a synaptically localized inhibitor of vulval muscle electrical excitability. **Kevin M. Collins**, Michael R. Koelle. Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

Neurotransmitters signal through heterotrimeric G proteins to alter cell excitability, often by modulating ion channel activities, and we seek to define the mechanism by which this occurs. In *C. elegans*, G proteins regulate specific behaviors that can be conveniently scored and quantitatively measured. One such behavior is egg laying, in which the HSN motor neurons release serotonin to activate postsynaptic $G\alpha_q$ and $G\alpha_s$ signaling, promoting vulval muscle contractility and egg laying. $G\alpha_o$ inhibits egg-laying behavior, although how it acts remains unclear.

I cloned the gene identified by new *C. elegans* mutants that display the hyperactive egg-laying phenotype characteristic of loss of $G\alpha_o$ signaling. The mutations affect *unc-103*, the *C. elegans* ortholog of the ether-a-go-go related gene (ERG), encoding a voltage-gated K^+ channel. Disregulation of human ERG causes Long QT syndrome, a sometimes fatal arrhythmia of the heart. ERG functions in neurons and muscle cells to regulate and limit their excitability. I found that single-copy transgene expression of ERG fused to GFP in the vulval muscles restores normal egg-laying behavior to *unc-103* null mutants. This ERG::GFP protein specifically localizes to postsynaptic muscle arms in the vm2 cells apposed to HSN presynaptic varicosities. Deletion of a predicted PDZ interaction sequence disrupts ERG::GFP enrichment at synapses and decreases phenotypic rescue. Thus, ERG acts postsynaptically to inhibit egg-laying behavior.

We hypothesize that synaptic localization has two roles in ERG function. It locally inhibits postsynaptic electrical activity, and it allows for local modulation by G protein signaling. To test this, we are using high-speed video recording and calcium imaging of freely moving animals to study how ERG mutants affect muscle contractility. We find that during locomotion, vulval muscles of wild-type animals show smooth contraction and relaxation with adjacent body wall muscles. During the active phase of egg laying, vulval muscles display occasional twitches that coincide with these ventral contractions. In animals lacking ERG, vulval muscles twitch during nearly all ventral contractions (even between egg-laying clusters), suggesting ERG regulates the egg-laying contraction program during locomotion. We are testing how disrupting ERG synaptic localization affects the kinetics of calcium transients and egg laying induced by HSN signaling. Together, our experiments will show how localization and G protein signaling regulate ERG activity to modulate synaptic electrical excitability and muscle contractility.

527C

Synaptic Change During *C. Elegans* Lethargus. **Nooreen S. Dabbish**[1], David M. Raizen[1,2]. 1) Department of Neuroscience, U Pennsylvania, Philadelphia, PA; 2) Department of Neurology and Center for Sleep, U Pennsylvania, Philadelphia, PA.

Approximately one fourth of the neurons in *C. elegans* adults are born during larval development, indicating tremendous plasticity in larval nervous system structure. We study plasticity at the neuromuscular junction during larval development using the acetylcholinesterase inhibitor aldicarb. During lethargus, a sleep-like state that occurs during larval transitions, there is an accelerated rate of contraction on aldicarb in comparison to mid L4 stage and to the adult stage. The rate of animal contraction when exposed to aldicarb is controlled by a balance between excitatory cholinergic and inhibitory GABAergic inputs on the muscle. Using mutant analysis, observations of drug sensitivity to the epileptogenic drug pentylenetetrazole, and optogenetic tools, we show that GABAergic synaptic transmission is reduced during lethargus. Worms in lethargus show resistance to $GABA_A$ receptor agonists, indicating that post-synaptic mechanisms contribute to lethargus-dependent plasticity. There is a reduction in synaptic protein expression and a change in splice form expression of the $GABA_A$ receptor UNC-49 during lethargus. Using genetic manipulations that separate the quiescent state from the developmental stage, we show that the synaptic plasticity is dependent on developmental time and not on behavioral state of the animal. We propose that the synaptic plasticity regulated by a developmental clock in *C. elegans* is analogous to synaptic plasticity regulated by the circadian clock in other species.

528A

Monoamine Oxidase Inhibitors in Monoamine Receptor Mutants. **Janet Duerr**, Nanda Filkin, Kiel Kreuzer, Joseph Ipacs, Theresa White. Dept Biological Sci, Ohio Univ, Athens, OH.

After monoamines (MAs) are released at the synapse, they are removed by the actions of re-uptake transporters. In vertebrates, once in the pre-synaptic cell the MAs may either be reused, or degraded by monoamine oxidases or catechol-*O*-methyltransferase. Monoamine oxidase inhibitors (MAOIs) raise the levels of MAs by inhibiting the degradation of MAs. MAOIs are used to treat atypical depression, Parkinson's disease, and some psychoses. Unfortunately, MAOIs have significant and varied undesirable side effects. We are using known MA mutants to identify targets for three MAOIs (phenelzine, selegiline, tranylcypromine) in *C. elegans*. As predicted, MAOIs cause inhibition of thrashing, while pumping may be either stimulated or inhibited. As described previously, the *amx-2;amx-1;amx-3* triple mutant, which has deletions in three putative monoamine oxidases, is partially (but not completely) resistant to the effects of the drugs. We are currently examining the effects of MA synthesis or MA receptor mutations on drug sensitivity. We examined tranylcypromine resistance in *dop-1, dop-2, dop-3* single, double, and triple mutants and have found that *dop-3* mutants (but not *dop-1* or *dop-2*) are partially resistant. Mutations in either *ser-1* or *ser-7* did not cause resistance. We are currently examining mutants with deletions in several octopamine or tyramine receptor genes (gifts of the Komuniecki lab). Our short-term plan is to determine if there is residual sensitivity to the drug when we simultaneously disrupt as many MAOI targets as possible. If there is residual sensitivity, we plan to use multiple mutants to identifying novels targets of MAOs that may be important for their affects on humans.

529B

The Role of Palmitoylation in Neuronal Exocytosis in *Caenorhabditis elegans*. **Matthew Edmonds**[1], Timothy Frost[1], Mary Doherty[2], Alan Morgan[1]. 1) Department of Cellular and Molecular Physiology, Institute of Translational Medicine, University of Liverpool, Liverpool, L69 3BX, UK; 2) University of the Highlands and Islands Department of Diabetes and Cardiovascular Science, Centre for Health Science, Old Perth Road, Inverness, IV2 3JH, UK.

Palmitoylation is a post-translational modification of proteins whereby palmitate, a C16 saturated fatty acid, is attached to cysteine residues through a thioester bond catalysed by palmitoyl acyl-transferases (PATs). Unlike other lipid modifications of proteins, palmitoylation is reversible, with palmitate removed by palmitoyl-protein thioesterases (PPTs). Classically, palmitoylation is involved in protein trafficking and interactions with membranes. Recent proteomic-scale analyses have shown exocytotic proteins such as SNAREs to be palmitoylated in both yeast and mammals, although functional consequences and specific enzyme-substrate pairs are largely unknown. There has been very little published about palmitoylation in *Caenorhabditis elegans*. Our aim is to elucidate the roles of palmitoylation in neuronal exocytosis using *C. elegans* as a model organism. Bioinformatic analysis shows that worms have 15 PATs and two PPTs, most of which are uncharacterised. Deletion strains were available for six PATs and both PPTs. These were characterised for behavioural phenotypes associated with neuronal function, initially looking for a phenocopy of a *dnj-14* deletion phenotype. DNJ-14 is the worm orthologue of cysteine string protein (CSP), the most palmitoylated protein known. Mouse and Drosophila knockouts of CSP show a neurodegenerative phenotype which is replicated in worms. None of the available deletion strains gave such a phenocopy, including the knockout of *tag-233*, the PAT most similar to the mammalian PAT for CSP, indicating there may be some functional redundancy in this system. Acyl-biotin exchange (ABE) chemistry coupled to mass spectrometry was applied successfully to detect over 200 palmitoylated neuronal proteins in rat brain homogenate, for example SNAP-25, VAMP-2, synaptotagmin-1. We are currently optimising this for use on worm lysates to identify the worm palmitoylome to add to published palmitoylomes of yeast and rat synaptosomes and cultured neurons. With this information we are hoping to systematically characterise neuronal palmitoylation *in vivo*.

530C

Suppressors of neuronal RHO-1 reveal multiple downstream pathways. **Muna Elmi**, Andrew Porter, Rachel McMullan, Stephen Nurrish. MRC LMCB, Univ College, London, United Kingdom.

The small GTPase RHO-1 is involved in regulation of adult neuronal activity. Expression of constitutively active RHO-1 in cholinergic motorneurons (N::RHO-1*) causes loopy locomotion and an increase in acetylcholine (ACh) release as shown by hypersensitivity to acetylcholinesterase inhibitors. RHO-1 acts in part via inhibition of the DAG kinase, DGK-1 resulting in accumulation of diacylglycerol, which stimulates neurotransmitter release. However, changes in RHO-1 activity in a *dgk-1* null mutant can still alter neuronal activity. Suggesting that in neurons there are RHO-1 effectors in addition to DGK-1. In order to identify other targets of RHO-1 we conducted a genetic screen for suppressors of the N::RHO-1* loopy locomotion. 2000 F1 N::RHO-1* animals mutagenized with EMS were screened for wild type locomotion. As a secondary screen RHO-1* was expressed from a heat-shock promoter that causes both neuronal and non-neuronal phenotypes. We then followed mutants suppressing the loopy locomotion but not the non-neuronal effects. This identified 20 strong N::RHO-1* suppressors. One suppressor, *nz94*, has a mutation in *unc-80*. UNC-80 along with UNC-79 are important regulators of the neuronal ion channel NCA-1/NCA-2, and mutations in any of these genes suppress the loopy locomotion of N::RHO-1*. Expression of UNC-80 in the cholinergic motor neurons is sufficient to rescue the loopy behavior demonstrating that UNC-80 acts in the same neurons as N::RHO-1* to regulate locomotion. Recent data in our lab suggest RHO-1 regulates the NCA-channel via the PI4P5 lipid kinase PPK-1. A second N::RHO-1* suppressor, *nz110*, has a missense mutation in the MHD domain of *unc-31*. A putative *unc-31* null mutation *(e928)* also suppresses N::RHO-1* loopy locomotion. UNC-31 is required for the release of dense core vesicles (DCV). Our results suggest that RHO-1 regulates both classical neurotransmitter

release and release of neuropeptides from DCVs. Interestingly previous *unc-31* mutations are paralyzed in the presence of food whereas our *unc-31 (nz110)* mutants move normally, suggesting the *unc-31 (nz110)* mutation only effects stimulated levels of DCV release. We assumed that suppressors of N::RHO-1* loopy locomotion would also suppress the increased release of ACh. However, many of our N::RHO-1* suppressors resulted in wild type locomotion but did not decrease ACh release. Our current model is that increased ACh release is necessary but not sufficient for loopy locomotion. We believe that an additional RHO-1 regulated signal from the cholinergic motorneurons is required for loopy locomotion. Our genetic screen suggests that the additional signal is released from DCVs, possibly a neuropeptide that acts on the muscle or other neurons.

531A

Circuits for Copulation in the *C. elegans* Male Tail. A. Bloniarz[1], T. Jarrell[1], Y. Wang[1], M. Xu[1], D. H. H. Hall[2], **S. W. Emmons[1,2]**. 1) Department of Genetics and; 2) Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Reconstruction of the posterior connectome of a *C. elegans* adult male is now essentially complete. The current dataset is denoted Release 2.2, which should be used for analysis. The total dataset includes processes of 171 neurons and 64 muscles joined by over 4000 chemical and 4000 electrical synapses. One hundred thirty-seven of the 171 neurons lie on short synaptic pathways between sensory input and endorgan (muscles and gonad) output. These neurons presumably control male mating behavior. Five neurons present in both sexes and 3 male-specific neurons (EF[1-3]) receive extensive input from male-specific neurons in the tail but have little output in the tail. They send processes through the ventral nerve cord into the nerve ring where their output may communicate circuit activity in the tail to the head. The remaining 26 neurons in the connectome are present in both sexes with similar connectivity and little or no interaction with male-specific circuits. The 137 neurons of the mating circuits form a highly interconnected neural network. The chemical network has a single giant component. It is a so-called small world network with short average minimum path length of 3.3 and a high clustering coefficient of 0.291 (probability that two neurons connected to a third are connected to each other). A corresponding random graph has a similar average minimum path length but a much smaller clustering coefficient (0.074, P = 0.005). Forty-five percent of the input to muscles comes directly in monosynaptic pathways from sensory neurons. The network is strongly recurrent, with 43% of the output of sensory neurons directed onto other sensory neurons, and 56% of interneuron output directed onto other interneurons. The high degree of cross-connectivity presents a challenge to identification of functional pathways controlling the separate sub-behaviors of mating. To identify such pathways, we employed published mathematical algorithms for optimal partitioning of networks into communities or modules, groups of nodes more highly connected to each other than to nodes in other communities. We found it is possible to partition the male mating network into 5 modules that seem to have biological significance. These modules appear to govern respectively the search for the vulva (backwards locomotion with unique posture), behavior when the vulva is detected (spicule prodding, spicule insertion, and ejaculation), locomotion, and, for two of the modules, body posture. Sensory input is partitioned among these modules into clear receptive fields. These findings provide a basis for reverse engineering the network through analyzing the effects of perturbations of various kinds. (The first three authors made equal contributions.).

532B

Function of VAV-1 in nervous system control of locomotion. **Amanda Fry**, 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 family GTPases and is homologous to the mammalian Vav proto-oncogenes. We found previously that VAV-1 regulates a variety of rhythmic activities in *C. elegans*, including pharyngeal pumping, ovulation and fertilization, and the defecation cycle, but its role in locomotion has remained unexplored. Interestingly, we have found that *vav-1* null animals display an elevated rate of locomotion and are hypersensitive to aldicarb (an acetylcholine esterase inhibitor), indicating heightened nervous system activity. The aldicarb hypersensitivity observed in *vav-1* mutants is rescued by pan-neural expression of VAV-1, and using further pharmacological assays, we have shown that this hypersensitivity is not due to altered response of *vav-1* mutant muscle cells to acetylcholine. Together, these data indicate that VAV-1 inhibits locomotion through a nervous system mechanism. Remarkably, Vav3 knockout mice are known to exhibit sympathetic nervous system hyperactivity, suggesting a conserved role of Vav proteins in negatively regulating nervous system activity, but the mechanism by which this regulation occurs is not fully understood (1-3). Since VAV-1 is known to be upstream of regulators of nervous system development (e.g. Rho/Rac GTPases), and impaired GABAergic neuron function can lead to aldicarb hypersensitivity, we examined the structure and function of GABA neurons by a combination of cell biological and pharmacological approaches. These analyses showed that both the development and function of GABA-releasing neurons is normal. To identify the cells that express *vav-1* and gain insight into the neural circuit that regulates locomotion, we have analyzed *vav-1* reporter expression in vivo and found that VAV-1 is expressed in a subset of neurons in the head ganglia of the worm, and highly expressed in the ALA neuron. Interestingly, the only known function of the ALA neuron is to regulate behavioral quiescence, the quantification of which is the rate of locomotion (4). We intend to elucidate the mechanism responsible for the control of locomotion by VAV-1 by determining the requirement of VAV-1 in the ALA neuron, as well as investigating the participation of VAV-1 in signaling pathways active in this cell. 1. Sauzeau et al. (2006). Nat. Med. 12, 841-845 2. Sauzeau et al. (2010). Mol Biol Cell. 21, 4251-63 3. Quevedo C. (2010). 15, 1125-39 4. Van Buskirk, C. and Sternberg, P.W. (2007) Nat. Neurosci. 10, 1300-07.

533C

The functional connectivity of male-specific CEM neurons in *C. elegans*. **Lan FU**, King L. CHOW. Division of Life Science, HKUST, Hong Kong.

CEMs are the only four male-specific cephalic neurons located in the head region of *Caenorhabditis elegans*. We have reported that CEMs along with a pair of amphid neurons AWA and interneurons AIZ are required for female sex pheromone perception (Chasnov *et al*. 2007; Chan, 2007). While EM sections of hermaphrodites revealed the synaptic formation between AWA and AIZ (White *et al*. 1986), the connectivity of CEM is still unknown. In order to define the functional connectivity of CEMs in the sex pheromone perception pathway, we recorded the physiological behavior of CEMs and AWAs upon different stimulations using GCaMP2. The activation of either AWA or CEM by excitation of an optogenetic reagent, ChR2, could induce the excitation of the other. It implies a bi-directional communication between CEM and AWA, even when the physical connectivity remains unclear. Although CEM could respond to sex pheromone with or without the input from AWA, it was not activated by sex pheromone to its full potential in the wild-type males. When additional activation of CEM by ChR2 was presented together with the sex pheromone, AWA exhibited a stronger influx of Ca^{2+}. Based on these observations and comparison with various controls, we hypothesize three different modes of CEM connectivity in the pheromone perception circuit for integrating input. (1) CEM and AWA are connected by gap junction while two different thresholds exist for AWAs' activation modulated by CEMs' input; (2) Same as (1) together with chemical synapses where two different thresholds for CEM to communicate with AWA electrically or chemically; (3) CEM, AWA and AIZ formed a classic tri-angular circuit for sex pheromone perception. The evaluation of these possibilities will be discussed. The future study of the CEMs' gene expression of components making up the chemical and electrical connections would provide more clues to the pheromone response circuitry in males. (The study is supported by Research Grants Council, Hong Kong.).

534A

Dopamine signaling for regulating experience-dependent odor avoidance. **Kosuke Fujita**, Kotaro Kimura. Department of Biological Sciences, Osaka University, Toyonaka, Osaka, Japan.

Dopamine signaling plays significant roles in regulating behavior and in learning. The underlying *in vivo* molecular mechanisms, however, are complex and still unclear. We have reported that the D2-like dopamine receptor DOP-3 is required in the RIC interneurons to regulate the enhancement to a repulsive odor 2-nonanone in worms (Kimura, Fujita and Katsura, J. Neurosci., 2010). Avoidance behavior of worms to 2-nonanone is significantly enhanced, rather than reduced, after 1 hr-preexposure to the odor. Unlike previously identified dopamine-regulated behavioral plasticities in worms, which require the presence of food for dopamine action (Sawin *et al*., Neuron, 2000; Hills *et al*., J. Neurosci., 2004; Sanyal., EMBO J., 2004; Kindt *et al*., Neuron, 2007), the enhancement of 2-nonanone avoidance is observed even after food absence for 2 hr, suggesting a novel role for dopamine signaling. In addition to the RIC-specific rescue of the *dop-3* phenotype, we recently found that RIC-specific RNAi of *dop-3* suppresses the enhanced avoidance behavior, indicating that DOP-3 activity in RIC is necessary and sufficient to regulate the behavioral plasticity (K. F. and K. K, unpublished).

How does dopamine signaling in RIC regulate the enhancement of 2-nonanone avoidance? The RIC neurons are known as octopaminergic, and octopamine release is suppressed by dopamine signaling via DOP-3 to regulate CREB expression in the SIA neurons (Alkema *et al*., Neuron, 2005; Suo *et al*., EMBO J., 2009). To test the involvement of octopamine signaling in the enhancement of 2-nonanone avoidance, we investigated if the *dop-3* phenotype is suppressed by null mutations in *tbh-1*, a tyramine beta-hydroxylase required for octopamine synthesis in RIC. The double mutants *tbh-1(n3247)dop-3(tm1356)* and *tbh-1(ok1196)dop-3(tm1356)* showed partial suppression of the enhancement-defective phenotype, suggesting that the antagonistic octopamine signaling is a part of, but not all of, the output for the dopamine signaling in RIC to regulate the enhancement. In addition to identifying the octopamine receptor(s) required for signaling, we are currently attempting to identify the other neuronal outputs from RIC by RIC-specific RNAi.

535B

A Co-operative Regulation of Neuronal Excitability by UNC-7 Innexin and NCA/NALCN Leak Channel. Magali Bouhours[1], Michelle Po[1,2], **Shangbang Gao[1]**, Lin Xie[1,2], Wesley Hung[1], Hang Li[1], John Georgiou[1], John Roder[1,2], Mei Zhen[1,2]. 1) Samuel Lunenfeld Research Institute, Mount Sinai H, Toronto, Ontario, Canada; 2) Department of Molecular Genetics, University of Toronto, Canada.

Gap junctions mediate the electrical coupling and intercellular communication between neighboring cells. Some gap junction proteins, namely connexins and pannexins in vertebrates, and innexins in invertebrates, may also function as hemichannels. A conserved NCA/Dmα1U/NALCN family cation leak channel regulates the excitability and activity of vertebrate and invertebrate neurons. In the present study, we describe a genetic and functional interaction between the innexin UNC-7 and the cation leak channel NCA in Caenorhabditis elegans neurons. While the loss of the neuronal NCA channel function leads to a reduced evoked postsynaptic current at neuromuscular junctions, a simultaneous loss of the UNC-7 function restores the evoked response. The expression of UNC-7 in neurons reverts the effect of the unc-7 mutation; moreover, the expression of UNC-7 mutant proteins that are predicted to be unable to form gap junctions also reverts this effect, suggesting that

UNC-7 innexin regulates neuronal activity, in part, through gap junction-independent functions. We propose that, in addition to gap junction-mediated functions, UNC-7 innexin may also form hemichannels to regulate C. elegans' neuronal activity cooperatively with the NCA family leak channels.

536C

ANALYSIS OF THE MOLECULAR MECHANISM OF SEROTONIN SIGNALING IN C. elegans. **Guliz Gurel**, Michael Koelle. Yale University, New Haven, CT.

Defects in serotonin signaling are hypothesized to cause depression. However, these defects remain undefined because the basic molecular mechanism of signaling through serotonin receptors remains unclear. We are studying the mechanism of serotonin signaling using a genetic approach in C. elegans. C. elegans uses serotonin to modulate certain behaviors and has close homologs of the human serotonin receptors. The first question we are addressing is how two receptors function together to create a response to serotonin. High levels of exogenous serotonin paralyze wild-type C. elegans. Knocking out either the G protein coupled SER-4 serotonin receptor, a homolog of the human 5HT1 serotonin receptor, or knocking out MOD-1, a serotonin-gated ion channel, renders animals almost completely unresponsive to exogenous serotonin (EC50 for N2= 10 mM, EC50 for mod-1 or ser-4 null mutants > 30 mM), suggesting both receptors are required to generate a response to serotonin. Overexpressing MOD-1 with a transgene bearing many copies of mod-1 genomic DNA produces animals extremely hypersensitive to exogenous serotonin (EC50=1 mM). Crossing this into a ser-4 mutant background produces animals with an intermediate level of serotonin response (EC50= 4 mM), suggesting the SER-4 and MOD-1 serotonin receptors act in parallel rather than in series to control locomotion. SER-4 appears to be expressed in a very limited number of neurons, of which only the AVF interneurons are known to affect locomotion. We are testing the hypothesis that serotonin acts on the AVFs via the SER-4 receptor to affect locomotion. We found that expressing SER-4 cDNA in the ser-4 null background in a subset of neurons including the AVFs partially rescues the exogenous serotonin resistance phenotype. Currently, we are working on identifying a more specific promoter to test whether expression of SER-4 only in AVFs will generate full rescue of this resistance phenotype. We are also examining whether MOD-1 affects locomotion in the same cell(s) as SER-4, or rather acts in different cells, such as the subset of ventral cord motorneurons in which it is expressed. The second question we are addressing is how serotonin travels to its site of action. SER-4 and MOD-1 are mostly expressed in neurons that are not postsynaptic to serotonergic neurons. We hypothesize that serotonin travels from the principle serotonin-immunoreactive neuron NSM to extrasynaptic sites of function where it signals through the SER-4 and MOD-1 receptors to control locomotion. To test this hypothesis, we are examining how mutants for MOD-1 and SER-4 respond to endogenous serotonin release from the NSM after Channelrhodopsin2 activation of this cell.

537A

Identification of chloride transporters that regulate GABA signaling. **B. Han**, A. Bellemer, M. Koelle. Molecular Biophysics and Biochemistry, Yale University, New Haven, CT.

The major inhibitory neurotransmitter in the adult brain, GABA, binds to and opens GABA-gated-Cl^- channels to allow Cl^- influx and hyperpolarization. This requires the high outside/low inside Cl^- gradient generated by Cl^- transporters. In embryonic brain, Cl^- transporters reverse the Cl^- gradient to change the direction of Cl^- flow; and GABA is excitatory rather than inhibitory. This switch in GABA signaling is critical for proper brain development and function. We are using *C. elegans* genetics to determine how Cl^- transporters control the cellular Cl^- gradient to regulate GABA signaling.

We found three Cl^- transporters that control the effects of GABA signaling onto the body wall muscles during *C. elegans* locomotion. KCC-2 (a K^+-Cl^- cotransporter) and ABTS-1 (a Na^+-dependent HCO_3^--Cl^- exchanger) function partially redundantly to extrude Cl^- and promote inhibitory GABA signaling. NKCC-1 (a Na^+-K^+-Cl^- cotransporter) accumulates Cl^- and promotes the excitatory effects of GABA by antagonizing KCC-2 and ABTS-1. The mammalian orthologs of KCC-2 and NKCC-1 are known to have analogous effects of GABA signaling in the brain, suggesting the control of GABA signaling in the two systems is analogous.

We are investigating the hypothesis that GABA switches from excitatory to inhibitory action on the body wall muscles during the L1 stage. This would be analogous to the GABA switch in the developing mammalian brain. However, the defined neural circuitry and genetics of *C. elegans* would allow us to understand the mechanism and purpose of the switch. In L1s, the GABAergic DD neurons synapse onto ventral body wall muscles, but the cholinergic neurons that will later excite these muscles have not yet developed. Nevertheless, L1s can execute ventrally-directed body bends. We hypothesize that the Cl^- transporters adjust the Cl^- gradient in L1 body wall muscles so that GABA is excitatory and causes ventral body bends. Our preliminary results are consistent with this model. In adults, the GABA agonist muscimol relaxes all the body wall muscles and causes the animal to lengthen. However, muscimol does not have this effect on L1s. Instead, it can shorten some L1s. This effect is enhanced in certain mutants with defects in acetylcholine or GABA synthesis. We are further characterizing how the muscimol response changes during L1 locomotor circuit rewiring as the GABAergic DD synapses onto the ventral muscles are replaced with cholinergic ones. Our results may account for how L1s can move. They could also provide the first understanding of how the GABA switch is useful by explaining how a specific neural circuit functions in a rudimentary way before most of its neurons have developed.

538B

Partners in Fatty Acid Beta Oxidation: Role of KLF-3 in Fat Burning and Reproductive Behavior of *C. elegans*. **Sarwar Hashmi**[1], Jun Zhang[1], Xiao-Liang Chen[1], Mahmood Hussain[2], I Dhansingh[3], Shahid Siddiqui[3]. 1) Developmental Biology Lab, New York Blood Center, New York, NY; 2) Dept. of Anatomy & Cell Biology, SUNY Downstate Medical Center, Brooklyn, New York, NY; 3) Dept. of Medicine, Pritzker School of Medicine, University of Chicago, Chicago, IL 60034.

Fat metabolism disorders may stem from an abnormal fatty acid oxidation, which limits energy production during periods of increased energy requirement, such as muscular exertion; gastrointestinal disease; exposure to cold and fasting. Thus, obesity leads to diabetes, cardio-vascular illness, hypertension, metabolic syndrome, polycystic ovary syndrome, Syndrome X and sleep apnea. During energy utilization, fatty acids are broken down through beta-oxidation to yield acetyl-CoA. We have discovered the exciting link of a member of Krüppel-like factors (KLF) in lipid utilization by analyzing *klf-3* mutants of *Caenorhabditis elegans* and found that many metabolic genes are potential targets under the control of *klf-3* in triglycerides (TG) derived FA beta-oxidation. Three lines of data suggest the role of KLF-3 in FA beta-oxidation (1), KLF-3 protein is primarily expressed in the intestine; (2), Large lipid droplets storing TG are seen in *klf-3* mutant, 3): KLF-3 binds to the promoters of *fat-7* (Stearoyl-CoA desaturases, SCD), *acs-1*, *acs-2*(Acyl CoA synthase), and *F08A8.1* (Acyl CoA oxidase) a set of genes essential for FA biosynthesis and beta-oxidation in *C. elegans*. The *klf-3* gene shows strong interaction with *acs-1, acs-2, F08A8.1* and *F08A8.2*. Mutation in *klf-3* (ok1975) causes impaired reproduction, suggesting that excessive fat deposition and reproductive defects may be intimately linked. To reconcile with these seemingly unrelated phenotypes of lipid utilization and reproductive behavior, we are examining *klf-3* gene function at biochemical, and developmental level, including genetic mosaic analysis of *klf-3* function in *C. elegans*. These results may elucidate the role of KLF transcription factors in fatty acid oxidation and reproduction.

539C

A new connectivity model for the locomotion network. **Gal Haspel**, Michael J. O'Donovan. Section on Developmental Neurobiology, NINDS, NIH, Bethesda, MD.

Seventy five motorneurons of eight classes innervate the body musculature that propels *Caenorhabditis elegans* with dorsoventral undulations. These motorneurons are interconnected by synapses and gap junctions to create a motorneuronal network. The nervous system of *C. elegans* has been reconstructed from electron micrographs and provides a connectivity data set that is unavailable for any other animal model. However, the dataset is incomplete. The ventral and dorsal nerve cords of a single nematode were reconstructed halfway along the body and the posterior data is missing, leaving 21 of 75 motorneurons of the locomotor network with partial or no connectivity data. Using a new framework for network analysis, the peri-motor space, we identified rules of connectivity that allowed us to approximate the missing data by extrapolation. We mapped the motorneurons in peri-motor space instead of the formerly used mapping coordinates that were either arbitrary or derived from connectivity. In the new peri-motor frame of reference, each motorneuron is located according to the muscle cells it innervates. In this framework, a pattern of iterative connections emerges which includes most (0.90) of the connections. We identified a repeating unit consisting of 12 motorneurons and 12 muscle cells. The cell bodies of the motorneurons of such a unit are not necessarily anatomical neighbors and there is no obvious anatomical segmentation. A connectivity model, composed of six repeating units, is a description of the network that is both simplified (omitting non-iterative connections and modular) and more complete (includes the posterior part) than the original data set. The peri-motor framework of observed connectivity and the segmented connectivity model give insights and advance the study of the neuronal infrastructure underlying locomotion in *C. elegans*. We are using the model of the motorneuronal network to give context to the recorded activity of motorneurons during locomotion. We expressed a calcium sensor in subsets of motorneurons to record their activity and correlate it with the locomotor behavior. We found that some classes of motorneurons are dedicated to either forward or backward locomotion, forming two overlapping motorneuronal networks. A neuronal network comprised of direction-specific classes of motorneurons, might be an ancestral form of locomotor control to which dedicated and multifunctional interneurons were subsequently added.

540A

Contribution of LEV-8 Subunit to the Kinetics of Activation and Desensitization of *C. elegans* Muscle Levamisole-Sensitive Nicotinic Receptors. **Guillermina S. Hernando**, Diego H. Rayes, Cecilia B. Bouzat. INIBIBB-CONICET, Bahia Blanca, Buenos Aires, Argentina.

Caenorhabditis elegans is sensitive to the majority of anthelmintic drugs that are used against parasitic worm infections of humans and livestock. The muscle levamisole-sensitive AChR (L-AChR) is a target of several anthelmintic drugs, such as levamisole, pyrantel and morantel. L-AChRs from *C. elegans* muscle appear to be composed of three essential (UNC-63, UNC-38, and UNC-29) and two accessory subunits (LEV-1 and LEV-8). We here explored at the single-channel and macroscopic current levels the contribution of the alpha-type LEV-8 subunit to the kinetics of activation and desensitization of L-AChRs from *C. elegans* muscle. To this end, we used a primary culture method that allows differentiation of *C. elegans* embryonic cells into larva 1 muscle cells. Single-channel activity of L-AChRs can be readily detected from muscle cells derived from LEV-8 null mutant strain (*lev-8(x-15)*), thus confirming that LEV-8 is not an essential subunit. Channel conductance is similar to that of wild-type L-AChRs (~36 pS). In contrast, the duration of the slowest open component differs from that of wild-type L-AChRs, being about 3-fold more prolonged

(0.28±0.06 and 0.91±0.3 ms for wild-type and mutant, respectively). A dramatic difference between recordings activated by ACh from LEV-8 null mutant and wild-type muscle cells is a time-dependent reduction in the frequency of opening events. Thus, whereas channel activity remains constant during the course of the recording in wild-type cells, it disappears within the first three minutes in the null mutant. Such a reduction is compatible with enhanced desensitization. To investigate this, we recorded macroscopic currents elicited by rapid application of ACh or levamisole in the whole-cell configuration. Our results show that the mean amplitude of currents from the null mutant is similar to that from wild-type strain. However, the decay time constant is ~4-fold reduced in the mutant, indicating faster desensitization. In addition, the steady state current, which represents receptors that remain active during the agonist-pulse, is smaller in the null mutant (41±10% and 19±10% of the peak current for wild-type and mutant, respectively). Taken together, our results reveal that L-AChRs lacking LEV-8 show increased open channel lifetime and enhanced desensitization. Thus, the properties of L-AChRs-mediated responses in muscle cells can be substantially changed whether or not the accessory LEV-8 subunit is incorporated into the pentameric receptor.

541B

Braking fusion: synaptotagmin relieves the complexin block on synaptic release. **RJ. Hobson**, S. Watanabe, Q. Liu, EM. Jorgensen. Dept Biol, HHMI, Univ Utah, Salt Lake City, UT.

The machinery that releases neurotransmitters is related to the machinery that fuses vesicles at the plasma membrane during constitutive secretion. The intertwining of SNARE proteins between lipid bilayers drives membrane fusion in both cases. However, they differ in one important aspect - synaptic vesicle fusion is tightly regulated by calcium. Two proteins play critical roles in conferring calcium sensitivity to synaptic vesicle fusion - complexin and synaptotagmin. Current models suggest that complexin blocks premature synaptic vesicle fusion, possibly by stabilizing the SNARE complex. Synaptotagmin, a calcium binding protein consisting of two C2 domains, may then relieve the complexin block upon calcium influx.

Analyses of complexin and synaptotagmin mutants in *C. elegans* are consistent with this model: Loss of complexin-1 (*cpx-1*) results in an increase in spontaneous release as assayed by the frequency of miniature currents (minis) caused by the release of neurotransmitter from a single vesicle. Synaptotagmin-1 (*snt-1*) mutants exhibit a >90% reduction in mini frequency. *cpx-1 snt-1* double mutants exhibit a severe reduction in mini frequency. These data suggest that synaptotagmin is required for fusion.

To test for the role of calcium sensing in fusion we mutated the calcium-binding sites in synaptotagmin. First, we used MosSCI to insert a single copy of the *snt-1(+)* gene into *snt-1* null mutants. This construct fully rescues the null phenotypes. We blocked calcium sensing by mutating the calcium coordinating aspartate residues in the C2A (C2A*), C2B (C2B*) or both C2 domains (C2A*B*) of *snt-1*. Each of the mutant proteins are trafficked to the synapse and partially rescue the locomotion phenotype of *snt-1* null mutants. Both the C2B* and C2A*B* mutants exhibit reduced neurotransmitter release, suggesting that C2 mutations are unable to detect calcium and drive fusion. In contrast to the *cpx-1 snt-1* double mutants, *cpx-1 snt-1* C2A*B* mutants exhibited elevated neurotransmitter release, similar to the cpx-1 null mutant. Thus, the ability of the C2 domains to bind calcium is not required for fusion, and when *cpx-1* is absent, the brake is removed. These results suggest that calcium-binding to SNT-1 may relieve the CPX-1 brake on synaptic vesicle fusion.

542C

Microbial proton pumps as hyperpolarizers complement the optogenetics toolbox in *Caenorhabditis elegans*. **Steven J. Husson[1]**, Jana F. Liewald[1], Jeffrey N. Stirman[2], Hang Lu[2], Alexander Gottschalk[1,3]. 1) Institute of Biochemistry, Goethe-University Frankfurt, Germany; 2) Interdisciplinary Bioengineering Program, School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA; 3) Frankfurt Molecular Life Sciences Institute, Goethe-University Frankfurt, Germany.

Optogenetic technologies use light to gain endogenous control of defined cells or tissues in a non-invasive manner. Neuronal activity can be manipulated at the millisecond timescale by expressing the light-activated depolarizing cation channel channelrhodopsin-2 (ChR-2) and subsequent illumination with blue light (1). In contrast, yellow light-triggered inhibition of neuronal activity can be achieved by activation of the hyperpolarizing halorhodopsin (NpHR) (2). However, only few successful attempts were undertaken to inhibit *C. elegans* neurons using NpHR, probably due to the insufficient trafficking to the plasma membrane, and thus need for high expression levels, or the limited hyperpolarizing power of this Cl- channel. An extensive screen of type I microbial opsins from archaebacteria, bacteria, plants and fungi recently revealed powerful outward directed proton pumps as valuable alternative hyperpolarisers (3). As cells and extracellular fluid are strongly buffered, shuffling protons across the membrane is not expected to cause any appreciable pH changes. The yellow-green light-sensitive archaerhodopsin-3 (Arch) from *Halorubrum sodomense* appears to be significantly more powerful than NpHR. Another proton pump from the fungus *Leptosphaeria maculans*, Mac, enables neuronal silencing by green-blue light. This opens the possibility to inhibit different neuronal populations, depending on the illumination wavelengths used. Here we present the use of these outward-directed proton pumps as potent circuit breakers in *C. elegans*. Electrophysiological recordings on dissected muscle cells allowed us to quantify the outward current evoked by either NpHR, Arch and Mac. As Mac can be stimulated using blue light, we can activate a subset of neurons using ChR2 while Mac could be used to simultaneously inhibit downstream neurons, using the same colour of light. Alternatively, illumination of predefined body segments with different colours of light using an integrated LCD projector as light source (4) allows using the more potent Arch (maximal hyperpolarizing power with green light) for neuronal inhibition and simultaneous ChR2-induced activation of other cells with blue light. A few examples for circuit dissection with either bacteriorhodopsin will be presented at the meeting. (1) G. Nagel et al., Curr. Biol. 15, 2279 (2005); (2) F. Zhang et al., Nature 446, 633 (2007); (3) B. Y. Chow et al., Nature 463, 98 (2010); (4) J. N. Stirman et al., Nat. Meth. 8, 153 (2011).

543A

C. elegans mutants defective in neuropeptide amidation enzymes are abnormal in egg-laying behavior. **Steven J. Husson[1,2]**, Liesbet Temmerman[2], Bart Landuyt[2], Ellen Meelkop[2], Niels Ringstad[3,4], H.Robert Horvitz[3], Alexander Gottschalk[1], Liliane Schoofs[2]. 1) Institute of Biochemistry, Johann Wolfgang Goethe-University Frankfurt, Germany; 2) Functional Genomics and Proteomics, Department of Biology, Katholieke Universiteit Leuven, Belgium; 3) Howard Hughes Medical Institute, Department of Biology, Massachusetts Institute of Technology, MA, USA; 4) Molecular Neurobiology Program, Skirball Institute of Biomolecular Medicine and Dept. of Cell Biology, NYU Medical Center, NY, USA.

Egg laying has mainly been studied at the behavioral, neuronal and neurochemical levels, but little is known about the biochemical control of the relevant neuropeptidergic signaling systems. Biosynthesis of endogenous peptides requires processing enzymes, such as proprotein convertase 2, which is encoded by *egl-3* (1, 2), and a carboxypeptidase encoded by *egl-21* (3, 4). Mutants defective in these genes have egg-laying defects, consistent with the finding that FMRFamide-like peptides (FLPs) have been linked to egg laying behavior. *C. elegans* enzymes that carry out the last step in the production of biologically active peptides, the carboxy-terminal amidation reaction, have not been characterized. This multistep reaction involves hydroxylation of the glycine α-carbon by a peptidyl-α-hydroxylating monooxygenase (PHM), followed by a cleavage reaction performed by peptidyl α-hydroxyglycine α-amidating lyase (PAL) to generate a glyoxylate molecule and the α-amidated peptide. In vertebrates, both enzymatic activities responsible for the carboxyterminal amidation reaction are contained in one bifunctional enzyme, peptidylglycine α-amidating monooxygenase (PAM). By contrast, invertebrates generally express two separate enzymes encoded by two different genes. Here we report the identification and characterization of *C. elegans* amidating enzymes using bioinformatics to identify candidate genes and mass spectrometry to compare the neuropeptides in wild-type and newly generated mutants. Mutants lacking a functional PHM displayed an altered neuropeptide profile, showed impaired egg laying behavior and had a decreased brood size. Interestingly, PHM mutants still displayed fully processed amidated neuropeptides, probably as a result of the presence of a bifunctional PAM, the main amidating enzyme in vertebrates. Our data indicate the existence of a robust complementation system for the amidation reaction of neuropeptides in nematodes and suggest the involvement of amidated neuropeptides in egg laying. (1) S. J. Husson et al., J. Neurochem. 98, 1999 (2006); (2) J. Kass et al., J. Neurosci. 21, 9265 (2001); (3) S. J. Husson et al., J. Neurochem. 102, 246 (2007); (4) T. C. Jacob, J. M. Kaplan, J. Neurosci. 23, 2122 (2003).

544B

Integration of temperature signals in interneurons of *C. elegans*. **Shingo Ikeda**, Tsubasa Kimata, Ikue Mori. Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ., CREST-JST.

Animals show complex behaviors as a consequence of integrating environmental information through neural circuits. In order to reveal the mechanism of integration, we are focusing on a neuronal circuit of *C. elegans*, composed of three interneurons, AIY, AIZ and RIA, which play an important role in thermotaxis behavior. The RIA neuron receives two upstream signals, one from AIY for thermophilic movement and the other from AIZ for cryophilic movement, and is supposed to integrate them to transmit to downstream motorneurons SMD and RMD, which connect with neck muscles directly (Mori and Ohshima, *Nature*, 1995; White *et al.*, *Phil. Trans. R. Soc. London*, 1986). To understand how RIA integrates two opposite signals from AIY and AIZ, thus reflecting in the behavior, we tried to monitor the activity of AIY, AIZ and RIA with Ca^{2+} imaging using calcium sensor, GCaMP3 (Tian *et al.*, *Nat Methods*, 2009). We so far observed significant responses of AIY and RIA to thermal stimuli, some of which were temperature-independent. The previous study using Cameleon showed that AIY responded significantly to thermal stimuli, while the response of RIA to thermal stimuli was minimal (Kuhara and Mori, *J. Neurosci.*,2006). Further, temperature-independent responses of AIY and RIA have not been reported, suggesting that GCaMP3 can detect different concentration range of intracellular Ca^{2+} compared to Cameleon YC2.12 or YC3.60. To further analyze how the circuit functions in integrating neural signals, we are currently trying to image the activity of AIZ and will perform simultaneous imaging of AIY, AIZ and RIA.

545C

Neuronal modeling toward quantitative understanding of nervous system of *C. elegans*. **Yuishi Iwasaki[1]**, Masahiro Kuramochi[1], Kazumi Sakata[2], Shigekazu Oda[3], Yuichi Iino[3], Ryuzo Shingai[2]. 1) Department of Intelligent System Engineering, Ibaraki University, Hitachi, Ibaraki 316-8511, Japan; 2) Department of Chemistry and Bioengineering, Iwate University, Morioka, Iwate 020-8551, Japan; 3) Department of Biophysics and Biochemistry, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan.

Our aim is to construct a neural model which quantitatively reproduces the experimental data and reliably predicts the neuronal dynamics in *C. elegans*. *C. elegans* shows various behaviors such as chemotaxis and thermotaxis. To understand these behaviors from the neurobiological viewpoint, the neuronal activity needs to be measured. The calcium imaging is a popular technique to visualize the neuronal activity. Since no evidence of Na^{+} current has been found in *C. elegans*, Ca^{2+} current is a key issue to the nervous system. Here

quantity to be measured in the calcium imaging is not the intracellular Ca^{2+} concentration itself but the fluorescence intensity. In addition to the membrane potential, therefore, our model includes the concentrations of Ca^{2+}, Ca^{2+}-buffering protein, fluorescent protein and Ca^{2+}-binding proteins as dynamical variables [Kuramochi & Iwasaki, 2010]. These concentrations are determined by chemical reaction equations. As ion channels, K^+ channel, Ca^{2+} channel and SK channel are considered. A calcium pumping mechanism which carries Ca^{2+} out of the cell across the membrane is also considered. The fluorescence intensity is calculated from the concentration of Ca^{2+}-binding fluorescent protein. The membrane potential and the fluorescence intensity are the observable variables which are comparable with the experimental data in *C. elegans*.

On the basis of the neuronal model, we carry out computational studies on the nervous system of *C. elegans*. Firstly, we study the electrical properties of a single neuron (ASE chemosensory neurons) and find that our results agree well with the experimental data [Goodman et al., 1998]. Secondly, we study a neural circuit for NaCl chemotaxis [Iino & Yoshida, 2009]. In *C. elegans*, the main chemosensory neurons for NaCl are ASEL/R. Here ASEL/R neurons exhibit the left/right asymmetric activities [Suzuki et al., 2008]. In this work, the asymmetric stimulations are considered. The responses of the membrane potential, the Ca^{2+} concentration and the fluorescence intensity to the NaCl stimulus are simulated. We find that the neuronal activity measured by the fluorescence intensity shows quantitatively different behavior from that measured by the membrane potential. The difference comes from the threshold dynamics of Ca^{2+} current.

546A

Stress response to food depravation is modulated by non-synthesizing 5-HT neurons that absorb non-synaptic 5-HT, via 5-HT transporter, MOD-5/SERT, in C. elegans. **Gholamali Jafari**, Bin Liang, Andrey Kullyev, Ji Ying Sze. Department of Molecular Pharmacology , Albert Einstein College of Medicine, New York, NY.202 Golding Building 1300 Morris Park Avenue Bronx NY 10461.

The significance of Selective Serotonin Reuptake Inhibitors (SSRIs) has been proven clinically in the treatment of depression. Although, it is believed that these drugs increase the level of synaptic serotonin (5-HT), the precise mechanism through which they act remains unclear. In C. elegans, we found that 5-HT is secreted from 5-HT producing neurons, NSMs, ADFs, and HSNs and then absorbed by specific 5-HT absorbing neurons, AIMs and RIH, which do not form any synapse with the 5-HT producing neurons. While AIMs and RIH are not able to synthesize 5-HT, they express the 5-HT transporter, MOD-5/SERT, to absorb 5-HT. Our genetic analyses show that 5-HT producing neurons secrete 5-HT via both synaptic vesicles (SVs), clustered at the synaptic sites, and dense-core vesicles (DCVs), clustered at dendrites, and axons, and somata. By ectopically expressing 5-HT transporter, MOD-5/SERT, we show that non-synaptic 5-HT is available throughout the nervous system and can be absorbed by non-serotonergic neurons as well. Finally, our behavioral study indicates that the 5-HT absorbing neurons represent a reservoir of 5-HT to fine-tune the level of 5-HT during and post stress conditions. The presence and roles of absorbing neurons in higher organism remain to be investigated. Knowing that the therapeutic effects of antidepressant, SSRIs, take place in two to three weeks in human, the immediate rise of synaptic 5-HT cannot fully explain the mechanism of action of those drugs. Our findings suggest that the 5-HT activity in neuronal circuits and consequently behavior may not be limited at the synaptic sites.

547B

Forward screen for mutants lacking a food block on olfactory adaptation. **Laine Janzen**[1], Amanda Cha[1], Ghazal Ghafari[1], Stephanie Summers[1], Sara Llamas[1], Pilar Stinson[1], Noelle L'Etoile[2,3], Jared Young[1]. 1) Mills Col, Oakland, CA; 2) Center for Neuroscience, University of California Davis, Davis CA 95817, USA; 3) Department of Psychiatry and Behavioral Sciences, University of California Davis, Davis CA 95817, USA.

Context cues play a role in compulsive and addictive behaviors by endowing accompanying stimuli with increased salience. For example, olfactory cues such as food odors accompany the reinforcing stimulus of the food itself, and thus stimulate cravings for the reinforcing stimulus. We suggest important modulation of odor processing may occur in olfactory sensory neurons, and indeed recent studies have indicated that responses to odors may be modified early in the olfactory pathway (1,2). Olfactory adaptation is a basic form of learning that causes desensitization of an organism to an odorant after a prolonged period of exposure. Studies have shown that adaptation to an odorant can be repressed by the presence of food in the model organism C.elegans (3,4). This block of adaptation involves inhibition of changes in the primary sensory neuron which are associated with olfactory adaptation. As part of a larger project to study the molecular, cell biological, and circuit level mechanisms by which food-derived signals affect odor signaling and long-term plasticity in the model organism C.elegans, this project will attempt to identify new genes involved in the inhibition of long-term neuronal plasticity by food signaling in the sensory neuron (AWC). To identify new genes involved in the food block on adaptation, we are using an unbiased forward genetic screen to isolate mutants. This foundational project will contribute to a larger endeavor to reveal how rewarding stimuli alter signal processing within the primary sensory neuron and may inspire therapies for controlling debilitating compulsive behaviors, such as over eating or drug addiction. 1. Li W, Howard JD, Parrish TB, Gottfried JA. Aversive learning enhances perceptual and cortical discrimination of indiscriminable odor cues. Science. 2008 Mar 28;319(5871):1842-5. 2. Doucette W, Gire DH, Whitesell J, Carmean V, Lucero MT, Restrepo D. Associative Cortex Features in the First Olfactory Brain Relay Station, Neuron. 2011 Mar;69(6):1176-1187. 3. L'Etoile ND, Coburn CM, Eastham J, Kistler A, Gallegos G, Bargmann CI. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in C. elegans. Neuron. 2002 Dec 19;36(6):1079-89. 4. Colbert HA, Bargmann CI. Environmental signals modulate olfactory acuity, discrimination, and memory in Caenorhabditis elegans. Learn Mem. 1997 Jul-Aug;4(2):179-91.

548C

Progress Towards a Reconstruction of a *C. elegans* Male Anterior Nervous System. **T. Jarrell**[1], Y. Wang[1], A. Bloniarz[1], M. Xu[1], C. Brittin[1], K. Nguyen[2], D. H. H. Hall[2], S. W. Emmons[1,2]. 1) Department of Genetics and; 2) Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

Having completed the connectivity of the posterior nervous system of a *C. elegans* male, we are now pursuing reconstruction of the male head. The anterior nervous system of the male contains about 200 neurons common to both sexes and only 4 male-specific CEM neurons as well as the processes of three EF neurons from the tail. This is in stark contrast with the posterior nervous system, which contains 85 male-specific neurons, 55 common neurons whose cell bodies are in the posterior and the processes of 18 common neurons running into the tail from the anterior. Our present connectivity results from the tail demonstrate that some common neurons have sexually dimorphic wiring. Therefore, we anticipate that common neurons in the head may also display different wiring in the male compared to the hermaphrodite. Male-specific EF neuron processes which run into the head will establish further differences. In addition to copulation, male behavior differs from that of the hermaphrodite in several general ways, such as locomotion, chemotaxis, and attraction to food and mates. Coordination of mating behavior with non-mating behavior likely is embedded in the nervous system wiring of the head. In order to compare male connectivity in the head to that of the hermaphrodite, and to complete the male connectome, a reconstruction of the anterior nervous system is necessary. Using traditional fixation, sectioning and staining methods, over 5,000 serial thin sections were obtained from a healthy male and then imaged using two TEMs. A Philips CM10 was used to image the ventral and dorsal nerve cords, and a Philips Tecnai 20 was used to generate montages of over 100 images necessary to cover each section in the nerve ring. Over 110,000 images in all were collected during a 17 month period. Images were digitally aligned using software designed by G. Hood and A. Wetzel at the Pittsburgh Supercomputing Center (see abstract). Aligned images were then entered into the software platform Elegance where reconstruction is under way. A full reconstruction is expected to take six to eight weeks. In addition, work has begun on an Elegance-based reconstruction of the hermaphrodite nerve ring from the Cambridge electron micrographs used for Mind of the Worm. Having the connectivity data in the same format will be necessary in order to make a rigorous comparison of male and hermaphrodite wiring, including relative synaptic strengths.

549A

Temporal processing of olfactory information in C. elegans chemosensory neurons. **Saul Kato**[1], Yifan Xu[2], Christine Cho[2], L. F. Abbott[1], Cornelia Bargmann[2]. 1) Department of Neuroscience, Department of Physiology and Cellular Biophysics, Columbia University College of Physicians and Surgeons, New York, NY; 2) Howard Hughes Medical Institute, The Rockefeller University, New York, NY.

Natural behaviors require sensory processing over a wide range of timescales. For example, the nematode Caenorhabditis elegans can track odor concentrations within a second for gradient climbing, escape a noxious stimulus within seconds, and evaluate the quality of its environment over many minutes. All of these behaviors are initiated by specific chemosensory neurons. What mechanisms support chemosensation at different timescales? To address this question we characterized the responses of three C. elegans sensory neurons, AWC, ASH and ASI, to rapidly fluctuating pseudo-random odor sequences. Precise control of input sequences was achieved by switching of laminar liquid streams within a microfluidic chip. All three neuron classes generate highly reliable calcium responses with distinct temporal selectivities. We applied reverse correlation techniques to extract the temporal filtering properties of each neuron. AWC can track odors at a sub-second time scale, consistent with its role in assessing gradient changes during chemotaxis, and the rapid response requires a specific G protein subunit. The ASH neuron acts as a slower integrator of nociceptive signals, including high osmolarity. Surprisingly, ASI also responded to the ASH stimulus, acting as a slow differentiator that subtracts stimulus history over the past few seconds from prior stimulus history. Both AWC and ASH operate in dual regimes with slow (~minute-long) odor responses superimposed on their rapid responses. Our results suggest that C. elegans chemosensory neurons are selective both for chemical identity and for specific temporal signatures.

550B

Characterization of the novel TOM-1 binding partner, VPS-39, in *C. elegans*. **Susan M. Klosterman**, Szi-Chieh Yu, Anna O. Burdina, Janet E. Richmond. Dept Biol, Univ Illinois Chicago, Chicago, IL.

Regulated exocytosis of secretory vesicles is critically dependent on the assembly of SNARE complexes between the plasma membrane SNAREs, synatxin and SNAP-25 and vesicle-associated synaptobrevin, which render vesicles fusion competent. Tomosyn, a syntaxin-binding protein, forms a complex with syntaxin and SNAP-25 in competition with synaptobrevin predicted to limit fusogenic SNARE complex formation. Consistent with these data, the highly conserved *C. elegans* tomosyn homolog, TOM-1, has been shown to negatively regulate both synaptic and dense-core vesicle release. The regulation and trafficking mechanism(s) for TOM-1 remain to be elucidated. A yeast-2-hybrid screen has revealed a novel TOM-1 binding partner, VPS-39, which could potentially play a role in these unknown processes. VPS-39 is a promising candidate because it is highly conserved among organisms and has previously been implicated in vesicle docking/fusion in other

model systems. Homozygous *vps-39* mutants from a balanced strain, grow to adulthood but lay dead embryos. A translational mCherry fusion construct capable of rescuing this embryonic lethality, indicates that VPS-39 is expressed in many tissues including body wall muscles, neurons, and coelomocytes. Consistent with this expression pattern, *vps-39* mutants are aldicarb-resistant and exhibit a reduced evoked postsynaptic response at the neuromuscular junction. These defects are not due to altered synaptogenesis as the neuronal architecture in *vps-39* mutants appears normal, based on pACR2::GFP expression and UNC-18 antibody staining. The presynaptic ultrastructure in *vps-39* mutants also resembles wild type levels for total number of synaptic vesicles and number of docked vesicles, although *vps-39* mutants exhibit a modest increase in cisternae. This phenotype may be indicative of a generalized endocytic defect, as the human homolog (VAMP6p) has been shown to regulate lysosomal clustering and fusion. NLP-21::Venus expression in coelomocytes also appears aberrant, providing further evidence of a possible vesicle recycling defect. Surprisingly, the expression of neuronal TOM-1 appears to be reduced in *vps-39* mutants, although *tom-1* mutants exhibit an opposing phenotype i.e. enhanced synaptic transmission due to an increase in morphologically docked vesicles. Together these data suggest that VPS-39 may have pleiotropic effects. Given the extent of the synaptic transmission defect, we are currently assessing the potential role of VPS-39 in other presynaptic and postsynaptic processes.

551C
AGS-3 alters *C. elegans* behavior after food deprivation via RIC-8 activation of the neural G protein $G\alpha_o$. Catherine Hofler, **Michael Koelle**. Dept. of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT.

Like all animals, *C. elegans* modifies a number of behaviors upon food deprivation in order to seek food. These behavioral changes depend on neurotransmitters and peptides that typically signal through the major neural G protein $G\alpha_o$, suggesting that signaling through this G protein ultimately mediates responses to food deprivation. Proteins containing the G Protein Regulator (GPR) domain bind to $G\alpha_o$ *in vitro*, but the biological functions of GPR proteins in neurons and the mechanism by which they might affect G protein signaling has remained unclear. We found that the conserved GPR domain protein AGS-3 activates $G\alpha_o$ signaling *in vivo* to allow *C. elegans* to alter several behaviors after food deprivation. These behaviors include octanol avoidance, area-restricted search, and egg laying. AGS-3 protein in whole worm lysates undergoes a progressive change in its biochemical fractionation pattern upon food deprivation, demonstrating that food deprivation changes the physical state of the protein. Cell-specific rescue and cell-specific inactivation experiments show that AGS-3 and $G\alpha_o$ act together in the ASH chemosensory neurons to allow food deprivation to modify response to octanol. Genetic epistasis experiments show that AGS-3 activation of $G\alpha_o$ in the ASHs requires the guanine nucleotide exchange factor RIC-8. Conversely, RIC-8 function in the ASHs also requires AGS-3. Using purified recombinant proteins, we characterized interactions of the proteins consistent with the genetic epistasis results. AGS-3 forms a complex with the the inactive, GDP-bound form of $G\alpha_o$, and RIC-8 can act on this complex to promote nucleotide exchange and dissociation of all the proteins, generating active $G\alpha_o$-GTP. These results identify a biological role for AGS-3 in response to food deprivation and indicate the mechanism for its activation of $G\alpha_o$ signaling in vivo.

552A
Oxidative stress induces loss of GFP labeled neurons and delayed development to adulthood in an *unc-13* mutant. Caitlyn McLarnon[1], Caitlin McGee[1], Lauren King[1], Christopher Frymoyer[1], Elizabeth Roth[1], Laura Gurenlian[1], Alicia N. Minniti[2], Nibaldo C. Inestrosa[2], **Rebecca E. Kohn[1]**. 1) Biol Dept, Ursinus Col, Collegeville, PA; 2) P. U. Católica de Chile, Santiago, Chile.

We are interested in understanding how changes in neurotransmitter release affect sensitivity of *Caenorhabditis elegans* to oxidative stress. Oxidative stress can occur when cells fail to neutralize reactive oxidative species. Nucleic acids, lipids, and proteins can be damaged in response to oxidative stress and affected neurons may undergo neurodegeneration. Activity in neurons can confer resistance to oxidative stress. Products of the *unc-13* gene regulate neurotransmitter release from all neurons in *C. elegans* and defects in this gene result in decreased abundance of neurotransmitters in synapses and paralysis. When wild type *C. elegans* are exposed to oxidative stress, there is a delay in the development of worms to adulthood. Following exposure of *unc-13* mutants to the chemical paraquat, which induces oxidative stress, we found that *unc-13* worms develop to adulthood later than wild type. Using strains with GFP labeled neurons, we examined the number of GABAergic, dopaminergic, and serotonergic neurons in worms with a normal functioning nervous system and with a mutation in *unc-13* following exposure to paraquat. There was a decrease in the number of GFP labeled neurons and more neurons were missing in the *unc-13* strain. These findings suggest that *unc-13* mutants with decreased neurotransmitter release have increased sensitivity to oxidative stress. We plan to perform experiments to determine whether the GFP labeled neurons are undergoing apoptosis or necrosis.

553B
Investigation of Anaphase Promoting Complex Function in Regulating Synaptic Transmission at the *C. elegans* Neuromuscular Junction. **Jennifer R. Kowalski[1]**, Hitesh Dube[1], Tina Jumani[2], Peter Juo[2]. 1) Biological Sciences, Butler University , Indianapolis, IN; 2) Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, MA.

Regulation of synaptic transmission is critical for proper nervous system function. Aberrant synaptic signaling is observed in many neurological and neurodegenerative diseases, ranging from epilepsy to Parkinson's Disease. The ubiquitin enzyme system controls the abundance of many synaptic proteins and thus plays a critical role in regulating synaptic transmission[1,2]. Misregulation of ubiquitin system function is also linked to neurological disorders. The Anaphase Promoting Complex (APC) is an ubiquitin ligase and a key regulator of protein turnover in the cell cycle[3]. Recent work in C. elegans, Drosophila, and mammals demonstrated novel roles for the APC in controlling synapse development and synaptic transmission through effects on pre- and postsynaptic protein abundance at glutamatergic synapses[4-7]. Much remains unknown, however, about how APC function is controlled in neurons and whether the APC may impact transmission at other synapse types. Here, we show that the APC regulates the balance of excitatory (acetylcholine) and inhibitory (GABA) signaling to control muscle contraction at a specialized synapse, the *C. elegans* neuromuscular junction (NMJ). Loss-of-function mutants in multiple APC subunits have increased excitatory transmission at the NMJ (*emb-30* APC4, *emb-27* CDC16, *mat-2* APC1, and *mat-3* CDC23), as indicated by their hypersensitivity to paralysis induced by the acetylcholine esterase inhibitor, aldicarb. Additional behavioral experiments, genetic analyses, and imaging studies support a presynaptic role for the APC in regulating signaling at this synapse. Rescue experiments in APC mutants, as well as assays testing responsiveness to a GABA antagonist, are consistent with a role for the APC in GABAergic neurons. Together, these data suggest a model in which the APC acts in GABAergic neurons to inhibit muscle contraction. Current studies are focused on confirming this site of action and testing whether the APC also functions in cholinergic neurons. Genetic and biochemical experiments to identify relevant APC substrates that mediate its effects at the NMJ are also ongoing. 1. Yi, J.J. and M.D. Ehlers. Pharmacological Reviews, 2007. 59:14-39. 2. Kowalski, J.R., et al, 2011. 31:1341-1354. 3. Peters, J.M. Mol Cell, 2002. 9:931-43. 4. Juo, P. and J.M. Kaplan. Curr Biol, 2004. 14:2057-62. 5. Yang, Y., et al. Science, 2009. 326:575-78. 6. van Roessel, P., et al. Cell, 2004. 119:707-18. 7. Fu, A.K.Y., et al., Nature Neuroscience, 2011. 14:181-9.

554C
An approach towards identification of cell recognition molecules involved in synapse specificity in the nematode *Caenorhabditis elegans*. **Maria I. Lazaro-Pena[1]**, Adam Bloniarz[1], Travis Jarrell[1], Scott W. Emmons[1,2]. 1) Department of Genetics and; 2) Neuroscience, Albert Einstein College of Medicine, Bronx, NY.

How neurons recognize their correct synaptic partners remains largely an unsolved problem. Additional synapse-level connectivity from the new field of connectomics reveal the complexity of this process. Neurons in the circuits of the C. elegans male tail that govern mating are joined in a complex network by over 4000 chemical and 4000 electrical synapses. Each neuron makes synapses with many other neurons (average 15-20). We measure the strength of these interactions from the number of serial EM sections over which they occur. Every neuron interacts strongly with some of its partners and weakly with others, forming a smooth distribution from strong to weak. Fifty percent of the total synaptic load is carried by the weaker set of interactions (<20 sections). To determine whether the weaker set was specific or random synaptic noise, we ranked the neurons by the similarity of their connectivity. Ranking neurons according to their weaker connections gave the same result as ranking them by their strong interactions: left/right homologs and other sets of neurons thought to be equivalent ranked as most similar in connectivity. Therefore, at least some of the weaker connections are specific. To determine whether the strength of synaptic interaction is a consequence of the amount of cell contact, we have begun a volumetric reconstruction of the neurons in the adult male pre-anal ganglion. Preliminary results indicate an absence of correlation between the amount of cell contact and the strength of synaptic interaction, implying the presence of cell-specific as well as neighborhood-specific recognition functions. A model to explain these observations hypothesizes that there are a large number of recognition molecules in every neuron mediating synapse formation, the number that match determining the strength of connection. Involvement of a large number of recognition molecules can explain why it has been difficult to find single gene loss-of-function mutations that alter connectivity. To test this hypothesis, we will select a pair of cells that are respectively synaptic and non-synaptic neighbors of the same cell. For example B-type ray neurons strongly synapse onto EF(1-3) interneurons while the A-type ray neurons contact EF(1-3) but do not synapse with them. Using cell-type gene expression profiling, we hope to identify genes expressed in the B-type neurons but not the A-type neurons that are candidates for mediating EF targeting. Their function can be tested by expressing them in the A-type neurons and examining whether this induces synapses with EF(1-3).

555A
ABI-1 controls acetylcholine receptor clustering and rpy-1 expression level. **Bo Yun Lee**, Seugnhee Nam, Junho Lee. Biophysics and Chemical Biology, Seoul National University, Seoul, Korea.

Rapsyn has a role in acetylcholine receptor clustering at neuromuscular junction. In mammalian cells, rapsyn interacts with actin cytoskeleton through utrophin. Although there might be many components in acetylcholine receptor (AChR) clustering, most of them are unrevealed. In this research, we tried to find the partners of rapsyn for acetylcholine receptor clustering using *C. elegans*. *rpy-1*, rapsyn homologue of *C. elegans*, interacts with *unc-29*, an AChR β subunit homologue of *C. elegans*, and controls AChR clustering. We conducted yeast two hybrid screenings and immunoprecipitation experiments, and found that ABI-1 binds to RPY-1. Abi, *abi-1* homologue of mammals, is a component of the SCAR/WAVE complexes which trigger actin polymerization at filopodia tips. Also, in *C. elegans*, SCAR/WAVE proteins are involved in apical F-actin enrichment. Furthermore, ABI-1 interacts with ARP2/3 complex to regulate actin cytoskeleton remodeling. To

identify where *abi-1* works in *C. elegans*, we checked the expression pattern of *abi-1* using *Pabi-1*::GFP construct. *abi-1* was expressed in muscles, neurons, hypodermis and many other cells. To verify that *abi-1* is related to AChR clustering, we treated *abi-1* mutant with 25 μM levamisole. While *rpy-1* or *unc-29* mutants were resistant to levamisole, *abi-1* mutant showed severer hypercontraction than wild type, N2. Also, when wild type ABI-1 protein was expressed in *abi-1* mutant, the phenotype was rescued. It means that *abi-1* would be involved in AChR clustering. Next, we tested *rpy-1* expression in *abi-1* mutant. Unexpectedly, the level of *rpy-1* expression was decreased in *abi-1* mutants. It means that *abi-1* is involved in the regulation of rpy-1 expression level. Our results suggest that *abi-1* has a role in AChR clustering and in the control of *rpy-1* expression.

556B

Short-term synaptic depression and vesicle pool refilling at the *C. elegans* neuromuscular junction. **Qiang Liu**, Shigeki Watanabe, Erik Jorgensen. Dept Biol, Univ Utah/HHMI, Salt Lake City, UT.

Short-term plasticity of synaptic transmission is recognized as an important element of information processing in neuronal networks. Acetylcholine release at the *C. elegans* neuromuscular junction is rapidly depressed upon repetitive stimulation and gradually recovers after a short rest. A simple model is that the readily releasable pool is depleted by extensive release and refilled from either newly endocytosed vesicles or from a large reserve pool. To determine the contributions of different pool refilling mechanisms and identify key molecular players, I use electrophysiological assays to screen candidate genes that function in endocytosis or other vesicle traffic pathways for altered synaptic depression and recovery. From this screen I expect to identify essential proteins that regulate synaptic strength and reveal mechanisms underlying synaptic depression. To target the screen specifically on the presynaptic components of synaptic depression, I built a reference strain to eliminate contributions from the postsynaptic acetylcholine receptors desensitization and the inactivation of channelrhodopsin-2. I used ChIEF, a channelrhodopsin-2 variant with less inactivation, in *acr-16*(-) background which has little receptor desensitization. Almost all the depression measured from this strain (*acr-16;Punc-17::ChIEF::mCherry*) is by presynaptic mechanisms. The time course of the depression and recovery measured from this control had two time constants indicating multiple underlying mechanisms. Candidate genes were cherry-picked based on their reported functions in endocytosis and vesicle trafficking steps including docking, priming, clustering and so on. Mutant strains of these candidate genes were crossed into the reference strain. Synaptic depression and recovery at the acetylcholine neuromuscular junction under 10Hz 30 sec stimulations were recorded. Preliminary recordings have been obtained for clathrin (*chc-1 ts*), dynamin-1 (*dyn-1 ts*), synaptojanin (*unc-26*), synapsin (*snn-1*), RIM (*unc-10*), liprin-alpha (*syd-2*), CDK5 (*cdk-5*), synaptotagmin-1 (*snt-1*), etc.. One interesting result emerged from the preliminary results is that *chc-1 ts* did not alter the depression nor the recovery rates at the restrictive temperature. This result calls into doubt endocytosis being the rate-limiting step for the synaptic depression and recovery. Alternatively, clathrin-mediated endocytosis may not be the essential endocytic pathway for synaptic vesicles in *C. elegans*. Clathrin and other mutants with either augmented or diminished depression or recovery rate will be further characterized using electron microscopy in the absence or presence of neuronal stimulation at different time points.

557C

C. briggsae puf-2 regulates pharyngeal muscle physiology and is essential for postembryonic development. **Qinwen Liu**, Eric Haag. Dept Biol, Univ Maryland, College Park, MD.

Proper control of larval physiology is necessary for postembryonic development. We have discovered that adequate pumping of pharyngeal muscle in newly hatched *C. briggsae* larvae requires the Puf family RNA-binding protein gene *Cbr-puf-2*. Mutants with a 1.7kb deletion in *Cbr-puf-2* gene fail to reach adulthood and arrest at the L2 stage. This unexpected phenotype has not been reported for any characterized *C. elegans* Puf family member. Larval arrest is rescued by a transgene bearing a wild-type copy of *Cbr-puf-2*. Mutants sustain a rate of pharyngeal grinding only one quarter that of wild-type animals at the same developmental stage. A feeding assay by using GFP-expression *E. coli* stain showed that mutants accumulate intact bacteria in their intestinal lumen. A *Cbr-puf-2* promoter reporter shows transient expression only in pharyngeal muscle 7 during a brief window from the late four-fold embryo to the early first larval stage. These data support a working model in which *Cbr-puf-2* functions transiently, but essentially, in pharyngeal muscle cells to promote food digestion during early larval development. Previous studies showed that *Drosophila* Pumilio mediates synaptic growth and plasticity at the postsynaptic side of the neuromuscular junction[1, 2]. Our finding suggests a functional conservation of Puf genes in postembryonic control of muscle physiology among ecdysozoan invertebrates. 1.Menon, K.P., et al., The translational repressors Nanos and Pumilio have divergent effects on presynaptic terminal growth and postsynaptic glutamate receptor subunit composition. *J Neurosci*, 2009. 29(17): p. 5558-72. 2.Menon, K.P., et al., The translational repressor Pumilio regulates presynaptic morphology and controls postsynaptic accumulation of translation factor eIF-4E. *Neuron*, 2004. 44(4): p. 663-76.

558A

Screening for ACR-16 clustering mutants. **Ashley A. Martin**, Feyza Sancar, Janet E. Richmond. Dept Biol, Univ Illinois Chicago, Chicago, IL.

At the *C. elegans* body wall neuromuscular junctions (NMJs) there are two cholinergic ionotropic receptor types, one that is heteromeric and activated by the anthelmintic levamisole (LAChR) and one that is homomeric and activated by nicotine (NAChR). Screens designed to isolate levamisole-resistant mutants in *C. elegans* have identified subunits of the LAChR as well as additional genes implicated in receptor function. These latter genes affect LAChR assembly and trafficking as well as receptor clustering at the NMJ. Specifically LEV-9, LEV-10, and OIG-4 have been implicated in the clustering of LAChRs, but remarkably the expression of the colocalized NAChR appears completely normal when these gene products are perturbed. The only receptor subunit known to be required for the *C. elegans* NAChR is ACR-16, which can form functional homo-pentameric receptors in a heterologous expression system. This receptor has been shown to contribute to the quickly desensitizing component of the NMJ evoked response that accounts for ~80% of the maximum evoked cholinergic response amplitude. At vertebrate skeletal NMJs the nAChR clustering mechanism is known to involve the proteins Agrin, MuSK, and Rapsyn. Similarly, the *C. elegans* ACR-16 receptor is regulated by the receptor tyrosine kinase, CAM-1, which is homologous to MuSK. However, the function of ACR-16 appears to be unaffected in *C. elegans* rapsyn mutants (*rpy-1*)(unpublished data JR), suggesting that CAM-1 may interact with other tethering factors to target ACR-16 to the NMJ. Forward and reverse genetic screens were therefore performed to isolate candidate genes involved in the clustering mechanism of ACR-16. The screens utilized a single-copy insertion of ACR-16::GFP in *acr-16* and *unc-63;acr-16* mutant backgrounds to isolate mutants that specifically impacted ACR-16::GFP function and expression pattern. From these screens, one gene candidate was identified by RNAi and 25 mutants were isolated following EMS mutagenesis. Mutants exhibited either a reduction in ACR-16::GFP expression at NMJs or ectopic ACR-16::GFP puncta. Preliminary electrophysiological recordings demonstrated a reduction in the evoked NMJ response in four of the EMS mutants as well as the RNAi construct. In all five cases this reduction corresponded to a similar reduction in ACR-16::GFP expression levels. Further characterization of these mutants will require an examination of synaptogenesis, muscle morphology, as well as an evaluation of the post-synaptic response to exogenous ACh. Those mutants that have normal synaptogenesis and muscle morphology will be SNP-mapped and genes of interest will be further characterized.

559B

The *sup-1* gene encodes a novel transmembrane protein that interacts with the UNC-17 vesicular acetylcholine transporter in *C. elegans*. **Ellie Mathews**[1], Greg Mullen[1], Jonathan Hodgkin[2], Janet Duerr[3], Jim Rand[1]. 1) Gen Models Disease Res Program, Oklahoma Med Res Foundation, Oklahoma City, OK; 2) Genetics Unit, Department of Biochemistry, University of Oxford, Oxford, UK; 3) Department of Biological Sciences, Ohio University, Athens, OH.

Acetylcholine (ACh) is a major neurotransmitter in both vertebrate and invertebrate nervous systems. A single phylogenetically conserved locus encodes both the ACh biosynthetic enzyme choline acetyltransferase (ChAT; *cha-1*) and the vesicular ACh transporter (VAChT; *unc-17*) proteins. To identify proteins that interact with UNC-17/VAChT, we screened for mutations that suppress the uncoordinated phenotype of *unc-17(e245)* mutants. Several *unc-17* alleles, including *e245* and *p300*, are associated with the same glycine-to-arginine amino acid substitution (G347R); this substitution introduces a positive charge in the middle of the ninth transmembrane domain of UNC-17. We identified both intragenic and extragenic suppressors, including dominant mutations in the *sup-1* locus. The *sup-1* gene encodes a novel single-pass transmembrane protein that is expressed in a subset of neurons and muscles. The *sup-1(e995)* mutation leads to a glycine-to-glutamic acid substitution (G84E), resulting in a negative charge in the middle of the SUP-1 transmembrane domain. We believe that electrostatic interactions between the UNC-17(G347R) and SUP-1(G84E) transmembrane domains may restore UNC-17 function; this is similar to the previously characterized interaction between UNC-17(G347R) and SNB-1(I97D) (Sandoval *et al.*, 2006). We identified a loss-of-function (putative null) *sup-1* mutant by mutagenizing suppressed animals and screening for re-emergence of the *unc-17* phenotype. The *sup-1* null mutant has no obvious deficits in cholinergic neurotransmission and does not suppress any of the *unc-17* mutant phenotypes. A transgenic SUP-1::GFP fusion protein is expressed in a subset of cholinergic neurons, as well as body wall muscles, and is enriched in neuronal processes. We used bimolecular fluorescence complementation (BiFC) analysis to look at association between SUP-1 and UNC-17 *in vivo*. BiFC fluorescence was observed in synapse-rich regions of the cholinergic nervous system, including the nerve ring and dorsal nerve cords. These observations suggest that UNC-17 and SUP-1 are in very close proximity at the synapse and, taken together with the genetic data, suggest that these proteins physically interact. (Supported by NIH grant GM038679).

560C

C. elegans Presenilin Regulates Muscle Cell Excitability. **Erin E McClure**, Ken R Norman. Cancer and Cell Biology, Albany Medical College, Albany, NY.

Mutations in the genes encoding presenilin-1 and presenilin-2 account for approximately 40% of all familial Alzheimer's disease cases. Despite the fact that altered presenilin activity has been known to have a role in Alzheimer's disease pathology for many years, the functional consequences of mutations in presenilins are controversial and not understood. In fact, mutations in presenilins have been implicated in such diverse functions as altered processing of β-amyloid precursor protein, Notch signaling, calcium entry, and calcium removal from the cytoplasm. Thus, the role of presenilins in Alzheimer's disease has remained elusive. Interestingly, we have found that mutations in a *C. elegans* presenilin homolog, *sel-12*, suppress erratic calcium signaling in body wall muscle. While investigating the role of SEL-12 in muscle function, we have found that *sel-12* mutants are hypersensitive to an acetylcholine receptor agonist, levamisole. Levamisole activates muscle cell contraction and causes paralysis independent of synaptic signaling.

Hypersensitivity to levamisole is due to hyperexcitability of the responding muscle cell, indicating that *sel-12* regulates muscle cell excitability. Additionally, we have found that this hypersensitivity can be suppressed by RNAi knockdown of the ryanodine receptor (RYR), UNC-68 and the inositol 1,4,5 trisphosphate receptor (IP3R), ITR-1. These results are consistent with presenilins playing a role in calcium regulation in the body wall muscle of *C. elegans*. Furthermore, since presenilins are known to be required for Notch signaling, we explored the role Notch signaling may play in the *sel-12* mutant levamisole hypersensitive phenotype. While *glp-1* mutants did not show levamisole hypersensitivity, *lin-12* mutants did display levamisole hypersensitivity similar to *sel-12* mutants. These data suggests that SEL-12 and LIN-12 activity is required for normal muscle cell excitability. Our future studies will include the analysis of tissue specific rescue, the expression levels of RYR and IP3Rs and calcium signaling in the body wall muscle in *sel-12* and *lin-12* mutant animals. Thus far, our results suggest that SEL-12 acts in a Notch/LIN-12 dependent manner to regulate muscle cell excitability.

561A

Epileptogenesis: Using *C. elegans* to elucidate the mechanism of a neural circuitry switch. **Allyson V. McCormick**[1], James H. Thomas[2], Brian C. Kraemer[3]. 1) SIBRC,Veterans Affairs Puget Sound Health Care System, Seattle, WA; 2) Department of Genome Sciences, University of Washington, Seattle, WA; 3) GRECC, Veterans Affairs Puget Sound Health Care System, Seattle, WA.

Epilepsy is a prevalent disorder of the brain caused by synchronizing neuronal circuitry. The pathway from normal to seizure-prone brain activity—epileptogenesis—remains elusive. CaMKII has seizure-protective properties in rodent epilepsy models and functional changes are evident in human epileptic brain tissue. We have used genetic approaches to characterize a *C. elegans* model of epilepsy using animals deficient in CaMKII/UNC-43. Neurostimulants pentylenetetrazole and pilocarpine potentiate worm clonic-like seizures while antiepileptic drugs (e.g. valproate) and loss of Ca^{2+} channels—*unc-2*, *unc-36* and *egl-19*—suppress them. CaMKII is required only in motor neurons to suppress what we have shown is an underlying defect in neuronal synchronizing activity. CaMKII further mediates a switch in adult neurons whereby temporary loss or presence of this kinase can reversibly interconvert animals between seizure-prone and normal states. This property of CaMKII allows a unique window into the process of epileptogenesis. Using an inducible promoter to express a potent and specific inhibitor of CaMKII, we can now conduct forward genetic screens in a free-living animal to isolate suppressors of induced seizures. Our analysis highlights both the key role that CaMKII performs in neuronal excitability and the clean utility of modeling epilepsy in worms to allow the molecular dissection of seizures.

562B

The DAF-7/TGF-β signaling pathway regulates the abundance of the glutamate receptor GLR-1 in the ventral nerve cord. **Annette McGehee**, Peter Juo. Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, MA.

The regulation of glutamate receptor (GluR) abundance and localization can be controlled by activity, and has been implicated in learning and memory. Thus, understanding the cell biological mechanisms that regulate GluRs is of significant interest. We use quantitative fluorescence microscopy to study genes and mechanisms involved in regulating the localization and abundance of GFP-tagged GluR GLR-1 (GLR-1::GFP) at synapses. GLR-1 is an AMPA-type GluR that is expressed in ventral nerve cord (VNC) interneurons where it localizes to sensory-interneuron and interneuron-interneuron synapses (1-3). Here we describe a novel role for the DAF-7/TGF-β signaling pathway in regulating the abundance of GLR-1 in the VNC. TGF-β signaling regulates multiple cell biological and developmental processes in many organisms, including several aspects of neuronal development. In *C. elegans*, the DAF-7/TGF-β pathway has been well studied as a regulator of dauer formation (4). We used temperature-sensitive (ts) mutants to show that multiple components of the DAF-7/TGF-β signaling pathway are involved in regulating GLR-1 levels in the adult VNC. Specifically, we found that the abundance of GLR-1::GFP increases significantly in *daf-7* (TGF-β/ligand), *daf-1* (type I receptor), *daf-8* (Smad), and *daf-14* (Smad) ts mutants compared to wild type animals. DAF-3 is a Smad whose transcriptional activity is antagonized by DAF-8 (5). We found that mutation of *daf-3* suppresses the increase in GLR-1::GFP observed in *daf-8* mutants, suggesting that the effect of DAF-8 on GLR-1 is mediated through DAF-3. The effect on GLR-1::GFP is specific for the DAF-7/TGF-β signaling pathway because animals with mutations in *sma-6*, a type I receptor that acts in a parallel TGF-β signaling pathway to regulate body size, showed no increase in the abundance of GLR-1::GFP in the VNC. We also found no change in the distribution of a presynaptic marker, GFP-tagged synaptobrevin, in *daf-8* or *daf-14* mutant animals suggesting that the number of presynaptic inputs is likely not affected by the DAF-7/TGF-β pathway. These results identify a novel function for the DAF-7/TGF-β signaling pathway in regulating the abundance of GLR-1 in the VNC, and suggest that an extracellular factor (TGF-β) is required to maintain GluR levels in the mature nervous system. (1) Hart et al. (1995). Nature 378:82-85. (2) Maricq et al. (1995). Nature 378:78-81. (3) Rongo et al. (1998). Cell 94:751-759. (4) Patterson and Padgett (2000). TIGS 16:27-33. (5) Patterson et al. (1997) Genes & Dev 11:2679-2690.

563C

Affinity Purification and Identification of Complexes of Interacting Proteins Directly from the *C. elegans* Nervous System. Christopher Hoover, Stacey Edwards, Michael Kinter, Caroline Kinter, **Kenneth Miller**. Oklahoma Medical Research Foundation, Oklahoma City, OK.

A strength of *C. elegans* is to the ability to use forward genetic screens to identify proteins that indirectly or directly contribute to synaptic function. The random nature of genetic screens can sometimes reveal novel proteins of essentially unknown function that are conserved in all organisms with a nervous system (see poster by Edwards et al.). Proteins in this class are especially interesting. Their conservation across animal phyla and their appearance in a synaptic function genetic screen suggest that they have a basic important function in all nervous systems. In some cases, they may represent windows into poorly understood synaptic functions. Because the sequences of these pioneer proteins offer no clues, it is often difficult to determine their role in neurons at a mechanistic level. However, if a pioneer protein is a component of a stable complex, then identifying the other components of the complex could yield functional clues. We have developed an affinity purification approach that allows us to purify complexes of interacting proteins directly from their native environment in the *C. elegans* nervous system. We begin by producing a transgenic strain, in a null or near-null background, that expresses a rescuing FLAG-tagged version of the protein of interest throughout the nervous system. After integrating the transgene, we produce a prep of 15 - 20 million eggs on 50 X 100 mm culture plates, using bleach to dissolve the adult bodies. Newly hatched larvae are rinsed from the plates, concentrated on a filter, and the larval paste is dripped into liquid nitrogen and ground to a powder. After homogenizing by low power sonication we run soluble or membrane fractions over small FLAG monoclonal antibody columns and wash extensively, including a final wash with scrambled FLAG peptide followed by a highly specific elution with authentic FLAG peptide. After quantifying the yield of the tagged protein using GST-FLAG standards, we run 25 pmoles 1 cm into an SDS-PAGE gel and process the gel for Mass Spec analysis. When we tested this method on RIC-8 (a former novel, conserved, protein of unknown function), the analysis revealed only 6 proteins that co-eluted with RIC-8. However, 4 of the co-eluted proteins were also identified in samples from at least one other FLAG-tagged protein of unrelated function, and thus were non-specific. The remaining 2 proteins were GOA-1 (Gαo) and EGL-30 (Gαq), which are the 2 Gα proteins that are known to physically interact with RIC-8.

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Forward Genetic Analysis of Dense Core Vesicle Maturation and Function. Stacey Edwards, Christopher Hoover, **Kenneth Miller**. Genetic Models of Disease Program, Oklahoma Medical Research Foundation, Oklahoma City, OK.

Recent studies suggest close interactions between the synaptic Gα signaling pathways and the functions of neuronal dense core vesicles (DCVs). We have found that the function of the neuronal Gαs pathway broadly overlaps with the function of UNC-31 (CAPS), which facilitates DCV docking and exocytosis.[1] A large forward genetic screen for suppressors of goa-1 (Gαo) null mutants recovered the expected mutants with impaired Gαq signaling but also produced mutations in proteins involved in DCV function (UNC-31) and maturation (UNC-108/ RAB2).[2] Interestingly, Rab2 is the most highly conserved Rab in the animal kingdom, and yet very little is known about its precise function. Recent studies have shown that *unc-108* null mutants send the same number of DCVs to axons as wild type; however, their vesicles are missing up to two-thirds of their soluble and transmembrane cargoes.[2,3] As a result of the loss of the DCV transmembrane cargo IDA-1-GFP in *unc-108* mutant somas, IDA-GFP accumulates in somas at levels about 2-fold higher than wild type. We have used this as the basis of a genetic screen to identify other players that act with UNC-108 in dense core vesicle maturation. We mutagenized a strain that expresses IDA-1-GFP in motor neurons as an integrated transgene. We then screened its grandprogeny for rare mutants with brighter cell somas. We also included another transgene in the strain that expresses soluble RFP from the same promoter to rule out gene expression changes. To screen animals we plated L4-stage F2s on 24-well culture plates (40 animals per well) and screened them using a fluorescence stereomicroscope with a high resolution objective. In a pilot screen (~20,000 F2s; ~4-fold coverage) we isolated 17 mutants of interest and have sorted them into complementation groups and mapped many of the new mutations. Among the interesting genes identified so far are three proteins that appear to be conserved in all organisms with a nervous system, but whose function is largely unknown. One of these proteins is HID-1, which was recently implicated in dense core vesicle maturation.[4,5] Since some of the genes are represented by only 1 or 2 alleles, we are doing more cycles. [1]Charlie et al., 2006. Genetics. 172(2), 943-961. [2]Edwards et al., 2009. J. Cell Biology.186(6):881-895. [3]Sumakovic et al., 2009. J. Cell Biology.186(6):897-914. [4]Mesa et al., 2011. Genetics. 187(2): 467-483. [5]Yu et al., 2011. Biochem J. 434(3):383-90.

565B

Functional imaging of neuronal activity for 2-nonanone stimulation. **Yosuke Miyanishi**[1], Junichi Nakai[2], Kotaro Kimura[1]. 1) Department of Biological Sciences, Osaka University, Toyonaka, Osaka, Japan; 2) Saitama University Brain Science Institute, Japan.

To better understand the neural basis that regulates a worm's sensory behavior and its modulation by learning, we are studying avoidance behavioral responses to 2-nonanone. We previously reported that the avoidance behavior to 2-nonanone is enhanced, rather than reduced, after preexposure to the odor, and this enhancement is acquired as a non-associative dopamine-dependent learning (Kimura et al., J. Neurosci., 2010; Fujita and Kimura, this abstract). In addition, we observed that worms respond to a spatial gradient of 2-nonanone (Yamazoe and Kimura, CE Neuro, 2010), which cannot be simply explained by the pirouette or weathervane strategies.

2-nonanone is mainly sensed by the AWB neurons, which have been shown to exhibit odor-OFF response in aqueous step stimulation with 2-nonanone (Troemel et al., Cell 1997; Ha et al., Neuron 2010). To understand how the neuronal circuits of worms regulate the characteristic 2-nonanone behavioral response, we are monitoring calcium changes in the

AWB and downstream neurons using G-CaMP 4 (Shindo et al., PLoS ONE, 2010). We thank Drs. S. Oda, K. Yoshida, and Y. Iino (U. Tokyo) for suggestions on microfluidics; M. Hendricks and Y. Zhang (Harvard) for aqueous 2-nonanone stimulation; and E. Busch and M. de Bono (MRC) for gaseous microfluidic stimulation.

566C

The Novel Conserved Gene *C44B9.1* Regulates *C. elegans* Behavior through G Protein Signaling and Likely Regulates Synaptic Vesicle Release. **N. Paquin**, A. Froehlich, D. Omura, H.R. Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

C. elegans modulates its locomotion and egg-laying behavior in response to environmental signals and its past feeding experience. For example, after encountering food animals slow their locomotion and lay eggs. In the absence of food, worms increase their locomotion and refrain from laying eggs. Animals that have been well-fed slow their locomotory rates when they encounter food (a behavior termed the "basal slowing response") less than do animals that have been food-deprived (termed the "enhanced slowing response"), presumably because food-deprived animals have a greater need for staying in the proximity of food.

We performed a mutagenesis screen and isolated mutations that cause well-fed animals to behave as if they had been food-deprived, thus modifying their behavioral state by uncoupling the extent of food-dependent slowing from past feeding experience. We isolated alleles of an uncharacterized gene that is highly conserved from *C. elegans* to humans, *C44B9.1*. Mutations in *C44B9.1* cause a severe locomotion defect of well-fed animals in the presence of food but have little effect on the locomotion of well-fed animals in the absence of food. Well-fed *C44B9.1* mutant animals also lay eggs at abnormally late developmental stages, as if they had been food-deprived. *C44B9.1* is expressed in most if not all neurons and is possibly neural-specific. The pharmacological sensitivity profile of *C44B9.1* mutant animals suggests a presynaptic role for *C44B9.1*. The behavioral phenotype and drug-sensitivity profile of *C44B9.1* animals are similar to those of mutants with defects in the regulation of synaptic vesicle exocytosis, such as *unc-64* (syntaxin) and *unc-31* (CAPS), suggesting that *C44B9.1* might regulate synaptic vesicle release. The C44B9.1 protein localizes to synapse rich-areas of neuron processes. Like synaptic vesicles, C44B9.1 fails to be transported and accumulates in neuronal cell bodies of animals mutant for the kinesin-like protein UNC-104/KIF1A. *C44B9.1* mutations suppress the egg-laying constitutive defect of mutants of the Gαo gene *goa-1* and its downstream effector diacylglycerol kinase gene *dgk-1*, suggesting that *C44B9.1* functions downstream of or in parallel to this inhibitory G protein signaling pathway. In short, these results suggest that *C44B9.1* and possibly its homologs are regulators of synaptic vesicle release.

We are currently investigating the pathway in which *C44B9.1* functions and identifying physical partners. In addition to characterizing a conserved protein likely involved in synaptic vesicle release, these studies of *C44B9.1* might also define a genetic pathway that regulates behavioral states associated with food experience.

567A

Mutants that fail to respond to exogenous serotonin identify new serotonin signaling genes. **Judy S. Pepper**, Michael R. Koelle. Department of Molecular Biophysics and Biochemistry Yale University, New Haven, CT.

Signaling by the neurotransmitter serotonin is thought to be reduced in the brain during depression, but the mechanisms that alter serotonin signaling remain unclear. We are using a genetic approach in *C. elegans* to delineate the molecules that mediate serotonin signaling to shed light on this issue. Several *C. elegans* behaviors are regulated by serotonin signaling, including locomotion, egg-laying, and pharyngeal pumping. Serotonin released when food-deprived animals encounter food slows locomotion, and high concentrations of exogenous serotonin paralyzes animals, presumably by exaggerating this response. To identify additional signaling molecules that mediate the effects of serotonin, we carried out a screen for mutants resistant to the paralytic effects of exogenous serotonin. Individual F1 progeny of mutagenized animals were cultured for one generation in 96-well microtiter dishes and 30 mM serotonin was added. This concentration paralyzes nearly all wild-type animals within minutes, and we looked for wells in which ~25% of the F2 progeny (mutant homozygotes) continued to thrash. We recovered 13 serotonin resistant mutants from a screen of 12,000 mutagenized haploid genomes. Using single nucleotide polymorphism (SNP) mapping and sequencing we identified the mutations causing serotonin resistance in the five strongest mutants.

Four mutations were in genes previously known to be required for serotonin response. One mutation was in the gene encoding G protein coupled receptor SER-4 and two were in the *goa-1* G protein gene, suggesting serotonin acts through SER-4 to activate GOA-1 signaling and slow locomotion. An additional mutation was in the serotonin-gated chloride channel MOD-1, suggesting serotonin has an additional role in slowing locomotion through this receptor. We found that the fifth strong serotonin resistance mutation, *vs119*, changes a glycine to a glutamate in the enzymatic core of the catalytic subunit of a conserved protein complex whose only known biochemical function is to post-translationally modify other proteins on lysine residues by acetylating them. Two additional alleles for this lysine acetylase gene as well as a mutation that disrupts another subunit of the complex all cause strong resistance to exogenous serotonin. We hypothesize that *vs119* may function in serotonin signaling by modifying one or more of the other proteins required for serotonin response: the SER-4 or MOD-1 serotonin receptors, or the G protein, GOA-1. We previously found that the GOA-1 protein runs a series of species of different charge on an isoelectric focusing gel, and we are currently testing the hypothesis that this is due to variable modifications of GOA-1 by the acetylase.

568B

An ADAMTS-Like protein is required for acetylcholine receptor localization. **Berangere Pinan-Lucarre**, Valerie Robert, Jean-Louis Bessereau. Institut de Biologie de L'Ecole Normale Superieure, Paris, France.

The *C. elegans* neuromuscular junction (NMJ) is a genetically tractable model to decipher the molecular mechanisms underlying synaptic formation. At the NMJ both heteromeric and homomeric ionotropic acetylcholine receptors (AChRs) mediate fast synaptic neurotransmission. The anthelmintic drug levamisole activates heteromeric AChRs (the levamisole-sensitive AChRs or L-AChRs) and causes muscle hypercontraction and death at high concentration. Screens for resistance to levamisole have been conducted for decades and identified genes required for biosynthesis, assembly, trafficking and localization of L-AChRs at the synapse. Specifically, screening for mutants that adapt after overnight exposure to levamisole identified 3 proteins, LEV-9, LEV-10 and OIG-4, which build an extracellular scaffold for clustering L-AChRs at cholinergic NMJs. However, how this scaffold localizes at synapses is not well understood.

To directly identify novel genes required for proper localization of L-AChRs, we recently conducted a visual screen for abnormal localization of fluorescently-tagged L-AChRs. Specifically, the TagRFP sequence was introduced in the L-AChR subunit locus *unc-29* using the MosTIC technique. Animals were mutagenized with EMS and the F2 progeny was screened for abnormal fluorescence pattern.

We screened 3,000 haploid genomes so far and isolated 29 mutants. Half were resistant to levamisole and represented alleles of genes encoding L-AChR subunits or their scaffolding proteins. In addition, the screen identified novel targets that are not resistant to levamisole and that have no obvious locomotory defects.

Among these novel genes, we characterized in more details a mutant displaying redistribution of L-AChRs at extrasynaptic areas of muscle cell membrane. By rough SNP mapping and whole genome sequencing, we identified a mutation in a gene coding for a non characterized ADAMTS-Like protein (A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif-Like protein). These proteins are predicted to be secreted and to interact with basal membrane components.

An overview of mutants retrieved during the screen together with the ongoing analysis of the ADAMTS-Like mutant will be presented at the meeting.

This work was supported in part by the Fondation pour la Recherche Medicale.

569C

ACR-12 receptor signaling complexes regulate motor circuit activity. **Hilary Prescott**, Marian Haburcak, Michael Francis. University of Massachusetts Medical School, Worcester, MA.

The anatomical basis for the generation of nematode movement has been well described. Excitatory cholinergic motor neurons (MNs) make synaptic contacts onto both muscle cells and GABA MNs that, in turn, make inhibitory synaptic contacts onto contralateral musculature. Several subunits of the nicotinic acetylcholine receptor (nAChR) superfamily are expressed in MNs, suggesting cholinergic signaling is important for coordinating MN activity. In previous work, we isolated several loss-of-function alleles of the nAChR subunit gene *acr-12* (Barbagallo et al., 2010) and we have found that *acr-12* is expressed in both GABA and ACh MNs. We are now studying the functions of *acr-12* signaling complexes in the motor circuit. While the movement of *acr-12* mutants is overtly normal, closer examination revealed that the amplitude of body bends during runs of forward movement was highly variable. Additionally, body bend amplitude was, on average, reduced and we observed hypersensitivity to the acetylcholine esterase inhibitor aldicarb. Cell specific expression of *acr-12* in GABA MNs but not ACh MNs was sufficient for rescuing each of these phenotypes. Analysis of the subcellular localization of the rescuing ACR-12::GFP fusion protein also suggested ACR-12 receptor complexes play distinct roles across the two motor neuron classes. Expression of ACR-12::GFP specifically in GABA MNs revealed fluorescence at discrete sites along neuronal processes, suggesting ACR-12 may cluster at synapses on GABA MNs. In contrast, we observed diffuse fluorescence upon cell specific expression of ACR-12::GFP in ACh MNs. Our behavioral, pharmacological and expression studies point to a key role for *acr-12* in the regulation of GABA MN activity. To test this hypothesis directly, we measured the frequency of GABA synaptic events in whole-cell patch clamp recordings from the muscles of *acr-12* mutants and observed a roughly 40% reduction in event frequency. In parallel with our loss of function approach we have pursued a gain of function approach. Transgenic animals expressing *acr-12(gf)* exhibit spontaneous muscle contractions, similar to strains carrying a previously characterized gain of function *acr-2* allele (Jospin et al., 2009). Cell specific expression of *acr-12(gf)* in ACh MNs was sufficient to generate this behavior, and spontaneous contractions were partially suppressed by a loss of function mutation in *acr-2*. Our analysis of *acr-12(lf)* and *acr-12(gf)* strains has revealed an interesting paradox: the loss of function analysis emphasizes a role for *acr-12* in GABA MNs while our gain of function work points toward an important role in ACh MNs. We are currently investigating the basis for this inconsistency and we will present our findings.

570A

A neuropeptide-mediated stretch response links muscle contraction to changes in neurotransmitter release. Zhitao Hu[1], **Edward Pym[1]**, Kavita Babu[1], Amy Vashlishan Murray[1,2], Joshua Kaplan[1]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Communication Sciences & Disorders, Emerson College, Boston, MA.

Neuropeptides represent a vast and chemically diverse set of neurotransmitters. Pro-neuropeptides are packaged into large dense core vesicle (DCV) precursors, where they are

processed into active forms by co-packaged enzymes. Many, and perhaps all, neurons express and secrete neuropeptides. Expression of specific neuropeptides is often utilized as a marker to distinguish subclasses of neurons. Despite their widespread expression, relatively little is known about how specific neuropeptides function within circuits. Secretion of neuromodulatory peptides has often been proposed as a mechanism for regulating synaptic efficacy and producing adaptive changes in behavior; however, genetic studies of neuropeptide function have primarily focused on endocrine functions. Much remains to be learned about how neuropeptides shape the function of these and other behavioral circuits. The nematode C. elegans has been utilized as a genetic model to study neuropeptide function. The genome sequence predicts 115 proneuropeptide genes, encoding 250 different mature peptides. Many of these predicted peptides have been confirmed by mass spectrometry and mutations have been described that disrupt proneuropeptide processing. neuromuscular signaling. We show that a brief treatment with the cholinesterase inhibitor aldicarb induces a form of presynaptic potentiation whereby ACh release at neuromuscular junctions (NMJs) is doubled. Aldicarb-induced potentiation was eliminated by mutations that block processing of pro-neuropeptides, by mutations inactivating a single pro-neuropeptide (NLP-12), and by those inactivating an NLP-12 receptor (CKR-2). NLP-12 expression is limited to a single stretch-activated neuron, DVA. Analysis of a YFP-tagged NLP-12 suggests that aldicarb stimulates DVA secretion of NLP-12. Mutations disrupting the DVA mechanoreceptor (TRP-4) decreased aldicarb-induced NLP-12 secretion and blocked aldicarb-induced synaptic potentiation. Mutants lacking NLP-12 or CKR-2 have decreased locomotion rates. Collectively, these results suggest that NLP-12 mediates a mechanosensory feedback loop that couples muscle contraction to changes in presynaptic release, thereby providing a mechanism for proprioceptive control of locomotion.

571B

A Gq and CaMKII-dependent serotonergic circuit in *C. elegans* aversive olfactory learning. **Yuqi Qin**[1,3], Xiaodong Zhang[2,3], Yun Zhang[1,3]. 1) Department of Organismic and Evolutionary Biology; 2) Molecular and Cellular Biology; 3) Center for Brain Science, Harvard University, Cambridge, MA 02138.

Serotonin regulates many animal behaviors across phylogeny. It has been shown that increase of serotonin level is correlated or causally linked with enhancement of neural plasticity. However, very little is known on how serotonergic system is modulated by experience. Compared with mammals, *C. elegans* has a simpler serotonergic system that serves as a good model to study this interesting question. Here we report that a sensory neural circuit engages CaMKII and Gq pathways to regulate serotonin induction for aversive olfactory learning of harmful food sources.

We previously showed that aversive training increases the serotonin content and the *tph-1* transcription in ADF serotonergic neurons. We also found that increased serotonin potentiates learning. To address how aversive training enhances serotonin signaling in ADF, we employed experimental approaches including neuronal-specific rescue, calcium imaging and behavior analysis. We found that food odors trigger calcium influx into ADF and this response is strengthened in trained animals. Because CaMKII is sensitive to calcium signal, we tested the role of CaMKII in the training process. We found that UNC-43(CaMKII homolog) is required for both serotonin induction and learning, and that UNC-43 functions cell-autonomously in ADF for these traits. In addition, constitutively activated form of UNC-43 generates higher level of *tph-1* transcription in ADF. These results are consistent with the possibility that training experience activates ADF neurons and increases calcium influx, which leads to calcium-dependent activation of UNC-43, resulting in elevated TPH-1 and serotonin. Gq has been shown to act in a CaMKII pathway for locomotion and egg laying. Consistently, we found that EGL-30(Gq homolog) and one of its downstream effectors UNC-73 are both required for the induction of *tph-1* transcription and serotonin. However, surprisingly, all other components in the CaMKII pathway including PLCβ, calcineurin and Go are not essential. These results suggest that UNC-43 and EGL-30 function in novel pathways for the modulation of serotonergic system by training. To further characterize the role of EGL-30, we identified that the function of EGL-30 in both AWB and AWC are required to rescue the mutant phenotype in *tph-1* regulation.

Together our results propose that aversive training activates ADF, leading to CaMKII-dependent induction of serotonin signaling, translating training experience into the alteration of serotonergic system through a novel signaling cascade.

572C

Transgenic expression of mammalian neuroligin rescues the phenotypes of *C. elegans* neuroligin-deficient (*nlg-1*) mutants. Jerrod Hunter[1,2], Greg Mullen[1], Ellie Mathews[1], Jessica Heatherly[1,3], **Jim Rand**[1,2,3]. 1) Gen Models Disease Res Program, Oklahoma Med Res Foundation, Oklahoma City, OK; 2) Department of Cell Biology, University of Oklahoma Health Science Center, Oklahoma City, OK; 3) Oklahoma Center for Neuroscience, University of Oklahoma Health Science Center, Oklahoma City, OK.

Neuroligins are postsynaptic cell adhesion proteins that bind specifically to presynaptic membrane proteins called neurexins. There are four neuroligin genes in humans, and mutations in the genes encoding neuroligin-3 and neuroligin-4 are associated with autism spectrum disorders (ASDs) in some families. *C. elegans* has a single neuroligin gene (*nlg-1*); null mutants are viable and are not deficient in any major motor functions. However, when we examined more subtle sensory behaviors, we found that *nlg-1* mutants have selective chemosensory deficits as well as deficits in the processing of thermosensory information. They also have difficulty integrating complex sensory inputs, such as simultaneous presentation of an attractant and a repellant. Although sensory problems are not part of the official diagnostic criteria for ASDs, difficulties with the processing and/or integration of sensory inputs are often part of the presentation. It is therefore particularly intriguing that *nlg-1* mutants have deficits in the processing of conflicting sensory inputs. *nlg-1* mutants are also hypersensitive to oxidative stress (*i.e.*, exposure to paraquat); this is an unexpected phenotype for a synaptic mutant. Like many other stress-sensitive mutants, *nlg-1* mutants also have a reduced lifespan and an increased level of oxidative protein damage. We obtained cDNAs for human neuroligin-4 (hNLGN4) and rat neuroligin-1 (rNLGN1) from Thomas Südhof (Stanford) and replaced the mammalian signal sequences and 3'UTRs with their nematode counterparts. We expressed these modified cDNAs in *nlg-1* mutants as stable transgenes under control of the *C. elegans nlg-1* promoter. We then compared the behaviors and paraquat sensitivity of animals expressing the mammalian transgenes to wild-type animals, *nlg-1* null mutants, and *nlg-1* mutants expressing a nematode neuroligin transgene. We found that transgenic expression of mammalian neuroligin rescued both the behavioral and oxidative stress phenotypes of *nlg-1* mutants. The *C. elegans* and mammalian neuroligins, therefore, appear to be functionally equivalent (including having the ability to prevent or counteract oxidative stress). This raises the possibility that neuroligin may play a role in the prevention of oxidative stress in mammals. (Supported by a grant from Autism Speaks).

573A

UNC-41/stonin functions with AP2 to recycle synaptic vesicles in *C. elegans*. Greg Mullen[1], Kiely Grundahl[1], Mingyu Gu[2], Shigeki Watanabe[2], Robert Hobson[2], John McManus[1], Ellie Mathews[1], Erik Jorgensen[2], **Jim Rand**[1]. 1) Gen Models Disease Res Program, Oklahoma Med Res Foundation, Oklahoma City, OK; 2) Department of Biology, University of Utah, Salt Lake City, Utah.

The recycling of synaptic vesicles requires the recovery of vesicle proteins and membrane. Stonin 2 is a mammalian synaptic protein that is thought to act as an adaptor to recruit the synaptic vesicle protein synaptotagmin to sites of synaptic vesicle endocytosis (the *Drosophila* stonin ortholog is STNB). To characterize the role of stonins in synaptic vesicle endocytosis, we examined *unc-41* mutants; the *unc-41* locus encodes the stonin/STNB ortholog in *C. elegans*. The *unc-41* gene encodes two protein isoforms that are differentially expressed in the *C. elegans* nervous system: UNC-41A is expressed in all neurons, while UNC-41B is expressed in a subset of neurons, including the GABAergic motorneurons in the ventral nerve cord. The UNC-41 proteins are localized to synapses, similar to the pattern seen with SNT-1. We examined the synaptic localization of GFP-tagged SNT-1 in *unc-41* mutants. Consistent with studies in *Drosophila*, tagged synaptotagmin is dimmer and mislocalized in *unc-41* mutants, and fluorescence is also observed in non-synaptic regions such as commissures. In contrast, GFP::UNC-41 is properly localized in *snt-1* mutants, demonstrating a unidirectional relationship for localization. Ultrastructural analysis indicates that *unc-41* mutants exhibit a defect in membrane retrieval: synaptic vesicle numbers are reduced by 50% compared to wild type. We conclude that UNC-41 is required to recruit synaptotagmin and recover membrane during synaptic vesicle endocytosis. *apm-2* mutants (pka *dpy-23*), lacking the μ2 subunit of AP2, exhibit a similar deficit in synaptic vesicle endocytosis. Because in mammals, both μ2 and stonin 2 can bind synaptotagmin, we considered the possibility that these proteins carried out overlapping functions in *C. elegans*. However, nematodes lacking both UNC-41 and μ2 (*unc-41 apm-2* double mutants) exhibit a decrease in synaptic vesicle number comparable to either single mutant. Our data suggest that UNC-41 and μ2 function in the same pathway for synaptic vesicle membrane recycling. (Supported by NIH grants NS034307 to E.J. and NS033187 and GM059642 to J.R.).

574B

The core apoptotic executioner proteins CED-3 and CED-4 promote initiation of neuronal regeneration in *Caenorhabditis elegans*. B. Pinan-Lucarre[1,2], C.V. Gabel[3], **C.P. Reina**[1], M. Driscoll[1]. 1) Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ; 2) Institut de Biologie de l'Ecole Normale Supérieure, Paris, France; 3) Department of Physiology and Biophysics, Boston University School of Medicine, Boston, MA.

How neurons in their native environments respond to localized physical disruptions such as axon severing is poorly understood. Femtosecond laser surgery allows precise cutting of individual axons within living transparent *Caenorhabditis elegans* such that regeneration can be observed in vivo. We applied this technology to investigate the role of the cell death machinery in the neuronal response to laser severing of ALM touch neurons visualized by Pmec-4GFP in young adult *C. elegans*. Unexpectedly, we found that CED-3 caspase, extensively characterized as the essential executioner protease in apoptosis, acts to promote early events in neuronal regeneration including outgrowth and reconnection. Time-lapse imaging of *ced-3* mutants revealed defects in the early stage of regenerative growth cone formation, i.e., the sprouting of short, often transient, exploratory processes. In addition, CED-3 also functions in early reconnection events as *ced-3* neurons are deficient in time to reconnection. The apoptotic caspase activator CED-4/Apaf-1 also is required for efficient regeneration, but the upstream apoptotic regulators CED-9/Bcl2 and the BH3 domain proteins EGL-1 and CED-13 are not required, indicating a regulatory mechanism distinct from the classical *C. elegans* apoptotic pathway. Regeneration also depends on the calcium-storing ER chaperone calreticulin *crt-1*, which contributes to the calcium flux that occurs immediately after axotomy.

Our initial work revealed an unexpected reconstructive role for proteins known to orchestrate cell death that may be conserved in higher organisms. A key question is how is CED-3 caspase locally activated and controlled to promote regeneration activities rather than acting in cell killing? Epistasis experiments suggest that *ced-3*, *ced-4*, and *crt-1* function within the same regenerative pathway; therefore, we propose the following

regeneration model: Axotomy induces a localized calcium influx amplified by CRT-1, calcium binds to putative CED-4 EF-hand calcium binding domains to activate CED-4 oligomerization, which promotes procaspase CED-3 binding and stimulates activating protease cleavage (proposed CED-4/CED-3 interaction similar to apoptosis). Current work is focused on determining whether CED-4 EF-hand domains bind calcium and whether they are required for CED-4 function in regeneration.

575C

Control of acetylcholine receptor maturation and ER homeostasis by a new transmembrane complex. **Magali Richard**[1], Thomas Boulin[1], Janet Richmond[2], Jean-Louis Bessereau[1]. 1) U1024, IBENS, PARIS, France; 2) Department of Biology, University of Illinois, Chicago, IL, USA.

Nicotinic acetylcholine receptors (AChRs) are ligand-gated ion channels that have been widely conserved throughout evolution and are implicated in numerous physiological and pathological processes in humans. In *C. elegans*, levamisole activates AChRs present at the neuromuscular junction causing death at high concentrations. Using *Mos1*-mediated insertional mutagenesis, we performed a large screen for mutants partially resistant to levamisole. Among 69 resistant strains, we identified one mutant allele of the previously uncharacterized gene, which we tentatively named *emc-6*. *emc-6(kr150)* mutants rapidly paralyze on levamisole but survive and adapt to prolonged exposure to the drug. This decreased levamisole sensitivity can be rescued by providing an *emc-6* genomic fragment. *emc-6* encodes a 111 amino-acid protein containing two predicted transmembrane domains. It is widely conserved from plant to human, but remains uncharacterized in any species. Based on immunostaining, *in vivo* imaging, western-blot analysis and electrophysiological recording, we demonstrated that AChR expression was reduced by 80 percent in *emc-6* mutants, yet the remaining functional AChRs were still clustered at neuromuscular junctions. Based on transcriptional reporter constructs, EMC-6 appears to be expressed ubiquitously. Using tissue-specific rescue experiments, we demonstrated that it acts cell autonomously in body-wall muscle to restore wild-type AChR function. *emc-6(kr150)* is likely a hypomorphic allele because *kr150* over deficiency is lethal and *emc-6* RNAi causes embryonic and larval lethality. Recently, the yeast ortholog of EMC-6 was identified as a member of the ER Membrane protein Complex (EMC). In *Saccharomyces cerevisiae*, loss of EMC leads to accumulation of misfolded proteins and activation of the Unfolded Protein Response (UPR). This complex is composed of six proteins highly conserved through evolution. Preliminary results of immunocytochemistry and colocalization experiments with known intracellular compartments markers indicate that *C. elegans emc-6* might localize in ER. RNAi targeting of additional *C. elegans* EMC members causes developmental defects and activation of stress reporters. These results suggest that EMC is required for ER homeostasis in metazoans, and especially for proper folding or assembly of AChR subunits. Current progress on *emc-6* function will be presented at the meeting. Specifically, AChR biosynthesis is being characterized as well as EMC function in UPR response.

576A

Single-neuron photoactivation by cell-specific expression of Channelrhodopsin-2 using the cre-lox system to analyze habituation in sensory neural circuits. **Cornelia Schmitt**[1], Alexander Gottschalk[1,2]. 1) Institute of Biochemestry, Goethe-University Frankfurt, Frankfurt am Main, Germany; 2) Frankfurt Institute for Molecular Life Sciences, Goethe-University, Germany.

Habituation is a simple form of non-associative learning, in which multiple repetitions of a stimulus cause a progressive reduction in response. Habituation of the withdrawal reflex in response to gentle touch or tap is well characterized, yet little is known about the mechanisms of habituation, and where in the signalling cascade leading to withdrawal habituation is effected: Does it occur at the level of the primary sensory receptors, signalling within the sensory neurons, at their output synapses or in the downstream interneuron network? Previously, we showed that Channelrhodopsin-2 (ChR2) mediated photostimulation of mechanosensory neurons induces withdrawal behaviour and habituation [1]. To this end, we expressed ChR2 in touch neurons. Expression of ChR2 in the downstream command interneurons also could induce withdrawal behaviour, yet, promoters active in these cells (e.g. pnmr-1, pglr-1) are not highly specific for the backward command neuron AVA. Thus, optogenetic stimulation will also affect other cells like the forward command neuron PVC, and consequently will interfere with the backward escape response. To achieve highly specific activation of ChR2 in the AVA neuron, we use the cre-lox system [2], combining two promoters to render expression at the intersection of the two expression domains. We tested the transgenic animals for photoinduced withdrawal and habituation of the response. Some habituation is apparent, though much less pronounced than in touch receptor neurons neurons (TRNs). An additional sensory neuron inducing withdrawals is the polymodal aversive neuron ASH, which detects harmful or toxic chemicals. A strain expressing ChR2 only in the ASH neuron [3] was recently generated using FLP recombinase [4] by the Schafer lab, who kindly provided this strain to us. Habituation in ASH neurons is also much less pronounced or absent, as could be expected for a nociceptor (similar findings were made for the PVD nociceptor neuron, see abstract by Husson et al.). We also test for potential cross-habituation of the two sensory pathways (TRNs and ASH), which will be assessed by combining photo-stimuli and 'natural' stimuli, and which should, if at all, occur at the level of the interneurons. To identify the synapses which are responsible for habituation effects we will specifically interfere with chemical and gap junction signalling and test for effects on withdrawal responses and habituation. 1. Nagel et al. (2005) Curr. Biol. 15: 2279-84 2. Macosko et al. (2009) Nature 58:1171-5 3. Ezcurra et al. (2011) EMBO J 1-13 4. Davis et al. (2008) PLoS Genetics 4: e1000028.

577B

Carbon dioxide avoidance is mediated by a diverse set of sensory neurons in *C. elegans*. **Z. Soltesz**, A. J. Bretscher, M. de Bono. MRC Laboratory of Molecular Biology, Cambridge, United Kingdom.

Monitoring carbon dioxide levels has a twofold importance for many living organisms: CO_2 can act as a sensory cue for food or other animals, while regulating internal CO_2 levels is an important part of homeostasis. *C. elegans* relies on diffusion for gas exchange, and avoids CO_2 levels as low as 1%. We are interested in the neural and molecular mechanisms underlying the *C. elegans* CO_2 avoidance behaviour.

Mutants defective in the tax-4 or tax-2 genes, which encode the α and β subunits, respectively, of a cGMP-gated ion channel, showed reduced CO_2 avoidance in behavioural assays[1,2]. By expressing tax-2 cDNA from neuron-specific promoters in tax-2 mutants to rescue the avoidance behaviour, and by imaging neurons using the genetically encoded calcium indicator YC3.60, we have shown that sensory neurons previously implicated in oxygen, temperature, and salt-sensing, including BAG, AFD and ASE, are CO_2 sensors as well[3].

We have observed both persistent and transient cell-intrinsic calcium-responses in several sensory neurons, suggesting that CO_2 stimuli could modulate neural activity in *C. elegans* in a complex manner. We are therefore investigating how CO_2 stimuli can affect neural processing in downstream neurons.

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[2] Elissa A. Hallem and Paul W. Sternberg, Acute carbon dioxide avoidance in Caenorhabditis elegans. *PNAS* 105(23):8038-8043

[3] Andrew Jonathan Bretscher, Eiji Kodama-Namba, Karl Emanuel Busch, Robin Joseph Murphy, Zoltan Soltesz, Patrick Laurent and Mario de Bono, Temperature, Oxygen, and Salt-Sensing Neurons in C. elegans Are Carbon Dioxide Sensors that Control Avoidance Behavior. *Neuron* 69(6):1099-1113.

578C

Notch signaling regulates synaptic transmission at the neuromuscular junction. **Altar Sorkaç**, Komudi Singh, Michael DiIorio, Anne Hart. Neuroscience Dept, Brown University, Providence, RI.

The role of Notch signaling during development has been characterized in many organisms, including the nematode *C. elegans*. In the canonical pathway, Notch receptors are cleaved by the γ-secretase/presenilin complex upon ligand binding, releasing the intracellular domain that enters the nucleus to act as a transcription factor. In *C. elegans*, Notch receptors LIN-12 and GLP-1 are activated by DSL ligands working with DOS-motif proteins that act as co-activators. Here, we show that Notch signaling impacts synaptic transmission. Loss of two DOS co-ligands, OSM-7 and OSM-11, resulted in hypersensitivity to inhibitors of cholinesterase (Hic) on aldicarb. *glp-1(gf)* or *glp-1(lf)* had no impact on aldicarb response; whereas *lin-12* null animals were Hic and *lin-12(gf)* mutants were Ric (Resistance to inhibitors of cholinesterase). These findings suggest a model in which OSM-7 and OSM-11 act on LIN-12 Notch receptors to regulate synaptic transmission at the neuromuscular junction.

579A

WDR-23 mediates synaptic transmission by regulating the transcription factor SKN-1 in motor neurons. **Trisha Staab**, Trevor Griffen, Derek Sieburth. Univ Southern California, Los Angeles, CA.

Oxidative and cellular stresses can contribute to neurodegeneration, but it is unclear how synaptic transmission is altered in response to stress. We show that a conserved stress-response pathway activated by the transcription factor SKN-1/Nrf2 regulates neurotransmission at the C. elegans neuromuscular junction. We found that the WD40 repeat protein WDR-23, which is reported to target SKN-1 for degradation, is required for normal neurotransmitter secretion. *wdr-23* null mutants are resistant to the paralytic effects of aldicarb, have defects in synaptic vesicle exocytosis and have reduced neuropeptide secretion from motor neurons. A WDR-23-GFP fusion protein localizes to presynaptic terminals in motor neurons. We found the primary function of WDR-23 in neurons is to negatively regulate SKN-1 activity—*skn-1* mutants are hypersensitive to aldicarb, whereas worms over-expressing functional SKN-1-GFP specifically in neurons are resistant to aldicarb. Furthermore, the aldicarb resistance of *wdr-23* mutants is completely dependent on the presence of SKN-1—animals lacking both *wdr-23* and *skn-1* are identical in their response to aldicarb as *skn-1* mutants. A transcriptional *skn-1* reporter reveals that SKN-1 is expressed in cholinergic motor neurons. Furthermore, a SKN-1-GFP fusion protein can be seen in both the cytoplasm and nuclei of motor neurons, where its distribution is regulated by WDR-23. To identify transcriptional targets of SKN-1 that specifically regulate synaptic transmission, we profiled the transcriptomes of *wdr-23* mutants compared to wild type controls using whole genome RNAseq. As expected, most genes that are up-regulated in *wdr-23* mutants are involved in detoxification, including the known SKN-1 target *gcs-1*. RNAi knockdown of the top 30 up-regulated genes has no impact on the aldicarb responsiveness of *wdr-23* mutants, suggesting that, independently, these genes do not regulate neurotransmission. Several genes with known roles in neuronal function are also up-regulated in *wdr-23* mutants. One of these is ACR-2, a subunit of a presynaptic acetylcholine receptor, which is specifically expressed in motor neurons and increases three fold by RNAseq. The *acr-2* promoter contains one predicted SKN-1 binding site that is conserved among nematodes. Using qRT-PCR and an *acr-2* reporter construct, we confirm that ACR-2 expression increases significantly in *wdr-23* mutants, and this increase is

abolished in *skn-1* mutants. Together, our results suggest a model in which the abundance of SKN-1 in neurons is regulated by the WD40 adaptor protein WDR-23. Furthermore, neurotransmission is mediated by SKN-1-dependent transcriptional programs that may include the regulation of the acetylcholine receptor, ACR-2.

580B

A conserved role of α-adducin (ADD-1) in memory, synaptic plasticity, and in AMPA-type glutamate receptor dynamics. Vanja Vukojevic[1,2], Leo Gschwind[1,3], Christian Vogler[1,4], Philippe Demougin[1,2], Dominique de Quervain[3,4], Andreas Papassotiropoulos[1,2,4], **Attila Stetak[1,2,4]**. 1) Univ. of Basel, Dept. of Psychology, Division of Molecular Neuroscience, Basel, Switzerland; 2) Univ. of Basel, Biozentrum, Life Sciences Training Facility, Basel, Switzerland; 3) Univ. of Basel, Dept. of Psychology, Division of Cognitive Neuroscience, Basel, Switzerland; 4) Univ. of Basel, University Psychiatric Clinics, Basel, Switzerland.

Identifying the molecular mechanisms that underlie learning and memory are one of the major challenges in neuroscience. Here, we investigated the role the actin-binding protein α-adducin (*add-1*) in synaptic plasticity during associative learning. We found that *add-1* loss of function mutant worms show normal chemotaxis, locomotor behavior, and associative learning but they have an impaired short- and long-term memory. We show that α-adducin has a crucial role in memory, but not in the learning process. Particularly, adducin is required *in vivo* for long-term consolidation of synaptic size expansion, changes in the post-synaptic density, and sustained increase of AMPA-type glutamate receptor (GLR-1) content in the synapses. ADD-1 also plays an important role in long lasting changes of GLR-1 turnover dynamics at the synapse. ADD-1 likely function through capping of the fast growing barbed end of actin filaments, as the actin polymerization inhibitor, cytochalasin B, suppressed the memory defect of *add-1(tm3760)* mutant worms. Finally, using tissue specific rescue experiments we demonstrate that α-adducin controls the storage of memories cell-autonomously in AVA command interneuron by consolidating altered synaptic structures, and through the maintenance of increased amount of AMPA-type glutamate receptor at synapses. In addition to *C. elegans*, genetic variability of the ADD1 (human adducin alpha) gene in humans was associated with episodic memory performance in 1086 healthy young adults. Specifically, carriers of the A allele of the intragenic single nucleotide polymorphism (SNP) rs10026792 had better performance than A allele non-carriers in a picture-based, delayed free recall task. Finally, expression of human α-adducin in *add-1(tm3760)* mutant worms efficiently compensated for loss of add-1 gene. Taken together our findings support a role for α-adducin in memory both in nematodes and in humans. ADD-1 likely acts through capping of actin filaments at the fast growing barbed-end in AVA neuron, suggesting that dynamic remodeling of the actin cytoskeleton in spines during learning has to be followed by stabilization of actin filaments for efficient memory storage.

581C

Subcellular localization and protein interactions of PTL-1 in *Caenorhabditis elegans*. **Kathleen M. Susman**, Joshua Sturm, Celia McKee, Eunice Chou, Nancy J. Pokrywka. Dept Biol, Vassar Col, Poughkeepsie, NY.

PTL-1, a microtubule-associated protein of the structural MAP2/tau family, is the sole member of this gene family in *Caenorhabditis elegans*. Structural MAPs of the MAP2/tau family include the vertebrate proteins MAP2, MAP4 and tau, and homologs like PTL-1 (Protein with Tau-like repeats-1) in other animals, including *Caenorhabditis elegans* (*C. elegans*) and *Drosophila melanogaster*. MAP2 and tau are expressed almost exclusively in neurons in mammals and are thought, by virtue of their microtubule-stabilizing activity in vitro, to regulate microtubule networks in dendrites and axons. MAP2, restricted to the somatodendritic compartment of vertebrate neurons, may be involved in the establishment or maintenance of dendritic polarity. Tau, localized to the axonal subcompartment in vertebrate neurons, may provide a similar function in axons. Tau hyperphosphorylation has been associated with a number of neurodegenerative conditions, so-called tauopathies, including Alzheimer's disease. In addition, this group of proteins may also function in the regulation, formation or maintenance of protein assemblies. Understanding the normal functions of this protein in less complex model organisms than mammals will shed light on the processes affected by its dysfunction in disease. Sequence analysis of available invertebrate genomes revealed a number of single, putative tau-like genes with high similarity to *ptl-1*. The *ptl-1* gene is expressed in a number of cells, most notably mechanosensory neurons and knockout worms exhibit a touch response deficit. *ptl-1* is also expressed in cells during the comma stage of development and knockout mutants exhibit an egg hatching defect. We examined the subcellular protein localization of PTL-1 using immunocytochemistry; PTL-1 protein is present both in the cytoplasm throughout the mechanosensory neuron, as well as localized to discrete puncta. Interacting proteins were identified using immunoprecipitation followed by MALDI-TOF analysis of trypsin-digested peptides.

582A

New Approach in Systems Neuroscience in C.elegans. **Hiroshi Suzuki[1,2]**. 1) Tanz Centre for Research in Neurodegenerative Diseases; 2) Dept Physiology, University of Toronto, Canada.

C.elegans offers us numerous advantages to use this model organism in systems neuroscience, amongst which is its small and well-defined neuronal circuit. Unlike the brains of overwhelming complexity in other animals, it is feasible to ask how the small "brain" of worms processes environmental cues to generate behavior. Our approach has been to monitor neuronal activity in live animals by using genetically encoded calcium sensors, starting with sensory neurons, such as mechanosensory neurons and salt-sensory ASEL & ASER neurons (2001 and 2003 Int'l worm meetings, respectively). Although this *in vivo* calcium imaging has been proved powerful, we have had two frustrating limitations: the number of target neurons (mostly one) and lack of concomitant behavioural data. In order to overcome the limitations, we have developed a new imaging system, which combines two independent imaging units to enable two simultaneous imaging, along with "worm tracker" functionality to perform imaging on a freely moving worm. The two imaging units are fully functional upright and inverted microscope units, and allow us various applications, such as simultaneous calcium imaging in two different targets or one calcium imaging at high-magnification on one side and capturing the whole-body image at low-magnification on the other side for fine analysis of behavior. The tracking is automated, and we can address the direct correlation of the neuronal activity and generated behavior from the simultaneously acquired data. We are applying the new method to further dissecting the salt-chemotaxis neuronal circuit by correlating the activities of the sensory ASEs and downstream interneurons to the behavior. Furthermore, we are pushing its limit by imaging the whole nervous system by expressing our probe (GCaMP + DsRed2 as a reference) using a pan-neuronal promoter. We have not achieved a resolution high enough to distinguish individual neurons, but observe the activation of a cluster of neurons, of which we have very limited or no knowledge, and concomitant behavior. We have found the resolution is significantly improved by targeting the probe in nuclei. We are also using different promoters to stain less number of neurons and also to observe a specific class of neurons (eg, *unc-17p* for cholinergic and *unc-47p* for GABAergic neurons). Moreover, in collaboration with Dr. Kevin Truong's group, we have generated Split-GCaMP to augment promoter-specificity by using two different promoters and reconstituting a functional probe in the neurons of overlapped specificity. Unlike previous approaches to rely on weak protein-protein interactions, our new probe is reconstituted by a covalent bond and functionally equivalent to an intact probe.

583B

MOD-5/SERT Function in 5-HT-absorbing Neurons Controls Behavioral Response to Food Deprivation. G. Jafari, Y. Xie, A. Kullyev, B. Liang, **J. Sze**. Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, NY.

In wild-type hermaphrodites, antibody raised against serotonin stains primarily four classes of neurons in the head region, NSMs, ADFs, RIH and AIMs, and a pair of HSN egg-laying neurons. We found that serotonin is produced only in NSMs, ADFs and HSNs, whereas RIH and AIMs use MOD-5/SERT to absorb serotonin from the extracellular space but cannot synthesize it. To distinguish between these two types of serotonergic neurons, we term NSMs, ADFs and HSNs as "5-HT-producing neurons", and RIH and AIMs as "5-HT-absorbing neurons". By expressing tph-1 specifically in ADFs, or in NSMs in tph-1 mutant background, we found that RIH and AIMs can absorb serotonin released from any of the 5-HT-producing neurons. RIH and AIMs are not connected to the 5-HT-producing neurons (White et al., 1986), suggesting that they absorb 5-HT from extra-synaptic space. Indeed, mutations in either dense-core vesicle release or synaptic vesicle release significantly reduced 5-HT in RIH and AIMs, and mutations affecting both forms of the release produced an additive effect. mod-5 mutants was initially identified in the Horvitz lab based on enhanced slowing locomotion following brief food deprivation (Sawin et al., 2000; Ranganathan et al., 2001). We found that transgenic expression of MOD-5/SERT in AIMs and several non-serotonergic neurons fully corrected the mod-5 mutant phenotype, whereas expression of MOD-5/SERT in NSMs and ADFs had a little effect. Experiments are under way to determine whether these 5-HT-absorbing neurons use serotonin as a "borrowed transmitter" to regulates their own targets, or they subserve to fine-tune the levels of extra-synaptic 5-HT, thereby modulating the targets remote from the serotonin release sites. References Ranganathan, R., Sawin, E.R., Trent, C., and Horvitz, H.R. (2001). Mutations in the Caenorhabditis elegans serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. J Neurosci 21, 5871-5884. Sawin, E.R., Ranganathan, R., and Horvitz, H.R. (2000). C. elegans locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. Neuron 26, 619-631. White, J.G., Southgate, E., Thomson, J.N., and Brenner, S. (1986). The structure of the nervous system of the nematode Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci B 314, 1-340.

584C

A genome-wide RNAi screen for levamisole sensitivity identifies new regulators of post-synaptic signaling. **Jessica E. Tanis**, Timothy Chaya, Todd Lamitina. Department of Physiology, University of Pennsylvania, Philadelphia, PA.

Multiple neurological disorders are caused by defects in acetylcholine (ACh) signaling. However, molecular mechanisms that regulate and maintain proper post-synaptic ACh signaling are not fully understood. At the *C. elegans* neuromuscular junction, the appropriate balance of ACh and GABA signaling is required for coordinated muscle contraction and movement. Wild-type animals exposed to levamisole, a pharmacological agonist of levamisole-sensitive ACh receptors on the body-wall muscles, undergo time-dependent, hyper-contracted paralysis. Resistance and hypersensitivity to levamisole-induced paralysis can be used to identify genes that regulate GABA or ACh signaling. In a prior forward genetic screen, mutations in 13 different genes conferring resistance to 1 mM levamisole were identified (Lewis et al., 1980). However, mutants with weak resistance or hypersensitivity, as well as sterile and lethal mutants, were not isolated. To identify additional factors required for ACh/GABA signaling we performed a genome-wide RNAi screen for gene knockdowns that cause hypersensitivity or resistance to 0.4 mM levamisole. We developed a liquid levamisole swim assay performed in 24 well plates and screened

each well at two time points in order to identify both hypersensitive and resistant mutants. This assay did not require picking or prodding animals and enabled us to screen 17,469 clones, representing 90% of the genome, in duplicate. Primary hits were retested in quadruplicate and validated hits tested positive in at least 4/6 trials. We identified 193 genes with altered levamisole response (25 resistant; 168 hypersensitive). Among our hits were several of the genes isolated in the original Lewis et al. screen, suggesting that our screen identified bona fide regulators of synaptic signaling. Of the 193 genes at least 53% have evidence for expression in muscle and 87% have human homologs. Many of the genes we identified have an annotated sterile or lethal phenotype and would not have been isolated in the Lewis et al. screen. The set of genes identified in our genome-wide levamisole screen does not show significant overlap with sets of genes identified in previous aldicarb resistant and hypersensitive screens (Sieburth et al., 2005; Vashlishan et al., 2008) indicating that these screens reveal distinct synaptic regulators. We are currently using genetic, cell biological, and optogenetic approaches to determine how these 193 genes influence synapse formation, maintenance, and/or signaling.

585A
Gustatory plasticity in C. elegans involves sensitisation of ASH nociceptive neuron and desensitisation of ASE gustatory neuron. **OLUWATOROTI O. UMUERRI**, Martijn Dekkers, Gert Jansen. CELL Biol, Univ ERASMUS Med Ctr, Rotterdam, Netherlands.

Neuronal plasticity allows an organism to alter its behavioural response to a stimulus depending on previous experiences. Although many molecular and cellular aspects of neuronal plasticity have been characterized, much is still unknown about how neuronal plasticity affects a neuronal circuit. We use behavioural assays and cell specific Ca2+ imaging to dissect a simple form of behavioural plasticity, gustatory plasticity, at the neuronal level in C. elegans. C. elegans is attracted to NaCl concentrations up to 200 mM, and avoids concentrations above 200 mM. However, these responses are plastic. After preexposing the worms to attractive concentrations of NaCl in the absence of food, they strongly avoid all previously attractive concentrations of NaCl. In naïve animals the response to low NaCl concentrations is predominantly mediated by the ASEL (ASE left, responds to an increase in NaCl concentration) and ASER (responds to a decrease in NaCl concentration) cells. In addition, ADF responds to a NaCl increase and ASH responds to a NaCl decrease. Both of these responses depend (at least partially) on input from other sensory neurons. Avoidance of high NaCl is mediated by the ASH neurons. We found that after 10 minutes exposure of C. elegans to 100 mM NaCl, ASEL was desensitized but ASER and ASH were sensitized. To test whether these (de)sensitization effects depend on signals from other cells, we tested the ASE cell fate mutant, che-1, and various neurotransmission mutants. We found reduced sensitisation of the ASH neurons after preexposure in the che-1 and tph-1 mutants compared to wildtype worm. These results suggest that sensitisation of ASH requires a signal from the ASE neurons and it involves serotonin. We are still testing other mutants to further characterise other proteins involved in this tractable behavioural switch.

586B
Modulation of Neuropeptide Release by Locomotion. **Amy B. Vashlishan Murray[1,3]**, Kavita Babu[1,2], Edward C.G. Pym[1,2], Zhitao Hu[1,2], Joshua M. Kaplan[1,2]. 1) Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Neurobiology, Harvard Medical School, Boston, MA; 3) Communication Sciences and Disorders, Emerson College, Boston, MA.

Neuropeptides represent a vast and chemically diverse set of neurotransmitters. Pro-neuropeptides are packaged into large dense core vesicle (DCV) precursors, where they are processed into active forms by co-packaged enzymes. Secretion of neuromodulatory peptides has often been proposed as a mechanism for regulating synaptic efficacy and producing adaptive changes in behavior; however, genetic studies of neuropeptide function have primarily focused on endocrine functions. In a few cases, the impact of specific neuropeptides has been explored in particular circuits, yet much remains to be learned about how neuropeptides shape the function of these and other behavioral circuits. We show that the neuropeptide, NLP-12, is expressed in a single neuron, DVA, and released in response to a brief treatment of the cholinesterase inhibitor aldicarb. This rapid release is dependent on dense core vesicle fusion, as it is suppressed in unc-13 and unc-31 mutants. It is also dependent on the DVA mechanoreceptor, TRP-4. By analyzing mutants that have either increased locomotion velocity (npr-1) or decreased locomotion velocity (mec-3), we show that NLP-12 release from DVA is regulated during normal locomotion.

587C
An Open-Source Neuromechanical Model of C. Elegans Locomotion. **Thomas Voegtlin[1]**, Netta Cohen[2]. 1) INRIA Lorraine, Vandoeuvre-les-Nancy, France; 2) School of Computing, University of Leeds, UK.

A neuromechanical model of locomotion in C. elegans was recently proposed by Jordan H. Boyle [1]. One of the main results is that both swimming and crawling can be generated by a single neural circuit, reflexively modulated by the environment. This supports the known experimental results showing that different forms of C. elegans forward locomotion (e.g., swimming and crawling) can be described by a modulation of a single biomechanical gait [2]. The modelling result illustrates the importance and the potential of neuromechanical simulations for the analysis of the worm's behaviour.

In order to continue this work, and to make it usable by a broader audience, we have developed a similar neuromechanical model of the worm using CLONES. CLONES (Closed Loop Neural Simulation) is an open source framework for neuromechanical simulations. CLONES implements a communication interface between a neural simulator, called BRIAN [3], and a physics engine for biomedical applications, called SOFA [4]. BRIAN and SOFA are open-source simulators that are easy to use and provide high performance.

Our implementation of the worm's locomotion reproduces the neural model described in [1]. However, there are two key differences between the original physical model and our implementation. Firstly, Boyle's model considers that the body of the worm has zero mass (a low Reynolds number approximation). In contrast, the SOFA simulator allows us to integrate equations with mass and inertia. Secondly, the original model uses rigid rods of fixed length orthogonal to the body axis (approximating the incompressibility of the body due to high internal pressure). In SOFA rigid rods are modeled as springs of very high stiffness.

The physical system simulated in SOFA is described using a XML syntax. The neural network model interpreted by BRIAN is written in Python, using MATLAB-like syntax. Thus, the model is completely interpreted, and it is possible to visualize/interact with the simulation during runtime. Physical environments containing obstacles or chemical concentration gradients can be defined easily.

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588A
Regulation of a rhythmic behavior in *C. elegan*s by neuropeptide signaling. **Han Wang**, Kelly Girskis, Krishnakali Dasgupta, Derek Sieburth. Zilkha Neurogenetic Inst, USC,Los Angeles, CA.

The defecation process in *C.elegans* is a rhythmic motor program that consists of three sequential muscle contractions: posterior muscle contraction (pBoc), anterior muscle contraction (aBoc) and enteric muscle contraction, which leads to the expulsion (Exp) of the gut contents. Previous studies have shown that the Exp step is regulated by a putative neuropeptide signaling pathway from the intestine that involves the activation of the GPCR *aex-2* expressed in a pair of GABAergic motor neurons(Mahoney, Luo et al. 2008). However, the neuropeptide has not been identified. Here we report that *nlp-40*, which encodes a conserved neuropeptide-like protein in nematodes, is the missing neuropeptide signal from the intestine that controls the Exp step. The *vj3* allele of *nlp-40*, identified in our lab, and *tm4085* allele from the knockout consortium are both putative null alleles, since both of them delete sequences in the putative mature NLP-40 peptide (Husson, Clynen et al. 2005). *nlp-40* mutants lack about 96% of Exp steps, although neither the cycle length nor the pBoc step of *nlp-40* mutants is affected. We found that *nlp-40* is exclusively expressed in all cells of the intestine. The Exp defects can be fully rescued by transgenes expressing NLP-40::GFP fusion protein under the endogenous *nlp-40* promoter or a heterologous intestine-specific promoter. The NLP-40::GFP fusion protein localizes to discrete puncta on the basolateral surface of the intestine. In addition, the fluorescence is also detected in coelomocytes. Together, these data indicate that NLP-40 is stored in dense core vesicles and is released from the intestine. Several results suggest that NLP-40 may be the ligand of AEX-2. First, both *nlp-40* and *aex-2* null mutants reduce expulsion frequency by about 96%. Second, both *aex-2* and *nlp-40* mutants are recessive, but double heterozygotes have a~50% reduction in Exp frequency. Finally, ectopic expression of *nlp-40* can partially rescue the Exp defects of *nlp-40* mutants but not those of *nlp-40; aex-2* double mutants. The NLP-40 pre-propeptide is predicted to be processed into at least three short peptides. We have identified a region of NLP-40 containing one of these peptides that is sufficient to rescue the Exp defects of *nlp-40* mutants. We are directly testing whether this peptide is the ligand of AEX-2 in vitro by testing whether a synthetic peptide can activate AEX-2 in HEK cells. We are also examining how NLP-40 release from the intestine is regulated and how its release controls the execution of Exp. Together, these results suggest that a neuropeptide signaling pathway initiated by the intestinal release of NLP-40 and activating AEX-2 regulates the execution of the rhythmic defecation motor program.

589B
The Role of Nicotinic Acetylcholine Receptors in Antipsychotic-induced Phenotypes in Caenorhabditis elegans. **T. Xu**, X. Wang, L. Hao, BM Cohen, EN Buttner. Pharmacogenomics & Molecular Pharmacology, McLean Hospital & Harvard Medical School, Belmont, MA.

The atypical antipsychotic clozapine has greater therapeutic efficacy than other medications in the treatment of schizophrenia, but the molecular mechanisms underlying clozapine's effects are poorly understood. The α7-nicotinic acetylcholine receptor (nAchR) has been implicated in the genetics of schizophrenia. Moreover, the heavy smoking behavior of schizophrenics may be a form of self-medication. Clozapine induces early larval arrest in C. elegans. A genome-wide RNA interference (RNAi) screen for suppressors of clozapine-induced larval arrest yielded acr-7, a homolog of the α7-nAchR. We obtained an acr-7 knockout strain and backcrossed this strain to wild type six times. We then confirmed our RNAi knockdown result by demonstrating that the acr-7 knockout suppresses clozapine-induced larval arrest. Interestingly, our preliminary observations suggest that knockout of acr-7 also suppresses clozapine-induced inhibition of locomotion in C. elegans. This finding

indicates that acr-7(lf) has pleiotropic effects and that acr-7 may mediate both developmental and behavioral phenotypes of antipsychotic drugs in C. elegans. Therefore, detailed characterization of the genetic, cell biological, and behavioral roles of acr-7 may provide insight into the effects of antipsychotic drugs on neuronal functions. Future experiments will include: 1) expression studies of acr-7 in C. elegans using GFP fusion constructs; 2) additional developmental and behavioral experiments to test whether acr-7 mediates the effects of antipsychotic drugs other than clozapine; 3) electrophysiological studies of acr-7 in heterologous systems; 4) epistasis studies of acr-7 and genes within the insulin sigaling pathway which also mediates clozapine's effects in C. elegans and mammals.

590C

Genetic analysis of acute ethanol responsive behaviors in *C. elegans*. **Joseph Alaimo**, Keith Shelton, Andrew Davies, Jill Bettinger. Virginia Commonwealth University, Richmond, VA.

Alcohol abuse is a complex disorder with a poorly understood etiology that includes both genetic and environmental influences. One factor found to influence drinking behavior and subsequent liability for dependence is variation in genes encoding ethanol metabolism machinery. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are enzymes involved in ethanol metabolism. We directly tested the effects of altering the function of these enzymes on ethanol responsive behaviors in *C. elegans*. We tested two ADH enzymes encoded by the genes *sodh-1* and *H24K24.3*. We have found that at 400 mM ethanol (worm cuticle is resistant to the passage of ethanol; N2 tissue concentrations at this dose are 50 mM) *sodh-1(ok2799)* animals are hypersensitive to ethanol's depression of locomotion relative to N2, but *H24K24.3(RNAi)* animals are not. We tested internal ethanol tissue concentrations and found that tissue concentration is increased in *sodh-1(ok2799)*, but not in *H24K24.3(RNAi)*. Importantly, we found that both strains develop robust acute functional tolerance (AFT) to ethanol, indicating that these enzymes are dispensable for this process. The nematode genome encodes 13 ALDH enzymes that are highly conserved compared with human ALDHs. Knockdown by RNAi of nine of these genes did not alter ethanol's depression of locomotion, but *alh-6(RNAi)* and *alh-13(RNAi)* resulted in hypersensitivity to this effect. Interestingly, internal tissue ethanol concentrations in these two strains appear to be slightly higher than in N2, suggesting that the lack of ALDH function may cause a buildup of acetaldehyde, which would be converted by ADH into ethanol. Collectively, these data suggest that altered ethanol metabolism in worms results in a mild but detectable effect on ethanol response behaviors. A second risk factor for alcohol dependence in humans is an individual's naive level of response to ethanol. This acute response to ethanol phenotype can be divided into at least two components, initial sensitivity and the development of AFT. The progression from initial ethanol responses to dependence is not well understood, but many studies have shown that acute ethanol administration induces changes in gene expression, which may contribute to later dependence behaviors. We will identify ethanol responsive genes by performing a series of microarray experiments at different times and doses of ethanol exposure. Candidate genes from this analysis will be tested for their functional relevance in ethanol responsiveness using an assortment of known ethanol-responsive behaviors. Our major goals are to identify genes that are important for ethanol responsive behaviors and to identify the molecular pathways involved in these responses.

591A

Calcium-dependent regulation of proton signaling during a rhythmic behavior in *C. elegans*. **Erik Allman**[1], Keith Nehrke[1,2]. 1) Department of Pharmacology and Physiology, University of Rochester School of Medicine and Dentistry, Rochester, NY; 2) Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY.

Both calcium levels and intracellular pH fluctuate during rhythmic execution of the defecation motor program (DMP). Cell-autonomous oscillatory Ca^{2+} signaling in the intestine times the defecation period and precedes cytoplasmic acidification. The first motor step of the DMP is a simultaneous contraction of the posterior body wall muscles (pBoc) that is triggered by bottom-up saltatory signaling through the intestinal Na^+/H^+ exchanger NHX-7. This exchanger extrudes protons through the basolateral membrane, causing a transient acidification of the pseudocoelom, which is sufficient to induce receptor-mediated muscle contraction. In addition to being the first example of Na^+/H^+ exchangers transmitting information between adjacent cells through regulated proton efflux, this behavior is the sole integrative model for studying Na^+/H^+ exchangers in a signaling context. To determine the relative contribution of Ca^{2+} signaling and cytoplasmic acidification on NHX-7 activity, we mutated predicted Ca^{2+} and pH regulatory motifs in the *nhx-7* coding region and assessed their ability to complement an *nhx-7(ok583)* null allele. Both behavioral and physiologic measurements of ion transients were made in live worms carrying variations of the mutant transgenes with fluorescent Ca^{2+} or pH biosensors. The results of these studies were compared with measurements of recombinant wild-type and mutant NHX-7 activity from cells in culture. Pharmacologic manipulation was used to further characterize the wild-type and mutant NHX-7constructs in a Na^+/H^+ exchange deficient mammalian cell line. The results of these experiments provide a platform for understanding how pH and Ca^{2+} signaling are integrated to foster Na^+/H^+ exchanger activity in a newly discovered proton signaling paradigm. Supported by Ruth L. Kirschtein National Research Service Institutional Training Grant T32 GM06841 (E.A.) and NSF IOS0919848 (K.N.).

592B

Investigating neural coding and interacting circuits. **Evan L. Ardiel**[1,3], Andrew C. Giles[1,3], Ithai Rabinowitch[2], William R. Schafer[2], Catharine H. Rankin[1]. 1) Brain Research Centre, University of British Columbia, Vancouver, BC, Canada; 2) Cell Biology Division, MRC Laboratory of Molecular Biology, Cambridge, UK; 3) authors contributed equally.

ASH mediates backward locomotion in response to osmotic pressure, nose touch, high ambient oxygen, and a variety of volatile and non-volatile compounds. We used optogenetics to simulate these stimuli to better understand their perception and how this changes with experience. Using an automated multi-worm tracker to measure behaviour, we found that a short light pulse (200-ms) of low intensity blue light elicited a reversal response of about 200-µm in 80-90% animals expressing ChR2 in ASH. A longer light pulse (2-s) elicited a larger reversal response (1-mm) that often ended in an omega turn. Previous work demonstrated that ASH-mediated responses to different stimuli were genetically dissociable (Mellem et al., 2002). We monitored the responses of *eat-4*, *glr-1*, *nmr-1*, and *glr-1;nmr-1* mutants and found that the behavioural impairments associated with nose touch and osmo-avoidance assays were also apparent in response to the short and long light pulses, respectively. This suggests that a part of the neural code distinguishing nose touch and chemoaversion is the duration of depolarization of ASH. We next tested habituation of ASH to our "nose touch" and "chemoaversive" light pulses. We found that both responses decremented from repeated exposure, but with differential kinetics. The proportion of worms responding to the longer light pulse decreased faster and further than responses to the nose touch-like stimulus. Disrupting neuropeptide signalling blocked this deep habituation. The interneurons downstream of ASH largely overlap with those mediating reversals to body touch. We were interested in addressing how activity in one circuit affected the other. We found that the tap response was decremented by a single light, but facilitated by repeated presentation of long-light pulses. We are currently investigating the cellular mechanisms underlying this cross-circuit plasticity.

593C

A Possible Role for *nhr-239* in Sensory Response. **G. Michael Baer**[1], Christopher Alvaro[1,2], Benjamin Perlman[1], Bruce Wightman[1]. 1) Biology Department, Muhlenberg College, Allentown, PA; 2) University of California, Berkeley, CA.

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*, the HR83 nuclear receptors of insects, and a related ortholog in the echinoderm *Strongylocentrotus*. The existence of *nhr-239* orthologs in both protostomes and deuterostomes suggests that this class could have an ancient origin, but has been lost from vertebrates. Unlike the insect HR83 genes, the *C. elegans* and *C. briggsae nhr-239* genes appear to have a truncated, degenerate ligand-binding domain.

Two deletions of the 5' region of the *nhr-239* gene have been recovered. *ok2526* deletes the entire *nhr-239* presumptive promoter region and first exon, plus the last exon of *feh-1*, the immediately adjacent gene. *tm4406* deletes a portion of intron one, but would not necessarily have any effect on exon sequences. Animals that are homozygous for the *tm4406* deletion have no obvious phenotype, but *ok2526* homozygotes display a behavioral phenotype that appears to be related to that of *npr-1*. These mutants avoid lawns of OP50 bacteria, but instead of bordering at the periphery of the food lawn, animals congregate a short distance outside of the lawn, creating a "halo" around the food lawn on crowded plates. Preliminary experiments indicate that the phenotype is observed on some bacterial strains, but not others, suggesting that it is not a simple physical response to the presence of bacteria. A null mutation in *feh-1* is lethal, but we cannot be sure at this time whether the *ok2526* phenotype is due to the loss of *nhr-239* or the last exon of *feh-1* (or both). An *nhr-239::gfp* transgene is expressed at low levels in two to three pairs of head neurons, at least one of which appears to be of a sensory type. We are exploring a possible role for *nhr-239* in the development or function of these neurons.

This work is supported by a grant from the NSF.

594A

Characterization of an evolutionary conserved vasopressin/oxytocin-like signaling system in *C. elegans*. **Isabel Beets**, Ellen Meelkop, Liesbet Temmerman, Tom Janssen, Marleen Lindemans, Liliane Schoofs. Laboratory of Functional Genomics and Proteomics, K.U.Leuven, Naamsestraat 59, 3000 Leuven, Belgium.

Neuropeptides represent a diverse and numerous class of signaling molecules in the nervous system, of which over 250 distinct sequences have been identified in the *C. elegans* genome so far. Through binding of G protein-coupled receptors (GPCRs), neuropeptides are thought to primarily transmit and modulate synaptic and endocrine functions. Therefore, they act as key players in the regulation of animal physiology including reproduction, locomotion, feeding and social behavior. Despite their clear role in neuronal signaling and behavior, neuropeptide functions and the underlying signaling pathways they govern are still not well understood. Identification of the receptors that bind neuropeptides should gain more insights into neuropeptidergic signaling. Over the years, our research group has successfully used expertise on GPCR deorphanization techniques to characterize several neuropeptide mediated signaling systems in *C. elegans* including gonadotropin-releasing hormone (GnRH), cholecystokinine (CCK) and pigment dispersing factor (PDF) signaling.

We now report the characterization of a novel vasopressin/oxytocin-like signaling system in *C. elegans*. Vasopressin (VP) and oxytocin (OT) are structurally related neurohypophysial peptides, first identified in vertebrates and more recently in some parts of the invertebrate lineage. By means of bioinformatic search methods, we have identified homologous genes for a VP/OT receptor and peptide precursor in the *C. elegans* genome. Structural features of the VP/OT superfamily have been generally conserved in *C. elegans* and other nematode species, both on the receptor and neuropeptide precursor level. Despite the overall conservation, the VP/OT-like peptide in *C. elegans* differs from the classical nonapeptide structure found in most vertebrate and invertebrate VP/OT peptides. We have cloned the *C. elegans* VP/OT receptor and expressed this GPCR in Chinese hamster ovary (CHO) cells upon characterization. The *C. elegans* VP/OT receptor was activated by the *C. elegans* VP/OT-like peptide through a Gαq protein (EC50 = 20 nM), but not by other members of the VP/OT family (e.g. inotocin, octopressin). Ongoing research focuses on the functional characterization of VP/OT signaling in nematodes. Preliminary localization studies indicate that the *C. elegans* VP/OT receptor is expressed in body wall muscles of all larval and adult stages as well as in vulval muscles of adult hermaphrodites, supporting an evolutionary conserved role of VP/OT signaling in muscle contraction and reproduction.

595B

A role for heterochronic genes in regulating C.elegans quiescence. **Heather L Bennett**[1], Komudi Singh[2], Edward Anderson[2], Mark E Corkins[2], Anne C Hart[1,2]. 1) Department of Molecular Biology, Cellular Biology & Biochemistry, Brown University, Providence, RI; 2) Department of Neuroscience, Brown University, Providence, RI.

Sleep is an essential biological process that is regulated by circadian rhythms, homeostatic regulation and arousal threshold. The nematode *Caenorhabitdis elegans* undergoes a sleep-like state called quiescence that accompanies each larval molt (Raizen *et al.,* 2008). Many pathways regulate larval development, including heterochronic genes and the Notch signaling pathway. Heterochronic genes control timing of cell differentiation and molting of larval cuticle. In *Drosophila*, circadian rhythm proteins cycle to regulate sleep behavior. In *C.elegans*, circadian rhythm homologs in the heterochronic pathway regulate the fusion of hypodermal seam cells during late development (Jeon *et al.,* 1999, Banerjee *et al.,* 2005). Recently, a temporal role in *C. elegans* molting was suggested for the Notch signaling pathway. Gain of function alleles of a Notch receptor gene, *lin-12*, result in precocious cell fate changes (Solomon *et al*., 2007). We have found that Notch also plays a role in the regulation of quiescence. Increased or decreased Notch signaling results in increased quiescence and altered arousal thresholds during the last larval stage (L4) to adult (A) transition (Singh *et al*., in press). However, the connection between circadian rhythm homologs, heterochronic genes, and Notch in the regulation of quiescence remains unclear. We are assessing gain and loss of function alleles of heterochronic genes acting in the L4/A transition to determine if they play a role in regulating quiescence. Demonstrating a genetic interaction between heterochronic genes, circadian rhythm proteins, and the Notch signaling pathway will be the first step in delineating mechanisms that coordinate cell fate and behavioral changes during development.

596C

Membrane lipid environment is important for the development of acute functional tolerance to ethanol. **Jill C. Bettinger**[1,2], Mia H. Bolling[2], Kapo Leung[2], Joseph T. Alaimo[2], Andrew G. Davies[1,2]. 1) Department of Psychiatry; 2) Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

There is substantial evidence from studies in humans that genetic variation that contributes to an individual's naïve level of response to ethanol strongly impacts their susceptibility to become alcoholic. Our laboratory uses worms to study the genetic influences on the level of acute ethanol response. There are at least two components to the level of response to ethanol, initial sensitivity (measured at 10 minutes exposure), and a robust development of acute functional tolerance (AFT, measured at 30 and 50 minutes). We have used a forward genetic screen to isolate mutants that fail to develop AFT, and have recovered 16 recessive mutations that identify at least 14 complementation groups. We cloned the first of these, *eg613*, which is a mutation in the gene *ctbp-1*, a transcriptional repressor. CTBP-1 acts with PAG-3 and ZAG-1 to repress transcription (Nicholas, H. *et al*., J Mol Biol 375:1-11). We also recovered alleles of *pag-3* and *zag-1* from our screen, demonstrating that we are able to identify genetic pathways using this approach. The gene encoding the triacylglycerol (TAG) lipase LIPS-7 has been shown to be inappropriately upregulated in a *ctbp-1* mutant strain, resulting in decreased TAG levels (Chen, S. *et al*., PNAS 106(5): 1496-501). We demonstrated that dysregulation of *lips-7* is at least partially responsible for the failure of *ctbp-1(eg613)* to develop AFT, and that loss of function of *lips-7* results in fast development of AFT. TAGs are used as fat stores in the worm, so we tested if other mutations that alter fat levels also impact AFT, and found that there was no correlation between overall fat levels and AFT. We hypothesized that *lips-7* might also be altering membrane composition and/or structure, which has been shown in worms and other systems to influence the function of membrane-bound proteins. Such modulation of function may represent a mechanism for developing tolerance to the depressive effects of ethanol. Supporting this hypothesis, we found that worms reared on cholesterol-depleted media were unable to develop AFT. We also found that altering *lips-7* levels modulated the function of two gain-of-function mutations in the ethanol-sensitive BK channel, SLO-1. Together, these data suggest a model in which the lipid bilayer plays an important role in the ability of proteins to modulate their function in response to ethanol treatment.

597A

Huffing and drinking are not the same: Screening for targets of toluene and ethanol. Ryan I. Friedberg[2], ChungLung Chan[2], Hersh Gupta[2], Victoria E. Brings[2], Charlotte M. Wincott[2], Keith L. Shelton[2], **Jill C. Bettinger**[1,2], Andrew G. Davies[1,2]. 1) Department of Psychiatry; 2) Department of Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA.

We are interested in understanding the mechanisms of action of drugs of abuse. Alcohol, as a pharmacological agent, is thought to have many molecular targets in the brain. Toluene is one of several volatile solvents that are abused by inhalation ("huffing"). As drugs with classical CNS depressant activities (low-dose activation, high dose sedation), it has been proposed that the molecular targets of alcohol and toluene are likely to overlap significantly although the scientific evidence for such overlap is limited. We have compared the behavioral responses of wild-type and mutant *C. elegans* to each compound. We found that the pattern of body bends made during crawling following a short toluene exposure (10 minutes) is distinct from our previously described acute effect of ethanol on crawling. This immediately suggests the possibility that ethanol and toluene have different mechanisms of action. At higher doses, both drugs cause a significant decrease in locomotor speed in the absence of food, however, at lower doses of toluene, but not ethanol, there is an increase in the average speed of locomotion. This is an effect that may be akin to the low dose locomotor activation that is seen with both toluene and ethanol in rodents. We have found that mutations in the *slo-1* and *rab-3* genes, which result in ethanol resistance and are likely to represent independent mechanisms of action of ethanol, do not alter sensitivity to toluene; this strongly suggests that toluene does not act through SLO-1- or RAB-3-dependent mechanisms, making the action of toluene distinct from the action of ethanol at least with regard to these pathways. To identify the targets of each of these abused drugs, we are pursuing genetic screens for new mutations that result in resistance to ethanol and/or to toluene with the goal of determining where the drugs' mechanisms of action overlap and where they do not. We have isolated new mutants of each class; to date we have not found mutations that confer resistance to both drugs, further supporting the hypothesis that these drugs act through distinct mechanisms. We are in the process of identifying the genes affected by these mutations. This research is supported by NIAAA (AGD).

598B

Lethargus is a highly regulated behavior, characterized by quiescence- and motion-bouts and a typical posture. S. Iwanir, S. Nagy, N. Tramm, C. Wright, **D. Biron**. Dept. of Physics, James Franck Institute & the Institute for Biophysical Dynamics, The University of Chicago, Chicago, IL.

C. elegans develops through four larval stages before it reaches adulthood. At the end of each stage it molts: it exhibits a quiescent behavior termed lethargus and subsequently sheds its cuticle. Raizen et al. demonstrated that lethargus bears behavioral similarities to sleep, such as reversibility, sensory gating and homeostatic control. We developed methods for quantifying lethargic behavioral patterns in more detail than previously reported, and assayed the consequences of several genetic perturbations and laser ablations. Our basic characterization of the microarchitecture of lethargus builds on the observation of individual bouts of quiescence and motion during its 2-3 hour duration. The duration of individual bouts ranges from a few to about 100 seconds. A two state Markov chain can be used to describe the statistics of these bouts. Its transition rates kq and km, denoting the rates of hopping out of the quiescent and motile states respectively, undergo slow modulation. The resulting statistics provide a robust set of phenotypic measures. We assayed the phenotypes of animals mutant in cAMP signaling genes. Specifically, we examined the adenylyl cyclase *acy-1* and the predicted PKA regulatory subunit of *kin-2*. Increased PKA signaling has been shown to cause hyperresponsivity to sensory stimuli and is predicted to result in reduced quiescence. Our data was consistent with these predictions. We have also begun to characterize the body posture of the worm during lethargus. We found that the curvature along the lethargic body is significantly lower than that of awake worms, and that quiescent worms typically exhibit a single (anterior) body bend, as opposed to 2-3 bends in motile animals. Van Buskirk and Sternberg showed that the activation of the EGFR LET-23 in the ALA neuron can suppress motion, and that EGF signaling promotes quiescence during lethargus. We ablated the ALA neuron and observed an increase in the mean duration of quiescence bouts. Expressing tetanus toxin in the AVE neurons (a kind gift from the lab of Shai Shaham), postsynaptic partners of ALA, resulted in the opposite effect. Our data thus suggests an inhibitory connection between ALA and AVE during L4 lethargus. In summary, we observe that lethargus is a complex and highly regulated behavior: it can be dissected to quantifiable elements, which exhibit complex temporal dynamics and are modulated by molecular and neuronal activity. This suggests that it is unlikely that lethargus is merely the result of mechanical restriction during molting.

599C

Spontaneous recovery from habituation to tap is interstimulus interval-dependent. **Ricardo Bortolon**, Catharine H. Rankin. Rankin Lab, Brain Research Centre, Vancouver, Canada.

Habituation is a fundamental form of learning throughout the animal kingdom but almost nothing is known about the mediating genes and proteins. The length of time between stimuli (interstimulus interval) is known to influence the rate and degree of both habituation and spontaneous recovery from habituation and whether long term memory is formed but little research has demonstrated its effect on other defining characteristics of habituation. Using a multi-worm tracker to measure habituation and recovery of both magnitude and frequency of reversals in response to tap, a 2-second interstimulus interval results in more rapid spontaneous recovery of both the magnitude and frequency of reversals from habituation than a 10-second interstimulus interval. This provides an opportunity to screen for genes which determine the mechanisms of spontaneous recovery from habituation and which mediate interstimulus-dependent differences.

600A

A *C. elegans* movement assay for the assessment of neurotoxicity and genetic mutations. **Windy A. Boyd**[1], Daniel W. Snyder[1], Grace E. Kissling[2], Jonathan H. Freedman[1,3]. 1) Biomolecular Screening Branch, National Toxicology Program, RTP, NC; 2) Biostatistics Branch, NIEHS, RTP, NC; 3) Laboratory of Toxicology and Pharmacology, NIEHS, RTP, NC.

Government agencies are using alternative toxicological model organisms to screen and prioritize chemicals to be used in traditional mammalian studies. The nematode *Caenorhabditis elegans* is a useful alternative model to characterize the behavioral effects of chemicals, because they possess a simple, well-characterized neuromuscular system. A movement assay has been developed to quantify *C. elegans* locomotion using a semi-automated motion tracking system. This system consists of an inverted fluorescence microscope equipped with a CCD camera, incubated motorized stage, and image analysis software. Using this system, a number of locomotion parameters were defined including curvilinear velocity, linearity, and amplitude of sinusoidal movement. RNA interference of a number of genes known to control normal nematode movement was used to evaluate the *C. elegans* movement assay. Changes in specific locomotion parameters indicated that the

assay was capable of characterizing various loss-of-function phenotypes, which included uncoordinated and slow movement. A transgenic *C. elegans* strain expressing a mutant human tau protein, which is associated with neurodegenerative diseases such as Alzheimer's, was also analyzed. Expression of the mutant tau transgene resulted in significantly impaired *C. elegans* movement, as determined by various motion parameters including decreased velocity. The phenotype was rescued to varying degrees by six different genetic suppressors. Finally, the effects of three chemicals, cadmium, chlorpyrifos and tebuconazole, on *C. elegans* movement were quantified. All movement parameters significantly decreased by 1 mM cadmium and 0.1 to 10 µM chlorpyrifos. Tebuconazole, however, did not affect nematode locomotion at the highest concentration tested (1 mM). Together, these results confirm the utility of the *C. elegans* movement assay as a robust endpoint for investigating the neurotoxicity of chemicals for toxicant screening and characterization.

601B

Maintenance of the identity of the chemosensitive BAG neurons requires an ETS family transcription factor. **Julia P. Brandt**[1], Sonya Aziz-Zaman[1], Jennifer K. Lee[1,2], Niels Ringstad[1]. 1) Molecular Neurobiology Program, Skirball Institute of Biomolecular Medicine and Department of Cell Biology; 2) Sackler Institute of Biomedical Sciences, NYU School of Medicine, New York, NY 10016.

Carbon dioxide (CO_2) is an environmental cue alerting animals to food, mates and danger. CO_2 is also a critical regulator of animal physiology. In many animals, including mammals, CO_2 is detected by specialized chemosensitive neurons. *C. elegans* possesses a pair of such chemosensitive neurons, the BAG neurons, which are highly sensitive to CO_2 and are necessary for behavioral responses to environmental CO_2. BAG neurons specifically express the guanylate cyclase genes *gcy-31*, *gcy-33* and *gcy-9*, and the neuropeptide gene *flp-17*. Here we show that the maintained expression of these BAG neuron genes requires the winged helix-turn-helix transcription factor ETS-5. Using both reporter transgenes and quantitative RT-PCR we found that expression of BAG-neuron-specific guanylate cyclase and neuropeptide genes is significantly reduced in *ets-5* null mutants as compared with the wild type. By identifying other genes that are regulated by ETS-5, we hope to discover new genes that function in neuronal chemosensitivity.

602C

Perception of bacteria by the AWC neuron. **Chantal Brueggemann**, Damien O'Halloran, Noelle L'Etoile. Center for Neuroscience University of California, Davis, CA.

Odor sensation and chemotaxis are essential cues that allow an organism to localize and tax towards food. However, the organism must also be able to ignore profitless odors. Thus, prolonged exposure of the olfactory sensory AWC neuron pair to benzaldehyde in the absence of food leads to desensitization of these sensory neurons, allowing the worm to ignore this odor [1, 2]. This adaptation process has to be modulated by the worm that the odor contained satisfying food remains attractive to the animal. Indeed, it is shown that in combination with food, adaptation to odor is blocked [3, 4]. In order to examine the molecular basis by which bacteria are able to block adaptation, we investigate bacterial molecules on cell surface or released into medium, which could allow the worm to distinguish between odor alone and odor with a nutritional benefit. One major molecule on the outer membrane of gram-negative bacteria is the strain specific lipopolysaccharide (LPS) structure, which acts as endotoxin and is able to induce strong immunological response in mammals and C. elegans [5-7]. We use E. coli strains with different LPS structures to ask how the LPS affect AWC during adaptation. Our results point out that altering LPS structure affects adaptation block. Interestingly, the ability of the bacteria to block adaptation depends on which strain the animals were grown on, indicating that there may be an `LPS memory'. In order to understand how growth on specific LPS containing bacteria creates this `LPS memory' we will determine which neurons within the animal are responsible for the memory. We are utilizing a combination of genetics and fluorescence based imaging to examine the calcium responses of the AWC neuron and its circuit to on-set and removal of bacteria or bacterial molecules. 1.Colbert, H.A. and C.I. Bargmann. Neuron, 1995. 14(4): p. 803-12. 2.Nuttley, W.M., S. Harbinder, and D. van der Kooy. Learn Mem, 2001. 8(3): p. 170-81. 3.Colbert, H.A. and C.I. Bargmann. Learn Mem, 1997. 4(2): p. 179-91. 4.Lin, C.H., et al. J Neurosci, 2010. 30(23): p. 8001-11. 5.O'Quinn, A.L., E.M. Wiegand, and J.A. Jeddeloh. Cell Microbiol, 2001. 3(6): p. 381-93. 6.Aballay, A., et al. Curr Biol, 2003. 13(1): p. 47-52. 7.Tenor, J.L. and A. Aballay. EMBO reports, 2007. 9(1): p. 103-109.

603A

Samamide is a new small-molecule probe of behavioural quiescence in *C. elegans*. **Andrew R. Burns**[1], Suji Tharmalingalam[2], William S. Ryu[1,3], Sean R. Cutler[4], David R. Hampson[2], Peter J. Roy[1]. 1) Department of Molecular Genetics, Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada; 2) Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada; 3) Department of Physics, Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, Canada; 4) Department of Botany and Plant Sciences, Center for Plant Cell Biology, University of California, Riverside, CA, USA.

C. elegans displays at least three distinct behavioural quiescent states. First, a sleep-like state is induced by epidermal growth factor signalling before each larval molt. Second, satiation induces a quiescent state that is regulated by both TGF-β and insulin signalling. Finally, adult worms cycle through periods of quiescence after prolonged swimming. In all three cases, the *C. elegans* cGMP-dependent protein kinase (PKG) homolog *egl-4* promotes quiescence. It has been shown that increased PKG activity in *Drosophila* promotes sleep, and PKG1 inhibition in mouse brain alters the sleep-wake distribution, suggesting phylogenetic conservation of factors that regulate sleep and perhaps behavioural quiescence in general.

In a screen of 4,000 drug-like chemicals we identified a previously uncharacterized molecule that we hypothesize is promoting quiescence during swimming. We call this molecule 'samamide'. Reduced swimming activity is observed in adult worms treated acutely with samamide, suggesting that the chemical alters the physiological state of the animal. Chemical-genetic analyses suggest that samamide promotes quiescence by modulating metabotropic glutamate receptor activity. Samamide holds promise as a unique tool to better understand behavioural quiescence in animals.

604B

Spatial memory in C. Elegans. **Adam Calhoun**[1,2], Tatyana Sharpee[2], Sreekanth Chalasani[2]. 1) Neurosciences Graduate Program, University of California San Diego, La Jolla, California 92093, USA; 2) The Salk Institute for Biological Studies, La Jolla, California 92037, USA.

Worms are known to increase the frequency of turning and reversal events in response to removal of a food stimulus. This keeps the animal in a small area near the last observation of food. The increase in turning frequency is known to be regulated by dopamine and glutamate (Hills et al 2004).

We have found that the search strategy is modified by the spatial pattern of food that is experienced. Worms adapt quickly to new food environments, learning spatial patterns on short timescales. The dopamine deficient cat-2 mutant is unable to learn different spatial patterns, suggesting that dopamine is required for spatial memory.

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605C

The paraoxonase-like protein K11E4.3 is needed for gentle touch and mec-4(d)-mediated neurodegeneration. **Yushu Chen**, Martin Chalfie. Biological Sci, Columbia Univ, New York, NY.

Paraoxonases (PONs) are a family of enzymes with various activities, including deoxidization of lipids, drug metabolism, and detoxification of organophosphates. Paraoxonases (PONs) have also been implicated in humans to protect against atherosclerosis, but the molecular role of the proteins is uncertain. In Caenorhabditis elegans, however, a PON-like protein MEC-6 is a necessary component of the MEC-4 mechanosensory ion channel complex in the six touch receptor neurons (TRNs), which transduces gentle touch. 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 is also expressed in the TRNs and a few other neurons. K11E4.3 co-localizes with MEC-6 in puncta along the TRN processes. K11E4.3 is required for touch sensitivity in sensitized backgrounds, specifically in mec-4 and mec-6 temperature sensitive alleles at the permissive temperature or mec-6 hypomorphic alleles that do not, on their own, produce touch insensitivity. Like MEC-6, K11E4.3 is required for neuronal degeneration caused by gain-of-function mutations in mec-4 [mec-4(d)]. Loss of either K11E4.3 or MEC-6 lowers the amount of MEC-4::YFP in the TRN process. K11E4.3 physically interacts with MEC-4(d) and increases MEC-4(d) channel activity in Xenopus oocytes. These data suggest that, as with the DEG/ENaC and stomatin-like proteins, the touch transduction complex has multiple PON-like proteins. These proteins may also be important in stabilizing the channel complex or in transporting it to the plasma membrane.

606A

Characterization of Electrotaxis Behavior in *C. elegans*. **Steven D. Chrisman**, Eric Foss, Lucinda Carnell. Biological Sciences, Central Washington University, Ellensburg, WA.

C. elegans will orient and travel in a straight uninterrupted path directly towards the negative pole of a DC electric field (Sukul and Croll, 1978: J Nematol 10:314-317). We have developed a population assay to measure electrotaxis behavior in a static electric field. Behavioral sensitivity to an electric field is determined by measuring average speeds and approach angles at different field strengths. During the course of these studies, we also uncovered two novel electric field responses in *C. elegans*: an initial increase in velocity to field stimulus, and an immediate reversal upon a decrease of field strength (off stimulus response). In examining the neural basis for this behavior we have identified a mutant, *eat-4*, that is severely disrupted in three aspects of electrotaxis behavior: velocity, directional behavior, and reversals. Testing the response of transgenic strains with neuron-specific rescue of the wild-type *eat-4* gene to an electric field stimulus has revealed a role for the amphid sensory neuron (AWC) in regulating aspects of velocity and directional sensing, but no role in the reversal behavior. In the neuron-specific rescue of the wild-type *eat-4* gene in ASK neurons, we observed an increase in directional sensing, but no recovery of increased velocity or reversal behavior. With neuron-specific rescue of wild-type *eat-4* in both sensory neurons, AWC and ASK in the same animal no recovery of velocity speeds or directional sensing was observed, suggesting that ASK may function to inhibit the AWC-dependent circuit responsible for electrotaxis behavior. We are currently generating neuron-specific rescue of wild-type *eat-4* in ASH and ASJ amphid neurons to explore the remaining aspects of electrotaxis behavior. To identify the neural circuits operating in electrotaxis, we also examined the role of two interneurons, AIY and AIB, which make synaptic connections to AWC. To test the role of AIY and AIB interneurons we examined two mutants, *glr-1* and

glc-3, which have defects in an AMPA-type glutamate receptor and a glutamate-gated chloride channel, respectively. We observed that *glc-3* mutant animals display defects in initiating velocity increases in response to an electric field, while *glr-1* mutant animals exhibited directional defects during migration in an electric field. Neuron-specific rescue of wild-type *glc-3* in AIY recovered the increase in velocity whereas rescue of *glr-1* in AIB recovered directional behavior to electric field stimulus. Our data suggests that EAT-4-expressing amphid neurons including AWC are coupled to regulate direction and velocity responses to a static electric field largely through the interneurons AIB and AIY.

607B

The rescue of ODR-1. **Kimberly D Collins**. Center for Neuroscience, UC Davis, Davis, CA.

Caenorhabditis elegans requires olfaction for food location and survival. C. elegans requires the protein ODR-1, a guanylyl cyclase, to interpret odor signals for all AWC sensed odors, to deem them nutritionally beneficial. ODR-1 is expressed in the chemosensory neuron AWC, where it is localized in the cilia. Odor sensation is a critical function of the ODR-1 protein. Persistent exposure to odors however, can decrease sensitivity and response to these odors. This phenomenon is deemed adaptation. Food can act as a signal to block the effects of adaptation when present at the time of prolonged odor exposure. Worms unable to respond to nutritionally beneficial odors are odr-1(n1936) (L'Etoile and Bargmann, 2000). As was previously seen (L'Etoile and Bargmann, 2000) a truncated (odr-1 short) version of this gene rescued chemotaxis in odr-1(n1936) worms. This version is missing the 3'UTR and possible coding for the last sixteen amino acids of the protein sequence. To determine the significance of the missing sequence in ODR-1's role in odor sensation, a full length odr-1 gene construct, the gene which codes for ODR-1 must be cloned. Key to the missing sequence could be important control elements, which may subtly alter ODR-1's ability to function in food block of adaptation. Once cloned, the full length odr-1 construct (odr-1 Full) will be used to determine chemotaxis effects, and GFP will be cloned in to determine the localization of ODR-1. Chemotaxis results from assays performed on odr-1(n1936) worms injected with odr-1 Full were found significantly different from the chemotaxis of the odr-1 null worms. To investigate further, we ask whether the extracellular domain (ECD) of the ODR-1 integrates food and odor signaling, and whether it is needed for food sensation. In our lab we have seen that the ODR-1 ECD may allow the AWC neuron to respond and express other molecules that respond to food. When odr-1(n1936) were injected with odr-1 short delta ECD DNA and subjected to prolonged odor with no food, prolonged odor with food, and to food alone, we showed little to no rescue of chemotaxis, leading to the conclusion that the ECD of ODR-1 is required for food block of adaptation. Cloning of an odr-1 Full, odr-1 Full + GFP, odr-1 Full delta ECD, odr-1 Full delta ECD + GFP constructs will allow us to re-perform these experiments to determine the significance of the ODR-1 ECD in food block of adaptation. Further investigation may be done to determine the localization and trafficking of ODR-1, ODR-1 expression levels, and nuclear localization of ODR-1 associated proteins such as EGL-4 which interacts with cGMP to aid in food block of adaptation.

608C

Dopaminergic receptor signaling in sex-muscles antagonizes cholinergic induced excitability post-eyaculation during male mating of C. elegans. **Paola Correa**[1], Garcia Rene[1,2]. 1) Texas A&M, College Station, TX; 2) Howard Hughes Medical Institute.

We want to address novel signaling pathways implicated in dopamine (DA) modulation of cholinergic circuits that control motor outputs. To understand the neuro-modulation of a cholinergic circuit we study a sub-step in male mating of Caenorhabditis elegans, spicule insertion. During this step a network of cloacal cholinergic sensory-motor neurons synapse onto the sex-muscles to control muscle contractile events. Tonic contraction of the sex muscles sustains full extension of copulatory spicules from the male tail until sperm transfer is completed. Sperm transfer initiates the relaxation of the protractor muscles and contraction of the retractor muscles which in turn regresses the spicules back into the male tail. The process from sex muscle contraction to spicule protraction is induced by nicotinic (nACh), muscarinic (mACh) and non-specific acetylcholine agonists. We find that exogenous DA can decrease nACh receptor-dependent excitability of the sex muscles when challenged simultaneously with cholinergic agonists. However, this inhibition is bypassed by mACh signaling during sequential exposure to DA followed by mACh agonists. In C. elegans, muscle excitability is decreased by the activation of DA 7-transmembrane heterotrimeric Gα-coupled D2-like receptors. These receptors are activated by DA released by sensory neurons located mainly in the head and tail ganglion. Through pharmacogenetics, we identified that simultaneous DA and ACh-agonists exposure can antagonize the excitability of the sex-muscles via DOP2 and DOP3 coupling to Gαo and GPA-7. Consistent with this data, the receptors and the G-protein α subunits are expressed in the sex muscles and/or neurons of the spicule circuit. Mating behavioral analysis of dop-2; dop-3 mutants indicate that, in wild type males, the effect of DA inhibitory signal is observed in the retractor muscles, where DOP2 and DOP3 are required for proper timing of spicule retraction. This may suggest a novel neuromuscular DA modulatory effect, where the inhibitory output of DA relies on the time of its secretion. To further address whether the timing of spicule retraction is coupled with activation of DA neurons during mating, we are currently performing calcium imaging of DA neurons in the male upon completion of sperm transfer into a hermaphrodite and spicule retraction.

609A

Dissecting predator/prey interactions in C. elegans. **Kevin Curran**[1], Ray Hong[2], Sreekanth Chalasani[1]. 1) Salk Institute , La Jolla, CA; 2) California State University, Northridge, CA.

A current challenge in neuroscience is to bridge the connections between genes, neurons, neural circuits, and behavior in a single animal model. We are exploring the mechanisms governing a social interaction between a predator and its prey. The predator, *Pristionchus pacificus*, is omnivorous in the wild yet will attack its prey, *Caenorhabditis elegans*, when the two species share an agar plate. Certain *Pristionchus* strains exhibit a carnivorous mouth morphology allowing them to slice open *C. elegans* cuticular exoskeleton and consume the underlying tissue. In turn, upon sensing this predatorial threat, *C. elegans* exhibits avoidance behavior, in the form of omega bends and reversals, so as to evade capture and consumption. We have developed assays to assess the degree of *C. elegans* avoidance behavior in response to a lethal predator. Initially, we qualitatively tested 8 *Pristionchus* strains for their propensity to attack *C. elegans* on a shared agar plate and observed a range of aggression. *Pristionchus* (Bolivia) proved to be the most aggressive *Pristionchus* strain while *Pristionchus* (Pasadena) demonstrated the least aggressive activity. Correspondingly, *Pristionchus* (Bolivia) also provoked a robust *C. elegans* avoidance response with our quantitative avoidance test, the single drop assay. Conditioned media pulled from a pellet of underfed *Pristionchus* (Bolivia) elicits robust avoidance, while supernatant from underfed *Pristionchus* (Pasadena) causes only moderate avoidance. Interestingly, *C. elegans* uses many of the classic neurotransmitter pathways found in vertebrate models (for ex. acetycholine, serotonin, dopamine) to execute cellular communication regarding behavior. Preliminary results reveal that *C. elegans* pre-treated with Fluoxetine, commonly known as Prozac, demonstrate a reduction in predator avoidance behavior, suggesting involvement of the serotonin pathway in predator avoidance behavior.

610B

Uncovering the molecular basis for ethanol activation of the BK channel using random mutagenesis. **Scott J Davis**[1], Sam Song[1], Kelly Milman[2], Jon Pierce-Shimomura[1]. 1) University of Texas at Austin, Austin, WI; 2) Brown University, Providence, RI.

Previous research has demonstrated a strong genetic component in the development of alcohol abuse. Specifically, people with a high resistance to intoxication at the time of their first drink are at risk. This suggests protein targets of alcohol or their downstream effectors that mediate behavioral intoxication are critical for the potential of an individual to abuse alcohol. A highly conserved target protein that is emerging as a key mediator for behavioral intoxication and tolerance is the big conductance potassium (BK) channel(1). The BK channel is activated by low levels of alcohol (~20 mM) across many species including *C. elegans*, rodent and human, which is equivalent to the legal level of intoxication. In mice, this channel contributes to both behavioral intoxication and tolerance. A gain-of function mutation in the BK channel results in hypersensitivity to alcohol in humans [2]. In addition, a genetic screen identified a null mutation in the slo-1 gene, which encodes the BK channel, as critical for resistance to behavioral intoxication in *C. elegans*[3]. In order to elucidate the interaction of ethanol with the BK channel at the molecular level, we are using a genetic screen to uncover novel non-null mutations throughout the SLO-1 channel that result in resistance to behavioral intoxication. In addition, we are using random mutagenesis to uncover novel non-null mutations in the C-terminus of the SLO-1 channel, a region previously implicated in the response to ethanol in mice[4]. Non-null candidates will be distinguished from null mutants by differences in locomotory pattern and subsequent sequence analysis. So far, one of the 20 mutants has been identified as a candidate non-null mutant. After a non-null mutant is identified, we will perform *in vivo* patch-clamp recordings to assess how the mutation alters basal BK channel function and the response to alcohol. This study may provide knowledge on how ethanol acts on the BK channel at the molecular level to induce intoxication across species.

1. Treistman and Martin. BK Channels: mediators and models for alcohol tolerance, Trends Neurosci. 2009. 32(12)629-37; 2. Du et al., Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder Nat Genet. 2005. 733-8; 3. Davies et al., A central role of the BK potassium channel in behavioral responses to ethanol in C. elegans. Cell. 2003. 115(6) 655-66; 4. Liu et al., Ethanol modulates BKCa channels by acting as an adjuvant of calcium. Mol Pharmacol. 2008. 74(3) 628-40.

611C

Behavioral characterization of BTBD9 homolog knockout in *C.elegans*. **Atbin Doroodchi**[1,2], Mark DeAndrade[2], Anthony Bucolo[1], Michael Miller[3], Charles Amsler[1], Qiang Ding[3,4], Yuqing Li[2]. 1) Department of Biology,University of Alabama at Birmingham, Birmingham, AL; 2) Department of Neurology,University of Florida, Gainesville, FL; 3) Department of Cell biology,University of Alabama at Birmingham, Birmingham, AL; 4) Department of Medicine,University of Alabama at Birmingham, Birmingham, AL.

Restless legs syndrome (RLS) and Tourette syndrome (TS) are two common movement disorders. RLS is a movement and sleep disorder with 10% prevalence in the general population and it is characterized by unpleasant sensations in the legs and an uncontrollable urge to move them. TS is a rare neurodevelopmental disorder that is characterized by physical tics. Two genome wide association studies have associated a common gene, BTBD9, to these two diseases. BTBD9 is a protein of unknown function that contains two highly conserved protein domains, a BTB/POZ domain and a BACK domain. The inrton variances in the BTB/POZ domain of BTBD9 are associated with RLS and TS. BTBD9 has several homologous proteins across species, including C05C8.6 in C. elegans, with a 40% protein homology. C05C8.6 is located on chromosome V and it consists of 5 exons and 4 intron, and it is expressed in the head neurons, intestine, pharynx and seam cells (Baillie's lab, unpublished data). The function of C05C8.6, like BTBD9, is unknown. A strain of worms containing a knockout of this gene, tm3719, has been previously generated by the National BioResource Project through chemical mutagenesis by removing part of the

second intron and second exon. After backcrossing three times with the wildtype N2 worm, we have found that the mutant strain have an egg-laying deficiency, hypoactivity, and higher rate of change in direction. The mutant worms' synaptic plasticity and chemotaxis will be further characterized. These findings will help to develop an understanding of the function of BTBD9 and potentially the underlying pathophysiology of RLS and TS.

612A

The functional importance of the MEC-4 transmembrane domain in force activation of the channel. **Amy L. Eastwood**, Miriam B. Goodman. Mol & Cell Physiol, Stanford Univ, Stanford, CA.

The C. elegans mechanoelectrical transduction channel MEC-4 is the most well-established force-gated ion channel to date. To gain insight into how it translates mechanical energy into ion flux, we incorporated single-copies of mutant MEC-4 genes into worms lacking the wild-type channel. We focused on two highly-conserved sequences in the channel: (1) the GxxxG motif in the pore-lining helix; and (2) the LxxxϕG sequence in the extracellular domain, which may act as a gating hinge by coupling the extracellular domain to the transmembrane domain. To study the role of the GxxxG motif, each glycine was mutated to the slightly larger alanine. Behavioral assays characterizing the responsiveness of mutant worms to gentle touch indicated that even these subtle mutations were enough to disrupt the touch sensitivity of the worm. To study the role of the potential gating hinge, the aromatic tyrosine in the LxxxϕG sequence, the asparagine at the top of the first transmembrane helix, and the glutamate at the top of the second transmembrane helix were ablated by mutating each to the much smaller alanine. In contrast to the GxxxG mutations, behavioral assays of these MEC-4 mutants suggested that these residues were not critical for MEC-4 channel function since all retained wild-type sensitivity. Together, these results indicate that force coupling through the transmembrane domain is crucial for the activation of the MEC-4 channel and the resulting aversive behavior to touch. We are working towards understanding how these mutations affect channel function using in vivo electrophysiology.

613B

Innexin gene *inx-4* mutant is defective in thermotaxis. **Taishi Emmei**[1], Nana Nishio[1], Hiroyuki Sasakura[1], Mari Akasaka[2], Ikue Mori[1,3]. 1) Graduate Sch of Sci, Nagoya University, Nagoya, Japan; 2) Dept of Genetics, Rutgers University, NJ; 3) CREST-JST, Japan.

Gap junctions are widely found in neural circuits, although their functional significance is poorly understood. Invertebrate gap junctions are formed by innexins and vertebrate gap junctions are formed by connexins. Although there is no significant amino acid sequence similarity between innexins and connexins, structurally compelling evidence indicates that innexins are functional analogues of the connexins. The mutant for innexin gene *inx-4* is abnormal in the ability of migration to the cultivation temperature on a thermal gradient. When wild type individuals are cultivated at a certain temperature with food and then placed on a thermal gradient, most animals migrate to the cultivation temperature. Intriguingly, *inx-4* mutants instead migrate to a slightly higher temperature than the cultivation temperature. To identify cells expressing *inx-4*, we constructed the fusion gene containing the *inx-4* genomic fragment and the 3.4kb upstream fragment fused to GFP. We observed the expression pattern of full-length *inx-4::gfp* in AFD, AWC, AIY, RIA, AIB, AVA, AVE, AVK, RIM, RMDV neuron etc. AFD, AWC, AIY, RIA are identified to regulate thermotaxis, especially AFD being a major thermosensory neuron. We introduced *inx-4* cDNA under the control of AFD specific promoter into the *inx-4(ok2373)* mutant at several doses. When *inx-4* cDNA was introduced at 20ng/µl, the thermophilic phenotype of *inx-4(ok2373)* mutants changed to cryophilic phenotype rather than normal phenotype. At 0.2ng/µl, phenotypes of transgenic lines were divided into three classes. Out of nine lines, two lines restored normal migration to cultivation temperature, three lines were thermophilic like the *inx-4(ok2373)* mutant itself and four lines exhibited cryophilic phenotype. These results indicate *inx-4* is involved in thermotaxis in a dose-dependent manner.

614C

Overexpression of APL-1 disrupts learning via the insulin/IGF-1 and TGF-β pathway. **Collin Ewald**[1,2], Ruby Cheng[1], Lana Tolen[1], Vishal Shah[1], Aneela Gillani[1], Afsana Nasrin[1], Chris Li[1,2]. 1) Dept Biol, City College New York, New York, NY; 2) Graduate Center, City Univ New York, New York, NY.

Disruption of the amyloid precursor protein (APP) gene is correlated with Alzheimer's disease (AD), a neurodegenerative disorder affecting over 5 million Americans. Accumulation of Aβ, a cleavage product of APP, into plaques is a hallmark of AD. However, overexpression of APP in mice can lead to behavioral deficits independent of neurodegeneration or Aβ accumulation. The pathways through which these behavioral deficits are activated are unknown. Here, we overexpress APL-1, the *C. elegans* orthologue of APP, in a variety of tissues using cell-specific promoters. Driving APL-1 overexpression by its endogenous promoter, which expresses in 50 neurons including ASJ, led to a diminished olfactory and gustatory chemotaxic response. Similarly, pan-neuronal promoter *snb-1* driving APL-1 led to a completely impaired olfactory and gustatory chemotactic response. However, pan-neuronal *rab-3* driven APL-1 animals showed a wild-type chemotactic response, but were impaired in olfactory and gustatory avoidance learning. Recent data indicates that the *rab-3* promoter is pan-neuronal, while the *snb-1* promoter drives expression in cell types besides neurons. Heat-shock induction of APL-1 overexpression in day one adults led to an impaired chemotactic response. To determine whether APL-1 overexpression affects other sensory modalities, we examined the touch habituation response, whereby animals no longer respond to repeated touch. APL-1 is not expressed in touch neurons under its endogenous promoter and animals overexpressing APL-1 showed wild-type habituation. By contrast, animals in which APL-1 is driven by the *snb-1* or *rab-3* promoters showed a slowed habituation rate compared to wild-type animals. The chemotaxis deficits and defective habituation response can be suppressed by mutations in *daf-16*, *daf-7* and *daf-12*. Although the mechanism of how APL-1 disrupts behaviors is still unclear, our results suggest that APL-1 might act upstream of the insulin/IGF-1 and TGF-β pathway and may be at the level of the sensory neurons.

615A

Modeling neuron, circuit and biology of Caenorhabditis elegans leads to a digital worm that conducts automatic locomotion, providing testable hypothesis regarding reversal control. Shijie Zhang[2], Beverly Piggot[3,4], Wei Jin[2], Shawn Xu[3,4], Jiong Yang[2], **Zhaoyang Feng**[1,2]. 1) Pharmacology, Case Western Reserve University, Cleveland, OH; 2) Electronic Engineering and Computer Science, Case Western Reserve University, Cleveland, OH; 3) Life Science Inistute, University of Michigan, An Arbor, MI; 4) Integrative Physiology, University of Michigan, Ann Arbor, MI.

We have constructed a virtual worm consisting of virtual neural circuits and a virtual body. The virtual worm body was simulated with a central pattern generator model powered with sine waves. Two layers of Markov processes were used to model the worm locomotion circuit, and the neurons in this circuit. Governed by its virtual neural circuits inferred with locomotion data obtained from biological worms, the virtual worm exhibits automatic locomotion which resembles the spontaneous locomotion of biological worms. The simulation results also suggested that AVA and other interneurons have distinct roles in regulating different aspects of spontaneous reversals. Using optogenetic methods that spatiotemporally control neuron activities together with neuron ablation, and a calcium imaging method to quantify neuronal activity in freely behaving worms, we experimentally confirmed these predictions. We concluded that construction of a virtual life by modeling biological laws is feasible and that cyber-experiments with a virtual life can make experimental confirmable predictions regarding novel mechanisms of behavioral regulation.

616B

Role of the 5-HT$_1$ receptor homolog, *ser-4*, and the CREB homolog, *crh-1*, in behavioral adaptation to chronic activation of serotonergic pathways in *C. elegans*. **Eric Foss**, Lucinda Carnell. Biological Sciences, Central Washington Univ, Ellensburg, WA.

We are investigating the molecular mechanisms by which neural signaling pathways are modulated by chronic elevations of serotonin (5-HT) levels. Acute 5-HT exposure decreases locomotory rate in *C. elegans*, while chronic long-term exposure to elevated 5-HT levels leads to a recovery of locomotory speed referred to as behavioral adaptation. A subsequent rebound or withdrawal occurs upon removal of animals from 5-HT, resulting in an increase of locomotory rate above that observed in untreated control animals. We initiated a study using a candidate gene approach to identify 5-HT receptors regulating these chronic responses to 5-HT in order to identify specific signaling pathways mediating these behaviors. We examined mutant animals with deletions in the five characterized 5-HT receptor orthologs for behavioral deficits and all of these single mutant animals underwent normal adaptation responses suggesting that multiple 5-HT receptors and neural pathways are involved. However, one mutant, *ser-4*, failed to display withdrawal responses. SER-4, which encodes for a 5-HT$_1$-type receptor ortholog, has previously been shown to couple to Gα_i and attenuate cAMP levels in cells (Olde and McCombie. 1997 J Mol Neurosci 8:53-62) suggesting a conserved function with higher organisms. Chronic 5-HT exposure could result in SER-4 receptor desensitization and thus elevated levels of cAMP. To further support a hypothesis for a role of cAMP in producing withdrawal responses, we tested a mutant defective in the *crh-1* gene, ortholog of the cAMP responsive element binding protein (CREB). We found that *crh-1* mutant animals are also defective in withdrawal supporting a role for a cAMP-dependent pathway in this process. Using *ser-4*::GFP reporter constructs, previous studies have demonstrated that SER-4 is expressed in a small subset of interneurons and other unidentified neurons (Tsalik et al. 2003 Dev Biol 263:81-102). We are currently attempting to identify these other neurons to determine whether or not SER-4 is acting on pre-synaptic serotonergic neurons or post-synaptic neurons. 5-HT$_{1A}$ receptors and their desensitization have been implicated in chronic effects associated with elevated serotonin levels that occur with treatment of selective serotonin reuptake inhibitors (SSRIs).

617C

Chemotaxis behavior is regulated by germline in *C. elegans*. **M. Fujiwara**, N. Sato, S. Maruyama, T. Akamine, T. 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, we analyzed a germline-ploliferation-defective mutant, *glp-1*, and found that this mutant shows a mild chemotaxis defect to diacetyl, an AWA-sensed volatile attractant, but not to other odorants including other AWA-sensed odorants. Ablation of the germline precursor cells (Z2, Z3) in wild-type animals with a laser microbeam confirmed the germline effect; those animals also showed the decreased chemotactic response specifically to diacetyl. 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. AWA neurons, in the control animals with normally developed gonads, responded to diacetyl presentation with sharp increases in calcium levels.

Interestingly, the AWA neurons in the animals without germline responded to diacetyl in the similar manner, suggesting that the behavioral difference is not due to the AWA sensitivity to diacetyl per se. To reveal the molecular mechanism of the chemotactic regulation by germline, we conducted a genome-wide RNAi screening, and found that RNAi knockdown of the genes encoding electron transport chain components and ATP synthase subunits restored the chemotactic response to diacetyl in the animals without germline, suggesting the important role of mitochondria. We are currently trying the developmental-stage specific and tissue specific RNAi analyses to address how mitochondria function is involved in the control of chemotactic behavior. We hope that further analyses will reveal the molecular and cellular mechanisms how gonad signal(s) control the olfactory response to a specific attractant.

618A

Automated and Quantitative Method to Identify Satiety Quiescence by Locomotion. **Thomas L. Gallagher**, Young-jai You. Biochemistry and Molecular Biology, Virginia Commonwealth University, Richmond, VA.

Satiated animals stop eating and often fall asleep. Previously we reported a new behavior, satiety quiescence, in worms which mimics the satiety behavior in mammals [1]. To date the behavior has been evaluated by eye and only at specific refeeding time points to not disturb worms. We have therefore been limited in investigating the time course of the behavior or whether certain behavioral states are ruled by changes in nutritional status during refeeding. In fact, several works analyzing worm locomotion have identified two states of locomotion, roaming and dwelling, affected by nutritional state and sensory perception [2,3]. This suggests locomotive activity can be readout of animals' nutritional states. However, satiety quiescence has not been identified using locomotive activity. We have developed an automated, quantitative, and unbiased system to monitor worm locomotive activity to identify satiety quiescence in addition to previously characterized roaming and dwelling behaviors. With our system, we found long periods of little or no movement after starvation and refeeding, distinct from dwelling, consistent with the previous method to evaluate quiescence. As with satiety quiescence, this inactivity state is dependent on nutritional status and food quality; worms are active on poor quality food but are inactive in high quality food. Worms that have experienced starvation show more pronounced and longer inactivity than worms that have not experienced starvation. Moreover, mutants of insulin, cGMP, and TGF-β signaling pathways that are known to regulate satiety quiescence lack this prolonged inactivity. We identify this inactivity in locomotion as satiety quiescence and provide a new and easy method to evaluate and analyze satiety quiescence. We are currently generating a mathematical model based on this data using a Hidden Markov Model to address whether there are specific patterns of activity and if so whether those patterns are cyclic. 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.Ben Arous, et al., Molecular and Sensory Basis of a Food Related Two-State Behavior in C. elegans. PLoS ONE, 2009. 4(10): e7584. 3. Fujiwara, et al., Regulation of Body Size and Behavioral State of C. elegans by Sensory Perception and the EGL-4 cGMP-Dependent Protein Kinase. Neuron, 2003. 36(6): 1091-102.

619B

Quantitative genetic dissection of a behavioral sequence in C. elegans. **Rajarshi Ghosh**[1], William S. Ryu[2], Leonid Kruglyak[1]. 1) Ecology and Evolutionary Biology Department, Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada.

Individuals within species exhibit heritable variation in sub-steps of a behavioral sequence. How are different parts of a behavioral sequence parsed at the genetic level? What genetic changes and architecture allow for natural variation in substeps of a behavioral sequence?

To address these questions we transiently raised the local temperature around a worm and recorded several aspects of the resulting escape response. Upon sensation of a thermal impulse worms exhibit a behavioral sequence in which they enter a pause state followed by reversals, omega turn and resumption of forward movement. We characterized several aspects of the escape response for the laboratory strain (N2) and a wild isolate (CB4856) of *C. elegans* for stimuli resulting in 0.3, 0.8, 3 or 7.5ºC rise in temperature. We found that various aspects of the response to 0.3ºC increase in temperature exhibited the greatest difference between these strains. We employed a quantitative trait loci (QTL) mapping approach and measured several traits including but not limited to speeds at every 0.18 seconds during the assay, probability of transition between various states, duration and number of reversals in recombinant inbred lines (RIAIL) made from N2 and CB4856.

We identified several QTL suggesting different aspects of the escape response are under distinct genetic control e.g. different phases of speed-profile during the assay were genetically separable. *npr-1*, shown to be responsible for variation in speed between N2 and CB4856, contributes to variation in pre-stimulus speed and deceleration. QTL on chromosome(chr) IV contributes to variation in acceleration to maximum speed after the thermal impulse.

Probability of responding by reversal is regulated by additive QTL on chr V and X. For this trait, F1 worms resulting from reciprocal crosses between N2 and CB4856, responded like CB4856 suggesting molecular loss of function allele(s) in N2. Using introgression lines we narrowed down the QTL interval on chr X to ~90 genes.

To determine if the pattern of genetic correlation between different aspects of escape behavior in the RIAILs reflect the broader population of *C.elegans* we quantified responses of 70 wild isolates of distinct haplotypes. Preliminary analysis suggests abundant phenotypic variation and a pattern of genetic correlation among the different sub-behaviors similar to RIAILs.

620C

Secondary Allele Screen for Extreme Habituation Phenotypes after High Throughput Behavioral Characterization of a Nervous-system-biased Mutant Library. **Andrew C. Giles**[1,2], Nicholas A. Swierczek[3], Rex A. Kerr[3], Catharine H. Rankin[1,4]. 1) Brain Research Ctr, Univ British Columbia, Vancouver, BC, Canada; 2) Graduate Program in Neuroscience, Univ British Columbia, Vancouver, BC, Canada; 3) Janelia Farm Research Campus, HHMI, Ashburn, VA, USA; 4) Department of Psychology, Univ British Columbia, Vancouver, BC, Canada.

Habituation is the most simple and fundamental form of learning and is measured as a decrease in response to a repeated stimulus. We assay habituation of the tap withdrawal response in *C. elegans* using the Multi-Worm Tracker (MWT), which allows rapid characterization of tap habituation and therefore enables the testing of large numbers of mutants. The assay consists of 10 minutes of recording prior to the stimulation protocol to assess locomotion and spontaneous reversal rates followed by thirty mechanical taps to the side of the plate at a 10 second inter-stimulus interval. In wild-type worms, the tap initially elicits a reversal response that gradually habituates with repeated presentations. We have collected and tested a nervous-system-biased mutant library (~700 strains) by cross-referencing a list of 2073 genes with predicted neural function based on domain structure (Sieburth *et al.* Nature:436, 2005) with the list of available strains at the *Caenorhabditis* Genetics Center. This identified a number of mutants with habituation phenotypes, but it was unclear whether these phenotypes were caused by the known mutation, or background mutations. We have now screened a set of secondary (and for some, tertiary) mutations to test if the phenotypes are consistent between alleles. The genes that cause the strongest effects on habituation and were consistent across all tested alleles encode the G-alpha-protein *goa-1* and the regulator of G-protein signaling (RGS) known as *eat-16*. We are currently following up on these genes as well as others to develop new insights into the molecular mechanism of habituation.

621A

Male mating potency decline during adulthood is correlated with the increase of sex muscles excitability in *C. elegans*. **Xiaoyan Guo**[1], Rene Garcia[1,2]. 1) Biol, Texas A&M Universtiy, College Station, TX; 2) Howard Hughes Medical Institute, Texas A&M University, College Station, Texas.

C. elegans contributes tremendously to identifying genetic and environmental factors which affect the lifespan. However, little is known how different behaviors differentially deteriorate during aging. In this study, we are interested in understanding the mechanism by which *C. elegans* male mating behavior changes during adulthood. Through mating potency analyses, we found that wild-type *C. elegans* virgin males' mating behavior begins to decline at day 3 of adulthood. 80%, 70% and 40% males can sire progeny at day 1, day 2 and day 3, respectively. 20 hrs of transient starvation during early adulthood or periodic mating over 2 days can extend male mating potency. To further determine the mechanism of mating potency decline, we observed and recorded the mating behavior of males at different ages. Day 3 males can sense the hermaphrodite as well as younger males. However, day 3 males showed spicule prodding at non-vulva regions during the backing step of mating behavior, which is rarely observed in younger males. This indicated that the excitability of neurons and sexual muscles involved in spicule insertion might increase during aging. We then determined the sex muscles' excitability during aging though pharmacological tests. The neuromuscular mating circuit of *C. elegans* males is mainly regulated by ACh signaling. We checked the sensitivity of acetylcholine signaling during aging by exposing males to ACh agonists such as levamisole and arecoline. The results showed that males become more sensitive to ACh agonists when they are aged. Interestingly, transient food starvation and periodic mating which prolong mating potency also attenuate the increase of male sex muscle excitability. We monitored the calcium transient using GCaMP expressed in the sex muscles of different aged males during both mating and non-mating states. Consistent with the pharmacological results, older males displayed more random calcium transients even during the non-mating state. During mating, we observed much higher calcium transients in older males. We then artificially decreased ACh signaling by reducing ACh expression via mutants to see if this can prolong mating potency. Indeed, males with one functioning copy of ACh receptors genes (*unc-29*, *acr-16*,*acr-18*, and *gar-3*) showed decreased excitability at day 3 and increased mating potency. These results suggested that the degeneration of male mating behavior during adulthood is correlated with an increase in sex muscle excitability. We are currently identifying the molecular factors that affect or maintain the sex muscles excitability during adulthood.

622B

Another PKG: C09G4.2. **Anu Gupta**. University of California, Davis, Davis, CA.

For worms, recognition towards certain odors and foods is a fundamental behavior in life. It is necessary to distinguish between profitless and beneficial volatile odors for survival. Odors can be sensed by sensory neurons within the Caenorhabditis elegan head region. Certain odors such as benzaldehyde, butanone, and isoamyl alcohol can be sensed by specific neurons, like the AWC. We are investigating the signaling pathway associated with the behavioral pattern of the worm when exposed to a volatile odor. Adaptation refers to when an organism learns to ignore a persistent, profitless odor. A PKG is a serine/threonine specific kinase activated by cGMP. We have identified egl-4 as a Caenorhabditis elegans PKG and have shown that long-term adaptation results in the translocation of EGL-4, from the AWC-cytoplasm to the AWC-nucleus. It is known that in an egl-4 null mutant,

adaptation behavior becomes defective. This suggests that adaptation is a cell intrinsic process initiated by cGMP (L'Etoile et al., Neuron, 2002). Because EGL-4 seems to be crucial in the adaptation to volatile odors, we wondered if there was another PKG. A second cGMP dependent protein kinase (PKG) was recently identified within the Caenorhabditis elegans genome. It is encoded by C09G4.2. We would like to determine if C09G4.2 is involved in AWC-mediated behaviors. In order to examine the role of this PKG, we are examining AWC-mediated behavior in ok966 and tm3878 deletion mutant strains. We have conducted chemotaxis, adaptation, and food block assays in order to check the role of this PKG in sensory neurons.

623C
Candidate mediators of the food block on olfactory adaptation. **Paula M. Gutierrez**[1], Jared Young[1], Joshua Braslow[1], Noelle L'Etoile[2]. 1) Biology Dept, Mills College Oakland, CA; 2) Center for Neuroscience, University of California Davis, Davis CA.

Animals sense a variety of environmental cues and can change their behavior as a consequence of the experience. For example, Caenorhabditis elegans is attracted to specific odors that are sensed by the paired AWC olfactory sensory neurons; however, the attraction decreases if exposure occurs in the absence of food, referred to as olfactory adaptation. We seek to further explore odor processing and its interaction with food sensory signals in the model organism C. elegans. Adaptation in C. elegans to particular odorants does not occur when exposure is accompanied by food. We are focusing on understanding the role of insulin signaling and oxygen sensing in the food block of adaptation by studying the behavior and neurobiology of mutant strains. Olfactory stimulation and the accompanying behavioral responses are important pathways for complex organisms and this fundamental project will contribute to its understanding.

624A
The Effect of Hermaphrodite Sperm Status on *C. elegans* Sexual Attraction. **Leonard A. Haas**[1], Natalia Morsci[1,2], Maureen Barr[1]. 1) Department of Genetics, Rutgers, The State University of NJ, Piscataway, NJ, 08854 USA; 2) Cell and Molecular Biology Program, University of Wisconsin-Madison, Madison, WI, 53706 USA.

Sexual behaviors are modulated by numerous sensory signals. We use *C. elegans* to study how hermaphrodite-derived signals regulate male mating behavior. Male-specific genes *lov-1* and *pkd-2* are homologous to human autosomal dominant polycystic disease (ADPKD) genes. *lov-1* and *pkd-2* mutants are defective in multiple male sensory behaviors, including contact-based response (Barr and Sternberg 1999; Barr et al. 2001). Strikingly, *lov-1* and *pkd-2* males exhibited a threefold increase in response to sperm-depleted N2 or sperm-less *fog-2* hermaphrodites. However, this improved response elicitation was decreased when *fog-2* hermaphrodites were supplemented with male sperm. Wild-type males exposed to a mixture of sperm-depleted and self-sperm containing hermaphrodites also prefer sperm-depleted hermaphrodites. We conclude that hermaphrodite sperm status affects male mating behaviors via an unidentified ADPKD-independent pathway.

We wanted to determine the nature of the signal that causes the increase in attractiveness to sperm-depleted hermaphrodites, using *pkd-2* males as a sensitized background. We considered three possible sources that caused the increased attractiveness of sperm-depleted hermaphrodites: spermatogenesis, sperm activation, or the presence of fertilized oocytes. Through experiments with *spe-19* and *spe-38*, we concluded that inactivated spermatids failed to inhibit hermaphrodite attractiveness; however, fertilization defective spermatozoa were capable of inhibiting hermaphrodite attractiveness. Although we do not know the molecular mechanism by which sperm signaling inhibits hermaphrodite mating cue, we show that it is triggered during or after sperm activation. We conclude that the reproductive state of the hermaphrodite impacts male behavior and mate choice. We are currently testing the different aspects of sperm activation for its effect on hermaphrodite attractiveness.

625B
Antipsychotic drug-induced developmental delay and lethality require the sphingomyelin synthase gene sms-1. **L. Hao**, B. Cohen, E. Buttner. Mailman Research Ctr, McLean Hosp, Belmont, MA.

We use C. elegans for pharmacogenomic studies to identify novel genes underlying the mechanisms of action of antipsychotic drugs (APDs). Many APDs delay worm development or cause lethality in a dose-dependent manner, but clozapine, which is uniquely effective clinically, also has unique effects on growth and behavior in worms. A genome-wide RNAi screen for suppressors of clozapine-induced larval arrest (Scla) yielded 42 genes, including the sphingomyelin synthase gene sms-1. Sphingomyelin synthases are critical for cell growth and survival, since they regulate important signaling molecules via conversion of ceramide and phosphatidylcholine to sphingomyelin and diacylglycerol. We validated our RNAi result by outcrossing the sms-1 deletion ok2399 six times and then confirming the Scla phenotype. In addition, sms-1(ok2399) suppresses the developmental effects of other APDs, such as chlorpromazine and fluphenazine. In contrast, deletions of the sphingomyelin synthase genes sms-2 and sms-3 fail to produce the Scla phenotype. sms-1(ok2399) is also slow-growing and uncoordinated (Unc). Since sms-1 encodes six isoforms, we made three transcriptional and three translational GFP fusion constructs of these isoforms in order to generate transgenic animals for analysis of their gene expression patterns and protein localization. We will test our translational constructs for rescue of the Scla phenotype. We will also test a variety of mutants involved in ceramide and diacylglycerol metabolism and signaling for the Scla phenotype. sms-1(lf) is expected to increase ceramide levels, and in other systems ceramide has been shown to antagonize PKB/Akt activity. Previous work by our laboratory1 and by Weeks et al.2 showed that APDs activate the insulin signaling pathway in C. elegans. Therefore, we are constructing an sms-1;daf-16::gfp animal to test the potential interaction of these pathways. The clinical effects of APDs arise slowly, probably through modulation of multiple cell signaling pathways. While many of the direct and immediate effects of APDs have been identified, their downstream effects on signaling pathways have not been defined. Because many of the genes in these signaling pathways are conserved across species, our studies may illuminate new pathways by which APDs produce their effects in humans, leading to improved or novel treatments for psychotic disorders. References: 1.Karmacharya, R. et al. Clozapine interaction with phosphatidyl inositol 3-kinase (PI3K)/insulin-signaling pathway in Caenorhabditis elegans. Neuropsychopharmacol 34, 1968-1978 (2009). 2.Weeks, K.R. et al. Antipsychotic drugs acivate the C. elegans Akt pathway vis the DAF-2/Insulin/IGF-1 receptor. ACS Chem Neurosci 1, 463-473 (2010).

626C
TYRA-3 mediates the tyraminergic "inhibition" of 5-HT-stimulated aversive responses in *Caenorhabditis elegans*. **Vera M. Hapiak**, A. Stein, A. Korchnak, R. W. Komuniecki. Dept Biological Sciences, University of Toledo, Toledo, OH.

Monoamines and peptides modulate neuronal plasticity and interact to define behavioral state in both vertebrates and invertebrates. In *Caenorhabditis elegans*, monoamines modulate aversive responses mediated by the polymodal, nociceptive ASH sensory neurons (Wragg *et al*., 2007; Harris *et al*., 2009, 2010). Previously, we have shown that food or 5-HT-stimulated aversive responses to dilute octanol are delayed by both octopamine (OA) and tyramine (TA) and demonstrated that the OA inhibition of ASH-mediated aversive responses is mediated by three different OA receptors that either modulate the ASHs directly or stimulate the release of an array of neuropeptides from the ADL, AWB and ASI sensory neurons that inhibit maximal ASH-mediated aversive responses through distinct peptide receptors expressed outside the ASH-mediated circuit (Wragg *et al*., 2010 *C. elegans* Neuro Meeting, #115). In the present study, we have dissected the role of TYRA-3, a $G\alpha_q$-coupled TA receptor, in the TA-dependent delay of 5-HT-stimulated aversive responses to dilute octanol. TA does not inhibit 5-HT-stimulated responses in *tyra-3* null animals and this TA "inhibition" can be fully restored by the expression of a full-length *tyra-3* transgene. *tyra-3* is expressed in the ventral ganglion, gonad, and a number of neurons in the head and tail, based on expression of full-length rescuing *ptyra-3::tyra-3:gfp* transgenes. Importantly, as described above for OA, the TA signal modulating ASH-mediated aversive behavior also appears to be amplified by the *tyra-3*-dependent release of neuropeptides that appear to differ significantly from those involved in OA-dependent "inhibition." These studies are continuing to define the relationship between monoaminergic and peptidergic signaling in the modulation of sensory-mediated locomotory behaviors.

627A
Oriental Beetle Pheromone Insensitive Mutants in Pristionchus pacificus. Jonathan Yaghoobian[1], Jessica Ciinkornpumin[1], Judy Salandanan[1], Neomal Muthumala[1], Jeffrey Shibata[1], Christoph Dieterich[2], **Ray Hong**[1]. 1) Dept Biol, California State Univ, Northridge, CA; 2) Max-Delbruck Center for Molecular Medicine, Berlin, Germany.

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 United States. Although biologists have been aware of such insect-nematode associations for some time, the details of these interactions remain largely unknown. In Pristionchus pacificus, the cGMP dependent protein kinase, Ppa-egl-4 is an important genetic modifier of insect pheromone reception. Upregulation of Ppa-egl-4 transcripts depends on intracellular cGMP levels and is highly variable among wild isolates from diverse host ranges. In particular, chemoattraction to the Oriental beetle sex pheromone is very strong in the Washington isolate (PS1843) but is elicited in the reference isolate California (PS312) only after a brief exogenous cGMP treatment. To obtain additional factors involved in insect pheromone attraction, we performed forward genetic screens in P. pacificus for mutants that do not show attraction toward the oriental beetle pheromone (Z-7-tetradecen-2-one, ZTDO) in both the natural isolates. From our behavior-based screens, we isolated two Oriental Beetle pheromone Insensitive mutants, obi-1 and obi-3 from the California strain, as well as two Washington obi's, wobi-2 and wobi-4. The chemosensory phenotype of obi-1 and obi-3 is strongly 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. The chemosensory profiles of wobi-2 and wobi-4 are also specific for ZTDO but show noticeable decrease in two other odors as well. We focused on the molecular cloning of obi-1 based on its strong and specific ZTDO attraction defect. In addition to the chemosensory defect, adult obi-1 hermaphrodites are ~20 percent longer than wildtype and moderately egg-laying defective. Using molecular SSLP markers, we were able to delineate the obi-1 lesion to a ~428 kb region represented by the ends of Supercontigs 1 and 15 on chromosome I. We further identified all polymorphic sites between PS312 and obi-1 in both Supercontigs by whole genome sequencing (Illumina) and found a single basepair deletion in the predicted coding region of a gene homologous to the C. elegans C06G1.1, an uncharacterized protein with a lipid-binding motif. Confirmation of this novel gene as a mediator of EGL-4-dependent insect pheromone sensing by transgenic rescue and RNAi knockdown is currently ongoing.

628B
Molecular mechanism governing interaction between nematodes and nematophagous fungi. **Yen-Ping Hsueh**[1], Erich Schwarz[1], Ry Forseth[2], Frank Schroeder[2], Paul Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125,USA; 2) Boyce Thompson Institute and Department of

Chemistry and Chemical Biology, Cornell University, Ithaca, NY 14853, USA.

C. elegans shares the same ecological niche with their predators, nematophagous fungi, which are ubiquitous in the soil. The predator-prey relationship between nematodes and nematophagous fungi makes them an attractive model to study co-evolution. When exposed to one of the most common nematophagous fungi, *Arthrobotrys oligospora*, both *C. elegans*, and other nematode species were attracted to *A. oligospora* in chemotaxis assays. Furthermore, chemotaxis assays performed on partition plates demonstrated that volatile compounds produced by *A. oligospora* contribute to worm attraction. Genetic analysis and cell-specific laser ablation showed that the AWC neurons are required for chemotaxis toward *A. oligospora* in *C. elegans*. Using gas chromatography-mass spectrometry (GC-MS), we identified volatile organic compounds (VOCs) produced by *A. oligospora* that could potentially mediate worm-attraction. We will use single neuron transcriptome profiling and comparative genomics to identify potential G-protein coupled receptors (GPCRs) involved in the detection of *A. oligospora*. The candidate GPCRs will be expressed in heterologous systems to test for potential ligand-receptor specificity, providing insights into the function and evolution of GPCRs in nematodes.

629C

An RNAi screen for defecation mutants. Andrew Burns, **Lois Immerman**, Jessica Bennett, Latarsha Porscher, Samuel McCright, Hillary Mullan, Monica Raible, Matthew Miller, Maureen Peters. Biology Dept., Oberlin College, Oberlin, OH.

Defecation in *C. elegans* is a highly regulated process that takes place approximately every 50 seconds in a feeding animal. The cycle is composed of three distinct muscle contractions: a posterior body contraction, an anterior body contraction, and an enteric muscle contraction. This one-minute biological rhythm is coordinated by a multi-tissue signaling system that utilizes several signaling molecules including calcium, protons, peptides, and classical neurotransmitters. The cycle's timekeeping mechanism is located in the intestine and requires calcium flux. Proton exchange at the intestinal membrane elicits contraction of the overlying body wall muscles. The subsequent contractions, anterior body and enteric muscle contractions, require the release of either peptidergic signals or a combination of peptidergic and GABAergic signals from neurons. Such signaling components have been identified primarily by forward genetic mutations that affect individual or multiple muscle contractions and/or cycle timing. Since these forward genetic screens have not been saturated, other critical components of the defecation cycle remain to be discovered.

A reverse genetic RNA interference (RNAi) screen may permit the identification of additional defecation cycle components. RNAi is induced in *eri-1(mg366)* animals by feeding bacterial strains from the ORFeome RNAi library. Animals are treated and grown on NGM plates with a transcriptional inducer present. F1 progeny are visually scored using a standard dissecting microscope. The primary phenotype of interest is constipation as evidenced by a distended intestinal lumen. Other traits are also noted. The swollen intestinal lumen is indicative of constipation and defective defecation and is common to cycle timing and contraction mutants. Approximately 6,000 of the approximately 11,000 genes in this RNAi library have been scored to date. Thirty-three gene knockdowns have resulted in a constipated phenotype. Moreover, our study has verified genes known to affect cycle timing, posterior body contraction, and joint processes required for both anterior and enteric body contraction. This provides an independent confirmation of our screening procedure, as it has identified successfully known defecation cycle mutants. New defecation mutants have been discovered. The majority of our constipated phenotypes can be attributed to knockdowns of genes not previously shown to act in defecation. Further analysis of candidate defecation genes will provide a greater understanding of the cell and molecular pathways governing defecation.

630A

CRF receptor-like GPCR mediated stress response and regulates behavioral states in *C. elegans*. **Changhoon Jee**[1,2], Jungsoo Lee[2], Steven McIntire[2], L. René García[1]. 1) Dept. of Biology, Texas A&M University, Howard Hughes Medical Institute, college station, TX; 2) Ernest Gallo Clinic and Research Center, Department of Neurology, Programs in Neuroscience and Biomedical Science, University of California, San Francisco, Emeryville, CA.

Motivation is a goal directed driving force based on physiological needs such as hunger, sexual arousal and drug craving. Neuroadaptive changes in brain reward and stress system of animals leads to compulsive behaviors such as binge eating, obsessive drug seeking and sexual behavior. Repeated consumptions of drug leads to neuroadaptive changes to balance the effect of it resulted in tolerance and withdrawal symptom. We isolated SEB-3, CRF receptor-like GPCR, in *C. elegans* that is a key mediator in stress response. Activation of brain stress system induces behavioral arousal and development of negative reinforcement mechanism that leads to powerful motivation. We found that CRF signaling positively regulates stress response that leads to enhanced active state of locomotion, behavioral arousal and alcohol tolerance and withdrawal behavior in *C. elegans*. To further understand how *seb-3* molecularly regulates the motivated behavioral state, we studied sex-specific mating behavior of *C. elegans*. The *seb-3* is expressed mainly in nervous system and male sex muscles. Normally, mating potency declines in aging wild-type old males and food deprivation stress during early adulthood leads to neuroadaptive changes resulting in maintaining potency in older animals. Interestingly, food deprivation could not enhance mating potency in aging *seb-3 (lf)* mutant animals. Additionally, we found that *seb-3 (gf)* enhanced potency in old males without food deprivation and did not increase life span, suggesting that CRF signaling mediates maintaining male mating behavior of old male after food deprivation in *C. elegans*. We are currently pursuing an answer of where and how SEB-3 regulates cellular excitability of behavioral circuit components.

631B

EGL-19, UNC-36 and CCB-1 underlie voltage-dependent calcium currents in *C. elegans* striated muscle. Viviane Lainé[1], Christian Frøkjær Jensen[2,3], **Maëlle Jospin**[1]. 1) CGphiMC, Université Lyon 1, UMR CNRS 5534, Villeurbanne, France; 2) University of Utah, Howard Hughes Medical Institute, Salt Lake City, UT, USA; 3) University of Copenhagen, Department of Biomedical Sciences and Danish National Research Foundation Centre for Cardiac Arrhythmia, Copenhagen, Denmark.

Voltage-gated calcium channels, which play key roles in many physiological processes, are composed of a pore-forming α1 subunit associated with three auxiliary subunits. In vertebrates, the role of auxiliary subunits has mostly been studied in heterologous systems, partially because of the severe phenotypes of knockout animals. The genetic model *Caenorhabditis elegans* has all main types of voltage-gated calcium channels and strong loss-of-function mutations in all pore-forming and auxiliary subunits; it is therefore a useful model to investigate the roles of auxiliary subunits in their native context. By recording calcium currents from mutants of the different subunits, we molecularly dissected the voltage-dependent calcium currents in striated muscle of *C. elegans*. We first showed that EGL-19 is the only α1 subunit that carries calcium currents in muscle cells. We then demonstrated that the α2/δ subunit UNC-36 modulates the voltage-dependence, activation kinetics and conductance of calcium currents, whereas another α2/δ subunit TAG-180 has no effect. Finally, we characterized mutants of the two β subunits, CCB-1 and CCB-2. We showed that (i) locomotion is impaired in *ccb-1* animals whereas it is similar to wild type for *ccb-2* mutants, (ii) *ccb-1* is expressed in most muscle and neuronal tissues whereas *ccb-2* is restricted to a few neurons of the head and of the ventral cord, and (iii) voltage-dependence and conductance of calcium currents are severely altered in striated muscle cells of *ccb-1* mutants whereas they are similar to wild type in *ccb-2* mutants. Altogether these results show that EGL-19, UNC-36 and CCB-1 underlie voltage-dependent calcium currents in *C. elegans* striated muscle.

632C

CNP-2 (Calcineurin Interacting Protein-2) modulates calcineurin activity in *Caenorhabditis elegans*. **Hana Jung**[1], Hyun-Ok Song[2], Weixun Li[1], Sunkyung Lee[1], Joohong Ahnn[1]. 1) Hanyang University, Seoul, Korea; 2) 2Department of Infection Biology, Zoonosis Research Center, Wonkwang University School of Medicine, Iksan, Chonbuk, 570-749, Republic of Korea.

Calcineurin is a serine/threonine phosphatase implicated in a wide variety of biological responses. Calcineurin consists of two subunits, catalytic subunit A(TAX-6) and regulatory subunit B(CNB-1). Previously, calcinuerin interacting proteins have been screened by yeast two-hybrid assays. As result, we found that CNP-2 interacted with TAX-6 in vivo, and we showed that CNP-2 binds to the catalytic domain of TAX-6. Phenotypes of calcineurin mutants are small body size, egg lying defect and defective brood size. Therefore, we investigate function of *cnp-2* in the calcineurin-mediated signal pathway. We have constructed several double mutants between *cnp-2* mutants and calcineurin mutants. Then, we characterized phenotypes associated with calcineurin mutants. The *cnp-2* gene is expressed in head neuron, posterior intestine and spermatheca. In particular, cell junction and valve in spermatheca. The *cnp-2* mutant does not show clear phenotypic defects. However, we suggest that *cnp-2* functions as a modulator of calcineurin in *C. elegans*..

633A

Role of ER resident proteins Calnexin and Calreticulin in Chemosensory Behavior in *C. elegans*. **Karunambigai Kalichamy**, Sunkyung Lee, Joohong Ahnn. Hanyang Univ, Seoul, Korea.

Calnexin and calreticulin, lectin chaperone proteins, are calcium binding proteins in Endoplasmic reticulum(ER). These proteins facilitate the proper folding and quality control of newly synthesized glycoproteins that exit from ER. *C. elegans*, a free-living soil nematode, can detect a wide variety of volatile attractants using their chemosensory amphid neurons. *C. elegans* homolog's calnexin and calreticulin are expressed in neurons. In order to study the function of calnexin and calreticulin in chemosensory behaviors, we investigated the behavioral phenotype of calnexin (*cnx-1*) and calreticulin (*crt-1*) mutants. Both *cnx-1* and *crt-1* mutants exhibited normal chemotaxis responding to AWC sensed odorants such as isoamyl alcohol, benzaldehyde and butanone. The *cnx-1* mutants failed to adapt to AWC sensed odors whereas *crt-1* mutants exhibited hyperadaptation, when compared with wildtype. The expression of str-2::GFP in *cnx-1* or *crt-1* mutant background showed morphological defects in neurons. We are further investigating how these ER resident proteins modulate to the chemosensory behavior and the underlying mechanism.

634B

C. elegans locomotory pattern, pausing frequency and speed is regulated by the CEP sheath glia. **Menachem Katz**[1], Francis Corson[2], Shai Shaham[1]. 1) Lab Developmental Genetics, Rockefeller Univ, New York, NY; 2) Lab Theoretical Condensed Matter Physics, Rockefeller Univ, New York, NY.

Neuronal circuits are tightly regulated to achieve proper animal behavior. Recent studies suggest that glial cells can influence neuronal functions and synaptic activities, and may, thus, contribute to behavior. However, the precise contributions of synaptic glia to animal behavior are poorly understood. *C. elegans* glia share morphological, functional, and genetic features with their vertebrate counterparts. However, in contrast to vertebrate glia,

C. elegans glia are not essential for neuronal survival, offering a unique arena for exploring the involvement of glia in neuronal functions in a live animal. The CEP sheath glia (CEPsh) are bipolar cells that ensheath the dendrites of CEP neurons, and envelope the nerve ring. Reminiscent of mammalian astrocytes, these cells extend processes that abut specific synapses within the nerve ring. We showed that ablation of the CEPsh glia during the first larval stage results in abnormal locomotory behavior. CEPsh-ablated animals display reduced locomotion speed and extended locomotory pausing, as well as exaggerated small-angle turns and frequent reversals that limit their dispersal. To understand how these behaviors are regulated by CEPsh glia, we focused on the ALA-AVE synapse ensheathed by these glia (White et al., 1986). In line with the previously described role of ALA in behavioral quiescence and locomotory pausing (Van Buskirk and Sternberg, 2008), we found that inactivation of ALA reduces the pausing frequencies of glia-ablated animals. In addition, the dispersal of these animals and their speed of locomotion are improved. Inactivation of AVE in wild-type animals, induces extended pausing periods comparable to those seen in glia ablated animals, and animal speed is reduced. Our results suggest that AVE is a key regulator of speed and pausing frequency in the *C. elegans* nervous system, and that ALA functions to inhibit the activity of AVE. Moreover, our results indicate that the CEPsh glia provide important negative regulation on the activity of this tripartite synapse. To understand the molecular basis of CEPsh glia function we have begun to identify genes expressed in these cells using an RNA-tagging method, with the aim of examining the roles of enriched genes in *C. elegans* locomotory behavior.

635C

Biomechanical analysis of *C. elegans* locomotion. **Daeyeon Kim**[1], Jin-Sung Park[2], David Weitz[2], Jennifer H. Shin[1]. 1) Mechanical Engineering Dept, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Korea; 2) Dept of Physics, School of Engineering and Applied Sciences (SEAS), Harvard University, MA, USA.

The characteristics of *C. elegans'* locomotion are sensitively regulated by mechanical environments. For example, the worm's wavelength and amplitude were shown to be altered in different gelatin concentrations and swimming velocity of a worm was enhanced in a granular medium compared to that in a simple fluid [1, 2]. Therefore, understanding the mechanism behind the differential responses of *C. elegans* in mechanically different environments is an intriguing problem to gain insight on mechano-sensation of the worm. In this study, we investigate the surface rigidity dependent crawling motion of a worm as well as swimming motion in colloidal suspensions. In crawling gait, the wild-type worm adaptively changes the shapes of its sinusoidal waveform when the surface rigidity of solid agar plates is varied. However, mechanosensation-defective worms (*mec-4* and *mec-10* mutants) show insensitivity to the changes in the surface rigidity. The wild-type worm modifies its waveform by decreasing the interval of muscle contractions and increasing the extent of them as the surface rigidity increases. This modulation pattern of the muscle contraction provides more propulsive force to the worm against the environments of higher loads.

In swimming gait, worms changed their swimming speeds in different volume percents of colloidal suspensions of 600 nm sized polystyrene beads. In low volume percents of colloids (0.4 ~ 8 %), the swimming speed was similar to that in the water whereas it significantly increased in 12 and 16 % of colloids. Through a detailed biomechanical analysis, we suggest that this enhancement in swimming speed results from the interplay between the shear thinning fluid properties of 12 and 16 % colloids and the unique stroke pattern of the worm.

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[1] Berri *et al*., HFSP J. 3, 186 (2009). [2] Jung *et al*., Chaos 18, 041106 (2008).

636A

Food Size and cGMP Affects Feeding Behavior in Pristionchus pacificus. **Silvina Kroetz**, Ray Hong. CSUN, Northridge, CA.

Hong Animals evolved different locomotory behaviors in order to find food in their environment. I studied the food seeking locomotion and pharyngeal pumping of nematodes Pristionchus pacificus on various food sources. For this study I used P. pacificus PS312, and the mutants Ppa-egl-4, which is a null mutation in the cGMP dependent protein kinase, and Ppa-obi-1, which is an oriental beetle pheromone insensitive mutant, and the double mutant Ppa-egl-4;obi-1. I tested these strains on plates containing no food and on E.coli OP50, HB101, Caulobacter crescentus (NA1000) and Bacillus subtilis. Locomotory behavior was analyzed using an automated tracking system, and pharyngeal pumping data was obtained by visually counting with a microscope at 80X magnification. I observed that locomotion of the strains differed on plates with no food and plates with food. On plates with no food, P. pacificus PS312 displayed a higher reversal rate compared to the Ppa-obi-1 strain. The double mutant egl-;obi-1 displayed similar locomotion patterns to Ppa-obi-1 on HB101. My results indicate that Ppa-obi-1 may act in either a parallel pathway, or upstream of Ppa-egl-4. Furthermore, when we compared PS312 pharyngeal pumping rates on and off food on two different size bacteria E. coli and C. crescentus, results showed a significant increased rate on PS312 on C. crescentus, which was the smaller bacteria. That is, PS312 raised on C. crescentus (NA1000) for 3 generations retained memory of the food experience regardless of whether they were removed from food or placed back on NA1000 as food. Increasing bacterial size using mutant C. crescentus strains seem to further decrease pumping rates off food. Our data suggest strong roles for food sizes and cGMP sensing proteins in maintaining feeding patterns in P. pacificus.

637B

Analysis of temperature memory in cultured thermosensory neuron AFD of *C. elegans*. **K. Kobayashi**, I. Mori. Group of Molecular Neurobiology, Nagoya University and CREST-JST, Japan.

A major goal of neuroscience is to understand how animals memorize a variety of information. The molecular mechanism on memory formation however is still largely unknown. To address this important question, thermotaxis of *C. elegans* provide a model system, when combined with powerful experimental methodologies. *in vivo* calcium imaging showed that the thermosensory neuron AFD responds to temperature above the threshold temperature that is set by previous cultivation temperatures: threshold temperature is lower when cultivation at lower temperature and higher when cultivation at higher temperature (Kimura *et al.,* 2004). This experience-dependent thermal response was unaltered in either *odr-7* mutants that disrupt AWA neuron presynaptic to AFD or *ttx-3* mutants that disrupt AIY neuron postsynaptic to AFD, and was still detected in the AFD sensory ending disconnected from the cell body, suggesting that the AFD neuron itself stores a temperature memory (Clark *et al.,* 2006). Toward further investigating temperature memory in AFD, we have started to conduct *in vitro* calcium imaging of cultured AFD neurons, aiming to exclude the effects of synaptic connections and secreted molecules on AFD. We are currently testing if experience-dependent thermal response is observed in the cultured AFD neurons. Once we could show that individual cultured cells maintain temperature memory, we are hoping to initiate pharmacological and electrophysiological analyses, thereby revealing principle of memory formation mechanism.

638C

A Deletion Mutation of the *C. elegans* CaMKII Gene *unc-43* Inhibits Associative Conditioning and Modulates Non-Associative Conditioning. **Sara M. Knauft**[1], Robert M. Bragg[2], Jade R. Brusseau[1], Jacqueline K. Rose[1,2]. 1) Behavioral Neuroscience Program; 2) Psychology, Western Washington University, Bellingham, WA.

Calcium-calmodulin protein kinase II (CaMKII) is widely expressed throughout the mammalian brain and has long been known to play a role in learning and memory. In *C. elegans*, the *unc-43* gene is an ortholog to mammalian CaMKII and although the role of UNC-43 has been well-characterized in basic worm function (e.g., egg-laying, defecation, etc.), studies investigating the role of UNC-43 in *C. elegans* learning has been limited due to the uncoordinated motor phenotype *unc-43* mutant strains exhibit. The current study utilized the *unc-43*(gk452) mutant strain (generated and identified by the *C. elegans* Knockout Consortium) as this strain is superficially wild-type displaying no obvious motor defects thus allowing for its use in behavioral paradigms. Associative learning was tested by employing both an associative chemotaxis assay and an associative chemoavoidance test. Both tests consisted of exposing worms to an attractant compound (i.e., NaCl) under "no food" conditions and then testing for either attraction to or avoidance of NaCl. Results from both associative protocols reveal that unlike wild-type, *unc-43*(gk452) worms continue to show a preference for NaCl after a one-hour "no food" pairing. To determine if this learning phenotype is specific for associative conditioning, *unc-43*(gk452) worms underwent habituation training (non-associative conditioning) that included repeated exposure to a low-intensity mechanosensory stimulus; locomotor reversal responses were scored. When habituation stimuli are delivered at a 60 second interstimulus interval, *unc-43*(gk452) worms show a slower rate of response decrement compared to wild-type, suggesting habituation is somewhat impeded, although not altogether absent. Taken together, it is concluded that the *unc-43*(gk452) strain displays an impairment in associative learning and possibly plays a role in non-associative conditioning as well. The *unc-43*(gk452) mutant strain consists of an insertion/deletion modification that includes the deletion of coding exon 5 of the UNC-43C isoform. Future work will assess if more specific mutations within this region account for this learning phenotype. *Support for this project provided by BRAIN funding in the form of new faculty start-up and stipend to JKR.*.

639A

Experience-dependent modulation of salt preference in *C. elegans*. **Hirofumi Kunitomo**, Ryo Iwata, Hirofumi Sato, Hayao Ohno, Yuichi Iino. Dept Biophys Biochem, Grad Sch Sci, Univ Tokyo, Tokyo, Japan.

Caenorhabditis elegans is attracted to a variety of chemicals including volatile odorants and water-soluble salts, and in many cases, the behavior is modified by experience. Sodium chloride is widely considered as an attractive cue for the animals. After exposure to NaCl with starvation, however, worms now avoid the salt, indicating that preference for salt is modified by the availability of NaCl and food (Saeki et al, 2001). To further clarify how salt preference is modulated by experience, we adopted a modified chemotaxis assay: animals were exposed to defined concentrations of NaCl with or without food and challenged with a chemotaxis test at a broad range of NaCl concentration. Using this assay, we found that worms are not merely attracted to salt, but they are attracted to the salt concentration at which they have been fed. In addition, worms avoid the salt concentration that they have experienced with starvation. Therefore, the behavior on salt gradient is based on salt concentration memory and depends on the food availability they experienced.

The ASE neurons, which consist of functionally asymmetric cells on the left (ASEL) and right (ASER), are known as the major gustatory neurons of this organism. Analyzing the mutants that have deficits in the function of specific sensory neurons revealed that both ASE cells are required for salt concentration-dependent behavior, although contribution of ASER is larger than that of ASEL. Cell-specific recovery of ciliary function revealed that input of NaCl only from ASER is sufficient for the experience-dependent salt preference. To elucidate how salt preference is molecularly regulated, we assessed the behavior of the previously characterized mutants that show defects in salt chemotaxis plasticity. Two

phospholipid signaling pathways, the diacylglycerol (DAG)/PKC pathway and the PI3K pathway are known to be involved in the control of the navigation modes (attraction or avoidance) in NaCl chemotaxis (Tomioka et al., 2006, Adachi et al., 2010). Activation and inactivation of the DAG/PKC pathway in ASER induced attraction to and avoidance of higher concentration of NaCl, respectively, irrespective of prior experience. Mutants for the insulin pathway, *ins-1*/insulin, *age-1*/PI3K and *akt-1*/AKT showed navigation defects only after starved conditions: they navigated to the salt concentrations they experienced even after starvation. This result is consistent with the idea that the insulin pathway is involved in switching the direction of chemotaxis toward the NaCl concentration which they have experienced.

640B

The Ca^{2+}-binding C2 domain of PKC-2 couples thermal activation of TAX-2/TAX4 to *C. elegans* behavior. **Marianne Land**[1,2], Charles Rubin[2]. 1) New York Institute of Technology, Old Westbury, NY; 2) Albert Einstein Col. Med.,Bronx, NY.

Physiological functions, effectors and upstream regulators of Ca^{2+} and diacylglycerol (DAG) activated, classical protein kinase C isoforms (cPKCs) are only partially understood. Either depletion or over-expression of PKC-2, the only cPKC in *C. elegans*, disrupts thermotaxis. pkc-2 null (*pkc-2(ok328)*), *tax-4(p678), tax-2(p671), pkc-2(ok328);tax-2(p671)* and *pkc-2(ok328);tax-4(p678)* mutants share an athermotactic (AT) phenotype. Mutant animals lose the ability to detect or localize at their previous cultivation temperature upon transfer to a thermal gradient. Instead, they move randomly, suggesting that TAX-2, TAX-4 and PKC-2 function together in a signaling pathway that couples changes in temperature to directed movement. *tax-4(p678)* and *tax-2(p671)* suppress cryophilic behavior induced by 12-fold overexpression of PKC-2 *(pkc-2(12X)*). Increased substrate phosphorylation was evident in transgenic animals (*pkc-2(12x)*) that sustain a supraphysiological PKC-2 concentration. However, phosphorylation of PKC substrates was suppressed below the basal WT level in TAX-2 or TAX-4 deficient animals *(tax-4(p678), tax-2(p671), tax-4(p678);pkc-2(12x*) and *tax-2(p671);pkc-2(12x))* expressing either normal or highly elevated amounts of PKC-2. A DAG mimetic, PMA, elicits a cryophilic phenotype in WT and TAX-2 deficient animals, whereas pkc-2 null, *tax-2(p671);pkc-2(ok328)* and *tax-4(p678)* worms are athermotactic. Thus, epistasis analysis places PKC-2 downstream from TAX-2. TAX-2 and TAX-4 form a multimeric, cGMP-regulated cation channel that transports Ca^{2+}. TAX-2 modulates the activity of TAX-4, which directly mediates cation influx. TAX-4, TAX-2 and PKC-2 are co-expressed in many neurons, including AFD, the predominant thermosensor, and other neurons in thermotaxis circuitry. The results suggest that Ca^{2+} entry mediated by the TAX-2/TAX-4 channel is critical for activation of PKC-2 and subsequent downstream signaling in the thermotaxis response. Expression of normal amounts of WT PKC-2 in the pkc-2 null background rescues the thermotaxis defect. A transgene encoding mutant PKC-2, in which the C2 (Ca^{2+} binding) domain is disrupted, failed to rescue either thermotaxis or substrate phosphorylation. This verifies that Ca^{2+} is essential for PKC-2 activation in thermotaxis and shows DAG binding via a C1 domain is insufficient for in vivo PKC-2 activation. TAX-4 may be a PKC-2 effector. A recombinant TAX-4-His-fusion protein was phosphorylated to high stoichiometry, *invitro* by PKC. Phosphorylated Ser/Thr in TAX-4 will be identified and expression of transgenes encoding phospho-mimetic and non-phosphorylatable TAX-4 mutants will be used to assess the physiological relevance of the PKC-2 target sites.

641C

Do ascarosides affect egg-laying behavior in Caenorhabditis elegans? **Circe Lassegue**, Ramadan Ajredini, Rathika Nimalendran, Arthur Edison. University of Florida, Gainesville, FL.

Caenorhabditis elegans, a small transparent nematode that lives in temperate soil environments, is one of the simplest eukaryotic organisms with a nervous system to be studied in great detail. Over recent years, a large number of ascarosides have been identified as signaling molecules in C. elegans (Edison, 2009). Ascaroside levels are affected by worm concentration and available food when developed in “worm water”. Ascarosides have been shown to regulate a large number of behaviors in C. elegans including dauer formation (Butcher, et al., 2007), mating behavior ((Srinivasan, et al., 2008), aggregation (Macosko, et al., 2009), and olfaction (Yamada, et al., 2010). Additionally, environmental and homeostatic cues are now being explored to see how these affect nematode egg-laying habits (Schafer et al., 2001). We studied the modulatory effect of several ascarosides on egg-laying behavior and brood size in adult female C. elegans. This study aims to determine the effect of ascarosides on egg-laying behavior in adult C. elegans. A range of concentrations of several synthetic ascarosides as well as natural worm water produced by C. elegans were studied. Standard egg-laying assays and known positive and negative controls were utilized (Koelle, 2004).

642A

K+ channel interplay modifies male mating response. **Brigitte L. LeBoeuf**, L. Rene Garcia. Howard Hughes Medical Institute Dept Biol, Texas A&M Univ, College Station, TX.

A large population of K+ channels provides a way for organisms to maintain and modify cell excitability, allowing for appropriate behavioral responses to various stimuli. *C. elegans* male mating is a complex behavior that requires precise regulation to be successfully completed. Males must locate a hermaphrodite, properly position their tales on the hermaphrodite cuticle, locate the vulva, insert their copulatory spicules, and transfer sperm. We focus on the spicule insertion step of mating, and have identified three K+ channels expressed in the sex circuit that regulate the timing of insertion: *ether-a-go-go* (EAG)/EGL-2, EAG-related gene (ERG)/UNC-103, and big current (BK)/SLO-1. 79% of a population of males lacking all three channels displays spontaneous spicule protraction (Prc) in the absence of mating cues. Our research indicates the function of these three K+ channels is modified by the presence or absence of the others. The loss of SLO-1 alone results in 70% instance of Prc, while a *slo-1(lf) egl-2(0)* double mutant is 30% Prc. This decrease in the mutant phenotype is due to an up-regulation of *unc-103* in the absence of *egl-2*, as a transgenically expressed dominant-negative form of *unc-103* returns the Prc phenotype to 72%. Additionally, the *rg432* allele of *slo-1* affects the Prc penetrance in an *unc-103(0);egl-2(0)* background. 58% of *unc-103(0);egl-2(0)* males are Prc; that percentage is reduced to 35% in *unc-103(0);slo-1(rg432) egl-2(0)* males. *slo-1(rg432)* is a point mutation in an intron located in a region that contains variable splicing and could result in up-regulation of specific isoforms that preferentially reduce male sex muscle excitability. In support of isoform-specific regulation, we determined that particular isoforms of *slo-1* are more capable of rescuing the Prc defect than others. Our work suggests that while the male sex muscles attempt to compensate for the loss of one K+ channel by up-regulating others, various factors exist that determine the success of such mechanisms. Future work will explore how K+ channel expression changes over time due to loss of channel function. This will allow us to dissect the relationship between the K+ channels that control appropriate sensory-motor responses.

643B

Tracing the genetic basis of the nictation behavior by QTL mapping. **Daehan Lee**[1], Junho Lee[1,2]. 1) Research Center for Functional Cellulomics, IMBG, School of Biological Sciences, Seoul National University; 2) WCU Department of Biophysics and Chemical Biology, Seoul National University, Seoul, 151-742, Korea.

Given harsh conditions such as high temperature, starvation and high population density, Caenorhabditis elegans enters an alternative developmental stage called dauer. Dauer larvae exhibit specific behavior called nictation - when a dauer confronts filamentous matter or rough surface, it stands and waves its body on the protruding ending. Nictation is a conserved behavior among nematodes, and may have been adopted as a strategy for phoresy, necromeny and parasitism. However, little is known about the genetic and neural basis of nictation. Unlike in laboratory conditions, wild strains of C. elegans habituate in fluctuating environments and they are usually isolated as dauer larvae even in a rich environment like compost soil. They are also often isolated from terrestrial isopod species, probably associated by nictation. Previously, we showed that mutants with defects in nictation could not be transported to new E. coli lawn by fruit flies in which condition these mutant dauers cannot recover from the dauer stage and reproduce. Nictation may facilitate transfer to new habitat in wild environment, which may greatly affect its survivorship and reproduction. Presumably, depending on its habitat condition, different wild isolates might have gone through different selection for nictation. In this work, we show phenotypic variations of nictation among wild isolates. By quantitative trait loci(QTL) mapping, we will trace the genetic basis of the phenotypic variation, and ultimately elucidate development and evolution of nictation.

644C

Neuronal Regulation of Nictation Behavior in Caenorhabditis elegans. **Harksun Lee**[1], Myung-kyu Choi[1], Daehan Lee[1], Hye-sung Kim[1], Hyejin Hwang[2], Heekyeong Kim[3], Sungsu Park[2], Young-ki Paik[3], Junho Lee[1,4]. 1) Research Center for Functional Cellulomics, IMBG, School of Biological Sciences, Seoul National University, Seoul, 151-747, Korea; 2) Department of Chemistry and Nano Sciences, Ewha Womans University, Seoul, 120-750, Korea; 3) Department of Biochemistry, College of Life Sciences and Biotechnology, Yonsei Proteome Research Center, Yonsei University, Seoul 120-749, Korea; 4) WCU Department of Biophysics and Chemical Biology, Seoul National University, Seoul, 151-742, Korea.

Many nematode species, including the free-living nematode Caenorhabditis elegans, exhibit an evolutionarily conserved, developmental stage-specific behavior called nictation, in which daurs stand and wave in three-dimensional loops on a projection. Here we show that nictation is necessary for nematode dispersal, which may be a critical survival strategy under harsh conditions. Using newly established assays for nictation. we showed that acetylcholine is the neurotransmitter required for nictation. By cell-type specific rescue experiments, we found that, among cholinergic neurons, IL2 head neurons were essential for nictation. Optogenetic activation of IL2 neurons using channelrhodopsin increased the nictation ratio and the genetic ablation of IL2 neurons abolished nictation. We also found the cilia structure of IL2 neurons was important for nictation by testing mutants that have defects in cilia structures. We finally demonstrated that nictation is required for transmission of dauers to a new niche using fruit flies as artificial carriers, suggesting a role of nictation as a dispersal and survival strategy under harsh conditions. We anticipate our data to be a starting point for further investigation into how external stimulus or conditions can evoke a specific responsive behavior via rewiring neural circuits at the cellular and molecular levels.

645A

STR-33, a novel G protein-coupled receptor that regulates locomotion and egg-laying in *Caenorhabditis elegans*. **J. E. Lee**[1], P. Y. Jeong[1], H. J. Joo[1], H. Kim[1], T. H. Lee[1], H. Koo[1], Y. K. Paik[1,2]. 1) Department of Biochemistry and Department of Integrated Omic for Biomedical Sciences, Seoul, Korea; 2) Yonsei Proteome Research Center, World Class University Program, Graduate School, Yonsei University, Seoul, Korea.

G-Protein Coupled Receptors (GPCRs) have variety functions in *C. elegans* as well as other animal models. These proteins mediate various sensing signaling by chemical, mechanical, temperature stimuli and so on. Most of GPCRs have seven trans-membrane domains and using this conserved structure, transmit the signaling by their specific ligands.

Signal transduction through GPCRs is conserved from yeast to mammals and mediates cellular processes as diverse as odorant detection, hormonal signaling, visions and drug response. We isolated nematode specific genes *str-33*, whose putative function was not assigned. Mutation of *str-33* gene shows deep body-bending movement and hyperactive egg-laying phenotype. This result suggests that this GPCR may act in neurotransmission system which is related to locomotion and egg-laying process in *C. elegans*. We identified that hyperactive egg-laying defect of *str-33* mutant is dependent on GOA-1 that is involved in Goα G-protein signaling. We speculate that STR-33 seven trans-membrane GPCR might be one of the neurotransmitter receptors that regulate locomotion and/or egg-laying in *C. elegans*. Endogenous acetylcholine suppresses egg-laying in C. elegans via Goα signaling pathway by inhibiting serotonin biosynthesis or releasing from the HSN neuron. In vivo expression of serotonin biosynthetic enzyme TPH-1 is up-regulated in *str-33* mutant, suggesting that STR-33 GPCR is related to neurotransmitter signaling which regulates locomotion and egg-laying of *C. elegans*. (This study was supported by a grant from the Korean Health 21 R&D Project, Ministry of Health, Welfare and Family Affairs, Republic of Korea, [A030003] and Korean Research WCU grant R31-2008-000-10086-0).

646B

Identification of Genes that Mediate Ethanol-Induced Acute Functional Tolerance in *C. elegans*. **Ka-Po Leung**, Mia Bolling, Gina Blackwell, Jennifer Gardner, Andrew Davies, Jill Bettinger. Virginia Commonwealth University, Richmond, VA, USA.

Alcohol abuse and alcoholism are prevalent diseases in our society that are physically and socially damaging. There are few treatments available today and these are largely inadequate, in part because the molecular mechanisms behind the development of alcoholism are still unclear. We know from human genetics studies that there is a significant genetic component that influences disease susceptibility, and that an individual's initial sensitivity and development of acute functional tolerance (AFT) after alcohol consumption are strong predictors of lifetime development of addiction. We have taken a genetic approach to study ethanol sensitivity and the development of tolerance in the worm. We performed a forward genetic screen for mutations in genes that are required for the development of AFT to ethanol. We identified a mutation, *bet11*, that causes mutant animals to be defective in the development of AFT in response to ethanol treatment. We have used genetic mapping to localize the gene that is disrupted by the *bet11* mutation to an approximately 80-gene interval on Chromosome I. We are currently using transformation rescue to identify the gene in this interval that is required for the development of AFT to ethanol. Additionally, a special feature of the *bet11* allele is that we have strong genetic evidence that the wild-type N2 allele of the gene that *bet11* disrupts participates in an interaction with an unlinked natural allele in CB4856. These two alleles can act together in a recessive manner to confer synthetic hypersensitivity to ethanol. Our model is that *bet11* is a strong loss-of-function allele of a gene that in wild-type N2 animals has a more modest partial loss-of-function allele, while CB4856 carries a separate low-function allele of an unlinked locus that synergistically sensitize an animal that is homozygous for both alleles to ethanol. We have localized the important CB4856 allele to a large region of Chromosome III and are currently refining our genetic mapping of this interval. Identifying and characterizing the genes that are responsible for alcohol-induced development of AFT will give us a better understanding of the neurobiological mechanisms that lead to alcohol abuse and alcoholism.

647C

Genetic and molecular investigation of PKD-2 and LOV-1 involved in male *C. elegans* perception of sex pheromone. **Rachel C.K. Li**, King L. Chow. Division of Life Science, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong.

Transient receptor potential polycystin (TRPP) complex PKD1/PKD2 in human are important in kidney's function, and their mutations are associated with polycystic kidney disease. The *C. elegans* counterparts, PKD-2/LOV-1 localized to the ciliated endings of the male specific neuron CEMs, however, are indispensable for the male response to sex pheromone.

In this study, we aim to identify additional PKD-2/LOV-1 polycystin signaling molecules required for sex pheromone perception. *atp-2* and *cwp* genes are potential *pkd-2* and *lov-1* interacting partners based on their roles in male sensory behavior and their co-expression in CEMs. In our pheromone assays, all *cwp* mutant males respond normally while males with *atp-2* knocked-down are defective, which suggest the distinct requirement of *atp-2* in this sex-specific behavior. The interdependence between *pkd-2*, *lov-1* and *atp-2* will be ascertained by testing *atp-2* RNAi males or its cell specific over-expression in *pkd-2* and/or *lov-1* mutants by pheromone assay. In addition, a *pkd-2* promoter deletion analysis has been performed to identify upstream regulatory components of PKD-2/LOV-1 TRPP channel synthesis. As genes co-expressed in a specific cell type may share a common cis-regulatory motif, identifying the signature motif for CEM-specific expression may help uncover potential *pkd-2* interacting partners by a genome-wide search for genes with a CEM-signature sequence.

For the cellular function of PKD-2/LOV-1 TRPP complex in CEMs, we hypothesize that this complex is directly activated by sex pheromone and the signal is relayed intracellularly. This hypothesis will be evaluated by an in vitro system using Drosophila Schneider cells to investigate if physiological response can be triggered via PKD-2/LOV-1 TRPP complex by sex pheromone stimulation. Results from this experiment would be informative in defining the functional activity of these TRPP channels in the sex pheromone perception, shedding light on the biology and signaling of polycystin complex. (This study is supported by Research Grants Council, Hong Kong.).

648A

The neural circuits underlying harsh touch sensation in *C. elegans*. **Wei Li**[1,2], Lijun Kang[1], Beverly Piggott[1,3], Zhaoyang Feng[4], Shawn Xu[1,3]. 1) Life Sci Inst, Univ Michigan, Ann Arbor, MI; 2) School of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei, China 430074; 3) Department of Molecular and Integrative Physiology, University of Michigan Medical School, Ann Arbor, Michigan; 4) Department of Pharmacology, Case Western Reserve University, Cleveland, OH.

Most animals can distinguish two distinct types of touch stimuli: gentle (innocuous) and harsh (noxious/painful) touch, but the underlying mechanisms are not well understood. *C. elegans* is a highly successful model for the study of gentle touch sensation, and work in *C. elegans* has derived a thorough understanding of the mechanisms of gentle touch sensation. However, little is known about harsh touch sensation. Here we characterized harsh touch behavior in *C. elegans*. *C. elegans* exhibits differential behavioral responses to harsh touch and gentle touch. Laser ablations identified distinct sets of sensory neurons and interneurons required for harsh touch sensation at different body segments. Optogenetic stimulation of the circuitry can drive behavioral responses. Both TRP family and amiloride-sensitive Na^+ channels regulate harsh touch sensation. Our results identify the neural circuits required for harsh touch sensation in *C. elegans* and establish *C. elegans* as a valuable model for studying this sensory modality.

649B

The robustness of *C. elegans* male mating behavior depends on the distributed properties of ray sensory neurons and their output through core and male-specific targets. Pamela Koo, Xuelin Bian, Amrita Sherlekar, Meredith Bunkers, **Robyn Lints**. Dept Biol, Texas A & M Univ, College Station, TX.

Many evolutionarily significant behaviors, such as mating, involve dynamic interactions with animate targets. What features of neural circuit design are essential to support such complex types of behavior? During *C. elegans* mating the male must establish and maintain tail contact with the hermaphrodite in order to search her surface for the vulva. Hermaphrodite movement during mating makes this task all the more challenging. The ray sensilla of the male tail mediate apposition of the tail against the hermaphrodite surface and control the search trajectory by modulating tail posture (1). How does the ray sensorimotor circuit simultaneously confer behavioral robustness and precision? The eighteen ray sensilla each contain the sensory endings of two morphologically distinct neurons, type A and B. We show through cell-specific ablations that the A-neurons are required for all appositional postures. A-neuron activity is instructive as artificial activation of these neurons with channelrhodopsin (ChR2; 2) can induce both scanning- and turning-like search postures. The B-neurons are only required for initial apposition of the tail where they enhance responsiveness to the hermaphrodite contact cue. When activated separately with ChR2, A- and B-neurons induce different types of tail ventral curls. When activated simultaneously an intermediate posture is formed. Together these data suggest a model in which A- and B-neurons act synergistically to promote initial tail placement in response to hermaphrodite contact while the A-neurons promote continued contact through appropriate induction of scanning and turning during the search. Significantly, males lacking the majority of rays retain a high degree of postural control indicating significant functional resilience in the system. Thus robust responsiveness stems from functional overlap between different rays while postural precision comes from subtle differences in the motor outputs of ray neuron types. Distributed processing is not limited to the sensory neurons of the circuit. It is also a feature of other circuit cells as eliminating many male-specific ray neuron targets only partially attenuates tail posture control. This observation also reveals that core (sex-common) tissues contribute to posture control and that many male-specific cells may serve to fine-tune circuit output. Taken together these results suggest that robustness may be a crucial feature of circuits underlying complex behaviors even in a simple animal like the worm. 1. Liu & Sternberg (1995) Neuron 14:79-89. 2. Nagel et al. (2005) Curr. Biol. 15:2279-84.

650C

Candidate screen for mutants defective in long-term memory of habituation. **Andrea McEwan**[1], Catharine Rankin[1,2]. 1) Brain Research Centre, Vancouver, B.C; 2) Department of Psychology, University of British Columbia.

Neurodegenerative-dependent memory loss is becoming increasingly relevant in today's aging population. The mechanisms that underlie memory are complex and, as yet, not fully understood. C.elegans offers a unique opportunity to study the biochemical pathways central to learning and memory. C.elegans has a simple, fully mapped nervous system and demonstrates several types of memory, including long-term memory for habituation. In the past, researchers have shied away from behavioural screens for long-term memory for habituation because of the labor intensive and time consuming methods used for data collection and analyses. Recently, a fully automated system called the multi-worm tracker has been developed and used to investigate genes involved in short-term habituation. The multi-worm tracker is able to record and analyze the movement of individual worms within a population in real time, greatly increasing the efficiency of behavioural studies. We have established a new protocol for investigating long-term memory for habituation on the multi-worm tracker and plan to perform a high-throughput candidate screen for mutants that fail to show long-term memory of habituation to mechanosensory stimulation. A screen for long-term memory for habituation could potentially identify novel players involved in aspects of memory, including genes involved in memory formation and memory storage.

651A

Humoral Cholinergic Signaling Augments Male Reproductive Motivation. **James T.**

Midkiff. Department of Biology, Texas A&M University, College Station, TX.

Mating in the male C. elegans worm is a complicated series of interrelated behaviors, all of which are controlled by a relatively few number of neurons. Previous studies have indicated that cholinergic signaling controls the mating circuit, although the source of the overall signal to mate has not been determined. These studies have focused on neurons and muscles in the male tail, but little has been done to examine if neurons in the head play a positive or negative role in mating behavior. We hypothesize that secretions from the head neurons attenuate the ability of mating-relevant neurons in the tail to fire under stimulation. To test this hypothesis, we have inserted the Channelrhodopsin-2 gene into various C. elegans cholinergic neurons, using a small unc-17 promoter that expresses primarily in the head neurons. We then used blue light to stimulate these neurons, and observed the effects on male behavior. Preliminary results indicate that the male's cholinergic head neurons, which were previously thought to facilitate locomotor behavior, regulate a number of mating behaviors in the male tail. Upon stimulation, the male worms stopped moving, stiffened the posterior half of their bodies, and began rapidly and repetitively curling their tails as if attempting to prod or insert into a hermaphrodite. A significant proportion of the stimulated males also began vulva-seeking behaviors on themselves with their tails. This study aims to identify the source, duration, and strength of the cholinergic signals that regulate male mating. First, we will identify the cholinergic neurons responsible for triggering the male mating response. This will be accomplished by laser ablating the cholinergic head neurons in groups, and then one at a time, and analyzing the behavioral responses after stimulation with blue light. Second, we will determine the duration of the mating-response behavioral effect by stimulating transgenic male worms under blue light for a short period (10 sec). After stimulation ends, we will measure how long mating behaviors are displayed. Third, we will determine if stimulating the cholinergic head neurons will increase the circuit's sensitivity to exogenous cholinergic stimulation. We will expose stimulated and nonstimulated males to varying concentrations of known acetylcholine receptor agonists, such as arecoline and levamisole. We hypothesize that stimulation will increase the sensitivity of circuit, such that the EC_{50} for stimulated worms will be significantly lower than for the controls. Last, we will test whether stimulating the head cholinergic neurons will override conditions that reduce sex drive in males, such as food deprivation and aging.

652B

Screen to identify mutants that fail to adapt to the inhibitory effects of serotonin on locomotory behavior. **Spencer Moen**, Eric Foss, Lucinda Carnell. Biological Sciences, Central Washington University, Ellensburg, WA.

Acute serotonin (5-HT) exposure decreases locomotory speed in *C. elegans* while chronic long-term exposure the 5-HT leads to a recovery of locomotory speed referred to as behavioral adaptation (Schafer and Kenyon Nature 375:73-78 1995). We have quantified this behavior by measuring average speeds of animal populations. In addition we have observed an increase of speed above untreated controls after removal from 5-HT; a behavior we referred to as withdrawal (Foss, E. and Carnell, L. unpublished data). Little is known of the underlying mechanisms that lead to adaptation and we therefore initiated a genetic screen to identify mutants that fail to adapt to the inhibitory effects of 5-HT on locomotion. We treated EMS-mutagenized animals overnight on plates containing 3mg/ml 5-HT and then tested for animals that continued to display inhibition of movement to 5-HT in liquid (5mg/ml). Our initial screen of 2400 haploid genomes identified two mutants that displayed characteristics of acute inhibition when chronically treated with 5-HT indicating a failure to adapt to 5-HT. Our mutant displayed adaptation speeds of 42 ± 8 μm/s versus 200 ± 10 μm/s for wild-type animals. Interestingly, the mutant animals while failing to adapt to 5-HT still displayed withdrawal upon removal from 5-HT. In addition to defects in adaptation to 5-HT, the mutant also shows decreased locomotion speeds on a bacterial lawn in the absence of exogenous 5-HT. As a population this mutant migrates across a bacterial lawn as a dense formation consuming the entirety of the bacteria as they move; we refer to this behavior as depressed foraging. We are currently mapping the location of the mutation in order to clone the gene responsible for this behavior.

653C

Assessing the neurotoxic effects of microcystins by developing a simple C. elegans model. **Caroline Moore**[1], Noelle L'Etoile[2], Birgit Puschner[1]. 1) UC Davis, Dept of Molecular Biosciences, Davis, CA; 2) UC Davis, Center for Neurosciences, Davis, CA.

Microcystins (MCs) are highly toxic, ubiquitous environmental contaminants produced by blue-green algae and found in water, sediment and food. With over 80 structural variants, the most commonly tested (MC-LR) has been found worldwide and has caused acute, lethal hepatotoxicoses in humans and animals. Produced in response to increased water temperatures, high phosphate and nitrate run off, stagnant water and other environmental cues, MC contaminations are predicted to increase with rising global water temperatures. MCs are resistant to degradation in water via chemical treatment and boiling, and pose a threat to both industrialized drinking water facilities and developing areas of the world where surface waters are untreated. While the acute hepatotoxic effects have been intensively studied, MCs toxic effects on other organs are not well understood. MCs cross the blood brain barrier and enter neurons, cause oxidative stress, and cause loss of memory in mammalian models. Case reports of human MC exposure include signs of neurotoxicity but to date the effects of MCs on specific brain cell types have not been evaluated. The proposed research study will establish a novel model system using Caenorhabditis elegans (C. elegans) to evaluate in vivo effects of MC on neurodevelopment. Although a simple organism, C. elegans are used to study neurodegenerative diseases and neurotoxicity. C. elegans are cost-effective, and allows for cutting-edge approaches to cellular, molecular and genetic analyses. Because their genome has extraordinary conservation with mammals and their nervous system shares similar complex physiological processes with higher animals, C. elegans offer a comparative platform to evaluate mammalian xenobiotic toxicity. The major goal of the proposed studies is to test the hypothesis that MCs interfere with neurodevelopment through their actions on known or alternative signaling pathways. To test this hypothesis, we will evaluate the effects of chronic exposure of C. elegans to MCs.

654A

Coordinated neural and mechanical modifications regulate sexual variation in *C. elegans* locomotor behavior. **William R. Mowrey**, Douglas S. Portman. Center for Neural Development and Disease, University of Rochester, Rochester, NY.

Animal species often exhibit distinct morphs with characteristic morphologies and behaviors. The relative contributions of morphological and neural specialization to establishing behavioral differences, however, is often unclear. To address this question, we are investigating the regulation of locomotor behavior by sexual modification of morphology and neural circuits in *C. elegans*.

The two sexes of *C. elegans* exhibit differences in locomotor speed that are supported by distinct locomotor kinematics. We find that these sex differences arise through the coordinated modification of neural signaling, muscle properties, and body mechanics. While the more slender body of the male decreases both internal and environmental resistance to body deformation, these mechanical differences are not sufficient to determine male-typical kinematics. On the contrary, we find targeted sex reversal of the nervous system can enforce opposite sex locomotor frequency in the absence of morphological change. Interestingly, the efficiency of locomotion is decreased in some sexually mosaic animals, suggesting that body mechanics may instead determine the effectiveness of specific motor patterns. Sexual modification of body-wall muscle also appears to be play an important, if more subtle, role in the regulation of this behavior, suggesting that adaptive variation in locomotion arises through coordinated modifications to diverse aspects of the motor system.

Though morphological differences are often correlated with changes in motor behavior, they may not fully explain their genesis. Our findings support the notion that adaptive variation in motor behavior is established through the coordinated modification of multiple tissues.

655B

Isolation and analysis of dauer pheromone response-defective (*phd*) mutants. **Scott J. Neal**[1], Kyuhyung Kim[1], Rebecca A. Butcher[2], Frank C. Schroeder[3], Piali Sengupta[1]. 1) Dept Biology, Brandeis Univ, Waltham, MA; 2) Dept Chemistry, Univ Florida, Gainesville, FL; 3) Boyce Thompson Inst, Cornell Univ, Ithaca, NY.

Dauer pheromone is comprised of a complex blend of derivatives of the dideoxysugar ascarylose. These so-called ascarosides have documented roles in dauer formation as well as in mediating sexually dimorphic adult behaviors in *C. elegans*. Our lab has described the functions of two chemoreceptors, SRBC-64 and SRBC-66, which are required for the response to specific ascarosides and signal via the GPA-2 and GPA-3 Gα proteins in the ASK neurons to regulate dauer formation. However, *srbc-64; srbc-66* double mutants remain responsive to other ascarosides, as does a strain in which the ASK neurons are genetically ablated, suggesting the presence of additional pheromone signaling pathways. Moreover, the mechanisms by which signals from ASK are transmitted to the ASI sensory neurons to regulate *daf-7* TGF-β expression, and thus dauer formation, are unknown. The expression of both *daf-7*p::*gfp* and *str-3*p::*gfp* in ASI is repressed by pheromone in wildtype worms. We reasoned that genes, mutations in which fail to downregulate expression of these reporters upon pheromone addition, might play roles in intra- or intercellular pheromone signaling. In a forward genetic screen, we isolated mutants that failed to repress *str-3*p::*gfp* when reared in the presence of dauer pheromone. Using dauer formation assays we quantified the ability of each candidate strain to form dauers in response to specific ascarosides. We selected five mutants (*oy103-oy107*) for further characterization on the basis of their ability to form dauers in response to at least one ascaroside while exhibiting defective dauer formation in response to other examined ascarosides. Genetic analysis suggests that these five alleles define four distinct loci which may be required for transduction of specific ascaroside signals to regulate downstream components such as *daf-7* expression in order to promote dauer formation. We are currently using traditional genetic mapping together with whole genome resequencing to clone the genes affected by these mutations.

656C

The role of cilia in cGMP signaling in the AFD thermosensory neuron. **Phuong Anh T Nguyen**, Jacque-Lynne Johnson, Michel R Leroux. Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

Primary cilia act as the antennae of eukaryotic cells, being able to transduce light, mechanical and chemical stimuli from the environment. The importance of these organelles in human physiology and development is increasingly recognized, given their involvement in different signaling pathways and implication in human disorders (ciliopathies) that affect essentially all organs.

We have just found through phylogenetic analysis that various cGMP signaling proteins are present in essentially all eukaryotes except for those that have secondarily lost cilia during evolution. As cyclic nucleotides are found in prokaryotes, this finding suggests that cGMP signaling could have been the first signal transduction pathway adopted by primary cilia during its emergence as a sensory device, preceding the advent of other ciliary signaling pathways in vertebrates. A prime example of the cilia-cGMP signaling connection can be gleaned in vertebrate photoreceptors; this specialized cilium concentrate cGMP

signaling proteins essential for phototransduction, including guanylyl cyclases that produce cGMP, phosphodiesterases that break down cGMP, and cGMP-dependent cation channels. How the cGMP signaling cascade is established in the ciliary compartment is not understood, however.

A well-studied behavior in *C. elegans* that involves cGMP signaling is thermotaxis. In this behavior, temperature is sensed mainly by AFD neurons, each of which possesses a cilium of unknown function. Our lab has showed that ciliary mutants are defective in thermotaxis. Because of the close link between cilia and cGMP signaling, we hypothesized that ciliary proteins are required to establish and maintain cGMP signaling in AFD. Indeed, we discovered that at AFD dendritic endings, cGMP signaling components are localized to different compartments, and proper localizations require specific ciliary proteins acting cell-autonomously. Microscopy studies of ciliary proteins in AFD suggest that its membranous 'fingers' are a bona fide ciliary compartment, because whereas most ciliary proteins are found in the canonical cilium, some are also present at the base of the fingers. This suggests ciliary proteins might form a barrier or 'transport hub' separating the finger membrane from the dendritic membrane. Our research will help establish the AFD neuron as a model system analogous to the ciliary photoreceptor, probing the important connection between ciliary trafficking and cGMP signaling.

657A

Analysis of molecular basis for thermotactic behavior regulated by CREB in *Caenorhabditis elegans*. **Yukuo Nishida**[1], Takuma Sugi[1], Mayu Nonomura[1], Ikue Mori[1,2]. 1) Graduate Sch of Sci, Nagoya Univ, Nagoya, Japan; 2) CREST-JST, Japan.

Animals cope with environmental stimuli by altering behaviors for ensuring survival and reproduction. Previous studies have characterized the thermotactic neural circuit, in which temperature is sensed and memorized through the coordination of thermosensory neurons and interneurons in *C. elegans*. Interestingly, the thermosensory neuron AFD stores temperature memory. In order to clarify the molecular basis for the memory formation, we focused on the possibility that CRH-1, an ortholog of memory-related transcriptional factor CREB, acts in a primary signaling pathway in AFD. The *crh-1* mutant cultivated at low temperature (17°C) normally migrated to cultivation temperature regions on the thermal gradient, whereas the mutants cultivated at high temperature (23°C) exhibited abnormal migration such as dispersing over on the gradient. This behavioral defect was recovered by cell-specific expression of *crh-1* in the AFD. These results underscore the importance of the CRH-1 transcriptional activity to higher temperature memory in AFD. To identify the downstream genes of CRH-1 which are expected to generate the temperature memory, we are conducting the whole-genome microarray analysis. Further analysis of *crh-1* and its downstream genes should shed a light on the molecular route from thermosensation to memory formation in *C. elegans*.

658B

Appetitive olfactory learning and associative long-term memory in *C. elegans* . **Saori Nishijima**, Ichiro Maruyama. Information Processing Biology unit, Okinawa Institute of Science and Technology, Okinawa, Japan.

C. elegans is an excellent model organism for the study of learning and memory at cellular and molecular levels because of the relative simplicity of the nervous system, which is invariant from animal to animal. Here we developed a paradigm for the study of associative learning and memory by classical (Pavlovian) conditioning of worms with nonanol, as a conditioned stimulus (CS), and potassium chloride (KCl) as an unconditioned stimulus (US). Before the conditioning, worms avoided nonanol, an aversive olfactory stimulus, and were attracted by KCl, an attractive gustatory stimulus, in chemotaxis assays. After massed training without an intertrial interval (ITI) or spaced training with 10-min ITI, by contrast, trained worms were attracted to nonanol. Memory induced by the massed training was extinguished within an hour, while that induced by the spaced training was retained for 24 hours. Worms treated with cycloheximide or actinomycin D failed to form the memory induced by the spaced training, whereas the memory induced by the massed training was not significantly affected by the mRNA and protein synthesis inhibitors. Furthermore, the memory induced by the massed training, but not that induced by the spaced training, was sensitive to experimental disruption by cold-shock anesthesia. By definition, therefore, the memories induced by the massed and spaced training are classified as short-term and long-term memories, respectively. Hence this appetitive olfactory conditioning with nonanol and KCl as CS and US, respectively, shares many of the defining features of associative learning as exemplified by classical conditioning: stimulus- and paring specificity, contiguity and contingency learning, and both short- as well as long-term retention. In support of this, *C. elegans* mutants defective in nmr-1 encoding an NMDA receptor subunit failed to form both of the short-term and long-term memory, while mutations in crh-1 encoding the CREB transcription factor affected only on the formation of the long-term memory.

659C

Quantitative analysis of exploratory patterns during thermotaxis. **Yuta Ochiai**[1], Yuki Tsukada[1,2], Ikue Mori[1,2]. 1) Graduate Sch Sci, Nagoya Univ, Nagoya, Japan; 2) CREST, Japan.

Exploratory behavior is essential for animals to obtain food, partners and other resources. When transferred from the plate with food to the plate without food, *Caenorhabditis elegans* shows several behavioral patterns, and the neural circuit that controls those patterns is identified (Gray *et al.,* 2005). While exploratory behaviors generally utilize various sensory cues, little is known about the exploratory behavioral patterns when specific sensory cues are available. Thermotaxis of *C. elegans* is a model of the environmental information-based exploratory behaviors because the neural circuit critical for thermotaxis is identified (Mori and Ohshima 1995; Kuhara *et al.,* 2008), and input and output of temperature information flow can be quantitatively measured in our system. Here, we aimed to assess the exploratory patterns during thermotaxis and clarify the neuronal mechanism regulating the exploratory patterns in accordance with sensory cues. We videotaped the freely moving individual animals on agar plate for up to 60 minutes using a computer-driven tracking system that keeps the animal in the microscopic field. Timings of reorientation turns were detected by analyzing the movies with image processing program. Then, we calculated the turn frequency for every ten minutes. We also analyzed the relationship between number of turns and temperatures. Wild-type N2 animals were cultivated at 17°C, 20°C and 23°C with food, OP50, and then were placed on the uniform 20°C 6×9cm agar plate without food. The turn frequency rapidly decreased within the initial 20 minutes and continued to gradually decrease until 30 minutes in 17°C, 20°C or 23°C cultivated animals. After 30 minutes, turn frequency of 20°C cultivated animals showed a constant turn frequency for another 30 minutes, but the turn frequency of 17°C or 23°C cultivated animals changed irregularly. By contrast, when the animals were placed on the plate with linear thermal gradient ranging from 15°C to 25°C, and with the temperature steepness of 1°C per 1cm, the turn frequency did not decrease rapidly in the initial 20 minutes. We classified the exploratory patterns on the thermal gradient into four classes according to the number of turns in relation to the temperatures. Mutants for cGMP-gated channel (*tax-4*) and AFD neuron-specific guanylyl cyclases (*gcy-23*, *gcy-8*, and *gcy-18* triple mutant) showed lower turn frequency on thermal gradient than wild type animals, thus showing different exploratory patterns during thermotaxis. Our results indicate that the change in turn frequency during exploratory behavior is modulated by temperature information. Our further analysis with thermotaxis mutants would be a key for clarifying this exploratory behavior mechanism.

660A

Biased motion in unbiased environments: are the worms navigating? **Margherita Peliti**, John Chuang, Stanislas Leibler, Shai Shaham. Rockefeller University, New York, NY.

C. elegans relies on sensory cues to find food. In the absence of such cues, the worm might display defined search patterns or other stereotyped behavior. We sought to characterize the motion of *C. elegans* in the absence of stimuli, over time scales comparable to that of starvation. To this end, we devised an imaging setup employing several flatbed scanners, which enables us to collect animals' paths over large (24 cm x 24 cm) surfaces. Wild-type worms display striking behavior in the absence of food. ~60% of the animals' paths display persistence in the direction of motion over length scales that are 50-100 times the body-length of C. elegans. The direction of movement differs from animal to animal, suggesting that the bias we observe might not represent a taxis towards an external cue. We confirmed these results with a camera imaging setup, in a more stringently homogeneous environment. Interestingly, directionality does not appear to arise from local correlation in the direction of motion. Furthermore, synthetic trajectories generated from the same angle and step distributions of individual trajectories indicate that the observed persistence in direction cannot be accounted for by simple random walk models. To determine whether sensory input is required for directionality, we analyzed the paths of sensory mutants. *che-2* animals, which display cilia defects, exhibit non-directional behavior. Surprisingly, however, ~20% of *daf-19* mutants, which lack cilia, display directionality. Mutations in *osm-9*, a TRPV channel required in several sensory neurons, do not affect behavior. By contrast, mutations in the cGMP-gated channel *tax-4* result in loss of directionality, indicating a requirement for the activity of sensory neurons. To further investigate the role of sensory perception we performed rescue experiments of TAX-4 function. No rescue is observed when driving TAX-4 in the thermosensory neuron AFD or in the olfactory neurons AWB and AWC. Moreover, mutants defective in signal transduction for thermotaxis, odortaxis, phototaxis, and aerotaxis do not display loss of directionality. We observe partial rescue of directionality when driving TAX-4 in a set of five cells: the oxygen-sensing AQR, PQR and URX neurons and the ASJ and ASK neurons. In conclusion, our results suggest that the motion of *C. elegans* in the absence of apparent sensory cues cannot be assimilated to a random walk, and raise the intriguing speculative possibility that *C. elegans* might achieve directional motion by relying solely on self-based information.

661B

A calcineurin homologous protein is required for multiple sodium-proton exchange events in the *C. elegans* intestine. Jamie Wagner[1], Erik Allman[2], Ashley Taylor[1], Kiri Ulmschneider[1], Timothy Kovanda[1], Bryne Ulmschneider[1], Keith Nehrke[3], **Maureen Peters**[1]. 1) Dept. of Biology Oberlin College, Oberlin, OH; 2) Pharmacology and Physiology Department, Univ. of Rochester, School of Medicine and Dentistry, Rochester, NY; 3) Department of Medicine, University of Rochester, School of Medicine and Dentistry, Rochester, NY.

Defecation in *Caenorhabditis elegans* is a rhythmic behavior driven by oscillatory calcium and proton signaling. The defecation mutant *pbo-1* encodes a calcium binding protein with homology to calcineurin homologous proteins, which are putative cofactors for mammalian sodium-proton exchangers. Loss of *pbo-1* function results in a weakened posterior body contraction and a caloric restriction phenotype. Both of these phenotypes also arise from dysfunctions in pH regulation due to mutations in intestinal sodium-proton exchangers. *pbo-1* is expressed in the developing and adult intestine as well as neurons. Adult, intestine-specific rescue of *pbo-1* ameliorates the posterior body contraction defects, suggesting that PBO-1 affects intestinal physiology rather than development. To analyze the periodic intestinal ion fluxes associated with the defecation cycle, *in vivo* imaging of

intestinal calcium and proton flux was performed. Cytoplasmic calcium flux of normal magnitude and frequency occurred in the mutant. By contrast, the proton movements associated with the intestinal sodium-proton exchangers, NHX-2 and PBO-4/NHX-7, are significantly reduced. The physiologic events associated with proton exchange events, namely nutrient uptake and initiation of the posterior body contraction, also appear compromised in the *pbo-1* mutant. We conclude that PBO-1 is required for normal sodium-proton exchanger activity and may couple calcium and proton signaling events.

662C

Influence of Body Mechanics on Force Thresholds for Touch Sensation in *C. elegans*. **Bryan C. Petzold**[1], Sung-Jin Park[1,2], Miriam B. Goodman[3], Beth L. Pruitt[1]. 1) Mechanical Engineering, Stanford University, Stanford, CA; 2) Disease Biophysics Group, Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA; 3) Molecular and Cellular Physiology, Stanford University, Stanford, CA.

We assessed the interplay between body mechanics and touch sensitivity by modulating muscle tone with Channelrhodopsin-2 and measuring force thresholds with a novel force-clamp metrology. Touch sensation is poorly understood despite the prevalence of disrupted touch and associated pain in pervasive diseases like diabetes. *C. elegans* is an ideal model for touch with its six touch receptor neurons (TRNs) and behavioral response to gentle touch. Force applied to the body results in stress/strain of nearby TRNs, triggering opening of force-gated ion channels, cellular depolarization, and an avoidance response for sufficiently large forces. Previously, we developed a behavioral force-clamp metrology capable of applying nN-µN forces to moving L4/young adult animals (Park et al, *Rev Sci Instr*, in press). Using this metrology, we showed that wild-type (N2) animals respond to forces ≥ 100 nN, revealing unprecedented mechanical sensitivity.

Previously, we showed that the three-layered outer shell (cuticle, hypodermis, and body wall muscle) plays a crucial role in filtering and transmitting applied forces (Park et al, *PNAS* 104:17376, 2007) and that body wall muscle tone regulates body mechanics (Petzold et al, *Biophys J*, in press). Now, we are testing the hypothesis that changes in body mechanics modulate touch sensitivity. To do this, we compare force-response curves in un-stimulated and hyper-contracted animals. Preliminary results show that larger forces are needed to evoke avoidance responses in hyper-contracted animals. We used light to manipulate body wall muscle contraction in transgenic animals expressing ChR2 under the control of a body wall muscle-specific promoter. This study provides a way to study the interplay between body mechanics and touch sensitivity in *C. elegans* and will further our understanding of the role of the outer shell in filtering and transmitting loads to the TRNs.

663A

Mammalian rhodopsin can functionally substitute for *C. elegans* photoreceptor to restore light sensitivity in worms. **Beverly J. Piggott**[1], Jingwei Gao[2], Jie Liu[1], X.Z. Shawn Xu[1]. 1) Life Sciences Institute, Department of Molecular and Integrative Physiology, and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109, USA; 2) College of Life Sciences, Wuhan University, Wuhan, Hubei 430072, China.

We have recently discovered that worms sense light and engage in phototaxis behavior that is essential to their survival. Our work has led to the identification of photoreceptor neurons and molecules in the *C. elegans* phototransduction pathway. Through electrophysiological interrogation, we discovered that LITE-1 acts in ASJ to transduce light signals through a G protein-mediated process which requires membrane-associated guanylate cyclases. This pathway shares striking similarities to those found in some vertebrate photoreceptor cells. Interestingly, *lite-1* belongs to the invertebrate taste receptor family. Discovering that a gustatory receptor could permit light sensation in worms, we wondered if expression of other known light sensing molecules, such as opsins, could function similarly in worms. In other words, could structurally distinct, mammalian opsins, hijack the *C. elegans* phototransduction machinery to restore photosensory behavior in *lite-1* mutants? To this end we made transgenic worms expressing bovine rhodopsin and discovered that it can restore light sensitivity in *lite-1* mutants. These findings demonstrate that divergent photoreceptor molecules can share functional homology.

664B

Hypoxia avoidance response in *C. elegans* requires *goa-1* and *dgk-1*. **Jason N. Pitt**, Mark B. Roth. Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA.

Oxygen (O_2) is essential for the growth and development nearly all metazoans. O_2 concentrations in the environment fluctuate and *C. elegans* exhibits an aerotaxis behavior dependent on the activity of soluble guanylate cylcases, *npr-1* and *glb-5*. We show that, in addition to the previously described aerotaxis pathway, C. elegans displays a genetically distinct acute hypoxia avoidance response (HAR). When worms are placed in hypoxic atmospheres containing less than 5% O2 they immediately switch from a dwelling to roaming behavior. Roaming speed is inversely correlated with %O_2 down to 1% O_2 and is positively correlated with O_2 concentrations <1%.

In addition to its requirement for oxidative phosphorylation, molecular O_2 is required for the synthesis of the monoamine neurotransmitters which regulate the switch from dwelling to roaming behaviors. The inhibition of roaming by monoamine neurotransmitters requires the activity of the heterotrimeric G-protein GOA-1. We find that loss of function mutations in *goa-1* eliminate HAR. *goa-1* animals also display a premature entry into suspended animation (SA) at O_2 concentrations ≤ 0.1%. GOA-1 negatively regulates the activity of the PLC-β EGL-8 through EGL-30. *egl-8* animals display a severely attenuated HAR and also prematurely enter SA. EGL-8 signaling through diacylglycerol (DAG) is negatively regulated by the DAG kinase DGK-1. In moderate hypoxia (≥5% O_2) *dgk-1* worms, like *npr-1* worms, show a pronounced decrease in locomotion. However, unlike *npr-1* worms, at < 5% O_2, *dgk-1* worms show an inverse HAR; locomotion is increased in room air but suppressed in hypoxia.

Hypoxia survival in *C. elegans* and other metazoans requires the transcription factor HIF-1. *hif-1* loss of function animals show no defects in HAR, however, animals that lack the function of the prolylhydroxylase EGL-9, the negative regulator of HIF-1, show an immediate arrest of locomotion when transferred to hypoxia. This arrest is transient and *egl-9* animals slowly increase speed during hypoxia eventually reaching wildtype levels of locomotion. Because *dgk-1* worms show a similar arrest immediately upon transition to hypoxia, we tested the affect of hypoxia on embryonic diapause, a phenotype which requires HIF-1. We found that *dgk-1* worms, like *hif-1* worms, exhibit a precocious diapause at 0.5% O_2. This suggests that the GOA-1/DGK-1 pathway might influence HIF-1.

665C

Is lethargus essential? **David M. Raizen**, Robert J. Driver, Annesia L. Lamb. 464 Stemmler, Univ Pennsylvania, Dept Neurology, Philadelphia, PA.

Lethargus is a 2-3 hour period that occurs at each of the four larval stage transitions during *C. elegans* development from an embryo to an adult. During lethargus, the animal secretes and assembles a new cuticle and escapes from the old cuticle via a process of apolysis (separation of the old from the new cuticle) and ecdysis. Animals in lethargus show reduced movement and an absence of pharyngeal pumping. Quiescence of locomotion is fully reversible upon strong mechanical stimulation. However, pharyngeal pumping is not increased by such stimulation nor is it increased by exogenous serotonin, which is stimulatory to pumping outside of lethargus. Moreover, dissecting lethargus pharynxes and bathing them in an artificial saline containing serotonin does not stimulate pumping. In addition to quiescence, animals in lethargus show the sleep-like properties of an elevated sensory arousal threshold, and a homeostatic response to deprivation of lethargus quiescence. While testing for the homeostatic response, we noticed that following a 30-minute period of forced swimming during lethargus, 11% of wild-type animals did not recover. Microscopic observations of these worms showed that their adult cuticle was secreted and separated from their L4 cuticle, but they failed to escape from the L4 cuticle. We found that *daf-16* mutants have a defective behavioral response to deprivation of lethargus quiescence and are hypersensitive to the lethal effects of deprivation. To deprive worms of lethargus quiescence genetically, we made strains carrying mutations in both *egl-4* and *kin-2*, each of which is partially required for normal lethargus quiescence and elevation of arousal threshold. While 100% of *egl-4* and 85% of *kin-2* single mutants reached adulthood, 0% of *egl-4; kin-2* double mutants reached adulthood. Instead, they arrested as early larvae with a molting-defective phenotype. We are currently testing whether this synthetic lethal phenotype can be explained by action of these genes in the nervous system. Given the tight association between lethargus and the molt, it might not be at all surprising that lethargus is required for the proper molt. But the precise function lethargus is serving remains a mystery. Given that total sleep deprivation in rats (Rechtschaffen et al, '83) and fruit flies (Shaw et al, '02) is also lethal, we are intrigued by the possibility that lethargus may serve a function similar to sleep.

666A

Uncovering the molecular and cellular basis for hygrosensation in *C. elegans*. **Josh Russell**, Jon Pierce-Shimomura. Section of Neurobiology, University of Texas at Austin, TX.

Each form of life has an optimum environmental humidity range and therefore many have evolved behavioral mechanisms to sense humidity and migrate to their own preferred levels of moisture. The ability to detect levels of moisture is called hygrosensation and the migration behavior towards preferred moisture levels is called hygrotaxis. Although the fundamental molecular bases for the detection most sensory stimuli ranging from light and pressure, to odors and toxins have been elucidated over the past half century, it remains unknown how humidity is sensed. We are using *C. elegans* to investigate the molecular and cellular basis for moisture sensation. We have discovered that *C. elegans* is especially well-suited for studying hygrosensation because it is capable of robust hygrotaxis in moisture gradients as small as 0.03% humidity per 1 mm. Moreover, humidity preference can be influenced by acute conditions as well as associative learning. Hygrotaxis is adaptive because extreme dry or wet conditions cause rapid desiccation or proliferation of worm's natural fungal/bacterial predators respectively. Through quantitative behavioral analysis of mutant worms we have gathered evidence that humidity sensation does not require conventional sensory signaling pathways. Instead, we have begun to uncover a new set of molecules which point to a physiological mechanism for hygrosensation as well as candidate hygrosensitive neurons.

667B

Sexual identity of core neuronal circuitry modulates sex-specific behaviors in *C. elegans*. Renee M. Miller[1], **Deborah A. Ryan**[1], Kelli Fagan[1], Douglas S. Portman[1,2]. 1) Center for Neural Development & Disease, University of Rochester, Rochester, NY; 2) Department of Biomedical Genetics, University of Rochester, Rochester, NY.

The two sexes of *C. elegans* exhibit common behaviors, but also engage in several sex-specific behaviors including egg-laying and mate searching. Sex-specific neurons, comprised of 8 neurons in hermaphrodites and 89 neurons in males, have traditionally been thought to underlie these sexually dimorphic behaviors. However, we have previously shown that the genetic sex determination pathway acts in subsets of core neurons to shape behaviors, presumably in a manner optimized for each sex. Here we investigate how shared circuits and sex specific neurons might interact to determine behavior. In particular, we want to learn how neurons present in both sexes are modified by the sex determination

pathway to enable appropriate and optimal sex specific behaviors such as egg-laying, mate searching, and male mating. To address this question, we examined animals in which the core nervous system is genetically sex-reversed; i.e. males with feminized neurons and hermaphrodites with masculinized neurons. We found that feminization of all core neurons reduced the efficiency of two steps of male mating behavior, diminished attraction of males to hermaphrodite conditioned media, and decreased the propensity of males to leave food in search of a mate. Similarly, masculinizing all core neurons in hermaphrodites led to pronounced egg-laying defects, attraction to hermaphrodite conditioned media, and robust food-leaving behavior. By sex-reversing progressively smaller sets of core neurons we show that the genetic sex of an individual neuron, AWA, influences the choice of both males and hermaphrodites to stay on or leave a food source. In hermaphrodites, we have found that masculinization of *tax-4*-expressing sensory neurons in the head interferes with normal egg-laying behavior. We are also examining the outcome of sex-reversing smaller sub-sets and individual core neurons in attraction to hermaphrodite conditioned media. We have demonstrated that the sex of particular core neurons is able to modify behavioral responses. Together these results indicate that sexual modification of the nervous system is important for generating sex differences in behavior.

668C

C. elegans uses distinct molecular mechanisms to respond to thermal impulse stimuli of different amplitudes. Rajarshi Ghosh[1], Aylia Mohammadi[2], Leonid Kruglyak[1], **William S. Ryu[2,3]**. 1) Ecology and Evolutionary Biology Department, Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ; 2) Department of Physics, University of Toronto, Toronto, ON, Canada; 3) Banting and Best Department of Medical Research, University of Toronto, Toronto, ON, Canada.

C. elegans responds to aversive stimuli by eliciting an escape response that typically consists of reversals and omega turns followed by a resumption of forward movement. Systematic neurogenetic analyses of responses to temperature have been restricted to thermotaxis and to a lesser extent thermal pain. How the nervous system responds to intermediate thermal stimuli is less well studied. We have developed an assay that allows simultaneous characterization of several aspects of the sensorimotor transformation elicited by thermal impulses of different amplitudes, including the speed of movement, and the duration and characteristics of a different behavioral states. We transiently raised the local temperature around a worm by an IR laser and recorded several aspects of the resulting escape response. Typically upon sensation of a thermal impulse worms exhibit a behavioral sequence in which they enter a pause state followed by reversals and an omega turn and resumption of forward movement. Though qualitatively similar, we found that these behavioral states were accompanied by large changes in speed of movement. We raised the temperature by 0.3, 0.8, 3 or 7.5C from the baseline and quantitatively characterized several aspects of the ensuing escape response for the laboratory strain (N2) as well as 55 strains bearing mutations in genes known to affect thermosensation and different motor aspects of the escape response. We employed a behavioral barcoding approach to quantify the behavioral patterns for each mutant relative to N2. Hierarchical clustering of the behavioral barcodes revealed distinct clusters for each temperature rise identifying the mutants that significantly deviate from N2. These mutants were distinct for each temperature rise suggesting that C. elegans discriminates between a 0.3, 0.8, 3 or 7.5C rise in temperature at the genetic level. The pattern of clustering implies that avoidance responses elicited by thermal impulses of different amplitudes are encoded by distinct molecular mechanisms converging onto qualitatively similar sequence of behavioral states. Our high- content behavioral profiling also allowed us to explore the neuro-genetic constraints imposed on the escape response. We find that different behavioral states of the escape behavior sequence could be decoupled at the genetic as well as neural circuit level.

669A

Microfluidics approach to study neurodegeneration in a *Caenorhabditis elegans* Parkinson's disease model. **Sangeena Salam[1]**, Pouya Rezai[2], P Ravi Selvaganapathy[2], Bhagwati P Gupta[1]. 1) Dept. of Biology, McMaster University, Ontario, Canada; 2) Dept. of Mechanical Engineering, McMaster University, Ontario, Canada.

C. elegans is an established model organism to understand the biology of neurodegenerative diseases and identify potential therapeutic targets. Parkinson's disease (PD) is one such disease that affects millions of people worldwide. A major cause of PD is the loss of dopaminergic (DA) neurons in the substantia niagra, a region of the brain that controls balance and movement. While genetic changes contribute to the development of PD, several chemical compounds, such as 6-hydroxy dopamine (6-OHDA) and methyl phenyl tetrahydropyridine (MPTP), have also been found to induce PD-like symptoms in humans by degeneration of DA neurons. *C. elegans* is sensitive to 6-OHDA and MPTP and exhibits movement defects. Toxin-induced worm PD models have been valuable in understanding the mechanism of neuronal degeneration and screening for potential neuroprotective compounds.

Movement assays in worms typically involves observing phenotypes on plates and counting animals manually. In some cases neuronal defects are visualized by fluorescent markers such as DAT-1::GFP. This manual process is slow, subjective and measurable parameters of behavior are limited. Also, it is difficult to account for experimental conditions that lead to variations between batches. Considering these limitations, we are taking a microfluidics approach to study neurodegeneration in worms exposed to 6-OHDA, MPTP and other neurotoxins. Microfluidics offers many advantages over conventional methods of worm handling. Our lab had previously shown that a low voltage DC electric field inside a microfluidic channel stimulates worms to move towards the cathode (termed electrotaxis) (Rezai et al., 2010). We found that the electrotactic response is instantaneous, extremely sensitive, robust and benign. Although the mechanism of electrotaxis remains to be determined, it appears to be mediated by certain amphid sensory neurons. We have found that neurotoxin-treated worms are defective in electrotactic behavior. They move with variable speed with intermittent pauses and abnormal body bends. Characterization of movement defects has revealed that our assay is much more sensitive than standard plate-based drug assays and requires low drug concentrations and short exposure times (typically few hours). Using the channel, we also tested the effect of neuroprotective compounds such as Acetaminophen and found that it efficiently suppresses 6-OHDA and MPTP toxicity. Currently, we are attempting to automate the entire process of drug exposure and phenotypic analysis. The progress on these fronts will be presented.

670B

How worms move up and down salt gradients. L Luo, M Hendricks, Y Zhang, **A Samuel**. Harvard University, Cambridge MA.

The behavioral and neural mechanisms by which C. elegans moves up NaCl gradients have been well characterized. The ASEL and ASER neurons are capable of sensing temporal upsteps and downsteps in salt concentration to bias a random walk towards regions with higher salt. By studying the movements of worms on linear salt gradients with defined slope and offset, we find that worms can both move up and down salt gradients in search of regions with salt concentrations that corresponds to their previous cultivation conditions. Using calcium imaging and quantitative behavioral analysis, we find that the ASEL and ASER neurons each have distinct roles in mediating movement up or down salt gradients when the worm navigates below or above the set-point of salt memory. Our results show how the salt-sensing circuit is modulated by experience to enable the worm to either ascend or descend salt gradients.

671C

Exploring the Function of TRF-1 in Polycystin-Expressing Sensory Neurons. **Dianaliz Santiago-Martinez[1]**, Maria Gravato-Nobre[2], Jonathan Hodgkin[2], Maureen M. Barr[1]. 1) Genetics Dept, Rutgers University, Piscataway, NJ; 2) Biochemistry Dept, University of Oxford, Oxford, UK.

The Tumor Necrosis Factor (TNF) receptor-associated factors (TRAFs) are adaptor/scaffold molecules that interact with members of the interleukin-receptor (ILRs) or TNFR families and function mainly in the immune system. TNF-alpha signaling promotes autosomal dominant polycystic kidney disease (ADPKD). In mammals, there are six TRAFs (1-6) that share a conserved C-terminal TRAF domain. The *C. elegans* genome does not encode a TNF Receptor but does encode a sole TRAF gene, *trf-1*. In humans, mutations in the polycystin-1 (PC-1) or polycystin-2 (PC-2) ciliary mechanosensory complex cause ADPKD. In *C. elegans*, the polycystins LOV-1 and PKD-2 localize on the ciliary membrane and are required for male sensory behaviors. Hence, the connection between the polycystins and cilia seems to be an ancient one.

We find that *trf-1* is co-expressed with *pkd-2* in the male specific CEM, RnB, and HOB neurons and is required for male mating behaviors. We are interested in exploring a potential connection between immune recognition and mate recognition. To this end, we examined the mating behavior of mutant males defective in the Toll pathway including *tol-1* and *ikb-1* (I kappa beta homolog). We performed four mating behavioral (leaving, retention, response, and vulva location). Like *pkd-2* mutants, *trf-1(nr2014)* mutant males have response and Lov defects. In contrast, *tol-1;him-5* and *ikb-1;him-5* are not statistically different from the wild-type *him-5*. *trf-1;him-5* and *pkd-2;him-5* males are leaving assay (Las) defective, whereas *tol-1;him-5* and *ikb-1;him-5* are not Las. All mutant strains seem to be normal for the retention assay, indicating that these males can sense the presence of a mate. We conclude that *trf-1* acts like *pkd-2*, suggesting the two function in a similar pathway. Genetic interactions between *trf-1* and components of the *C. elegans* PKD pathway (*lov-1*, *pkd-2*, *klp-6*, and the *cwp* genes) are being examined.

To ascertain the site of TRF-1 action, we generated transgenic animals expressing TRF-1::GFP. Although dimly expressed, TRF-1::GFP localizes to cell bodies of CEM and HOB neurons, and in rays 3, 8 and 9, TRF-1 localizes to cell bodies and ray dendrites. TRF-1::GFP is not detectable in sensory cilia. We are currently determining whether *trf-1* is required for PKD-2 localization or abundance. We are also testing the hypothesis that TRF-1 physically associates with the intracellular domains of the membrane proteins LOV-1, PKD-2, and CWP-5, or with the kinesin-3 motor protein KLP-6.

672A

Regulation of thermosensation by SRTX-1(GPCR) in AFD thermosensory neurons. **Hiroyuki Sasakura[1,2]**, Keita Suzuki[1], Hiroko Itoh[1], Ikue Mori[1,2]. 1) Nagoya University; 2) CREST, JST, Japan.

C. elegans senses the environmental temperature by AFD and AWC sensory neurons. Genetic analysis suggested that signal transduction of thermosensory system in AFD and AWC is similar to that of olfactory and visual system in *C. elegans* and other animals (Mori et al., 2007; Kuhara et al., 2008). Based on the analogy to olfactory and visual system, it is plausible to hypothesize that G protein-coupled receptors (GPCRs) sense the environmental temperature. Previous reports showed that *srtx-1* encoding GPCR is specifically expressed in sensory ending of both AFD and AWC neurons (Colosimo et al., 2004; Biron et al., 2008). We evaluated the expression of *srtx-1* in detail by comparing fluorescence intensities of *srtx-1promoter::GFP* in AFD and AWC. Our results suggest that *srtx-1* is expressed strongly in AFD, very weakly in AWC OFF neuron, and not expressed in AWC ON neuron. We generated two deletion mutants, *srtx-1(nj62)* and *srtx-1(nj63)*, and found that both mutants exhibited impaired thermotaxis. Wild type animals migrated to the previous cultivation temperature after cultivation at 17, 20 or 23 degree on a temperature gradient.

Both *srtx-1* mutants however migrated to colder region than the cultivation temperature after cultivated at 23 degree, and slightly but significantly migrated to colder region than the cultivation temperature after cultivation at 20 degree. By contrast, *srtx-1* mutants migrated to higher region than the cultivation temperature after cultivation at 17 degree. The expression of *strx-1cDNA* only in AFD restored normal thermotactic behaviors. When *strx-1cDNA* was overexpressed in AFD of wild type animals, the distribution, on a temperature gradient, of the animals cultivated at 17 and 20 degree was shifted to higher region. Although *srtx-1* mutants exhibited poorer Isothermal Tracking (IT) behavior, they retained the ability of IT behavior. Calcium imaging revealed that *srtx-1* mutants showed lower response to temperature change but retained the response to temperature. These results suggest that thermosensory function of AFD is not completely but partially impaired in *srtx-1* mutants. According to all these results, we propose that STRX-1 (GPCR) is a key component for thermosensation in AFD, somewhat regulates temperature sensing range. Given the recent finding that Rhodopsin, photoreceptor GPCR, functions in temperature discrimination in Drosophila larva (Shen et al., 2011), GPCR may be throughout evolution important for thermosensation. We thank D. Garbers for *gcy-8p* promoter, H. Oliver for *ceh-36* partial promoter, C. Bargmann for *str-1* and *str-2* promoter H. Inada for TMV-UV library, T. Kohashi for improvement of assay systems.

673B

High alkaline pH sensation in *C. elegans*. **Toshihiro Sassa**, Takashi Murayama, Ichiro Maruyama. Information Processing Unit, Okinawa Institute of Science and Technology, Onna, Japan.

High alkaline pH is harmful to organisms including *C. elegans*. Wild-type worms avoid higher alkaline pH than pH 11 as a noxious stimulus. *C. elegans* mutants defective in gustatory sensory neurons, *che-2*, *dyf-3*, *osm-1* and *osm-3*, showed abnormal behaviors in high alkaline pH avoidance assay. Mutants in *osm-9* and *ocr-2* genes encoding transient receptor potential (TRP) channels also had abnormal behaviors in the assay. The expression of OSM-9 in the ASH sensory neurons rescued the defect in *osm-9* mutants. Upon stimulation with high alkaline pH, Ca^{2+} concentration increase was observed in wild-type worms by Ca^{2+} imaging using G-CaMP as a Ca^{2+} indicator, while no Ca^{2+} increase was observed in *osm-9* and *ocr-2* mutants. This demonstrates that ASH is responsible for the high alkaline pH sensation.

Furthermore, the following *C. elegans* mutants also showed abnormal behaviors in the high alkaline pH avoidance: *goa-1* and *egl-30* defective in G-protein coupled receptor (GPCR)-mediated signaling cascades, and *egl-3* and *egl-21* defective in vesicular neuropeptide processing. The increase of Ca^{2+} concentrations in ASH neurons of *goa-1* and *egl-3* mutants was normal upon high alkaline pH stimulation, indicating that Ca^{2+} influx into ASH is not affected by these mutations. These results suggest that signaling cascades that include GPCR and neuropeptides are responsible for the processing of high alkaline pH avoidance.

674C

Experience-dependent modulation of the neuronal response in the gustatory circuit. **Hirofumi Sato**, Hirofumi Kunitomo, Shigekazu Oda, Yuichi Iino. Department of Biophysics and Biochemistry, Graduate School of Science, the University of Tokyo, Tokyo, Japan.

Animals remember past experience and change their behaviors based on this memory. *Caenorhabditis elegans* utilizes several sensory cues to search for foods and interact with its environment. We have recently found that *C. elegans* is not merely attracted to salt (NaCl), but it approaches the salt concentration to which they were previously exposed along with food. Therefore, it appears that *C. elegans* is able to memorize a particular salt concentration. Although the memory of NaCl concentration must be stored somewhere in the neural circuit that regulates salt chemotaxis behavior, it is unknown which neurons change their response depending on past experience. Therefore we searched for the neurons whose response is regulated by previously exposed NaCl concentrations.

First, we focused on the ASER gustatory neuron and AIB interneurons. ASER plays a major role in salt attraction behavior. AIB is a postsynaptic neuron of ASER and promotes turning behavior. We monitored the activity of these neurons using the calcium indicator G-CaMP and PDMS microfluidic device to immobilize the animals. Before each experiment, we cultivated worms for more than 12 hours on different concentrations of NaCl. Both ASER and AIB are OFF-cells, so we used a down-step of NaCl concentration from 50 mM to 25 mM as a stimulus. We found that both ASER and AIB neurons change their responses depending on the NaCl concentration at which the worms had been cultivated. The amplitude of ASER response decreased as the salt concentration during cultivation was decreased. Meanwhile, the response of AIB disappeared when the worms were cultivated at 25 mM NaCl or lower, suggesting that there is a transformation of the signal between ASER and AIB.

To determine whether inputs to other sensory neurons are required for the changes of ASER response, we used cell-specific rescue of sensory functions. DYF-11 is required for formation of ciliary segments in sensory neurons and thus *dyf-11* mutants show defects in salt perception. ASER neurons of *dyf-11* mutants showed a tiny response to a downstep of NaCl regardless of prior experience. In contrast, when ASER was solely rescued in the *dyf-11* mutant, its response patterns were similar to the wild type, indicating that proper sensory input to ASER is sufficient for the plasticity of its response. These results suggest that the ASER gustatory neuron has a capacity to memorize salt concentration.

675A

Changes in responsivity of olfactory neurons to odor during olfactory adaptation and recovery in *Caenorhabditis elegans* . **E. Sawatari**, A. Inoue, T. Teramoto, T. Ishihara. Dept. Biol., Kyushu Univ., Fukuoka, Japan.

Animals acquire tremendous quantity of information from environment, and process them in their nervous systems. In this process, forgetting is important to prevent excess memory capacity or interference between old and new memories. However the mechanisms of forgetting processes are not elucidated at the molecular and neuronal circuit level. In *C. elegans*, we found that mutants of P38/JNK pathways regulate forgetting processes for the adaptation to diacetyl. In wild-type animals, the memory for the adaptation to diacetyl is fully recovered within hours, but in the mutants of P38/JNK pathways, the memories were extended to more than one day. The prolonged retention of memory of these mutants was rescued by expressing the wild-type gene product in AWC sensory neurons. In addition, the *ceh-36* mutants that have no functional AWC sensory neurons show prolonged retention of memory, suggesting that AWC sensory neurons may secrete molecule inducing forgetting. However, how and where the memory for the adaptation to diacetyl is retained have not been revealed.

To examine whether the memory for adaptation to diacetyl is retained in peripheral sensory neurons or interneurons, we analyzed the diacetyl-evoked Ca^{2+} response in AWA sensory neurons of naïve, adapted and recovered animals, by expressing a Ca^{2+} indicator, YC3.60, in AWA sensory neurons. Ca^{2+} transient was measured in the PDMS microfluidic device by using same animals for naïve, after adaptation and after recovery. In naïve animals, diacetyl stimulus induced a Ca^{2+} increase in the AWA sensory neurons. After continuous perfusion of diacetyl for 1.5 hrs, the Ca^{2+}-responses to the odor were completely diminished, suggesting that the AWA neurons were adapted. Interestingly, after continuous washing out of the odor for 2-4 hrs, odor-evoked increase of Ca^{2+} was re-emerged. These results suggest that AWA sensory neurons are recoverd to an active state and this time course of the Ca^{2+} response is very similar to that of the behavioral response to diacetyl. We are currently analyzing P38/JNK mutant to see the neuronal activities during the recovery process from the olfactory adaptation.

676B

Mapping the neural circuits that function downstream of the sensory rays in C. elegans male mating behavior. **Amrita Sherlekar**, Pamela Koo, Xuelin Bian, Robyn Lints. Department of Biology, Texas A&M University, College Station, TX.

During mating the male presses his sensilla-laden tail against the hermaphrodite cuticle and moves backwards along her surface in search of the vulva. We have shown that establishment of tail contact and modulation of tail posture during the search is controlled by the sensory neurons of the male ray sensilla (see Koo et al. abstract). As in the natural behavior, stimulation of the ray neurons, using channelrhodopsin-2(ChR2), induces ventral bending of the tail and backwards locomotion. Our objective is to define the circuitry and signaling molecules involved in controlling these two aspects of mating behavior. Cell ablation experiments suggest that control of tail posture is distributed and involves both male-specific and core (sex-common) cell types (see Koo et al abstract). In contrast to the posture control, the circuitry controlling ray-regulated locomotion may be less distributed and more straightforward to dissect. A few ray neurons make direct connections with the core command interneurons AVAL/R, which are required for backwards locomotion in the hermaphrodite (2). However, the major pathway to these core interneurons appears to be via two male-specific interneurons, PVY and PVX, of the pre-anal ganglion. We find that PVY/PVX ablation blocks backing during mating and, conversely, ChR2-mediated activation of these neurons induces backing without influencing posture. Defects in DA/VA motor neuron specification (as a consequence of unc-4 loss of function) block PVY/PVX-induced backing consistent with the model that the rays regulate backing through modulation of the core locomotory circuitry. In hermaphrodites AVAL/R express numerous glutamate receptor genes and mutations in some of these genes affect spontaneous- and stimulus-induced reversal (3). We find that the receptor genes are also expressed in the same core neurons in the male and we are currently testing whether mutations in AVA-expressed glutamate receptors affects PVY/PVX-induced backing. If glutamate transmission is required for PVY/PVX-mediated control of AVAL/R then we would predict that PVY/PVX should synthesize glutamate. Interestingly, we find that PVY/PVX do not express eat-4 reporters (a marker for glutamatergic fate) and consistent with this mutations in eat-4 do not significantly affect PVY/PVX- or ray-induced backing. We are currently investigating whether two putative eat-4 paralogs (vglu-2 and -3) are expressed in PVY/PVX and whether their loss of function disrupts mate-induced backwards locomotion. 1. The Male Wiring Project: http://worms.aecom.yu.edu/pages/male_wiring_project.htm 2. Chalfie et al (1985) J Neurosci. 5:956-64. 3. Brockie. & Maricq (2006) http://www.wormbook.org.

677C

Notch signaling regulates chemosensation and behavioral adaptation to stress. **Komudi Singh**, Anne C. Hart. Dept of Neuroscience, Brown University, Providence, RI.

A role for Notch signaling in development is well established, but several studies now support a role for Notch signaling in the adult nervous system. The molecular mechanisms and targets critical for Notch function in neuronal signaling and behavior are unclear. OSM-11 is one of the DOS (Delta and OSM) motif proteins that act with DSL domain ligands to activate LIN-12 Notch receptors in vulval cell fate specification (Komatsu et al., 2008). Loss of osm-11 causes defective osmotic avoidance (Wheeler & Thomas, 2006) and, as we report here, impairs avoidance of 1-octanol and increases dispersal due to increased locomotion speed and decreased spontaneous reversal rates. The role of osm-11 and Notch pathway genes in octanol avoidance response was addressed in depth and suggested a redundant role for lin-12 and glp-1 Notch receptors in this chemosensory behavior.

Furthermore, dissection of cellular site of action for Notch receptors suggested that the two receptors functioned in different/non-overlapping population of neurons. Studies in the past have shown that increased external osmolarity (hyperosmotic stress) leads to increased synthesis of the osmolyte glycerol in adult C. elegans (Lamitina, et al 2006). Under these unfavorable conditions, animals might also be expected to alter their behavior. We find that adaptation to hyperosmolarity impairs octanol response and increases dispersal, which are defects observed in osm-11 animals. Additionally, we find that the physiological and behavioral consequences of hyperosmotic stress adaptation are genetically separable. The role of Notch pathway genes in these potentially adaptive changes is under investigation.

678A

Notch signaling regulates many aspects of quiescence during C. elegans lethargus. **Komudi Singh**, Heather L. Bennett, Altar Sorkac, Michael A. Dilorio, Anne C. Hart. Dept. of Neuroscience, Brown University, Providence, RI.

Sleep/sleep-like behavior is universal across the animal kingdom. Yet, the molecular mechanisms underlying this behavior are not well understood. During the molt between each larval stage, C. elegans shed their cuticle and exhibit behavioral quiescence. Work from the Raizen laboratory has shown that quiescence shares many characteristics with sleep including diminished activity, rapidly reversibility, and decreased responsiveness to sensory stimuli. We examine here that the impact of Notch signaling on C. elegans quiescence. osm-11 encodes one of the five DOS family proteins that, with C. elegans DSL family ligands like LAG-2, activate LIN-12 and GLP-1 Notch receptors. Over-expression (OE) of osm-11 in adults induced anachronistic quiescence that was similar to molting quiescence and genetic epistasis studies suggested that osm-11(OE) mediated adult quiescence acts via Notch receptors and via pathways known to regulate C. elegans molting quiescence. To determine if the Notch pathway regulates larval molting quiescence, we developed a simple assay system using a microfluidic chip designed by Shawn Lockery to measure quiescence during L4-to-adult (L4/A) lethargus. Analysis of gain of function, loss of function, and transgenic animals revealed that altered Notch signaling impacts multiple elements of L4/A quiescence including amount of quiescence, arousal threshold, basal activity, and duration of lethargus. Our on-going studies suggest that C. elegans quiescence is a complex behavior composed of multiple elements and that Notch receptors may act in various sites to independently regulate these behavioral components of quiescence. For example, GLP-1 Notch receptor functions in sensory neurons to regulate arousal, but may act elsewhere to regulate basal activity. Notch signaling mediated regulation of quiescence in C. elegans suggest a similar role for Notch in other species that remains to be explored. Identification of Notch targets and further analysis of the cellular site of action of Notch pathway genes will help reveal the molecular mechanisms underlying quiescence and the circuitry of quiescence, respectively.

679B

Variation in temperature-dependent behaviours among natural isolates of *Caenorhabditis briggsae*. **Gregory Stegeman**[1], Jiwon Shin[2], Nan Lin[2], Matthew Bueno De Mesquita[2], Asher Cutter[1], William Ryu[2,3]. 1) Department of Ecology and Evolutionary Biology, University of Toronto; 2) Department of Physics, University of Toronto; 3) Banting and Best Department of Medical Research, University of Toronto.

Natural genetic variation allows the discovery of new gene functions and novel alleles for genes already known to act in biologically important processes. We are applying this approach to temperature-dependent behaviours in nematode worms in order to better understand the genetics behind behaviour. We focus on *Caenorhabditis briggsae* because most wild caught individuals fall into two genetically distinct clades that correspond approximately with northern temperate or with tropical latitiudes. Interestingly, strains from the tropical clade have higher fecundity when reared at higher temperature than do the temperate strains, suggesting local adaptation to climate variables like temperature (Prasad et al. 2011). Movement through its thermal landscape is the main way for nematodes like *C. briggsae* to regulate body temperature, so we also expect to see heritable differences in temperature-dependent behaviours. Here we quantify for the first time classic thermal-response behaviours among several *C. briggsae* wild strains from different haplotype groups using assays like accumulation on a linear thermal gradient, isothermal tracking, and a new droplet based thermal gradient assay. We demonstrate that *C. briggsae* shows thermotaxis and isothermal tracking similar to *C. elegans* but with some differences. We also identify heritable differences among strains from wild genetic backgrounds within *C. briggsae*. We will continue to develop higher throughput assays for temperature-dependent behaviour in order to carry out a quantitative trait loci mapping project using recombinant inbred lines derived from tropical and temperate parental strains. Prasad, A., M. Croydon-Sugarman, R.L. Murray & A.D. Cutter. 2011. Temperature-dependent fecundity associates with latitude in *Caenorhabditis briggsae. Evolution.* 65: 52-63.

680C

Temporal and molecular characterization of short-term associative memory in *C. elegans*. **Geneva M Stein**, Amanda L Kauffman, Coleen T Murphy. Princeton University, Princeton, NJ.

Current evidence suggests that the molecular basis of short-term associative memory (STAM) arises from changes in protein dynamics that increase the strength of synaptic signaling; however, many of the fundamental molecular mechanisms underlying STAM, particularly in *C. elegans*, remain unknown. Our lab developed a short-term associative memory assay in which worms learn to associate food with an odor and remember this association for over one hour. Here we use this assay to characterize molecules involved in short-term associative memory in *C. elegans*, testing candidate mutant strains from both canonical memory pathways and other *C. elegans* learning paradigms. We show that there are unique molecular characteristics for different temporal phases of STAM, which include learning, memory formation, maintenance, and extinction. As has been shown in higher organisms, we find that *C. elegans* STAM relies on calcium signaling. For example, the *C. elegans* adenylate cyclase homolog, ACY-1, is required for STAM formation, while the *C. elegans* CaMKII homolog, UNC-43, is necessary for STAM maintenance. STAM maintenance is also dependent on protein translation but, as in other organisms, not gene transcription. Additionally, STAM can be distinguished from olfactory adaptation, a paradigm in which worms are trained to disregard an inherently attractive odor, as adaptation mutants show variable responses to short-term associative memory training. Adaptation genes *egl-4* and *fbf-1* are required for STAM extinction. These mutants remember the association for longer than 6 hours after training. Another adaptation gene, *nos-1*, is necessary for STAM formation. These data suggest that adaptation and associative memory differentially employ the same molecular machinery. Although further studies are needed to confirm and expand on these findings, we have shown that *C. elegans* short-term associative memory is a distinct behavior that relies on conserved memory mechanisms for specific temporal phases of memory. We aim to use this system to identify novel regulators and effectors of short-term associative memory.

681A

Regulation of behavioral plasticity by systemic temperature perception in Caenorhabditis elegans. **Takuma Sugi**[1,2,3], Yukuo Nishida[1,2], Ikue Mori[1,2]. 1) Grad School of Sci, Nagoya Univ, Japan; 2) CREST, Japan Sci and Tech Agency, Japan; 3) Institute for iCeMS, Kyoto Univ, Japan.

Animals cope with environmental changes by altering behavioral strategy. Environmental information is generally received by sensory neurons in the neural circuit that generates behavior. However, environmental temperature inevitably influences animals' entire body, although systemic temperature perception remains largely unknown. We show here that systemic temperature perception induces change of a memory-based behavior. During behavioral conditioning, somatic cells such as body wall muscles and intestine respond to cultivation temperatures through heat-shock transcription factor that drives expression changes of genes involved in estrogen synthesis. The systemic signaling, via an estrogen signaling pathway, regulates cell non-autonomously the AFD thermosensory neuron by modulating the downstream of the cGMP-dependent temperature signaling for thermotactic behavior. We provide a link between systemic environmental recognition and behavioral plasticity by the nervous system.

We further identified AFD as a unit of CREB-dependent temperature memory within a neural circuit. CREB is expressed in nearly all cells and best known for its involvement in learning and memory via interactions with the estrogen receptor. We however found that *C. elegans* CREB ortholog, CRH-1, acts only in the one thermosensory neuron for thermotactic behavior. Surprisingly, restoration of *crh-1* in AFD of CRH-1-depleted *C. elegans* completely rescues its behavioral defect, whereas restorations in other neurons do not. Calcium imaging indicated that CRH-1 is responsible for the responsiveness of AFD neuron to the temperature above a threshold that is set by temperature memory. This presents a novel platform to analyze the mechanism for behavioral memory at the single cellular resolution within the neural network.

682B

Bold Text Effects of ionizing radiation on pharyngeal pumping in *Caenorhabditis elegans*. **Michiyo Suzuki**[1], Tetsuya Sakashita[1], Yuya Hattori[1,2], Toshio Tsuji[3], Yasuhiko Kobayashi[1]. 1) Microbeam Radiation Biology Group, Japan Atomic Energy Agency, Japan; 2) Graduate School of Engineering, Hiroshima University, Japan; 3) Faculty of Engineering, Hiroshima University, Japan.

Caenorhabditis elegans is a good *in vivo* model system to examine radiobiological effects. Recently, we found that locomotion caused by body-wall muscles was reduced in a dose-dependent manner after gamma-ray irradiation, and that the locomotion was eventually restored (1-2). However, it is not known whether the same effects are observed in other types of movements in *C. elegans*. The combination of muscles and motor neurons used for locomotion is different from that used for other movements such as chewing and swallowing (pumping motion). Therefore, a study of radiation effects on different types of movements might help to identify those regions among muscles and the nervous system that are strongly related to radiation responses. Here, we examine the radiation effects on pumping by the pharyngeal muscles in particular.

In the experiments, 50 or more well-fed adult *C. elegans* were placed on an agar dish with a bacterial lawn (food) and irradiated with a graded dose of ^{60}Co gamma rays. Pharyngeal pumping in 5 or more animals was recorded using a high-speed camera every 2 h from 0 h to 8 h post-irradiation. The frequency of pharyngeal pumping was counted using 60 continuous recording images of 3 s duration. We found that irradiated animals could be classified into 2 groups. One group stopped pumping immediately after irradiation and the other showed normal pumping activity. This tendency of the 2 groups was distinctly different from that of locomotion using body-wall muscles, wherein the motility of the irradiated animals decreased uniformly in a normal distribution wholly in a dose-dependent manner. In addition, the pumping activity was completely restored up to the level of the non-irradiated animals within 2 h, and the restoration level was higher than that in locomotion. Our findings indicate that whole body irradiation reduced the pumping and locomotion and both movements were restored within several hours, although there was an obvious difference in the aspect of the reduction between pumping and locomotion. This difference might depend on the difference of the number and/or type of neurons controlling the pharyngeal muscles for pumping and body-wall muscles for locomotion. Further study

on the mechanism underlying the irradiation-induced reduction and restoration in each movement will be required.

(1) Sakashita, T. *et al.* (2008) J. Radiat. Res. **49**, 285. (2) Suzuki, M. *et al.* (2009) J. Radiat. Res. **50**, 119.

683C

Revealing the promiscuous nature of pigment dispersing factor signaling. **Liesbet Temmerman**, Ellen Meelkop, Annelies Bogaerts, Liliane Schoofs, Tom Janssen. Laboratory of Functional Genomics and Proteomics, K.U. Leuven, Naamsestraat 59, B-3000 Leuven, Belgium.

In addition to the classical neurotransmitters, *C. elegans* uses neuropeptides as messengers or modulators in the nervous system, the latter which mainly act upon G protein-coupled receptors (GPCRs). 1300 GPCRs have been predicted in *C. elegans*, of which the majority is uncharacterized and for which the ligand is unknown.

Not so long ago, our group identified a GPCR and three corresponding neuropeptide ligands of particular interest. These molecules comprise the *C. elegans* pigment dispersing factor (PDF) signaling system, which resembles both the invertebrate PDF system as well as the widely studied vertebrate VIP signaling system. In insects, PDF function has been and still is profoundly studied with regard to molecular dissection of circadian clock mechanisms and the effects on (mainly) locomotion. The drawback of this focus is a remaining ignorance on possible other functions for PDF, which are better described for the vertebrate homolog VIP. Using the molecular toolkit available for *C. elegans*, we have broadened the scientific view on invertebrate PDF signaling systems and observed additional resemblances to the vertebrate VIP system.

Here, we combined top-down and bottom-up approaches to identify novel actions of the PDF signaling system. Apart from the observed sex-specific spatial expression profiles for *pdf-1* and *pdfr-1*, quantitative expression experiments indicated a higher expression of *pdf-1* and *pdf-2* in male individuals. Complementary behavioral assays showed that mutant males perform less efficiently when fertilizing hermaphrodite partners. Certain male-specific functions have also been proposed for VIP.

Performing a combination of differential transcriptomic and proteomic experiments furthermore strongly indicates a modulatory role for the PDF signaling system in energy metabolism, especially under starvation. This aspect of PDF signaling again finds its counterpart when regarding the VIP system, which also is involved in fatty acid metabolism and seems to be needed under starvation. Other modulating functions of the PDF signaling system in resistance to stress and development emerge from our bottom-up approaches and are under current investigation.

Taken together, our data support the hypothesis that the PDF system in *C. elegans* modulates a broad range of processes, rather than being confined to the regulation of circadian rhythms.

684A

Analysis of molecular mechanisms that integrate sensory perception of nutrient with rhythmic motor output. **Baskaran Thyagarajan**, Keith W. Nehrke. Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY.

The rhythmic defecation motor program (DMP) is executed on average once every ~ 45-seconds in well-fed worms. The defecation period is timed by cell-autonomous oscillatory calcium signaling in the intestinal epithelium through activation of the inositol 1,4,5-trisphosphate receptor¬ ITR-1. Normally, worms don't defecate in the absence of food. However, a weak reduction-of-function mutation in the nematode ortholog of Ca2+/Calmodulin dependent protein kinase II causes worms to continue to defecate even while off food. In addition, the unc-43(sa200) mutant worms reiterate a partial DMP in between cycles. These behavioral repetitions have been termed "shadows". The interval between the DMP and shadow, or latency, is quite regular at ~ 15-seconds. The idea has been put forth that unc-43 normally suppresses the DMP, and perhaps coordinates food availability with motor output. Our objective here was to answer whether unc-43 acts directly by regulating ITR-1 in the intestine and to determine the mechanism whereby the recognition of food availability is integrated with motor output. We found that serial food dilution significantly lengthened the defecation period of N2 worms; at the highest dilutions the worms ceased to defecate entirely. Interestingly, there was an abrupt cutoff where further diluting food by just 2-fold caused nearly all of the worms to cease defecating. However, the unc-43(sa200) mutant's cycle period was not significantly affected by food dilution, even at the highest dilutions. Instead, the shadows themselves became less frequent, though when they occurred their latency was still ~15-seconds. Using this model, we combined unc-43 cell-specific RNAi and unc-43(rf) complementation to ask whether regulation was cell autonomous. We then combined mutant alleles of unc-43 and itr-1 with RNAi to assess whether they operated through the same pathway. We also targeted intestinal cmd-1 (calmodulin ortholog) as an alternative way of inactivating unc-43. Remarkably the cmd-1 RNAi-treated worms phenocopied the unc-43(rf) mutant, and after prolonged treatment began to execute a full DMP at ~15-second intervals. Finally, we employed dynamic fluorescent imaging to determine how each of the mutations affected calcium oscillations in the intestine as a function of food availability. Our results provide insight into the molecular mechanism linking nutrient availability to rhythmic motor output.

685B

Ca^{2+}-CaM-dependent protein Kinase I is required for short- and long-term mechanosensory habituation. **Tiffany A. Timbers**, Jing Xu, Catharine H. Rankin. Brain Research Centre and Dept. of Psychology, University of British Columbia, Vancouver, BC.

Ca^{2+}-CaM-dependent protein kinases CaMKI and CaMKIV are abundant in the brain and are activated in response to elevated intracellular Ca^{2+} levels by Calmodulin and phosphorylation by the upstream kinase CaMKK. Studies of CaMKIV and CaMKK in knockout mice show that these genes are critical for learning and memory. Curiously, CaMKI, a widely expressed CaMK known to be critically important for the developing nervous system, has received little attention on its role in learning and memory. We tested whether the *C. elegans* CaMKI homologue, CMK-1, was necessary for short- and long-term mechanosensory habituation. Worms were habituated to mechanical stimuli (taps to the side of the Petri dish) using different stimulation protocols known to induce short-term habituation, 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. cmk-1 mutants cannot form long-term memory for habituation training. No deficits in either short or long-term habituation were found in animals with a mutation in ckk-1 (homologous to CaMKK), although we did find long-term memory deficits in a well known downstream target of CMK-1; the transcription factor CREB (CRH-1). Currently we are using cell-specific rescues and cell-specific RNAi knockdown to investigate which neurons require functional cmk-1 for wild-type habituation at a 60s ISI and which are require functional cmk-1 and crh-1 for long-term habituation. This work was supported by operating grants from NSERC to CHR and by Graduate Fellowships from NSERC to TAT.

686C

Role of dopamine in locomotory transitions in C. elegans. **S. Topper[1]**, A. Vidal-Gadea[1], L. Young[1], A. Gottschalk[2], J. Pierce-Shimomura[1]. 1) Institutes of Neuroscience & Cell and Molecular Biology, The University of Texas at Austin, Austin, TX 78712 United States of America; 2) Institute of Biochemistry, Johann Wolfgang Goethe-Universität Frankfurt am Main Germany.

Many animals switch behavioral and locomotory patterns in order to move efficiently in different environments. We have found that the nematode C. elegans displays alternate forms of locomotion; crawling on firm substrates and swimming in liquid environments. These forms of motion appear to represent gaits because when we constrain worm speed with viscous solutions, worms swim at low viscosity, crawl at high viscosity and switch between crawling and swimming at intermediate viscosity. Neuromodulators such as dopamine have conserved roles in switching between locomotory forms as evident in human Parkinson's disease patients who have trouble initiating voluntary motor commands. We investigated a role for dopamine in C. elegans locomotory transitions. Ablation of dopamine neurons, reduced dopamine synthesis, or deletion of the D1-like dopamine receptors (dop-1 or dop-4) impaired the transition from swim to crawl, but not crawl to swim. Furthermore, photoactivation of channelrhodopsin2 in the dopamine neurons was sufficient to induce crawl-like behavior in the swimming animal. Loss of the dop-1 receptor significantly reduced the amount of crawl-like behavior after photoactivation of dopamine neurons, while loss of dop-4 entirely eliminated photo-induced crawl-like behavior . Further investigation into dop-4 will elucidate downstream components that have a role in the swim to crawl transition.

687A

Molecular mechanism of salt taste. **OLUWATOROTI O. UMUERRI**, GERT JANSEN. CELL Biol, Univ ERASMUS Med Ctr, Rotterdam, Netherlands.

NaCl is essential for salt and water homeostasis and it is important for physiological functions in many organisms. However, the detailed molecular mechanism of NaCl sensation is not well known. In mammals, the epithelial Na+ channel (ENaC) has been shown to be involved in NaCl detection but there is also an ENaC channel-independent salt taste mechanism. Several genes involved in NaCl chemoattraction in C. elegans have been identified. These include 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, these mutants still showed significant attraction to 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 use cell specific rescue of the mutant genes and neuronal calcium imaging to find out where in the neuronal circuit of C. elegans these genes function. The involvement of the main salt sensing neurons, ASE, has been confirmed. In addition, we found that the Gα protein, odr-3, functions in chemosensation in the ADF neurons. We have recently performed a forward genetic screen to identify additional genes that play a role in NaCl chemotaxis. We found 16 independent mutants among 20,000 haploid genomes screened. 6 mutants have completely lost chemosattraction to NaCl and 10 mutants showed highly reduced chemoattraction. One of the 6 identified mutants is a mutation in the che-1 gene (allele gj1010). We are currently using SNP-mapping and sequencing to identify the genes affected in two other mutants, gj1008 and gj1009 . gj1008 mapped to the left arm of chromosome I and gj1009 mapped to the middle of chromosome III. This will help in deciphering the signal transduction pathway that mediates NaCl sensation.

688B

Glial regulation of chemosensation. **Sean W Wallace**, Shai Shaham. Laboratory of Developmental Genetics, The Rockefeller University, New York, NY.

Glia play active roles in regulating nervous system development and function; however,

little is known about the regulation of neuronal sensory transduction by glia at sensory organs. Ablation of glia in the *C. elegans* amphid results in functional defects in the associated sensory neurons, suggesting glia-derived factors regulate sensory neuron function (Bacaj *et al*., 2008). We aim to identify and characterize the functions of such factors. Previous work from our lab showed that the amphid sheath glial gene *fig-1* is required for amphid sensory neurons to take up lipophilic but not water-soluble dyes from the extracellular media, raising the possibility that *fig-1* may alter neuronal membrane properties. Furthermore, *fig-1* mutants display defects in 1-Octanol avoidance behavior. *fig-1* is predicted to encode a secreted protein containing two thrombospondin domains. Glial proteins with similar domains play roles in synapse establishment in vertebrates.

To understand how FIG-1 protein functions, we plan to use genetic screens to determine how FIG-1 secretion from glia is regulated, and to identify neuronal proteins that may be targets of its activity. To identify additional glial regulators of sensory neuron function, 298 amphid sheath glial genes previously identified in our lab will be screened by RNA interference (RNAi) for defects in chemotaxis and avoidance behaviors.

Sensory function is critical for animal survival, and loss of sensory function results in decreased quality of life in humans. The presence of glia at sensory structures is a conserved feature of animal evolution, yet their contribution to sensory function is poorly studied. Furthermore, the receptive endings of chemosensory neurons resemble synapses in that they contain dendrites that respond to similar chemicals and that are intimately associated with glia. Thus, our studies are likely to inform us about the roles glia play at sensory structures, and have the potential to provide general insights into the synaptic functions of glia.

689C

GRK-2 regulates serotonin metabolism and egg-laying behavior in *C. elegans*. **Jianjun Wang[1]**, Jiansong Luo[1], Dipendra Aryal[2], William Wetsel[2], Jeffrey Benovic[1]. 1) Dept Biochem & Mol Biol, Thomas Jefferson Univ, Philadelphia, PA; 2) Psychiatry And Behavioral Scis, Duke Univ, Durham, NC.

Many C. elegans behaviors are regulated by G protein-coupled receptor (GPCR) signaling including egg-laying, locomotion, feeding and chemosensation. Agonist binding to a GPCR promotes interaction with three protein families, heterotrimeric G proteins, GPCR kinases (GRKs) and arrestins. G proteins function to regulate downstream effector enzymes and signaling while GRKs and arrestins function to positively or negatively modulate GPCR signaling and trafficking. C. elegans contain two GRKs, GRK-1 and GRK-2, and a single arrestin, ARR-1. Previous studies have revealed an essential role for GRK-2 in chemosensation and suggested that it plays a positive role in G protein signaling while ARR-1 plays a role in adaptation and longevity. Here, we evaluated the role of GRK-2 in egg-laying. Egg-laying is stimulated by GPCRs coupled to EGL-30 (Gαq) and inhibited by GPCRs coupled to GOA-1 (Gαo). We found that loss-of-function mutants of grk-2 were egg-laying defective and retained more eggs in uteri than wild type animals and laid late stage eggs. Interestingly, the constitutive egg-laying phenotype of animals that have activated Gαq signaling [egl-30(ep271), eat-16(ep273), dgk-1(sy428)] or inhibited Gαo signaling goa-1(n1134) were suppressed by grk-2 mutation suggesting that GRK-2 may function downstream of diacylglycerol kinase. Since exogenous serotonin was able to rescue the egg-laying defect in grk-2 mutants, we evaluated serotonin levels and the results revealed that endogenous serotonin levels are significantly decreased in grk-2 mutants. However, the expression analysis of tph-1 suggests there is no detectable change in tph-1 expression. Then, we measured the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA), and found that it was significantly increased in grk-2 mutants suggesting that serotonin metabolism may be increased in the grk-2 mutant strains. To inhibit serotonin metabolism, we treated animals with the monoamine oxidase (MAO) inhibitor pargyline and found that it could rescue the egg-laying defect of grk-2 mutants. Similarly, knockout of the MAO orthologs amx-1 and amx-2 also rescued the egg-laying defect of grk-2 mutants. Since the egg-laying defect of grk-2 mutants is stronger than the knockout of tph-1, we evaluated whether 5-HIAA had a direct effect on egg-laying. Interestingly, exogenous 5-HIAA effectively inhibited egg-laying in wild type animals but had no effect on ser-1(ok345) or goa-1(n1134) mutants. Overall, these results suggest that GRK-2 plays a role in regulating serotonin metabolism and 5-HIAA may negatively regulate SER-1 signaling.

690A

Bending waves during *C. elegans* locomotion are driven by proprioceptive coupling. **Quan Wen[1]**, Elizabeth Hulme[2], Sway Chen[1], Xinyu Liu[2], Marc Gershow[1], Andrew Leifer[1], Victoria Butler[3,4], Christopher Fang-Yen[5], William Schafer[3], George Whitesides[2], Matthieu Wyart[6], Dmitri Chklovskii[4], Aravinthan Samuel[1]. 1) Dept of Physics, Harvard University, Cambridge, MA; 2) Dept of Chemistry, Harvard University, Cambridge, MA,; 3) MRC Laboratory of Molecular Biology, Cambridge University, Cambridge, England; 4) Janelia Farm Research Campus, HHMI, Ashburn, VA; 5) Dept of Bioengineering, University of Pennsylvania, Philadelphia, PA; 6) Dept of Physics, New York University, New York, NY.

Locomotion requires mechanisms for coordinating motor activity throughout an animal's body. Here, using microfluidic devices to bend specific body regions while monitoring the consequences on the rest of the body, we show that proprioceptive coupling drives and organizes forward locomotion in *C. elegans*. Proprioceptive feedback compels each body region to bend after and in the same direction as the bending in the anterior neighboring region. To understand how proprioception is integrated into the neuromuscular network, we performed calcium imaging of muscle cells to directly visualize the motor activity along the body of a worm trapped in these microfluidic devices. We used optogenetic stimulation of the motor circuit to interrogate the cellular mechanism of proprioceptive feedback. We found that the cholinergic motor neurons both generate and propagate the proprioceptive signal from anterior to posterior body regions. Moreover, body wall muscles in *C. elegans* can sustain contraction without synaptic input, thereby requiring motor neurons only to trigger changes in bending states but not to maintain bending. Quantifying our experimental observations enabled us to build a simple mathematical model of locomotion, in which we show that proprioceptive coupling not only organizes the bending wave during locomotion, but also explains gait adaptation when the external load on a moving worm is gradually increased.

691B

Acetylcholine-Gated Chloride Channels (ACCs) may be involved in development and behaviour, suggesting a central role for fast inhibitory cholinergic neurotransmission in *C.elegans*. **Claudia M. Wever**, Patrick Janukavicius, Jin-Kyung Chang, Danielle Farrington, Julian Gitelman, Igor Putrenko, Joseph A. Dent. McGill University, Department of Biology, Montreal, QC, Canada.

Acetylcholine is an abundant neurotransmitter in *C.elegans* that is involved in many of the organism's behaviours including feeding, locomotion and egg-laying. Over a third of the neural cells in *C.elegans* release acetylcholine and acetylcholine is the only essential neurotransmitter in *C.elegans*. We previously cloned and characterized members of the acetylcholine-gated chloride channel (ACC) family in *C.elegans* (Putrenko et al., 2005) and the discovery of these channels was the first evidence that *C.elegans* employs acetylcholine as a fast inhibitory neurotransmitter. We have shown that ACC-1, ACC-2, ACC-3 and K10D6.1 subunits form homomeric acetylcholine-sensitive channels in *Xenopus Laevis* oocytes and that ACC-1 and ACC-3 subunits interact in oocytes to form a heteromeric acetylcholine-sensitive channel. Expression data from promoter-GFP fusion constructs revealed that ACC-1 and F47A4.1 are co-expressed in M3 neurons. Supporting these results, when co-expressed in oocytes, the ACC-1 and F47A4.1 subunits form a functional heteromeric chloride channel that is less sensitive to acetylcholine than the ACC-1 homomer. Interestingly, the membrane currents generated by the ACC-1/F47A4.1 heteromer are over five times larger in magnitude than those generated by the ACC-1 homomer. In addition to characterizing the electrophysiological properties of the ACC channels, we have obtained strains with deletions in the various ACC genes in order to begin to understand their functions in vivo. Worms with deletions in ACC-2 have delayed developmental timing, taking longer to reach gravidity compared to N2 worms. F47A4.1 promoter-GFP fusions are expressed in HSN neurons, which are a central component of the egg-laying circuit. We therefore examined egg-laying behaviour in the F47A4.1 and ACC-1 deletion strains. Both strains lay fewer eggs than N2. The decrease in the number of eggs laid appears to be the result of an ovulation defect as opposed to a deficit in the egg-laying circuit itself. Rescue experiments as well as experiments to find and characterize new phenotypes associated with deletions in the ACC genes are ongoing. We are also currently generating double and triple mutant strains to uncover any phenotypes that are only apparent when multiple members of the ACC family are affected. (Putrenko et al., 2005, J. Biol. Chem. 280, 6392-6398) This work is supported by NSERC and Chemtura Co.

692C

In vivo structure-function analysis of *C. elegans* GRK-2. **Jordan Wood[1]**, Jeffrey Benovic[2], Denise Ferkey[1]. 1) Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, NY (USA); 2) Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA (USA).

G protein-coupled receptor (GPCR) signaling mediates an array of physiological processes, including several modalities of sensory signaling. G protein-coupled receptor kinases (GRKs) are key regulators of signal transduction that specifically phosphorylate activated GPCRs to terminate signaling. There are 7 mammalian GRKs. *C. elegans* have single orthologs of the GRK2/3 and GRK4/5/6 families, GRK-2 and GRK-1, respectively. Extensive biochemical and crystallographic study has provided insight into mammalian GRK2/3 structure and interactions: the N-terminal α-helix stabilizes GRK interaction with ligand-bound GPCRs, the regulator of G protein signaling homology (RH) domain binds activated Gαq and the pleckstrin homology (PH) domain mediates membrane localization via Gβγ and phospholipid interactions. Despite extensive *in vitro* characterization, little is known about the *in vivo* contribution of these described GRK structural domains to proper GRK function in signal regulation. Although GRK activity typically downregulates GPCR signaling, *C. elegans* lacking GRK-2 function are not hypersensitive to chemosensory stimuli. Rather, *grk-2* mutant animals fail to avoid aversive stimuli or chemotax toward attractive stimuli. To discern the interactions required for proper *in vivo* GRK-2 function, we took advantage of the disrupted chemosensory behavior characteristic of *grk-2* mutant animals. Informed by mammalian crystallographic and biochemical data, we introduced amino acid substitutions into the *grk-2* coding sequence that are predicted to selectively disrupt GPCR phosphorylation, Gα binding, Gβγ binding or phospholipid binding. Each construct was tested for its ability to restore chemosensory behavior in animals lacking endogenous GRK-2. Our preliminary data indicates that changing the most N-terminal residues, which have been shown in mammalian systems to be required specifically for GPCR phosphorylation, but not phosphorylation of alternative substrates or recruitment to activated GPCRs, disrupts the ability of GRK-2 to restore chemosensory avoidance of the odorant 1-octanol, which is detected primarily by the nociceptive ASH sensory neurons. Thus, although GRKs can interact with and phosphorylate a wide variety of other proteins in addition to GPCRs, including signaling molecules, it appears that the primary role of *C. elegans* GRK-2 in the regulation of chemosensory behavioral responses is the phosphorylation of putative chemosensory GPCRs. Analysis of the Gα, Gβγ and phospholipid binding mutants of GRK-2, as well as additional ASH, AWA and AWC chemosensory stimuli, is underway.

693A

Worms maintain their response to the repulsive odor 2-nonanone during avoidance behavior. **Akiko Yamazoe**[1], Yuishi Iwasaki[2], Kotaro Kimura[1]. 1) Department of Biological Sciences, Osaka University, Toyonaka, Osaka, Japan; 2) Department of Intelligent System Engineering, Ibaraki University, Ibaraki, Japan.

Animals can maintain their behavioral response to environmental stimuli even under unstable environmental conditions and during various animal movements. To investigate neural mechanisms for such robust behavioral responses, it is necessary to quantitatively analyze the time-course changes in the correlation between the stimulus and behavioral response. For this, we quantitatively analyzed stimulus as well as behavior of worms' avoidance response to repulsive odor 2-nonanone. When animals migrate away from a source of repulsive signal, their avoidance response is likely weakened. In a previous study, however, we have shown that worms exhibited a constant average velocity of avoidance from 2-nonanone for 10 min (Kimura et al., J. Neurosci., 2010), suggesting a neural mechanism for such constant avoidance.

In addition to the quantitative analysis of avoidance response to 2-nonanone (Yamazoe & Kimura, CeNeuro, 2010), we recently developed a technique to measure the concentration of 2-nonanone at specific spatial and temporal points of gas phase in the assay plate. By using a highly sensitive gas chromatograph, we observed a clear gradient of 2-nonanone, of which concentration increased with time. Based on this measured gradient of 2-nonanone, we determined the 2-nonanone concentration that each worm experienced during the avoidance assay (C_{worm}) and observed the following: (1) During the first 2 min of the assay worms did not initiate avoidance response and migrated randomly, and C_{worm} increased continuously up to the order of μM at 2 min. (2) After 2 min, worms started to migrate farther away from the odor source, and C_{worm} was maintained around the concentration, despite increase in the concentration gradient. (3) C_{worm} decreased effectively during runs, while it increased and decreased largely during pirouettes. (4) When compared between the early and late phases of the assay, the maximum dC_{worm}/dt in each run decreased several fold along with the avoidance behavior, even though the orientation directions did not change considerably; that is, even when the gradient of 2-nonanone became shallower, the accuracy of worm orientation appeared maintained. These results suggest that worms may increase sensitivity to dC/dt during exposure to a certain concentration of 2-nonanone. We are currently conducting computer simulation to test this hypothesis. Further analysis may help us uncover the mechanism of maintaining proper behavioral responses.

694B

Olfactory preference switch depending on odor concentration is mediated by combinatorial change of acting sensory neurons. **K. Yoshida**[1], T. Hirotsu[2], T. Tagawa[1], S. Oda[1], Y. Iino[1], T. Ishihara[2]. 1) Dept Biophys & Biochem, Tokyo Univ, Tokyo, Japan; 2) Dept Biology, Kyushu Univ, Fukuoka, Japan.

Many organisms exhibit olfactory preference towards most odorants. It is empirically known that the perceived quality of an odorant changes depending on its concentration in some organisms including humans. However, the neuronal basis of the preference change depending on odor concentration is still elusive. Here we show *C. elegans* also shows changes of olfactory preference depending on odor concentration.

When concentrations of various attractive odorants such as isoamyl alcohol are increased, animals become to show avoidance of them. Behavioral analyses and computer simulation revealed that the behavioral change depending on concentration of an odorant is mainly mediated by one of two known behavioral strategies, klinokinesis (the pirouette mechanism). Namely, the dependence of the frequency of pirouette behavior on temporal changes in odor concentration is reversed in different concentration ranges. On the other hand, the other behavioral strategy klinotaxis (the weathervane mechanism) is employed in the direction to drive attraction to the odorant regardless of its concentration.

We next examined what molecules mediate these behaviors, and found that ODR-3 G protein α subunit mediates klinokinesis and klinotaxis in the concentration-dependent chemotaxis to isoamyl alcohol. Moreover, ODR-3 transmits the olfactory signaling depending on isoamyl alcohol concentration by acting in different sensory neurons.

We further show that distinct combinations of sensory neurons function at different concentrations of isoamyl alcohol: AWC sensory neurons are known to mediate attraction to lower concentration of isoamyl alcohol, whereas ASH, AWB and ADL sensory neurons are required for avoidance of higher concentration of it. Calcium imaging revealed that activity patterns of these sensory neurons dramatically change depending on isoamyl alcohol concentration: the higher concentration of isoamyl alcohol fails to elicit responses of AWC, which strongly responds to lower concentration of the odorant, and elicits particular activity patterns of AWB and ASH. Hence, our study shows that the information processing which regulates olfactory preference change depending on odor concentration is clearly explained by the labeled-line theory.

695C

Calcium-activated chloride channels (CaCCs) act as ciliary sensory receptors in C.elegans. **Yuxia Zhang**, Jinghua Hu. Mayo Clinic, Rochester, MN, USA.

Members of the Anoctamin (Ano)/TMEM16A family have recently been identified as Ca(2+)-activated chloride channel (CaCCs) and are shown to correlate with various human diseases. However, how CaCCs function in vivo remains poorly understood. Using bioinformatics, we identified the only two CaCC homologues in C. elegans genome, cacc-1 and cacc-2. Promoter analysis indicated these two genes are specifically expressed in ciliated neurons. GFP-tagged proteins strongly labeled sensory cilia, indicative of the primary roles for CaCCs as sensory receptors. Loss of either cacc-1 or cacc-2 leads to significant olfactory defects mediated by AWA and AWC neurons. cacc-1 and cacc-2 mutant animals are otherwise wild-type. Our data suggest that CaCCs act as major ciliary sensory receptors in worm chemosensory neurons and point out an ancient correlation between CaCC family members and cilia-related function.

696A

Two homeodomain transcription factors, MLS-2 and CEH-6, promote tube development in the excretory system. **Ishmail Abdus-Saboor**, Craig Stone, John Murray, Meera Sundaram. Gen, Univ Pennsylvania, Philadelphia, PA.

Tubes are an important component of many organs, but the processes by which cells adopt tube fates and organize into complex tubular shapes are only beginning to be elucidated. The C.elegans excretory system consists of three unicellular tubes (duct,pore, and canal) that connect in tandem. Excretory system development consists of distinct stages including but not limited to de novo epithelial polarization, tubulogenesis, and proper stacking of the three tubes. After the three tubes are stacked in tandem, terminal differentiation occurs and epithelial junctions that connect the cells to each other must be maintained. This developmental paradigm mirrors those of more complex organs such as the mammalian kidney. In screens for excretory defective mutants we identified two homedomain transcription factors, MLS-2 and CEH-6. MLS-2 and CEH-6 are both expressed in the duct and pore lineages, with MLS-2 expression coming on in these lineages prior to CEH-6. CEH-6 is also expressed in the canal cell, and others have shown that CEH-6 is required for canal cell development. *mls-2* mutants fail to express duct differentiation markers and excretory lethality is exacerbated at low temperatures. *mls-2* and *ceh-6* each lack a pore auto-cellular junction. In addition, *mls-2* and *ceh-6* each interact genetically with the EGF/Ras signaling pathway. We are currently investigating how *mls-2* and *ceh-6* interact with each other and the EGF/Ras signaling pathway.

697B

The *C. elegans* Wnt Effector TCF/LEF/POP-1 Acts to Protect the Signalled Fate by Restricting β-catenin/WRM-1 to the Nucleus in Stem-Like Seam Cells. **Peter J. Appleford**, Charles Brabin, Alison Woollard. Laboratory of Genes and Development, Department of Biochemistry, Univ Oxford, Oxford, United Kingdom.

The *C. elegans* seam provides a paradigm for the stem cell mode of division, dividing asymmetrically during larval development to produce anterior daughters that differentiate and posterior daughters that retain the stem cell-like fate. As is common in a wide range of biological systems, division asymmetries are controlled by Wnt signalling; in *C. elegans* a variant Wnt pathway, known as the Wnt/β-catenin asymmetry pathway, predominates. Where POP-1 is high in the nucleus relative to the atypical β-catenin SYS-1, it acts to repress transcriptional targets, whereas low POP-1/high SYS-1 ratios enable POP-1 to act as a transcriptional activator. Here, we show that POP-1:SYS-1 reciprocal asymmetry is required to control the anterior-posterior asymmetry of seam cell divisions, as lowering the level of POP-1 causes seam cell hyperplasia. Paradoxically, however, over-expression of POP-1 has the same effect, due to symmetrisation of seam cell divisions towards the seam fate. We show that POP-1 overexpression hyperplasia results from a negative feedback mechanism, whereby WRM-1 (a β-catenin required for POP-1 nuclear export) is retained in the nucleus, causing enhanced nuclear export of POP-1 from both daughters. The acquisition of the hypodermal fate at the expense of the seam fate can only be achieved when POP-1 is driven into the nucleus by addition of an NLS and export prevented via downregulation of LIT-1/WRM-1. Thus, there is a very robust homeostatic mechanism operating in this stem-like cell lineage that acts to protect the seam fate from aberrant *pop-1* expression.

698C

A focused reverse genetic screen for regulators of Distal Tip Cell specification. **Austin T. Baldwin**, Bryan T. Phillips. University of Iowa, Iowa City, IA, IA.

Asymmetric cell division allows the two daughters of a mitotic division to give rise to different developmental lineages, allowing organisms such as C. elegans, which undergo repeated asymmetric divisions, to generate a multitude of different cell types by the adult stage. During larval development, the Distal Tip Cells (DTCs) of the forming gonad are specified via asymmetric cell division of the somatic gonadal precursors, Z1 and Z4. The DTCs function in gonad arm migration and maintain a germline stem cell population. The DTC fate is specified by the Wnt/β-catenin Asymmetry pathway, which activates Wnt target genes through reciprocal asymmetric distribution of SYS-1 and POP-1, C. elegans homologs of β-catenin and TCF, respectively. The mechanism controlling POP-1 asymmetry has been well studied, but the mechanism controlling SYS-1 asymmetry is not well understood. Since SYS-1 is a cryptic β-catenin regulated posttranslationally at the level of protein stability and because other β-catenins are targeted for degradation via phosphorylation, we have undertaken a reverse genetic screen of known and predicted kinases using the feeding RNAi library. To increase throughput we have utilized a strain expressing GFP in the DTCs (DTC::GFP) that can be visualized under low magnification. We also screened using two sensitized strains. rrf-3;DTC::GFP provides general enhancement of RNAi efficacy. Additionally, pSYS-1::YFP::SYS-1;DTC::GFP, which expresses higher than normal levels of SYS-1 protein, acts a sensitized strain for loss of SYS-1 negative regulation. In our primary screen, synchronized L1 worms of the above strains were subjected to feeding RNAi targeting specific C. elegans kinases and then screened for DTC number as L4s. To determine the extent to which defects in DTC specification result from aberrant Wnt/β-catenin asymmetry signaling during asymmetric divisions, expression of SYS-1, POP-1 and their target gene CEH-22 will be examined cytologically in SGP daughter cells shortly after division. These analyses will identify new regulators of the Wnt/β-catenin asymmetry pathway and DTC fate. A benefit to this screen is that both positive and negative regulators of DTC fate can be identified. We screened approximately 250 kinases and found both positive and negative regulators of DTC fate, the strongest of which are currently being screened cytologically.

699A

Towards a quantitative understanding of the vulva cell fate patterning network. **Michalis Barkoulas**, Marie-Anne Félix. Institut Jacques Monod, 15 rue Helene Brion, 75205 Cedex 13, Paris, France.

The *C. elegans* vulva is the egg-laying and copulatory organ of the adult hermaphrodite that is specified during the third larval stage of postembryonic development from a row of six vulva precursor cells (P3.p-P8.p). These six cells are all competent to adopt vulva fates, but only three (P5.p-7.p) will normally generate vulva tissues, by adopting two distinct cell fates: the 1° cell fate, which is acquired by P6.p, and the 2° fate which is adopted by P5.p and P7.p. Two major signalling pathways have been shown to be critical in specifying the vulva precursor cell fate pattern. Firstly, an inductive signal from the uterine anchor cell activates the 1° cell fate in P6.p through EGF/LIN-3 -Ras-MAP kinase signalling. Secondly, a Delta-Notch/LIN-12 pathway is involved in lateral signalling among the Pn.p cells, resulting in acquisition of the 2° cell fate in P5.p and P7.p. Extensive genetic analyses over the last thirty years have revealed both the key molecular components of these two pathways, and also how these two pathways interact within a single or neighbouring Pn.p cells. Nonetheless, even in such a well-studied system, a quantitative understanding of the molecular network is still lacking. For example, although the vulva cell fate pattern is known to be very robust to environmental and genetic variation, the exact range of this robustness with regard to changes of EGF or Notch pathway activity remains unclear. We have started addressing such questions experimentally by systematically varying the doses of EGF and Notch pathway activities in the vulva precursor cells by using tissue-specific transgenic manipulations. We present here our results on the first errors of the vulva cell fate pattern that we observed in these transgenic lines. Our aim is to establish complete EGF and Notch pathway dose-response curves and study their interaction, combining our experimental results with a computational model of the vulva cell fate network that we recently developed in the lab. Finally, we would like to transfer the quantitative knowledge of the *C. elegans* network to other wild isolates and species to better understand the evolution of the vulva patterning system.

700B

gad-1 RNAi Leads to a General Increase in Cell Lifetimes During Development. **Max E. Boeck**, Robert Waterston. Dept Genome Sci, Univ Washington, Seattle, WA.

gad-1 (gastrulation defective) was first described for its role in E lineage gastrulation. A temperature sensitive mutant and *gad-1* RNAi both lead to a failure to gastrulate and eventual arrest prior to hatching (Knight and Wood 1998). The cellular phenotypes described for *gad-1*i are similar to those that seen in *end-3*(-) embryos. These phenotypes include delayed gastrulation, abnomral axis of the E2 division and decreased lifespan of E lineage cells. Both *end-1* and *end-3* are GATA transcription factors essential for E lineage fate specification in early embryogenensis. To understand *gad-1*'s role in E lineage specification we used 4D imaging and automated cell tracing to examine *gad-1* RNAi-treated embryos. In *gad-1*i embryos *end-1* and *end-3* RFP reporters had a normal onset of expression even when the gastrulation defect was most severe, indicating *gad-1* does not directly activate *end-1* and *end-3*. However, expression of an *elt-2* reporter - a downstream GATA transcription factor known to be a target of *end-1/3* which is also essential for E lineage activation - was delayed in *gad-1*i embryos. Further, all phenotypes seen for *gad-1*i, including *elt-2* activation, were variable in their penetrance, similar to *end-3*(-) embryos. When we examined the cellular phenotypes throughout the rest of the embryo, however, we found a variable, but reproducible delay in the cell lifetimes of all lineages. This delay increases the average cell lifetimes at the 50 cell stage by 12% when excluding the E lineage and coincides with both gastrulation and the onset of zygotic expression. Subsequently the delay became more pronounced and eventually leads to the AB cells at the 250 cell stage living 40% longer when compared to WT. After the initial decrease in lifespan seen in E2 cells the E lineage also shows extended lifespans with the E8 cells living 20% longer in *gad-1*i embryos. An RFP reporter of *gad-1* is expressed ubiquitously beginning at the E4-cell stage. These phenotypes, including the failure to gastrulate and a general extension of lifespans, closely resemble those seen in RNAi knockdowns of the RNA polymerase II subunit *ama-1*, supporting *gad-1* playing a more general role in zygotic transcription. Many of the phenotypes observed in *ama-1*i may be a result of a failure to activate *gad-1*. While it is clear that *gad-1* plays a role in E lineage specification, the embryo-wide defects observed point towards it having an important role in general embryonic development. The variable nature of these phenotypes indicates *gad-1* has an important role in maintaining robustness during development.

701C

A regulatory network of homeobox genes is required for the function of the Caenorhabditis elegans excretory cell. Yong-Guang Tong, Krai Meemon, **Thomas R. Burglin**. Biosciences & Nutrition, Center for Biosciences, Karolinska Institutet, Huddinge, Sweden.

Homeobox genes play important roles in the development and differentiation of animals. We have previously found that the POU-III class homeobox gene, ceh-6, functions in the excretory cell (Bürglin et al. 2001). We have been studying the three C. elegans otd/Otx homeobox genes, ceh-36, ceh-37 and ttx-1 (Tong et al., IWM 2007). Using mutant alleles and RNAi we have found that ceh-37 and ttx-1 act redundantly in the excretory cell (Meemon et al., EWM 2008). Double mutants arrest with excretory cell defects.

Furthermore, we have found that the prospero homeobox gene ceh-26 is expressed in the excretory cell and loss of function of ceh-26 causes early larval lethality consistent with excretory cell defects. We further examined the effects of knocking down ceh-6, ceh-26, and ceh-37/ttx-1 in combination by looking at GFP reporters expressed in the excretory cell. For example, knock-down of all genes downregulates the expression of clh-4::GFP in the excretory cell. In contrast, expression of sulp-4::GFP is suppressed in ceh-26(RNAi) and ceh-6(mg6) animals, but not in ceh-37(RNAi)/ttx-1(RNAi) animals. Further, only ceh-6(RNAi) completely abolishes GFP expression of pgp-12, but ceh-26(RNAi) and ceh-37(RNAi)/ttx-1(RNAi) only weakly reduce the level of GFP. We also investigated the regulation of the homeobox genes amongst each other using RNAi and homeobox::GFP integrated lines. ceh-6(RNAi) nearly totally suppresses the GFP expression of both ceh-37 and ceh-26. ceh-26 (RNAi) also abolishes ceh-37::GFP expression. ceh-37/ttx-1(RNAi) of ceh-26::GFP showed that 42%; of the arrested worms still had GFP expression, indicating a partial downregulation, possibly a regulatory feedback loop. Based on our findings, we propose a regulatory hierarchy with ceh-6 at the top, ceh-26 in the middle, and the otd/Otx genes downstream. Yet, not all excretory cell genes depend on this ceh-6 cascade, suggesting the other factors remain to be uncovered.

702A

A Genetic Screen for Kinases regulating Body Size with calcineurin in *C. elegans*.. **Tae-Woo Choi**, Joohong Ahnn. Hanyang Univerisity, Seoul, Korea.

Calcineurin is a calcium/calmodulin-dependent Serine/Threonine protein phosphatase and is well known to have diverse cellular functions in different organisms. The previous studies have shown that calcineurin (TAX-6/CNB-1) is involved in body size regulation in *C. elegans*. However, the exact mechanism is still unknown. In order to identify specific kinase(s) antagonizing calcineurin in regulating body size, we performed targeted RNAi screening. Since tax-6 is predominantly expressed in neurons and the neuronal expression of *tax-6* is sufficient to rescue the small body size of *tax-6* mutant, we selected kinase candidates, which have shown neuronal expression. Candidates were further selected by availability for bacterial feeding RNAi clone in RNAi library. For the feeding RNAi, we used VH624 strain. We fed to VH624 strains which are more efficient to RNAi in neuron, with bacteria expressing RNAi of kinase candidates and *tax-6* RNAi. In F2 generation after RNAi feeding, we measured body length of one day old adults and selected candidates which rescued small phenotype of *tax-6* mutants. We found that RNAi feeding of 22 kinase candidates rescued *tax-6* phenotype of small body size. To confirm whether positive hits, we generated RNAi plasmids containing partial cDNA of each candidates. We showed that all candidates rescued *tax-6* RNAi small phenotype.

703B

Identification of genes that genetically interact with *pha-4*/FoxA to regulate pharynx development. **Youngeun Choi**[1], Trisha J. Brock[1], Dustin L. Updike[2], Susan E. Mango[1]. 1) Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA; 2) Department of Molecular Cell and Developmental Biology, University of California, Santa Cruz, CA.

The forkhead box A (FoxA) transcription factor plays a critical role in endoderm development in all animals examined to date (1,2). Its *C. elegans* orthologue, *pha-4*, is the organ selector gene for pharynx development, and tight regulation of temporal, spatial and degree of *pha-4* activity is essential for successful pharynx formation (3,4). Therefore, identification of molecules that genetically interact with *pha-4* will inform us of additional regulatory mechanisms that control pharyngeal cell fate specification and differentiation. To this end, we performed EMS mutagenesis and RNAi screening to look for *pha-4* suppressors and enhancers. For the screens, we generated and used *pha-4* (ts) strains that rapidly degrade *pha-4* transcripts at restrictive temperature through NMD pathway and thus fail to grow to adulthood. Our screening identified 250 genes, including *ruvb-1*, that enabled *pha-4* (ts) animals to survive at restrictive temperature (5,6). Conversely, we undertook a *pha-4* RNAi suppression assay to verify the positive hits from the initial screens and to expand the repertoire of potential suppressors and enhancers in candidate pathways; weak *pha-4* RNAi treatment causes defects in pharynx development, which leads to lethality in approximately 80% of wild-type worms. We found that some mutants are able to enhance or suppress the lethality associated with a partial loss of *pha-4*. The candidate genes for *pha-4* enhancers and suppressors will be further investigated to determine how they interact with *pha-4* and modulate pharynx development.

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704C

Interaction of TGFβ regulators, LON-1 and LON-2 with its downstream target, DBL-1. Y.J. Ang, J.C.C. Lee, C.K.H. Wong, M. Mok, **K.L. Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

In *C. elegans*, TGFβ pathway impacts on the body size of the organism in addition to a myriad of other developmental features. The body size of a worm is primarily controlled by endoreduplication that affects the cell size instead of the cell number. Mutants of Lon genes, e.g., *lon-1, lon-2* and *lon-3*, produce worms 1.5 times longer than wild type. These Lon gene products act in the extracellular matrix regulating the body size, and they encode a PR-related protein, a glypican and a collagen, respectively. Their regulatory mechanism, however, remains unclear. Here, we are particularly interested in *lon-1* and *lon-2* genes which act as negative regulators of the BMP-ligand, DBL-1 in the TGFβ pathway. In *C. elegans*, the effect of LON-1 exerts on DBL-1 was likely through the BMP ligand facilitator, CRM-1, possibly through physical interaction. We mapped the interaction domain between LON-1 and CRM-1 and demonstrated that the LON-1 SCP domain interacts with CR domains on CRM-1. In addition, lon-2 null mutant allele attributed to two large deletions was characterized. While glypican has the ability to bind BMP ligand, we would investigate this interaction between LON-2 glypican and DBL-1 ligand *in vivo*. Tagged version of both LON-2 and DBL-1 are expressed in transgenic animals for co-immunoprecipitation and domain mapping. The data from these experiments and the implication will be discussed in this poster. (This study is funded by the Research Grants Council, Hong Kong.).

705A

Geometric modeling of *C. elegans* vulval patterning. **Francis Corson**, Eric Siggia. Center for Studies in Physics and Biology, The Rockefeller University, New York, NY.

The conventional approach to the modeling of gene networks begins with a list of molecular parts and interactions, which are translated into a description of the dynamics, e.g. a set of differential equations. While the behavior of idealized networks comprising a small number of elements can be analyzed systematically in this way, actual biological networks are far more intricate, making it much less straightforward to relate model structure and observed behavior.

The geometrical theory of dynamical systems suggests an approach to gene network modeling that constructs a representation of their “effective dynamics” in terms of a small number of variables. In this minimal description, the many molecular constants collapse onto a few parameters that have a natural relation to the dynamics, and whose effect on the behavior of the system can easily be visualized.

We have applied this approach to the development of *C. elegans* vulva, a well-studied model system for which abundant experimental data are available in the literature. The vulva forms from a row of six vulval precursor cells (VPCs), which adopt an invariant pattern of fates in wild type. The three possible fates of the VPCs are specified by two signaling pathways (inductive signaling from the neighboring anchor cell and lateral signaling between VPCs), suggesting a two-dimensional representation of their dynamics. Qualitatively distinct dynamics, embodying different “scenarios” for vulval patterning, can be enumerated and discriminated by experimental observations. A more quantitative representation is obtained by formulating a generic model of the dynamics (the simplest equations with three stable states corresponding to the three fates), which is then fit to experimental data (proportions of different fate patterns under given conditions). This minimal model is found sufficient to account for several non-intuitive mutant phenotypes, which are given simple interpretations in terms of the geometry of the trajectories taken by the cells, and can be used to generate novel predictions, e.g. double mutant phenotypes.

This approach, by its generic nature, could readily be transposed to other systems, and, by providing a representation of dynamics that is abstracted from the details of its implementation on the molecular level, points to the possibility of discussing the organization and evolution of developmental mechanisms on a phenotypic level.

706B

Dormant Self-renewal Program of *C. elegans* Early Blastomeres Controlled by Protein Turnover. **Zhuo Du**, Anthony Santella, Zhirong Bao. Sloan-Kettering Institute, New York, NY.

An intrinsic property of many master transcription factors is their transient activity during development. While how to activate master regulators with temporal and spatial specificity is subject to intensive studies, the developmental significance of the turnover process is largely unexplored. Using the endomesoderm (EMS) lineage as a model we examined the developmental role of the turnover of SKN-1, a transiently activated master transcription factor crucial for EMS lineage specification and differentiation. Through systematic analysis of cell lineage, fate patterning, single-cell behavior and morphogenesis, we found, interestingly, that turnover of SKN-1 is required for EMS differentiation. When the activity of SKN-1 is maintained, the EMS blastomere, progenitor of endomesoderm, undergoes multiple rounds of asymmetric self-renewal in which the anterior daughter (MS) or anterior granddaughter (MSa) of EMS blastomere maintains EMS fate and differentiates into both mesoderm and endoderm. Mechanistically, the self-renewal is achieved by inducing a poised state of differentiation: when SKN-1 is sustained, transcription of its target genes, which are normally activated by SKN-1 and are required for differentiation, is delayed. During normal development, however, this self-renewal program is kept dormant by tightly regulated protein turnover cascades. Specifically, the activity of SCF^{LIN-23} and $CUL\text{-}2^{ZYG-11}$, two conserved E3 ubiquitin ligase complexes, is required to suppress self-renewal and initiate differentiation by promoting SKN-1turnover. Furthermore, depletion of SCF^{LIN-23} also induces self-renewal in the germline progenitor cells, though the underlying mechanisms are different from that of EMS renewal. Our findings reveal a dormant self-renewal capacity of transient progenitor cells. We propose that turnover of master transcription factor can function to balance the choice between self-renewal and differentiation, and more generally, protein turnover is required for developmental state transition.

Poster | Development and Evolution: Cell Fate Patterning (Embryonic and Postembryonic)

Program number is in **bold** above the title. Presenting author is noted in **bold**.

707C

Use of Single-molecule FISH To Determine Expression Patterns and Functions of *C. elegans* Lysine Methyltransferases. **Christoph G Engert**[1], Alexander van Oudenaarden[2], Bob Horvitz[3]. 1) Computational & Systems Biology, Massachusetts Institute of Technology, Cambridge, MA; 2) Departments of Physics and Biology, Massachusetts Institute of Technology, Cambridge, MA; 3) Department of Biology and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA.

The family of lysine methyltransferases (KMTs) can regulate access to the genome by methylating lysine residues in the N-terminal tails of histones. In humans, several KMTs have been implicated in disease, such as cancer. In the *C. elegans* genome, 41 putative KMTs have been identified. Four KMTs are essential for viability. Five have been shown to act to generate or maintain the germline, or to inhibit ectopic vulval development. A systematic analysis of the remaining KMTs revealed few gross defects after mutation or RNAi inactivation of individual KMT genes.[1] Three-quarters of the *C. elegans* KMTs have plausible mammalian orthologs.

To investigate KMT function in *C. elegans*, we have determined the cell-specific expression patterns of the 41 KMT genes in the L1 and L3 larval stages using single-molecule fluorescence *in situ* hybridization (smFISH). smFISH enables the determination of expression of single molecules of endogenous mRNAs throughout the entire animal.[2] Our analysis showed that ten KMTs are expressed throughout the animal. Fifteen KMTs are expressed in a single tissue, such as *set-6* in muscle and *set-20* in the hypoderm. Six and four KMTs are expressed exclusively in the germline or the muscle, respectively. In addition, several KMTs are expressed in the seam cells but not in other hypodermal cells. Based on these observations, we are designing experiments to identify the functions of KMTs in these tissues. We will also use these KMT expression patterns to investigate whether functional redundancy among certain KMTs can account for the apparently wild-type phenotypes of animals lacking the functions of many individual KMT genes.

We hope our analysis will define KMT functions and interactions in specific *C. elegans* tissues and suggest possible roles of worm KMT homologs in mammalian biology.

1 Andersen and Horvitz, Development, 134, 2991-9, 2007.

2 Raj et al., Nat Methods, 5, 877-9, 2008.

708A

Multiple Routes to Suppressing *aph-1(zu147)* Embryonic Lethality. **Victoria Fang**, Valerie Hale, Caroline Goutte. Biology, Amherst College, Amherst, MA.

aph-1 encodes one of the four essential components of the gamma secretase complex, which is essential for Notch signaling. *aph-1* null mutations cause fully penetrant maternal-effect embryonic lethality and egg laying defect. The leaky *aph-1(zu147)* mutation does not compromise egg laying and causes a less severe defect in embryogenesis in which 1% of the embryos survive to adulthood. The *aph-1(zu147)* mutation introduces a stop codon 33 residues prematurely. We have found that this mutant mRNA is destabilized by nonsense-mediated mRNA (NMD) decay. Interrupting NMD with a *smg* mutation increases mRNA levels of *aph-1(zu147)*, and allows for successful embryogenesis in virtually all embryos. This result suggests that it is the decreased levels of *aph-1* activity that are the cause of failed Notch signaling in the embryos from *aph-1(zu147)* mutant hermaphrodites. We have identified two different extragenic suppressors of *aph-1(zu147)* that do not appear to act on mRNA levels, but may instead regulate protein stability through ubiquitin-mediated proteosomal degradation. One of the suppressors encodes a novel gene product that interacts with an E3 ubiquitin ligase, and the other may itself function as an E3 ubiquitin ligase. We do not yet know whether the relevant target protein of these suppressors is APH-1 itself, or other components of the Notch signaling pathway. To this end we have investigated the ability of these suppressors to influence Notch signaling in worms that have compromised Notch receptors or compromised LAG-1, the downstream transcritption effector of the Notch signaling pathway. At least one of the suppressors can influence GLP-1 activity in embryos as well as in gonads, but has no significant effect on LIN-12 function during vulval morphogenesis. Ubiquitin-mediated degradation of Notch receptor and presenilin have previously been demonstrated for the E3 ubiquitin ligase SEL-10, which itself was identified as a suppressor/enhancer of LIN-12 (Hubbard et al., 1997, Wu et al., 2001, Li et al, 2002). We have found that the *sel-10(n1077)* missense mutation (Jager et al, 2004) can partially suppress *aph-1(zu147)* embryonic lethality, suggesting that elevation of Notch and/or presenilin may overcome the *aph-1(zu147)* defect, or that SEL-10 E3 ubiquitin ligase might also target APH-1. We are investigating whether our two suppressor genes act in the same, or in distinct pathways as that of the SEL-10 E3 ubiquitin ligase to modulate embryonic Notch signaling.

709B

Rab-8 functions to mediate excretory cell development in *C. elegans*. **D. A. Fantz**[1], K. Kornfeld[2], D. Smith[1]. 1) Dept of Chemistry, Agnes Scott College, Decatur, GA; 2) Dept of Developmental Biology, Washington University School of Medicine, St. Louis, MO.

The conserved Ras signaling pathway regulates multiple cell fates during *Caenorhabditis elegans* development. During vulval development, the downstream ERK MAP kinase directly regulates the ETS transcription factor, LIN-1 which plays a key role in vulval fate specification. In a genetic screen to identify suppressors of the *lin-1(n383)* multivulval phenotype, we identified the *C. elegans rab-8* gene as a novel, positive regulator of Ras-mediated development. In addition to its role during vulval development, Ras signaling is thought to regulate excretory cell divisions. Deletion mutants of *rab-8* exhibit a partially penetrant lethality with animals exhibiting a rod-like death suggestive of excretory cell defects. To determine the role of *rab-8* during excretory cell development, a set of genetic experiments were conducted using transgenic animals that expressed a fluorescent reporter, *lin-48::GFP* in excretory cells. Excretory cell fluorescence in animals lacking *rab-8* function was compared to controls, and a significant number of mutants demonstrated abnormal transgene expression. These results indicate that *rab-8* does play a role in excretory cell development and is a key regulator of the Ras signaling pathway in during the development of multiple tissues.

710C

Identification and characterization of cell type-specific Wnt pathway-regulated genes in C. elegans. **Lakshmi Gorrepati**, David.M. Eisenmann. Biological Sci, UMBC, Baltimore, MD.

The Wnt signaling pathway is a key extracellular pathway involved in many developmental processes in vertebrates and invertebrates. In the nematode C. elegans, there is a BAR-1/β-catenin mediated Wnt signaling pathway and a divergent Wnt/β-catenin asymmetry pathway. Both are involved in cell fate 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 and Wnt/β-catenin asymmetry pathway functions in the specification of seam cells. Seam cells are specialized epithelial cells that lie along the apical midline of the worm hypodermis and divide in a stem cell like manner during larval life. We propose to identify the targets of the Wnt pathway functioning in the VPCs and seam cells using Affymetrix microarray analysis and quantitative real time PCR.

The Wnt pathway was alternately activated by utilizing worms expressing a heat shock inducible stable variant of BAR-1/β-catenin (pathway over-activation), or a dominant negative variant of POP-1/TCF (pathway under-activation).The transcript pools differentially activated in the VPCs and seam cells were enriched for by the mRNA-tagging method. In this method, transcripts are 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.

We identified 32 putative Wnt pathway targets showing average fold change ≥ 1.5 fold in the Wnt pathway over-activation samples compared to the under-activation samples via microarray analysis and qRT-PCR. Of these, 19 genes were found to be expressed in the cell and tissue types of interest. Reduction of function of 3 cell cycle regulators by RNA interference revealed a previously unreported defect in the formation of alae, an epidermal structure secreted by seam cells. RNA interference of 6/19 genes showed defects in the formation of vulva. We also identified GATA transcription factors and known embryonic regulators of seam cell specification *egl-18* and *elt-6*. Preliminary epistasis analysis suggests these factors could be acting downstream or parallel to the Wnt pathway in specifying seam cell fate during larval life. This is the first report of identifying a possible interaction between Wnt signaling and GATA factors in seam cell development.

711A

Exploring Presenilin Differences in *C. elegans*. **Caitlin Greskovich**, Rebecca Resnick, Valerie Hale, Caroline Goutte. Biology, Amherst College, Amherst, MA.

Throughout development, Notch signaling requires a functioning gamma-secretase complex responsible for the intramembranous cleavage of the Notch receptor protein upon ligand interaction. In *C. elegans* the components of gamma-secretase are encoded by the genes *aph-1, aph-2, pen-2, sel-12* and *hop-1*, where the latter two genes are functionally redundant and encode two slightly different versions of presenilin protein. These gene products must be supplied maternally in order to mediate early embryonic events; if mothers are homozygous mutant for any one of these genes (both for the presenilins), embryos arrest with characteristic Notch signaling defects. Singly mutant *sel-12* or *hop-1* hermaphrodites yield viable embryos, indicating that either SEL-12 or HOP-1 presenilin is fully capable of mediating embryonic Notch signaling. However, we have uncovered a difference between the ability of these two presenilin genes to supply gamma-secretase activity in mutant *aph-1* backgrounds. In general, we find that worms that have reduced *aph-1* activity, are far more dependent on *sel-12* function than on *hop-1* function. We present our analysis of these differences in three different *aph-1* mutant backgrounds: *aph-1(zu123), aph-1(zu147)*, and *aph-1(zu147); smg-3(r930)*. In order to compare the roles of *hop-1* and *sel-12* in the context of a developmentally distinct Notch signaling event, we have turned to the Notch-induced process of germline proliferation during larval development. By comparing the size of the distal proliferative zone of the gonad, we have detected a slight difference in the effect of removing *sel-12* vs. *hop-1* function, and we present our analysis of this effect in an *aph-1(zu147)* mutant background compared to a wild type background.

712B

Environmental flexibility of the genetic network underlying *C. elegans* vulval development. **Stephanie Grimbert**, Christian Braendle. Institute of Developmental Biology and Cancer, CNRS - University of Nice Sophia-Antipolis, Nice, France.

We are interested in how environmental variation affects the functioning of developmental systems and their underlying genetic networks. As a model system, we study the process of *C. elegans* vulval patterning - a mechanistically well-defined system involving a signalling network of Ras, Notch and Wnt pathways. We previously showed that vulval patterning generates an invariant phenotypic output across various environments although the underlying signalling pathways turned out to be highly environmentally sensitive. In particular, our results and these of others suggest that development in starvation conditions or dauer passage strongly suppresses the Vulvaless phenotype of the reduction-

of-function mutations in the Egf/Ras/Mapk pathway. These observations suggest an upregulation of the vulval inductive signal through Ras and/or Wnt pathways in these conditions. We are now characterizing the mechanisms by which environmental signals alter activities and interaction of vulval signalling pathways, and how such changes impact the precision of developmental outcomes. In particular, we focus on the starvation suppression of *lin-3(e1417)* and *lin-3(n378)*, which cause a strong hypoinduced vulval phenotype under normal conditions. Our genetic analyses indicate that (a) internal physiological cues rather than externally perceived sensory cues are responsible for the observed starvation suppression, (b) dauer formation induced by mutation rather than by the environment is insufficient to suppress *lin-3(rf)* mutations, and (c) starvation effects suppressing the penetrance of *lin-3* mutations are unlikely due to the extensive developmental delay (~48h) caused by the starvation treatment. Previous results further indicated that starvation significantly increases Ras pathway activity, which seems to partly explain *lin-3(rf)* suppression. However, the Wnt pathway may be also involved because *lin-3(rf)* starvation suppression is abolished in the double mutant *lin-3(rf);bar-1(null)*. We are therefore currently testing to what extent the relative contribution of Ras and Wnt pathways to the vulval inductive signal changes with the environment. Ultimately, we aim to understand whether and how such environmental variability of molecular signals may contribute to phenotypic robustness.

713C

A role of the LIN-12/Notch signaling pathway in diversifying the non-striated egg-laying muscles in *C. elegans*. **Jared Hale**, Carolyn George, Leila Toulabi, Zachary Via, Nirav Amin, Jun Liu. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY 14853.

Our lab aims to understand the mechanisms underlying the diversification of the postembryonic mesoderm lineage, which arises during embryogenesis from a pluripotent cell known as the M mesoblast. During postembryonic development in *C. elegans*, the M mesoblast divides to generate fourteen body-wall muscles, two coelomocytes, and two sex myoblasts (SMs). The SMs further divide and differentiate into sixteen non-striated egg-laying muscles: four each of type I and type II vulval muscles and uterine muscles, respectively. Very little is known about how these different types of vulval and uterine muscles are specified. In an RNAi screen for transcription factors important for M lineage development, we found that *lag-1*(RNAi) led to the production of extra type I vulval muscles. Similar phenotypes were also observed in animals with reduced functions of the Notch receptor LIN-12 and its ligand LAG-2. The extra type I vulval muscles in animals with reduced LIN-12/Notch signaling did not appear to be a result of extra SMs or extra SM proliferation, rather a fate transformation from other egg-laying muscles to type I vulval muscles. We are currently examining the expression patterns of *lin-12* and *lag-2* relative to SM lineage development, and further determining the origin of the extra type I vulval muscles in LIN-12/Notch signaling pathway loss-of-function mutants. Results from these studies will provide insight into how different types of non-striated egg-laying muscles are specified.

714A

Single-blastomere transcriptomics elucidates the *C. elegans* E-lineage gene regulatory network. **Tamar Hashimshony**, Florian Wagner, Itai Yanai. Technion - Israel Institute of Technology, Haifa, Israel.

Genetic analyses have identified key developmental regulators, yet even well characterized networks may not be complete due to functional redundancies which are not easily identifiable. For a more unbiased and systematic approach, embryonic gene networks have been studied using whole transcriptome methods, however these have lacked the spatial and temporal resolution required for addressing the intricate events occurring in the early embryo. Here we determine the whole-transcriptomes of precisely staged single blastomeres spanning the entire development of a single cell lineage, the E-lineage, encoding the endoderm in *C. elegans*. We hypothesized that such data would allow us to recover the underlying network components, both known and uncharacterized. Accordingly, we find that early uncommitted blastomeres are extremely similar to one another, in comparison with blastomeres in which cell fate is being specified, suggesting that the transcriptional variation found is related to the specification process. We find that the transcriptome is induced significantly earlier and sharper in the E lineage relative to its sister lineage MS, consistent with E's earlier lineage specification. The known endoderm GATA regulators are highly expressed and E-lineage specific, providing evidence that the lineage gene network is captured by our blastomere transcriptome data. Surprisingly, additional transcription factors, including a dozen members of the nuclear hormone receptor family, are also E-lineage specific, hinting at a more complex network than previously known. In order to understand the functional relevance of the uncharacterized factors, we are repeating our analysis in the *C. japonica* E-lineage, which may reveal which factors are conserved and consequently play a role in the network. Our results show that given precisely staged single cell transcriptome data, the gene regulatory networks may be determined.

715B

Identification of genes that function with *dsh-2* to regulate asymmetric cell division. Kyla Hingwing, Tammy Wong, Jack Chen, **Nancy Hawkins**. Dept Mol. Biol & Biochem., Simon Fraser Univ, Burnaby, BC, Canada.

Asymmetric cell division is essential to generate cell diversity during development. In *C. elegans*, Wnt signaling regulates many asymmetric cell divisions. We have focused our analysis on DSH-2, a key Wnt signaling pathway component. Loss of both maternal and zygotic *dsh-2* function results in asymmetric neuroblast division defects and embryonic/early larval lethality, while loss of zygotic *dsh-2* function disrupts asymmetric cell division of the somatic gonadal precursor cells, Z1 and Z4. To identify genes that function with *dsh-2* in asymmetric division, we undertook a genetic screen to isolate suppressors of *dsh-2* lethality. This screen was highly successful and we isolated over 60 suppressors, all of which are dominant. These suppressor mutations could be activating mutations in downstream pathway components or in parallel signaling pathways that function in concert with DSH-2. We have focused our characterization on *Sup245*, *Sup305* and *Sup327*, which suppress all *dsh-2* defects examined thus far. We have further characterized suppressor function in Z1/Z4 division. Asymmetric division of Z1/Z4 involves the reciprocal asymmetric localization of POP-1/TCF and SYS-1/β-catenin in the proximal and distal daughters resulting in a high ratio of SYS-1 to POP-1 ratio in the distal daughter and the specification of distal cell fate. *dsh-2* mutants disrupt both POP-1 and SYS-1 asymmetry (1, 2). To determine if *Sup245*, *Sup305* and *Sup327* suppress *dsh-2* by restoring asymmetric localization of POP-1 and/or SYS-1 we have analyzed the expression of two GFP reporters, *Psys-1::SYS-1::GFP (qIs95)* and *Psys-1::POP-1::GFP (qIs74)* in the *dsh-2; Sup* strains. All three suppressors partially re-establish both POP-1 and SYS-1 asymmetry. Thus, a sufficient SYS-1 to POP-1 ratio is likely restored to specify distal cell fate. Genetic mapping experiments have placed both *Sup305* and *Sup327* on chromosome I to the left of *lin-17*. Based on their genetic map position, and similar phenotypes they are likely to be allelic. *Sup245* has been mapped to the left of *unc-54* on the right arm of chromosome I. To determine the molecular identity of the suppressor mutations, two strains were sent for whole genome sequencing; one containing *Sup305* and a second strain containing both *Sup327* and *Sup245*. We are currently analyzing the resulting sequence and testing potential candidates. These studies will identify novel genes that function with the Wnt signaling pathway to control asymmetric cell division. 1. Chang et al., (2005) *Mech. Dev.* 122:781-789 2. Phillips et al., (2007) *PNAS* 104:3231-3236.

716C

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 temporal and spatial control of PAL-1 expression in the *C. elegans* early embryo. 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 becomes depleted in the posterior by the 4-cell stage. Normal MEX-3 patterning requires the CCCH Zn-finger protein MEX-5, the RNA Recognition Motif protein SPN-4, and the serine/threonine kinase PAR-4. Genetic and biochemical evidence suggests 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.

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. Full-length MEX-3 expressed in this manner can functionally replace endogenous MEX-3. Results indicate that the N-terminus of MEX-3, which contains two KH domain RNA binding motifs, confers unusual stability to GFP. In contrast, the C-terminus of MEX-3, which contains potential phosphorylation sites, causes GFP to be degraded over the same timeframe as endogenous MEX-3. Furthermore, a region in the C-terminus containing a glutamine-rich region and just a few potential phosphorylation sites is required for timely degradation. Research on neurodegenerative diseases resulting from expansion of polyglutamine tracts, including Huntington's Disease, indicates that polyglutamine regions can serve as sites for protein cleavage and that polyglutamine proteins can be ubiquitinated and targeted to the proteosome. This raises the possibility that MEX-3 is inactivated by phosphorylation, then ubiquitinated and targeted to the proteosome for degradation. Intriguingly, the four human Mex-3 homologs and the apparent ascidian homolog all contain a RING finger motif, which can serve as an E3 ubiquitin-protein ligase. By creating additional fusion proteins that are missing potential ubiquitination and/or phosphorylation sites, we are determining which residues are required for inactivation and degradation.

717A

The CEH-20/UNC-62 transcriptional partnership regulate seam cell proliferation by controlling division asymmetry. **Samantha L. Hughes**, Charles Brabin, Alison Woollard. Deptartment of Biochemistry, Oxford University, Oxford, United Kingdom.

During development, stem cell-like seam cells undergo asymmetric divisions producing an anterior daughter cell that fuses with the hypodermal syncytium and a posterior daughter that retains the seam fate and has the ability to undergo further proliferation. The balance between proliferation and differentiation is controlled, in part, by *rnt-1* and *bro-1*. We have performed a whole genome RNAi by feeding screen for novel regulators of seam cell divisions that function in the *rnt-1/bro-1* pathway and isolated *ceh-20* as a negative regulator of seam cell proliferation. The *ceh-20* RNAi induced seam cell hyperplasia is absolutely dependent upon the function of *rnt-1* and *bro-1*. In addition, we observed that the

expansion of the number of seam cells is exacerbated when both *ceh-20* and its DNA binding partner *unc-62* are silenced. We will show that CEH-20/PBX and UNC-62/MEIS appear to act in a complex to regulate seam cell number via the perturbation of asymmetric divisions during development.

718B

Geometric Comparisons of the *EMS* Blastomere in *Caenorhabditis elegans* and *C. briggsae*. **Corey R Johnson**[1], Scott Thatcher[2], Timothy D Walston[1]. 1) Biology, Truman State University, Kirksville, MO; 2) Math and CS, Truman State University, Kirksville, MO.

The four-cell stage of *Caenorhabditis elegans* and *C. briggsae* can facilitate analysis of the forces acting upon blastomeres during early embryogenesis. During the 4-cell stage, the *EMS* blastomere exhibits a dynamic protrusion towards *ABa* in both species. The mechanism and purpose of this protrusion remain unexplained. Using differential interference contrast microscopy and measurement tools developed within MATLAB, the length and height of the *EMS* protrusion and total cell width was measured to quantify the size of the protrusion. Further, three-dimensional measures of size and shape of the 4-cell stage blastomeres were analyzed. This was facilitated by semi-automated image segmentation techniques that use watershedding algorithms to identify cell boundaries and region-based active contours to identify eggshell boundaries. Measurements between the two species were compared to determine the differences that exist in cell size and shape, particularly in *EMS* and the protrusion it forms. Quantification of this phenomenon and statistical differences may support further exploration into differences in cellular and molecular mechanisms that affect the magnitude of this cellular protrusion in the two species.

719C

Transcriptional control of dorsal-ventral polarity. **Rossio K. Kersey**, Thomas Brodigan, Tetsu Fukushige, Michael Krause. Molecular Biology, 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. 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 requires 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 and maintained, we have analyzed the regulation of *unc-130* expression and the distribution of UNC-130 protein. We found *cis*-acting elements in the promoter that drive ventral body wall muscle expression when isolated and that are sufficient to impose ventral polarity on the *unc-54* promoter that normally is expressed uniformly in all body wall muscle. We have also defined the temporal distribution of UNC-130 in body wall muscle cells during embryogenesis. Our results clarify the mechanisms by which *unc-130* regulates *unc-129* expression and contributes to our understanding of the logic behind the generation of polarity cues.

720A

Study on localization mechanisms of maternal *mex-3* mRNA. **Hiroyuki Konno**[1], Koki Noguchi[2], Yuji Kohara[1,2]. 1) Dep. Genetics, Graduate Univ Adv Studies, Mishima, Japan; 2) Natl Inst Genetics, Mishima, Japan.

In early stages of embryogenesis, the spatially regulated expressions of maternal mRNAs are essential for proper development. One system to achieve the spatial regulation is the localization of maternal mRNA to a subset of blastomeres, which is used in diverse organisms. Many localized maternal mRNAs have also been detected in *C. elegans* by *in situ* hybridization, yet mechanism and function of the localization remain largely unknown.

Aiming at elucidating the mRNA localization mechanism, we are focusing on maternal *mex-3* mRNA as a model mRNA localized to anterior blastomeres. After fertilization, the *mex-3* mRNA is gradually localized to the anterior half during the one-cell stage, and is predominantly localized to the anterior AB cell at the two-cell stage. Localization pattern of MEX-3 protein, an RNA binding protein containing the KH domain, is similar to that of the *mex-3* mRNA (1). In the anterior blastomeres, MEX-3 represses translation of the *pal-1* mRNA that encodes a transcription factor specifying the fate of the posterior blastomeres (2).

To determine cis-regulatory element for the *mex-3* mRNA localization, coding sequence of VENUS, a GFP variant, was joined to fragments of *mex-3* gene, and the constructs were introduced into worms by bombardment to generate transgenic lines. mRNAs derived from the constructs were detected by *in situ* hybridization with a probe of the VENUS antisense sequence. As a result, we found 179-nt sequence in the 3' untranslated region (3'UTR) of *mex-3* is required and sufficient for mRNA localization to the AB cell. Moreover, we found the 179-nt sequence is also required for MEX-3 protein localization to anterior blastomeres. Further deletion analysis revealed that 35-nt sequence of the 179-nt sequence is required for the mRNA localization. The 35-nt sequence is well conserved among Caenorhabditis species. But the 35-nt sequence is not sufficient for the mRNA localization.

(1)Bruce W. Draper *et al.* Cell. 1996. 87, 205-216

(2)Craig P. Hunter *et al.* Cell. 1996. 87, 217-226.

721B

Region II of the *C. elegans* Transcription Factor LIN-31: Site-Directed Mutagenesis and Transcription Assay Development. **Sarah Mathes**, Leilani Miller. Santa Clara University, Santa Clara, CA.

The goal of this ongoing research project is to determine the specification of vulval cell fate in the model organism *Caenorhabditis elegans*. LIN-31, a transcription factor in a conserved RTK/Ras/MAP kinase signaling pathway, is required for proper vulval development in *C. elegans*. In addition to a winged-helix DNA-binding domain, the LIN-31 protein contains four MAP kinase consensus phosphorylation sites, an acidic domain, and a region conserved among other transcription factors, Region II. Region II is currently the only major region in the LIN-31 protein whose effects on vulval cell fate are completely unknown. To better understand how Region II affects LIN-31 function, conserved sites within Region II are being altered using PCR-based site-directed mutagenesis. Transgenic animals carrying the mutated version of the protein will be created using germline microinjection techniques, and the resulting vulval phenotypes will be analyzed. Based on those results, we hope to achieve a better understanding of the contribution of each amino acid to LIN-31 function. Region II in some other winged-helix homologs acts as a transactivation domain. We are also developing a transcription assay in order to investigate this as a possible function for Region II in the LIN-31 protein.

722C

Role of PHA-4/FoxA binding in cell fate specification during organogenesis. **Christina K. McPhee**, Susan E. Mango. Dept of Molecular and Cellular Biology, Harvard University, Cambridge, MA.

Embryonic cells are born pluripotent, but their cell fate choices become restricted during development, and they differentiate. What mechanisms mediate the transition from plasticity to differentiation in the developing embryo? The *C. elegans* embryo is an excellent model to study the transition from plasticity to differentiation *in vivo* because the stages of plasticity versus commitment are well defined temporally. FoxA transcription factors control gut development in all animals studied to date (1). *C. elegans pha-4/FoxA* is critical to specify foregut/pharynx fate during early embryogenesis and to drive gut differentiation and morphogenesis at later stages (2). PHA-4 accomplishes these tasks in a changing nuclear environment, in which chromatin in early embryos is in a more open, permissive state compared to older embryos (3, 4). We are currently testing models of cell fate commitment and plasticity by probing PHA-4/FoxA association with its target genes during development, and assessing cell fate specification. We are examining the interaction of *C. elegans* PHA-4/FoxA with its target genes beginning at the onset of gastrulation, when developmental plasticity is lost. 1.K. H. Kaestner, Curr Opin Genet Dev 20, 527 (Oct, 2010). 2.S. E. Mango, Annu Rev Cell Dev Biol 25, 597 (2009). 3.T. Yuzyuk, T. H. Fakhouri, J. Kiefer, S. E. Mango, Dev Cell 16, 699 (May, 2009). 4.H. Niwa, Genes Dev 21, 2671 (Nov 1, 2007).

723A

ACE-like non-peptidase (acn-1) as a new suppressor of *let-7*. **Chanatip Metheetrairut**, Frank Slack. Dept. of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT.

The *let-7* microRNA (miRNA) is an important regulator of developmental timing in the heterochronic pathway in the seam cell lineage of *C. elegans*. It is also a key regulator of cell differentiation in various organisms, and the loss of *let-7* expression has also been associated with human cancer [1]. In *C. elegans*, *let-7* controls cell division and terminal differentiation at the transition from the fourth larval stage (L4) to adult stage. Known targets of the *let-7* miRNA include *lin-41* and *hbl-1* genes, which are regulated via the interaction of their 3'UTRs with *let-7*.

Even though these two targets of *let-7* have already been identified, it is still unclear how the functions of these genes give rise to the observed phenotypes. In order to understand how these key regulators of developmental timing actually effect cell fate outcomes, we aimed to identify additional downstream targets and effector genes. Loss of *ACE (angiotensin converting enzyme)-like Non-peptidase (acn-1)* function lead to a suppression of the retarded phenotypes resulting from the loss of *let-7* expression, both in the hypodermal seam cells and in the vulva. However, loss of *acn-1* by itself did not appear to cause any precocious heterochronic defect in the seam cells and alae. Previous research has examined the expression pattern of *acn-1* and found that it is expressed in hypodermal seam cells, vulval cells, and the ray papillae of the male tail during all larval stages, but not in adulthood [2], meaning that it is down-regulated during the L4 or young adult stage, approximately the same time that *let-7* is being expressed. However, its 3'UTR does not have any predicted binding site for the *let-7* miRNA, making it unlikely to be a direct target of *let-7*. Brooks et al. also reported that knock-down of *nhr-25*, which is part of the nuclear hormone receptor protein family, by RNAi can silence the expression of ACN-1::GFP fusion protein expression in the seam cells [2]. This nuclear receptor gene *nhr-25* was later identified by our lab to play multiple roles at the larva-to-adult transition [3]. Our hypotheses is that *acn-1* is not directly regulated by *let-7*, but functions further downstream. We aim to further elucidate how this new gene fits into the heterochronic pathway.

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2. Brooks, D.R., et al., **J Biol Chem.**, 2003. *278(52)*.

3. Hada, K., et al., **Dev Biol.**, 2010. *344(2)*.

724B

Understanding the Role of MAP Kinase Phosphorylation in *C. elegans* Cell Signaling. **Fernando Meza Gutierrez**, Misbah Palla, Leilani Miller. Department of Biology, Santa Clara University, Santa Clara, CA.

LIN-31, a member of the winged-helix family of transcription factors, acts as a tissue-specific effector of the conserved RTK/Ras/MAP kinase signaling pathway and is required for the proper specification of vulval cell fates in the nematode *Caenorhabditis elegans*. In the current model for LIN-31 function, 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 signaling events, it promotes a vulval cell fate in other cells. To understand how MAP kinase regulates LIN-31, different combinations of LIN-31's four MAP kinase consensus sites were altered using PCR-based site-directed mutagenesis (SDM) to prevent phosphorylation. While elimination of the first three individual phosphorylation sites has no obvious effect on LIN-31 function, preliminary SDM experiments show that elimination of the M4 consensus phosphorylation site seems to partially disrupt LIN-31's ability to promote vulval cell fates--transgenic animals are ~29% vulvaless (Vul). In combination with other previous results (M1M4 knockouts are ~42% Vul and M1M2M3M4 knockouts are 62% Vul), our current model suggests that M4 seems to be essential for vulval function (M1M2 and M1M2M3 mutants are essentially WT), presence of additional sites seems to have an additive effect on vulval function. In addition, yeast two-hybrid assays have shown that constitutive phosphorylation of site M1 (by replacing a threonine in the consensus site with a glutamic acid) partially disrupts interaction of LIN-31 with LIN-1. This result is consistent with the model that phosphorylation of LIN-31 disrupts the LIN-31/LIN-1 heterodimer.

725C

Mutational and yeast two-hybrid analysis of the *C. elegans* winged-helix transcription factor LIN-31 reveal functionally-distinct domains. Corey Morris[2], David Doroquez[3], Scott Montgomery[4], E. Lorena Mora-Blanco[2], Misbah Palla[1], **Leilani Miller[1]**. 1) Dept Biol, Santa Clara Univ, Santa Clara, CA; 2) Dept Cell Biol, Harvard Medical School, Boston, MA; 3) Dept Biol, Brandeis University, Waltham, MA; 4) Saint Louis University School of Medicine, Saint Louis, MO.

LIN-31, a member of the winged-helix family of transcription factors, acts as a tissue-specific effector of a conserved RTK/Ras/MAP kinase signaling pathway and is required for the proper specification of vulval cell fates in the nematode *Caenorhabditis elegans*. An understanding of how cells choose their fates in this simple model system may provide insight into the developmental processes of more complex organisms, including humans. LIN-31 is believed to play two roles during specification of *C. elegans* vulval cell fates: (1) to promote non-vulval cell fates (when bound to the Ets transcription factor, LIN-1), and (2) to promote vulval cell fates (when not bound to LIN-1). According to the current model, the LIN-31/LIN-1 heterodimer is disrupted when phosphorylated by MAP kinase. In addition to a DNA-binding domain, the LIN-31 protein contains four MAP kinase consensus phosphorylation sites, a small acidic region, and a region conserved among other transcription factors. In order to elucidate the function of these domains, site-directed mutations were introduced into the gene at these regions of interest, and the phenotypes of transgenic animals were analyzed. The results of these experiments demonstrated functionally-distinct domains for the vulval and non-vulval functions of LIN-31. While the DNA binding domain was required for both cell fates, the MAP kinase consensus phosphorylation sites were required for vulval cell fates (although there is some redundancy to the sites), and the acidic domain was required for non-vulval cell fates. In addition, yeast two-hybrid experiments revealed that the small acidic region is a LIN-1 interaction domain, consistent with the mutagenesis experiments showing it to be required for non-vulval cell fates. Yeast two-hybrid experiments also showed that constitutive phosphorylation of one of the MAP kinase consensus sites partially disrupts the LIN-1/LIN-31 interaction, consistent with the current model that phosphorylation of some combination of those sites disrupts the LIN-31/LIN-1 dimer.

726A

RalGEF (RGL-1) signaling performs dual/antagonistic functions in vulval patterning. **Kimberly B. Monahan**, Tanya P. Zand, Channing J. Der, David J. Reiner. Pharmacology, Lineberger Comprehensive Cancer Center, UNC-Chapel Hill.

The Ras small GTPase and oncoprotein that is mutationally activated in approximately 30% of all cancers. Ras and Ras-related small GTPases are involved in a myriad of cellular processes. Most studies have focused on how activated Ras utilizes two main effector signaling pathways, Raf-MEK-ERK and PI3K-AKT, in cancer initiation, development and progression. Recent studies have established a role for the non-canonical Ras effector pathway, Ral guanine nucleotide exchange factor (RalGEF) activation of-Ras-like (Ral) small GTPases, in pancreatic and other cancers. However, the mechanisms by which Ral GTPases are controlled in this pathway are still largely unknown, and how RalGEFs regulate the activation of Ral GTPases is now being explored. While there has been research focused on the GEF activity of RalGEFs, GEF- independent functions have been observed that remain poorly understood. Here, we use vulval patterning in C. elegans to study the GEF-independent functions of RalGEF (RGL-1). The canonical Ras effector in 1° fate induction is the LIN-45/Raf S/T kinase. However, we recently showed that the RGL-1/RalGEF-RAL-1/Ral signaling pathway functions in an antagonistic capacity relative to the Ras-Raf pathway, where Ras-RalGEF-Ral promotes 2° fate. Additionally, we found that RGL-1 function in the vulva is non-equivalent to that of RAL-1, suggesting RGL-1 has Ral-independent activity. ral-1-or rgl-1-directed RNAi enhanced the vulval hyper-induction of the activating Ras let-60(n1046gf) mutant, consistent with RGL-1-RAL-1 antagonism of LET-60/Ras-LIN-45/Raf. But surprisingly the three independent deletions of rgl-1 did not enhance. However, a rgl-1 deletion enhanced the let-60(gf) ectopic excretory duct cell defect, showing that the rgl-1 null mutation still antagonized Ras-Raf signaling. We hypothesize that in the vulva, RGL-1 encodes two opposing signaling activities.We used a RGL-1 GEF-dead mutant construct to rescue rgl-1 pro-1° but not pro-2° activity in a let-60(gf); rgl-1(0) background. Animals expressing the GEF-dead allele had an increase in pro-1° vulva formation when compared to animals expressing wild-type RGL-1, suggesting that the GEF-independent activity of RGL-1 is necessary to promote 1° fate. We further confirmed that this GEF-independent function is RAL-independent. Consistent with its pro-2° function, the ral-1 promoter drives GFP expression mainly in 2° cells during induction. However, we observed that the rgl-1 promoter drives GFP expression in both 1° and 2° vulva precursor cells. These results suggest that RGL-1 has dual, opposing functions in vulva cell fate patterning. A switch in RGL-1 signaling activity may contribute to robustness of vulval patterning.

727B

Automated phenotyping of *Caenorhabditis elegans* embryogenesis at the single cell and cellular group levels. **Julia Moore[1,2]**, Zhou Du[1], Anthony Santella[1], Christian Pohl[1], Zhirong Bao[1]. 1) Developmental Biology Dept, Sloan-Kettering, New York, NY; 2) Tri-Institutional Training Program in Computational Biology & Medicine, Weill Cornell Graduate School, New York, NY.

Recent advances in imaging technology have allowed scientists to observe biological dynamics at improved temporal and spatial resolutions. These improvements have facilitated studies directly observing *C. elegans* embryogenesis at the single cell level while maintaining viability. We have developed an automated analysis pipeline to translate time-lapse image series into extensive, easily interpreted information describing embryonic phenotypes.

C. elegans' invariant lineage provides the opportunity to compare individual cells with the same identity in different embryos. Nuclei are visualized with a histone-GFP fusion protein and imaged each minute to track every cell throughout embryogenesis. We quantified each cell's wild type proliferation, differentiation, and morphogenesis, then compared RNAi-treated embryos to wild type values. With this method we can detect aberrant behavior in single cells the first minute they are abnormal.

To assist our interpretation of abnormalities that disrupt wild type grouping (such as cells of the same lineage or fate) we identify groups of cells that divide synchronously, take on the same fate and/or migrate together. Once we uncover new groups, we can hypothesize which biological process was disrupted to produce the observed reclassification. Using this method we found interesting embryonic RNAi phenotypes for many genes including par-2, pie-1, pop-1, skn-1 and mex-5.

728C

Regulation of Asymmetric Seam Cell Division by the Non-Receptor Tyrosine Kinase, FRK-1, during Post-embryonic Development in Caenorhabditis elegans. **Kelsey Moore**, James Bour, Sherri Smith, Aaron P Putzke. Department of Biology, Hope College, Holland, MI.

Stem cell maintenance is a critical process that when perturbed results in serious negative consequences in a variety of contexts from embryonic development to tumor formation. The importance of stem cell regulation has been widely recognized in the last decade, and although many signaling pathways have been associated with stem cell maintenance, much remains unknown. We have characterized FRK-1, a homologue of the mammalian Fer non-receptor tyrosine kinase, and found it to be required for differentiation and maintenance of epithelial cell types, including the stem cell-like seam cells of the hypodermis in *Caenorhabditis elegans*. FRK-1 is localized to the plasma membrane, where it stabilizes cellular adhesion complexes. In the absence of maternal and early zygotic FRK-1, hypodermal cells are specified but not fully differentiated indicating that FRK-1 is required for maintaining hypodermal differentiation in developing embryos. Furthermore, we have demonstrated a requirement for FRK-1 during endoderm proliferation, where in the absence of FRK-1, endoderm cells hyperproliferate via an ectopic Wnt signaling mechanism through the β-catenin homologue, HMP-2. More recently, we have characterized a genomic knockout of *frk-1* (*frk-1(ok760)*) which eliminates only zygotic FRK-1 expression and results in lethality at the L1 larval stage. In mutants homozygous for the *frk-1(ok760)* deletion we have observed an excess number of lateral hypodermal cells which appear to have lost the asymmetry in the stem cell-like divisions of the seam cell lineage. More specifically, our data shows a loss of seam cell specific markers, such as *scm::gfp*, as well as loss of seam-specific cuticular alae formation. Immunostaining the *frk-1(ok760)* mutants with the epithelial adherens junction marker, MH27, shows that the lateral hypodermal cells are smaller in size, similar to the anterior daughter of a normal asymmetric seam cell division. Furthermore, the lateral hypodermal cells appear to have differentiated as non-seam based on the presence of non-seam hypodermis markers such as *elt-3::gfp*. We are currently investigating the mechanism by which the loss of FRK-1 causes this apparent cell fate switch and whether FRK-1 translocation to the nucleus during mitosis is required for the stem cell-like self-renewal exhibited by seam cells during post-embryonic development in *C. elegans*.

729A

Genetic analysis of vulval development in *Caenorhabditis briggsae*. **Devika Sharanya P**

Premkumar, Bavithra Thillainathan, Bhagwati P Gupta. Department of Biology, McMaster University, Hamilton, Ontario, Canada.

Vulval development in *C. elegans* is an established model to study conserved genes and signaling pathways that control cell proliferation, differentiation and morphogenesis. With the release of its genome in 2003, *C. briggsae* has become a promising system to decipher mechanisms of evolutionary changes in gene function and regulation. Comparative studies have revealed that although *C. briggsae* is phenotypically very similar to *C. elegans*, differences exist in processes such as vulval precursor competence, brood size, sheath-contraction rate, and reproductive efficiency. Thus there are significant differences in the underlying function and regulation of gene networks during vulval morphogenesis in these two species.

To study changes in the molecular mechanisms of vulva formation, standard F2 screens were carried out in *C. briggsae* to isolate Egl (egg-laying defective) mutants. Here we summarize progress on 18 such mutants that define at least 11 different genes. Based on their phenotypes the mutants can be placed into four distinct categories. Class I mutants exhibit wild type vulval induction and morphology suggesting that these genes control other components of the egg-laying system. Class II mutants frequently lack some or all of the vulval precursors (VPCs) due to a failure of P cells to migrate into ventral hypodermal region. Class III mutants affect VPC competence since VPCs in these animals fuse to hyp7 prematurely. Finally, the class IV mutants show defects in vulval invagination and morphology. The adults have protruding vulva (Pvl) phenotype.

We have further characterized Class III and IV mutants and show that they encode orthologs of *C. elegans* Homeobox genes *lin-11* (LIM family) and *lin-39* (Deformed/Sex combs reduced (Dfd/Scr) family). The molecular and genetic analyses of *Cbr-lin-11* and *Cbr-lin-39* have revealed that the function of both these genes in the egg-laying system is evolutionarily conserved. We are continuing to study vulva mutants and attempting to clone other loci. A detailed analysis of vulva formation in *C. briggsae* will form the basis of future studies to examine developmental mechanisms and compare gene function with *C.elegans* counterparts. The findings will improve our understanding of signaling pathway regulation and crosstalks in organ formation and diseases.

730B

Screening for Inhibitors of Cellular Reprogramming. **Tulsi Patel**[1], Baris Tursun[2], Dylan Rahe[3], Oliver Hobert[2]. 1) Department of Genetics and Development, Columbia University, New York, NY; 2) Department of Biochemistry, Columbia University, New York, NY; 3) Department of Biological Sciences, Columbia University, New York, NY.

As cells differentiate, they lose the ability to be converted into other cell types; however, this terminally differentiated state is not necessarily irreversible. For instance, ectopic expression of transcription factors (TFs) like MyoD, the master regulator for skeletal myogenesis, can convert other differentiated cells into muscle (Weintraub et al.,1989). The efficiency of conversion by master regulators, however, is dependent on cellular context. While certain cells like fibroblasts convert with high efficiency, others do not. We hypothesize that some cells are refractory to reprogramming because of factors that inhibit an ectopically expressed master regulator from exerting its activity. In order to find factors that inhibit reprogramming, we have conducted a pilot RNAi screen and are following it with a clonal genetic screen. To study these reprogramming efforts, we use neuronal terminal selectors that are essential for inducing specific neuronal types. One of these factors, *che-1*, a Zn-finger TF, is required for the terminal differentiation of ASE neurons, a pair of gustatory neurons in the head of the worm (Hobert,2008). Similar to other master regulatory factors, the ability of ectopically expressed *che-1* to induce ASE neuron fate in other cell types is limited. Transgenic animals containing the *che-1* gene driven by a heat shock inducible promoter and a GFP reporter driven by the promoter of an ASE marker are being used for the screen. Under non heat-shocked conditions, these animals express GFP in one ASE neuron. Upon heat shock, CHE-1 is ubiquitously expressed, however GFP is only turned on in the ASE and a few other neurons, indicating that CHE-1 is not sufficient to induce ASE-like fate. We are screening for factors that when knocked down or mutated, would allow CHE-1 to induce expression of the ASE marker in other cell types. This method has already turned out to be successful, as we have discovered that knockdown of *lin-53*, a histone chaperone and a component of histone modifying complexes, allows ectopic CHE-1 to reprogram mitotic germ cells into ASE like neurons based on morphology, marker expression and antibody staining of fate specific proteins (Tursun et al.,2010). Here I will be presenting data for other candidates isolated from these screens. Some of the observed phenotypes include expression of an ASE marker in different areas of the germline, muscle, pharynx and hypodermis. Initial characterized of these putative mutant phenotypes will include scoring for the expression of more ASE and pan-neuronal markers and for the loss of tissue specific markers.

731C

Components that Transduce Extracellular Matrix Signals Are Required for Developmental Commitment of *C. elegans* Embryonic Progenitor Cells. **Misty R Riddle**, Nareg J Djabrayan, Joel H Rothman. University of California, Santa Barbara. CA.

Embryonic cells in *C. elegans* remain pluripotent through the end of gastrulation, as demonstrated by their ability to adopt endodermal, mesodermal, or ectodermal fates when presented with the appropriate tissue-promoting transcription factor. Following this period of developmental plasticity, they become refractory to such reprogramming. We found that knocking down an intracellular effector of cell-matrix interactions, PAT-4 integrin linked kinase (ILK), causes post-gastrulation embryonic cells to adopt endodermal or mesodermal fates when confronted by ectopic expression of *end-3* (endoderm-promoting GATA transcription factor) or *hlh-1* (the myogenic factor MyoD), respectively. Further, we found that other components known to associate with PAT-4, including alpha-parvin (PAT-6), mitogen inducible protein (UNC-112), and alpha integrin (INA-1) apparently have a similar role in restricting developmental potential of cells following the period of developmental plasticity. PAT-4 is thought to function strictly as an adaptor molecule within integrin adhesion complexes; thus, it is conceivable that simply anchoring cells to the ECM via PAT-4 function may be required to maintain a differentiated state. Worms carrying a kinase-dead form of PAT-4 appear wild-type; however, PAT-4 kinase activity might have a previously unknown function in “locking down” cell fate, which would be revealed if cells carrying kinase-dead PAT-4 were coaxed into adopting an alternative fate. PAT-4 has a known role in muscle cell development; however, *pat-4* transcripts are detected as early as the 4-cell stage, so it is possible that PAT-4 functions to inhibit developmental potential in many cell types. Numerous studies in mammalian systems have suggested that ECM-to-cell signals are critical both for regulating pluripotency and maintaining differentiated cell types. Understanding when and where PAT-4 acts to restrict developmental potential and how its action is translated into nuclear modifications that allow or restrict changes in gene expression may therefore be informative to regenerative medicine.

732A

The chromo-helicase domain protein CHD-7 is a putative regulator of WNT-ligands. **Daniel Roiz Lafuente**, Ivo Rimann, Steffanie Nusser-Stein, Alex Hajnal. Institute Molecular Life Science, University of Zurich, Zurich, Zurich, Switzerland.

WNT mutants are defective in cell competence as well as cell fate specification in different tissues of C. elegans. In the vulva, a WNT signal from the tail maintains the Vulval Precursor Cells (VPCs) unfused and renders them competent to differentiate into vulval cells by inducing the expression of the hox gene lin-39. We have identified in an EMS mutagenesis screen for mutants defective in VPC specification a mutation in the chromo-helicase domain protein chd-7. Mutations in the human homolog of chd-7 lead to CHARGE Syndrome, which is characterized by malformed cranio-facial structures, defects in the peripheral nervous system, in the eyes, ears and the heart. Recent evidence suggests that mammalian CHD-7 is essential for the specification of neural crest cells (Bajpai et al 2010). In C. elegans chd-7(lf) mutants, the anterior VPCs P3.p, P4.p and P5.p often lose their competence and fuse with the surrounding hypodermis hyp7 (the 4° cell fate). We are therefore investigating the role of CHD-7 in the WNT signaling pathway using cell lineage analysis, genetic epistasis tests and transcriptional gfp reporters for chd-7 and the wnt genes. chd-7 and the wnt ligands cwn-1, cwn-2, lin-44 and egl-20 are expressed in the same tissues in the tail from the L1 stage until adulthood. Based in our data, we propose that chd-7 might regulate VPC competence by regulating the expression of WNT ligands in the tail.

733B

Evolution of embryonic development in nematodes. Jens Schulze, **Einhard Schierenberg**. Zoologisches Institut, Univ Cologne, Koeln, Germany.

Nematodes are well suited for a comparative study of early embryogenesis. Analyzing development of a single model system like Caenorhabditis elegans does not shed any light on the degree of evolutionary modifications within the taxon Nematoda. For better understanding evolution of development among nematodes including the identification of plesiomorphic and apomorphic characters, we compared early embryogenesis of representatives from all 12 nematode clades (phylogeny after Holterman et al., 2006; Mol. Biol. Evol. 23:1792-1800). Our data reveal that embryogenesis is unexpectedly variable with floating transitions that can be interpreted as frozen images of evolutionary change. Particularly, members of clade 1 and 2 differ massively from the standard C. elegans. Nevertheless, some basic developmental similarities appear to be common among all nematodes, e.g. the general existence of at least partial cell lineages and the influence of Polarity Organizing Centers (POCs). These POCs are required for generating a linear sequence of cells along the a-p axis which constitute the ventral midline. Depending on phylogenetic position fewer or more cells of this midline divide into left and right daughters this way establishing bilateral symmetry within individual lineages. Our studies reveal the stepwise emergence of founder cells and dramatic fate shifts during evolution. The comparison between embryogenesis of the basal nematode Tobrilus stefanskii and the tardigrade Hypsibius dujardini (Gabriel et al., 2007; Dev. Biol. 312, 545-59) with respect to the early cell division pattern revealed surprising similarities between these two. This may be considered as embryological support for the Ecdysozoa hypothesis.

734C

Plectus - a stepping stone in embryonic cell lineage evolution of nematodes. Jens Schulze, Jana Uenk, **Einhard Schierenberg**. Zoologisches Institut, Univ Cologne, Koeln, Germany.

The outstanding feature of Caenorhabditis elegans is its invariant cell lineage generating blastomeres of fixed identity. In contrast, the early embryo of the basal nematode Enoplus brevis shows only symmetric early cell divisions and the only identifiable lineage generates the gut (Voronov and Panchin, 1998; Development 15:143-150). How an organism with invariant development like C. elegans can evolve from an ancestor with an indeterminate mode remains a central question. Our lineage analysis of Plectus sambesii offers an important piece of the puzzle. Unexpectedly, cell arrangements in the AB lineage of this species are rather variable but merge into six different stereotyped patterns which all are compatible with normal development. However, one of the six patterns is prevalent and shows the same early cell arrangement as found in C elegans. This observation can be

interpreted as an evolutionary trend from an indeterminate to an invariant pattern of cells. Furthermore, our cell lineage analysis of hypodermal and pharyngeal cells reveals that AB cell fates in all six stereotyped patterns are determined in a position-dependent rather than a lineage-dependent manner resulting in a single distinct right-handed juvenile pattern. This differs fundamentally from the lineage-dependent C. elegans mechanism where a left-handed situs inversus can be generated experimentally by switching positions of AB cells in the early embryo (Wood, 1991; Nature 349:536-38).

735A

The Panacea Consortium: Identification of new Modulators of EGFR/RAS/MAPK and WNT signaling during vulval development. **Tobias Schmid**, Fabienne Largey, Alex Hajnal. Institute of Molecular Life Sciences, University of Zurich, Switzerland.

Complex human diseases such as cancer are responsible for the death of more than 50% of the adult human population. They arise from interactions between different signaling networks and the environment. Previous successful studies were mainly focused on the identification of genes involved in the development of rare monogenic diseases. Nevertheless, investigations of the more common complex, polygenic diseases are required. The genetic background has a considerable effect on the pathogenesis of many complex diseases, but further investigation is needed to decipher the underlying genetic risk factors. Cancer-related signaling networks can be investigated in the *C. elegans* vulva with the aim to expand the knowledge about complex signaling pathways. The development of the *C. elegans* vulva depends on the interplay between the EGFR/RAS/MAPK, the Notch and the Wnt signaling pathways, all of which play a central role in the development of cancer in humans. As part of the Panacea Consortium, we are addressing the question of how the genetic background influences the output of these signaling networks. We therefore crossed mutants in the RAS, Notch and WNT pathways originally isolated in the N2 Bristol background into CB4856 Hawaii and selected for suppressors or enhancers of the mutant phenotypes to establish recombinant inbred (RIL) lines. A RIL set containing the *let-60(n1046)* allele in a mixed CB4856/N2 background exhibits a strongly enhanced Multivulva (Muv) phenotype when compared to *let-60(n1046)* in the "pure" N2 background. We have identified by QTL mapping genomic regions that contribute to the enhanced Muv phenotype and are investigating candidate genes in these regions for their role in EGFR/RAS/MAPK signaling. Another RIL set generated with the *bar-1(ga80)* allele using the same approach exhibits severe larval lethality and an enhancement of the *bar-1(ga80)* vulval and gonad migration phenotypes when compared to the N2 background. The quantification of these phenotypes is underway and will be combined with the genotype data to predict regions containing modifiers of the WNT pathway.

736B

The histone acetylation, methylation, and H2A.z are involved in the maintenance of cell fates in *C. elegans*. **Yukimasa Shibata**[1,2], Hitoshi Sawa[2,3], Kiyoji Nishiwaki[1]. 1) Dept.of Biosci., Sch. of Sci. and Tech., Kwansei Gakuin Univ., Sanda, Hyogo, Japan; 2) CDB, Riken, Kobe, Hyogo, Japan; 3) NIG, Mishima, Hyogo, Japan.

Epigenetic mechanisms, including histone modifications, are likely to play crucial roles in the cell-fate maintenance. Recently, we found that the *C. elegans* acetylated-histone-binding protein BET-1 and MYST family histone acetyltransferases (MYST HATs) are required for the maintenance of cell fates in various cell lineages, indicating that histone acetylation plays a crucial role in the cell-fate maintenance. BET-1 prevents the ectopic expressions of cell-type-specific genes, such as *mec-3*, suggesting that BET-1 and MYST HATs maintain the transcriptional repression.

To further elucidate the mechanism to maintain cell fates, we screened genes that phenocopy, enhance and suppress the *bet-1* mutant phenotype by RNAi. RNAi screening revealed that *bet-1* phenotype was phenocopied by RNAi of *ssl-1* and *ekl-4* that are required for the deposition of histone H2A variant, H2A.z/HTZ-1. We also found that the disruption of *htz-1* enhanced the phenotype of a weak *bet-1* allele, but not that of a null allele, indicating *htz-1* and *bet-1* functions in the same genetic pathway for the maintenance of cell fates. One of the suppressors was UTX-1 that demethylates a silencing mark, methylation on H3K27. *utx-1* RNAi suppressed the defect of the cell-fate maintenance in multiple lineages of *bet-1* mutants. These genetic interactions suggest that histone acetylation, methylation and H2A.z cooperate to maintain the stable cell-fates. We speculate that, when UTX-1 removes the silencing mark, H3K27Me, BET-1 and HTZ-1 maintains the transcriptional repression of cell-type-specific genes.

737C

The β-catenin HMP-2 functions downstream of Src in parallel with the Wnt pathway in early embryogenesis of *C. elegans*. **Eisuke Sumiyoshi**[1,2], Sachiko Takahashi[1], Hatsue Obata[1], Asako Sugimoto[2,3], Yuji Kohara[1]. 1) Genome Biology Laboratory, National Institute of Genetics, 1111 Yata, Mishima, 411-8540, Japan; 2) Laboratory for Developmental Genomics, RIKEN Center for Developmental Biology, 2-2-3 Minatojimaminamimachi, Chuo-Ku, Kobe, 650-0047, Japan; 3) Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai, 980-8577, Japan.

The Wnt and Src pathways are widely used signal transduction pathways in development. β-catenin is utilized in both pathways, as a signal transducer and a component of the cadherin cell adhesion complex, respectively. A *C. elegans* β-catenin HMP-2 is involved in cell adhesion, but its signaling role has been unknown. Here, we report that in early embryogenesis HMP-2 acts as a signaling molecule in the Src pathway.

During early embryogenesis in *C. elegans*, the Wnt and Src pathways are redundantly involved in endoderm induction and spindle orientation in ABar blastomere. By a series of RNAi experiments, we found that, in both processes, HMP-2 functions in the Src pathway, but in parallel with the Wnt pathway. At the four-cell stage, P2 induces EMS to undergo asymmetric division and produce an anterior descendant, MS, which produces primarily mesoderm, and a posterior descendant, E, that gives rise to the entire endoderm. Whereas RNAi of either *hmp-2* or Wnt pathway genes partially did not cause severe phenotypes in endoderm induction, when both genes were knocked down, E descendants transformed into MS descendants and caused loss of endoderm. This functional redundancy of HMP-2 and the Wnt pathway was also observed for ABar spindle orientation. In early embryos, HMP-2 localized at the cell boundaries and nuclei including those of E and MS cells. We found that SRC-1 negatively regulates the localization of HMP-2 at the cell boundary, and positively regulates nuclear localization of HMP-2. Furthermore, HMP-2 was Tyr-phosphorylated in a SRC-1-dependent manner *in vivo*.

Taken together, we propose that HMP-2 functions downstream of the Src signaling pathway and contribute to endoderm induction and ABar spindle orientation, in parallel with the Wnt signaling pathway.

738A

Nhr-67/tailless Functions in the AC-VU Decision, AC Differentiation, and Expression of *lin-12* in the pre-VU Cells. **Tara Tappen**, Lauren Pioppo, Brittany Sanford, Jenny Hall, George McClung, Rachel Summer, Alex Breiding, Sheila Clever, Bruce Wightman. Biology Department, Muhlenberg College, Allentown, PA.

Genes encoding the *tailless* family of nuclear receptors are highly conserved among animals where they play various roles in regulating development. The *C. elegans tailless* ortholog, *nhr-67*, is expressed in a dynamic pattern in pre-uterine cells: initially in the 4 pre-VU cells during the L2 stage, then at high 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, and later only in the UTSE syncytium.

We have found that *nhr-67* is required for the AC-VU decision. A loss of *nhr-67* results in a two AC phenotype, indicating that nhr-67 is required for execution of the VU fate in response to *lag-2/lin-12* signaling between AC and VU. But *nhr-67* is also required for differentiation of the AC, including expression of *lag-2* and *zmp-1*, a phenotype that is very similar to that of *egl-43* mutants. *nhr-67* is also required for execution of the π cell lineages. Epistasis analysis indicates that this requirement depends on *nhr-67* function in the VU lineages rather than the AC. Finally, *nhr-67* is required for expression of *lin-12* in the pre-VU cells and later VU lineages, suggesting that a major function of *nhr-67* may be differentiation of the pre-VU cells, one of which becomes the AC. The loss of *lin-12* expression may be sufficient to account for both the AC-VU defect and the π lineage defect. The presence of a conserved canonical NHR-67 binding site in the *lin-12* promoter raises the possibility that *nhr-67* may directly control the transcription of *lin-12*.

We are currently evaluating a 250 bp fragment of the *nhr-67* promoter that contains six evolutionarily-conserved candidate cis-acting sites. Deletions of this region of the promoter cause a strong loss-of-function for *nhr-67* function in the AC and VU lineages. The 250 bp region is sufficient to drive expression of GFP in the AC, indicating that it contains promoter elements for cell-specific expression. Among these six sites are two predicted binding sites for HLH-2, suggesting that HLH-2 may be a direct regulator of *nhr-67* expression. We are also exploring the functions of other candidate upstream regulators of *nhr-67*, such as *egl-43*. This project is supported by a grant from the NSF.

739B

Quantifying gene expression dynamics of LIN-3/EGF targets during vulva induction. **Jeroen S van Zon**, Alexander van Oudenaarden. Department of Physics and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA.

How equipotent cells reliably interpret morphogen gradients to establish a precise spatial pattern during development is a general and unresolved question. During *C. elegans* vulva development, a spatial LIN-3/EGF gradient from the anchor cell (AC) induces different cell fates in the vulva precursor cells P3.p to P8.p in a distant-dependent manner: P6.p, closest to AC, adopts the 1° fate, whereas the more distant neighbors P5.p and P7.p adopt the 2° fate. It has been suggested that LIN-3/EGF induces cell fate patterning in a graded manner, with 1° cell fate markers such as the Notch ligands *lag-2*, *apx-1* and *dsl-1* initially expressed most highly in P6.p and at lower levels in the more distant P5.p and P7.p, reflecting the underlying morphogen gradient. Lateral LIN-12/Notch signalling subsequently restricts expression of these LIN-3/EGF targets to P6.p, while inducing 2° cell fate in P5.p and P7.p. We quantitatively study the expression dynamics of LIN-3/EGF targets during vulva induction using single molecule Fluorescence In Situ Hybridization, a novel technique that allows visualization of endogenous mRNA with single molecule precision. We find that some LIN-3/EGF targets, such as *apx-1*, are indeed expressed initially in a graded pattern in P5.p, P6.p and P7.p before becoming confined to P6.p. Surprisingly, we find that other LIN-3/EGF targets, such as *lag-2* and *egl-17*, are significantly more precisely controlled, being expressed almost exclusively in P6.p at all stages of vulva development. We show that the confined expression pattern of *lag-2* and *egl-17* is due to the fact that the readout of the LIN-3/EGF gradient, in the form of upregulation of LIN-3/EGF targets, occurs on a much slower timescale than lateral inhibition of LIN-3/EGF targets by LIN-12/Notch signalling. Moreover, our experiments together with mathematical modelling suggest that the combination of different modes of Notch ligand upregulation during vulva induction -

graded versus exclusively in P6.p - confers robustness against fluctuations in the LIN-3/EGF morphogen gradient.

740C

RNAi screening in *Caenorhabditis elegans* Ras mutant identified *blmp-1* as a putative Ras cooperating tumor suppressor gene. **Nattha Wannissorn**, Andy Fraser. Department of Molecular Genetics and Donnelly Centre, University of Toronto, Toronto, ON, Canada.

The key to successful diagnosis and treatment of cancer is not only to identify the individual mutations that drive tumorigenesis but also to understand how multiple mutations cooperate. Activating mutations in the Ras oncogene are found in a high proportion of human tumors; however, other mutations are required for full-blown cancer and identifying these additional mutations is crucial. In the first part of my project, I tackled this challenge by using RNAi screens to identify genes whose losses enhance phenotypes associated with activating mutations in *let-60*, the *C. elegans* ras orthologue. Human orthologues of these genes might thus play a similar cooperative role in ras-driven tumors. I found that RNAi knockdown of the *PRDM1* ortholog *blmp-1* strongly enhanced the activated Ras vulval phenotype. Although PRDM1 regulates several critical developmental processes and is a tumor suppressor gene, it has not been previously described as a regulator of Ras signaling. To dissect the mechanism by which *blmp-1* loss cooperates with activated *let-60* mutations, I carried out a second RNAi screen for genes that suppress the *blmp-1; let-60* enhanced phenotype. These genes may be required for the cooperation between *blmp-1* and *let-60*. I identified the Paired-box transcription factor gene *pax-3* as a strong suppressor of the enhanced activated Ras phenotype. The data presented here suggests that BLMP-1 may repress *pax-3* transcription directly and that activated Ras leads to attenuation of this repression. My future works are directed towards testing this model. In summary, I have shown that loss of blmp-1 strongly cooperates with activating mutations in let-60 and that this cooperation requires the direct BLMP-1 target *pax-3*. We predict that similar cooperation will occur between mammalian Ras and *PRDM1* in human cancer.

741A

Comparing gene expression pattern in the skn-1 intestinal developmental network in C. briggsae, C. remanei, and C. elegans to gain insights into the dynamical functional roles of orthologous genes. **Allison Wu**[1], Lawrence Du[3], Scott Rifkin[2]. 1) Bioinformatics and Systems Biology Graduate Program, University of California, San Diego, La Jolla, CA; 2) Section of Ecology, Behavior, and Evolution, Division of Biological Sciences, University of California, San Diego, La Jolla, CA; 3) Division of Biological Sciences, University of California, San Diego, La Jolla, CA.

Previous studies have shown that, in the C.elegans skn-1 network, the most downstream gene elt-2 determines the onset of gut development while end-3, end-1, and med-1/2 constrain the gene expression variability. Inboth C. briggsae and C. remanei, there has been a large amount of copy number evolution in this network: there are 5 functionally similar med paralogs in C. remanei , 4 med paralogs in C. briggsae and two orthologs of end-3 in C. briggsae. These potentially functionally similar homologs add extra connections to this intestinal specification network. We want to know how and why these redundant genes are preserved, whether these seemingly redundant connections are really redundant, and whether these connections add to or strengthen specific dynamical network properties, such as buffering of noise or environmenal variation or feedback strength. In this study, we use single-molecule fluorescence in situ hybridization (smFISH) to measure the expression pattern of genes in the skn-1 network in these three species, including end-3 orthologs, end-1 and elt-2. By quantitatively measuring the expression dynamics of these genes, we willustrate the dynamical roles these orthologs play in the intestine development in different nematode species and reveal the effects of gene duplication on dynamical network properties.

742B

Direct in vivo Cellular Reprogramming Involves Transition Through Discrete, Non-Pluripotent Steps. **S. Zuryn**, J. P. Richard, N. Fischer, V. Pavet, N. Vaucamps, S. Jarriault. IGBMC, Strasbourg, France.

Cells can adopt a new identity during normal development, in response to tissue damage or defined artificial treatments, or during disease processes such as cancer. Remarkably, cells can not only undergo reprogramming to an embryonic stem cell-like state, but can also undergo direct conversion from one cell type to another. Direct cell type conversion represents an attractive strategy for regenerative medicine, but is poorly understood. For example, the cellular steps undertaken as a change of identity occurs are unknown. Furthermore, it is not understood whether enhanced cellular potency accompanies the direct conversion process, a property that may be hazardous in a therapeutic context. Here we have used the Y-to-PDA model system in *C. elegans* to discern the cellular mechanism of direct natural reprogramming. The Y cell is initially a fully differentiated rectal epithelial cell in early larval development. However, it later retracts from the rectum, migrates anterior-dorsally and transdifferentiates into the PDA motoneuron. After exploring factors pertaining to competence, lineage and local environment, we present an in-depth analysis of the cellular transformations involved in this event. We found that as the Y cell undergoes transdifferentiation, it adopts a temporary state where it lacks the characteristics of both its initial and final cellular identities. The cell then undergoes stepwise redifferentiation into the PDA motorneuron, a process requiring the conserved COE transcription factor UNC-3. The complete erasure of the cells initial identity, although temporary, is reminiscent of dedifferentiation that occurs when cells are reprogrammed to an embryonic cell-like state in vitro. This process is accompanied by increased developmental plasticity, so much so, that the cells can be induced to change into different types. Unlike this process in vitro, we found that reversion to a dedifferentiated identity does not lead to an increase in cellular potential in a natural, in vivo context. Together, these findings suggest that direct in-vivo cellular reprogramming in *C. elegans*, and possibly other species, involves transition through discrete, non-pluripotent steps, a conclusion with important and advantageous implications for regenerative medicine.

743C

Genome wide RNAi screen to study uv1 cell necrosis. **Awani Awani**, Matt Crook, Wendy Hanna-Rose. Pennsylvania State University, state college, PA.

Necrosis is more than a phenomenon of uncontrolled cell death. There is increasing evidence to suggest that necrosis is genetically programmed. We are exploiting a new model to investigate necrotic cell death. In worms that lack pnc-1 nicotinamidase, the four uv1 cells die by necrosis (1). We want to investigate why uv1 cells die in pnc-1 mutants and to identify genes that promote or inhibit uv1 cell necrosis. In pnc-1 mutant animals with a uv1 cell specific GFP transgene, we can screen for rescue of uv1 cell necrosis by screening for animals that retain the uv1 GFP signal. We aim to screen using the genome-wide RNAi library and expect to gain insight into how and why uv1 cells die in pnc-1 mutants. We are using a split GFP system to engineer animals with uv1 cell specific GFP expression for this screen (2), and we will discuss progress in engineering the strain and preparing for the screen. Reference: 1. EGF signaling overcomes a uterine cell death associated with temporal mis-coordination of organogenesis within the C. elegans egg-laying apparatus. Li Huang , Hanna-Rose Wendy 2. Antiparallel Leucine Zipper-Directed Protein Reassembly: Application to the Green Fluorescent Protein. Indraneel Ghosh , Andrew D. Hamilton , Lynne Regan.

744A

Apoptosis-mediated toxicity of water stable carbon nanoparticles in *Caenorhabditis elegans* . **Shin S. Choi**, Yoon J. Cha. Department of Food and Nutrition, Myongji University, Yongin, Gyeonggi, Korea.

The application of nanoparticles in various fields has been evolved rapidly due to its authentic physicochemical properties. The potential biological or environmental toxicity of nanoparticles, however, has been frequently reported using in vitro systems. In this study, the toxicity of fullerene (nC60) and fullerol (C60-OH) in Caenorhabditis elegans was assessed through the measurement of lifespan, body size, and brood size. The water-stable colloids form of nC60 are prepared through the long-term exposure of fullerene, C60 in THF. We confirmed that the oral-administrated carbon nanoparticles were accumulated in the animals for several days after feeding the mixture of bacterial food and nC60 or C60-OH to the L4 larvas of C. elegans. The C60-OH reduced the viability of animals while no decrease in survival was found in the animals fed with nC60. We have also found that both nC60 and C60-OH reduced the reproduction of C. elegans. In order to illuminate the genetic mechanism of toxicity induced by those nanoparticles, the assessment of viability and fertility was carried out using the strains mutated in oxidative stress or programmed cell death regulators.

745B

Exploring alternative means to induce axonal injury in *C. elegans* neurons. **Sean Coakley**, Massimo A. Hilliard. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland, Australia.

Current studies of processes such as axonal regeneration and degeneration in *C. elegans* rely heavily upon recent advances in *in vivo* optical surgery, such as UV- and femtosecond-laser axotomies. These technologies, despite being very successful, impose time-bottlenecks when performed on large numbers of animals, which are only partly circumvented by the development of microfluidic devices and automation software. In an effort to perform large-scale forward genetic screens and isolate axonal regeneration/degeneration mutants, we sought to design alternative axonal damage strategies that are suitable for use on large populations of animals.

We are using chemically based approaches, as well as a genetic-based approach to identify conditions that are able to transiently-induce damage to axons. We tested different toxic compounds linked to neurodegeneration symptoms in humans for their efficacy in transiently inducing axonal damage in the mechanosensory neurons of *C. elegans*.

Wildtype *C. elegans* expressing a *Pmec-4:GFP* transgene to label their mechanosensory neurons, were grown on NGM plates with the neuropathic compounds colchicine, paclitaxel, vincristine or β-methylamino-L-alanine across a range of concentrations at different larval stages. These animals were then analysed for indicators of axonal degeneration such as axonal thinning, beading and fragmentation, as well as evidence of regeneration such as growth cones and sprouting.

Our results show that animals grown in the presence of paclitaxel (2μM) undergo fragmentation of the ALM and AVM mechanosensory neurons indicative of neurodegeneration. The PLM and PVM mechanosensory neurons in these animals show no axonal degeneration phenotype.

We are currently exploring if the effects of these drugs are altered in sensitised genetic backgrounds by screening several mutants in candidate genes, such as the drug sensitive mutant *bus-17(e2800)*, or a mutant in the neuroprotectant *sir-2.1(ok434)*. We plan to use these mutants in combination with drug treatments to optimise an axonal damage strategy that will facilitate the study of axonal regeneration and degeneration on large populations of animals *in vivo*.

746C

Neuron specific knock-down of *smn-1* causes neuronal degeneration and death. I. Gallotta, P. Bazzicalupo, **E. Di Schiavi**. IGB, CNR, Naples, Italy.

Spinal Muscular Atrophy (SMA) is one of the most common genetic causes of infant mortality and consists of a degeneration of spinal cord motoneurons eventually resulting in the atrophy of the innervated muscles. All classical forms of SMA are associated with mutations of the Survival of Motor Neurons gene (*Smn1*) which is involved in the biogenesis of small RNAs. *Smn1* is expressed in all tissues, but its loss of function affects almost exclusively spinal cord motoneurons. Albeit *Smn1* has been extensively studied, it is still unknown why only motoneurons degenerate. *smn-1*, the *C. elegans* ortholog of *Smn1*, is ubiquitously expressed and reduction of its function results in pleiotropic phenotypes, sterility and lethality which have made very difficult so far its study via traditional mutations or RNAi (Miguel-Aliaga et al., 1999; Briese et al., 2009; Dimitriadi et al., 2010; Sleigh et al., 2010). To focus on the effect of *smn-1* loss of function in neurons we have used a simple reverse genetics approach to reduce the function of specific genes in chosen neurons (Esposito et al., 2007). By expressing sense and antisense RNA corresponding to a gene of interest under cell-specific promoters, we obtain an efficient, heritable and cell autonomous knock-down of the targeted gene function in specific neurons. Transgenic animals in which *smn-1* is silenced specifically in GABA motoneurons are viable, fertile and largely normal except for a reduction of visible GABA motoneurons and a defect in backward movement. Using SYTO11 staining we determined that motoneurons die. The loss of visible motoneurons and neuronal death increase with age, a finding consistent with the progression expected for a neurodegenerative process. In addition we found that factors of the classical cell death pathway are involved. We have also shown that there is no spreading of the silencing effect to other neurons of the ventral cord. In order to address the question of motoneuron specificity we silenced *smn-1* in a different class of neurons. Also in this case we observed functional alteration of the targeted neurons and their age-dependent disappearance. However the degenerative process was slower than in GABA motoneurons and we also observed alterations in axon morphology. This suggest that, as it happens in humans, degeneration and cell death progress differently depending on intrinsic features of the affected neurons. We have established a workable model of neurodegeneration due to *smn-1* cell-specific knock-down, which we will use to understand the molecular pathways critical for the pathology and to identify genes as well as small molecules capable of favouring or contrasting the progression of SMA neurodegeneration.

747A

Elucidating the genetic and molecular mechanisms underlying *smn-1* synaptic defects. **M. Dimitriadi**, G. Kalloo, A.C. Hart. Neuroscience, Brown University, Providence, RI.

Spinal Muscular Atrophy (SMA) is the most common genetic cause of infant mortality and is caused by diminished function of the Survival Motor Neuron (SMN) protein. SMN plays a major role in spliceosome assembly and is involved in additional mRNA regulatory processes. The scientific community still does not understand why decreased SMN function causes selective neurodegeneration nor is there consensus on the cellular and molecular pathways that are critical for SMA pathology. To better understand the pathological mechanisms underlying this disease, we utilized the *C. elegans* SMN ortholog, *smn-1*. We and others have shown that *smn-1(lf)* causes neuromuscular defects that are followed by larval lethality (Dimitriadi *et al.*, 2010; Briese *et al.,* 2009). As loss of neuromuscular function is an early event in SMA pathology, we tested whether *smn-1(lf)* impacts synaptic function. We found that *smn-1* animals are resistant to inhibitors of cholinesterase, but sensitive to levamisole activation of post-synaptic receptors, suggesting that *smn-1* loss causes pre-synaptic neuromuscular defects. Also, a subset of pre-synaptic markers are not properly localized in *smn-1(lf)* animals. Genetic studies suggest that the *smn-1(lf)* synaptic defects are not likely caused by defects in synaptic vesicle exocytosis. Ongoing studies are examining a deficiency either in clathrin-mediated endocytosis or bulk endocytosis. Delineation of the genetic and molecular mechanisms that underlie *smn-1* synaptic defects will likely provide new insights into the neuromuscular role of SMN and reveal previously unsuspected modifiers of synaptic function. References: Dimitriadi M, Sleigh JN, Walker, *et al.* (2010) PLos Genet. 6(10): e1001172 Briese M, Esmaeili B, Fraboulet S, *et al.* (2009) Hum Mol Genet, 18, 97-104.

748B

vha-12, a catalytic subunit of the V-ATPase, is required for apoptotic corpse clearance. **Glen Ernstrom**[1], Robby Weimer[1,3], David Greenstein[2], Erik Jorgensen[1]. 1) Department of Biology & HHMI, University of Utah, Salt Lake City, UT; 2) Department of Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN; 3) Department of Biomedical Imaging, Department of Neuroscience, Genentech, South San Francisco, CA.

An essential function of the V-ATPase proton pump is to acidify organelles in secretory and endocytic pathways of all cells. Though the architecture of the multisubunit V-ATPase is highly conserved, plants and animals express a variety of subunit variants that suggest V-ATPases can be tailored to specific functions. The pump is composed of two protein complexes, the transmembrane V_o complex and the cytoplasmic V_1 complex. Analyses of mutants in the V_o subunit *a* have identified specific roles for the subunit. However, the V_1 subunits have been more difficult to examine in adult tissues either because of multiple redundant genes or because mutations are lethal at an early developmental stage. In the *Caenorhabditis elegans* genome, there are two V_1 B-subunit genes, *vha-12* and *spe-5*. Two mutations, a weak loss-of-function mutation and a strong loss-of-function mutation, mapped to the *vha-12* gene locus. The weak *vha-12* mutation was identified in a screen for uncoordinated movement, and also displayed mild defects in the epidermis and the gonad. The strong mutation is embryonic lethal. The *vha-12* gene is expressed in all somatic tissues starting at mid-embryogenesis and is highly expressed in the nervous system. Consistent with the embryonic expression, strong *vha-12* mutants displayed two phenotypes. First, strong *vha-12* mutant embryos developmentally arrested during late morphogenesis and did not hatch. Second, close examination of the strong *vha-12* mutant embryos with time lapse confocal imaging revealed that removal of apoptotic cell corpses was significantly delayed during early embryogenesis. Impaired clearance of apoptotic cells is consistent with recent observations that link the V-ATPase to the effective clearance of engulfed cellular material.

We conclude that the *vha-12* V-ATPase B subunit functions in several tissues but also has prominent functions in embryonic development and neuronal function.

749C

Identification of modulators of RNA-dependent toxicity in Myotonic Dystrophy. **Susana M. Garcia**[1,2], Guinevere F. Lourenço[1,2], Gary B. Ruvkun[1,2]. 1) Dept Molec Biol, Massachusetts Gen 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 repeat (CTG) expansions in non-coding regions in which pathogenesis was shown to be mediated by RNA. The toxic effect caused by these mutant RNA transcripts highlighted the capability of RNA to act as a dominant pathogenic species. Expansions of CTG or CCTG repeats in non-coding regions of unrelated genes were 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 trans-dominant 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 identification of 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 RNA toxicity. To identify the complement of factors that modulate CUG transcript toxicity and lead to cellular dysfunction, we have generated *C. elegans* strains expressing GFP containing different CTG-repeat lengths in its 3'untranslated region (UTR), in body wall muscle cells. Animals expressing GFP containing expanded CTG repeats in their 3'UTR exhibit toxic phenotypes. Furthermore, animals expressing expanded CTG repeats display a fainter GFP expression compared to control strains and GFP levels decrease progressively with ageing. We have taken advantage of the age-dependent GFP fluorescent phenotype observed in animals expressing expanded CTGs to screen, by RNAi, for genes that cause a specific increase in GFP fluorescence. The genes identified by RNAi are predicted to be modulators of CTG RNA dysfunction. So far we have identified different categories of genes and are currently characterizing them for their function in CTG RNA toxicity. The identification of these factors will allow a better understanding and characterization of the underlying RNA toxic mechanisms associated to RNA toxicity.

750A

Growth cone guidance receptors modulate mitochondrial morphology and function via a secreted moiety of the VAPB/ALS8 protein, the major sperm protein domain. **S. Han**[1], H. Tsuda[2], Y. Yang[1], J. Vibbert[1], P. Cottee[1], C. Haueter[3], J. Prasain[4], H.J. Bellen[2,3,5], M.A. Miller[1]. 1) Department of Cell Biology University of Alabama School of Medicine Birmingham, AL; 2) Department of Molecular and Human Genetics Baylor College of Medicine, Houston, TX; 3) Howard Hughes Medical Institute; 4) Department of Pharmacology and Toxicology University of Alabama School of Medicine Birmingham, AL; 5) Program in Developmental Biology Baylor College of Medicine Houston, TX.

Mitochondria are obligate endosymbionts that provide cells with energy and metabolic functions critical for their survival. Mitochondrial morphology and subcellular localization can be coordinated among different cells, but the mechanisms are not understood. Here we show that C. elegans, Drosophila, and human VAPB/ALS8 major sperm protein domains (MSPs), which are implicated in amyotrophic lateral sclerosis and spinal muscular atrophy, comprise a hormone class that modulates mitochondrial position, morphology, division (fission), and function. MSPs transduce signals to mitochondria through Roundabout and Lar-like growth cone guidance receptors that remodel the actin cytoskeleton. We show that neurons secrete MSPs to control actin-related protein 2/3 complex activity in striated muscle, stabilizing mitochondria at actin- rich I-bands. Our results indicate that secreted MSPs promote energy production and metabolism by regulating mitochondrial localization and function. Hence, extracellular signals that influence the actin cytoskeleton may be important regulators of mitochondrial activity.

751B

The Sp1 Family Transcription Factor SPTF-3 Promotes M4 Sister Cell Death through *egl-1* Expression in the M4 Sister Cell. **Takashi Hirose**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA.

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.

By a genetic screen and a subsequent candidate-gene approach, we have identified seven genes required for M4 sister cell death: *ceh-32*, *ceh-34*, *eya-1*, *sptf-3*, *gcn-1*, *abcf-3* and *pig-1*. Here we describe our studies of the Sp1 family transcription factor SPTF-3, which promotes M4 sister cell death through *egl-1* expression in the M4 sister cell. From our genetic screen, we recovered *n4850*, an allele of *sptf-3* that causes a defect in the death of the M4 sister cell but not of other cells, including the I1 sister cells, the NSM sister cells or the VC homologs of the ventral nerve cord. This result indicates that *sptf-3* is specifically involved in M4 sister cell death rather than in all programmed cell deaths. *sptf-3* encodes an Sp1 family transcription factor that contains three zinc finger domains. The *sptf-3(tm607Δ)* deletion lacking two of the zinc finger domains causes a defect not only in M4 sister cell death but also in pharyngeal morphology, leading *sptf-3(tm607Δ)* animals to die by the early L1 stage. A translational *sptf-3::gfp* transgene is expressed during embryogenesis in most cells, including those of the pharynx. These results suggest that *sptf-3* is required for both pharyngeal development and the regulation of M4 sister cell death.

We found that *sptf-3* is required for expression of the pro-apoptotic BH3-only gene *egl-1* in the M4 sister cell. While *egl-1* is known to function in the M4 sister cell, our mosaic analysis indicates that the *sptf-3* function is required at or later than the stage of the great-great-grandmother cell of the M4 sister cell. One possibility is that SPTF-3 likely regulates *egl-1* expression indirectly through other genes. We previously reported that the *C. elegans* Six family homeodomain proteins CEH-32 and CEH-34 directly activate *egl-1* expression in the M4 sister cell. *ceh-32(ok343Δ)* and *sptf-3(tm607Δ)* animals share a "pharynx unattached" (Pun) phenotype, suggesting that *sptf-3* and *ceh-32* regulate pharyngeal development in the same pathway. We are currently testing whether SPTF-3 promotes *egl-1* expression through *ceh-32* expression in the M4 sister cell.

752C

sli-1 Cbl Opposes the Engulfment of Apoptotic Cells. **Michael E. Hurwitz**[1], Courtney Anderson[1], Bob Horvitz[2]. 1) Yale Cancer Center, Yale University School of Medicine, New Haven, CT; 2) HHMI, Dept. Biology, MIT, Cambridge, MA.

In *C. elegans*, apoptotic cells are engulfed by neighboring cells. Three parallel and partially redundant core pathways comprising at least nine genes (*ced-1, ced-6, ced-7, dyn-1* and *ced-2, ced-5, ced-10, ced-12* and *abi-1*) function in this process. Loss-of-function (lf) mutations in these genes cause the persistence of unengulfed cell corpses. CED-7 is an ABC-type transporter that appears to signal through CED-1, a transmembrane receptor and CED-6, an adapter protein predicted to bind the phosphotyrosines of CED-1. CED-6 activates the dynamin, DYN-1, which regulates membrane dynamics. CED-2 is an adapter that contains an SH2 and two SH3 domains and activates the CED-5/CED-12 heterodimeric guanine nucleotide exchange factor (GEF). The CED-5/CED-12 GEF, in turn, activates CED-10, a small Rac GTPase. ABI-1 is a cytoskeletal regulator found in several protein complexes. One protein that interacts with Abi proteins in mammals is the c-Cbl oncoprotein, an adaptor and E3 ubiquitin ligase. In *C. elegans*, the c-Cbl homolog SLI-1 inhibits LET-23 EGFR/LET-60 Ras signaling by downregulating LET-23 through ubiquitination. We tested whether mutations in *sli-1* modify engulfment pathway gene loss of function defects. Loss of *sli-1*(lf) suppressed the engulfment defects of strong loss-of-function mutations in the *ced-1/6/7* pathway but not of the *ced-10* Rac pathway, suggesting that *sli-1* opposes these pathways but not by acting on the *ced-1/6/7* pathway. Instead, *sli-1* might act upstream of the *ced-10* Rac pathway or in parallel to both pathways. To further investigate the role of *sli-1*, we determined the effects of *sli-1*(lf) on another phenotype associated with engulfment mutants. The *ced-10* Rac pathway genes are required for normal distal tip cell (DTC) migration and, therefore, normal gonad morphology. By contrast to its effects in engulfment, *sli-1*(lf) suppressed the DTC migration defects of strong loss-of-function alleles of all the *ced-10* Rac pathway genes, suggesting that *sli-1* acts in parallel to that pathway. To determine whether SLI-1 acts through the LET-23/LET-60 pathway to inhibit engulfment we assessed the effect of a *let-60*(gf) mutation on engulfment. *let-60*(gf) did not cause an engulfment defect and did not enhance or suppress the engulfment defects of genes from either the *ced-1/6/7* pathway or the *ced-10* Rac pathway. We propose that *sli-1* suppresses a genetic pathway parallel to *ced-10* Rac for apoptotic cell engulfment and DTC migration independent of its known function to inhibit the LET-23 EGFR/LET-60 Ras pathway. We are currently testing whether *sli-1* suppression of engulfment and DTC migration happens in *abi-1* mutant animals to assess whether *sli-1* acts through *abi-1*.

753A

A Screen for Genes Involved in Engulfment-Dependent Cell Death. **Holly L. Johnsen**, H.R. Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 01239 USA.

Programmed cell death occurs during the normal development of many organisms. The *C. elegans* cell-death pathway has been extensively studied for a variety of cells that are fated to die. During programmed cell death, caspases are activated in the dying cell. The cell corpse is then engulfed by a neighboring cell and degraded. In most cases, cell death is cell-autonomous and caspase-dependent, and can occur even in engulfment-defective animals.

During development of the *C. elegans* male, the cells B.alapaav and B.arapaav are generated during the late L3 stage. During the early L4 stage one of these cells undergoes caspase-dependent programmed cell death and the other survives. The decision of which cell dies and which survives is stochastic. The cell that dies is engulfed by the neighboring cell P12.pa. If engulfment is blocked through a mutation in one of the genes in the engulfment pathway or by ablating P12.pa, both B.alapaav and B.arapaav survive. If one cell is ablated by laser microsurgery, then the other cell will invariably survive. These cells form an equivalence group: both have the potential to either survive and form part of the male tail or undergo programmed cell death. These observations suggest that cell interactions between B.alapaav and B.arapaav and between B.al/rapaav and P12.pa are involved in this cell death.

We will investigate the regulation of this unusual programmed cell death. To this end, we will perform a genetic screen to identify genes that function in B.al/rapaav cell death. *cog-1::GFP* has been reported to be expressed in B.alapaav and B.arapaav. Using this reporter strain, we will screen for mutants in which neither or both B.alapaav and B.arapaav are present because of misregulation of fate determination or of engulfment-dependent cell death. This screen might identify new genes involved in fundamental mechanisms of

programmed cell death, cell-cell signaling and fate determination within equivalence groups.

754B

In vitro and *in vivo* investigation of modulators of hyperactivated ion channel induced necrosis in *C. elegans*. **Shaunak Kamat**, Shrutika Yeola, Wenying Zhang, Monica Driscoll. Molecular Biol & Biochem, Rutgers Univ, Piscataway, NJ., New Jersey.

Exposure of tissue to extreme temperature, corrosive chemicals, ischemia or other noxious stimuli can induce necrotic degeneration that is characterized by cellular swelling, loss of organellar integrity, accumulation of reactive oxygen species, activation of proteases and ultimately cell lysis. Necrosis can be genetically induced in *C. elegans* neurons by expression of mutant hyperactive ion channels. Mutant DEG/ENaC channels cause entry of excess calcium into the cell, triggering release of ER calcium stores and subsequent activation of calpain proteases. Due to the role of calcium in the induction and progression of this type of necrotic cell death we screened genes that encode calcium binding proteins and assayed for potential modulation of the cell death phenotype. RNAi and deletion mutant data indicate the involvement of EF hand-containing proteins localized to ER and mitochondria in the progression through necrosis. We will report on progress in deciphering details of their contributions to cell death. To extend mechanistic dissection, we have also been studying properties of hyperactivated ion channel-induced necrosis in *C. elegans* embryonic cell culture and find that cultured neurons behave similarly to their counterparts *in vivo*. We have been pioneering the use of total internal reflection fluorescence microscopy to measure the MEC-4/MEC-10 channel at the neuron surface. We find changes in the surface expression and distribution of mechanotransduction complex proteins when necrosis is induced using a temperature-sensitive *mec-4(d)* allele. We are also studying the effects of mutant DEG/ENaC channels on mitochondrial morphology by imaging mitochondrially localized GFP *in vitro* and *in vivo*, as data suggest a relationship between maintenance of mitochondrial integrity and susceptibility to necrotic stimuli.

755C

Genetic Control of Neurodegeneration by an Evolutionary-conserved Heterotrimeric G Protein Signaling Pathway. **Bwarenaba B Kautu**, Matthew L Hicks, Adam J Harrington, Kim A Caldwell, Guy A Caldwell. Biological Sciences , The University of Alabama, Tuscaloosa , AL.

Accumulation of the α-synuclein (α-syn) protein is a pathological hallmark of Parkinson's disease (PD). Multiplication of the human α-syn locus, as well as several studies in animal models of PD, revealed that overexpression of α-syn can lead to the progressive degeneration of dopaminergic neurons. Whether or not overabundance of α-syn on the dopaminergic cells is mediated by select signal transduction pathways is not well understood. Our model overexpresses human α-syn in the eight dopaminergic neurons of *C. elegans*, which results in the age-and dose-dependent degeneration of these cells. Here, we show that the heterotrimeric G proteins, Gαo (GOA-1) and Gαq (EGL-30), play a fundamental role in controlling the survival of dopaminergic neurons in the presence of α-syn. These two G proteins have previously been shown to have opposing actions on dopamine-mediated behaviors such as locomotion (Chase et al., 2005). Our genetic analysis also shows that they act oppositely with respect to the neurodegeneration of dopaminergic neurons. Loss-of-function mutation of EGL-30 led to enhanced neurodegeneration, while inactivation of GOA-1 protects dopaminergic neurons against α-syn toxicity. Furthermore, we showed that overexpression of EGL-30 is significantly protective against α-syn induced neurodegeneration. Such protection likely requires activation of downstream factors of EGL-30 such as EGL-8 and PKC-1. This hypothesis is supported by evidence that inactivation of these downstream factors all enhanced dopaminergic neurodegeneration. More interestingly, cell-specific depletion of major components of this canonical pathway by RNAi suggests that the specific gene products may function in concert within the dopaminergic neurons to regulate neurodegenerative/neuroprotective mechanisms in response to the overexpression of α-syn. In all, our findings strongly suggest a role for an evolutionary-conserved signal transduction pathway in the genetic control of neurodegeneration. Moreover, these data demonstrate the utility of the *C .elegans* model for elucidating functional relationships that may be significant for future therapeutic intervention against Parkinson's disease.

756A

An MLL-type H3K4 methyltransferase complex is required for linker cell death. **Maxime J Kinet**, Shai Shaham. The Rockefeller University, New York, NY.

Programmed cell death plays a crucial role in development and disease; however, apoptosis and its variations may not account for all such cell deaths. Recently, our lab uncovered a new cell death program in the *C. elegans* male-specific linker cell. In contrast to most other cell deaths in *C. elegans*, which occur in undifferentiated young cells, the linker cell dies after a relatively long, differentiated life spent elongating the male gonad. The linker cell's death and removal connects the vas deferens to the cloaca, enabling sperm exit. Linker cell death is non-apoptotic by morphology and is independent of all known *C. elegans* cell death genes. Ultrastructural features of this death, such as nuclear envelope indentation and open chromatin, have been described in developing vertebrate nervous systems and in polyglutamine-repeat neurodegenerative diseases. Strikingly, *pqn-41*, a polyglutamine-repeat gene, promotes linker cell death (Blum et al, unpublished).

Here we present evidence that a highly conserved histone H3K4 methyltransferase complex is also involved in linker cell death. Targeting several predicted subunits of this complex by RNAi results in a death defect phenotype. RNAi against core structural and regulatory subunits *swd-2.2, rbbp-5, wdr-5* and *ash-2*, as well as a potential targeting mediator, *pis-1*, resulted in the survival of 10-17% of linker cells examined. Importantly, targeting an MLL-type catalytic H3K4 methyltransferase gene, *set-16*, but not the SET1-like gene *set-2*, blocked linker cell death in 50% of animals. Finally, knockdown of *utx-1*, encoding a catalytic histone H3K27 demethylase that in other systems associates with MLL-type complexes, also blocked linker cell death (18%). To determine how this putative complex might act in linker cell death, we used cell-specific RNAi to show that at least two genes, *swd-2.2* and *set-16* can act cell-autonomously in linker cell death. Consistent with a cell-autonomous role for a *set-16* complex, both genes are expressed in the linker cell. While *set-16* genetic mutants die embryonically, mosaic analysis of mutants rescued with a genomic *set-16* clone confirms that *set-16* acts in the linker cell to promote its death.

Interestingly, *set-16* is homologous to human MLL3, a gene found mutated in a variety of cancers. MLL-type H3K4 methyltransferase complexes function as transcriptional coactivators controlling a finite set of targets. In light of this role, we are currently undertaking cell-specific transcriptome studies to identify genes that are regulated by *set-16* and may contribute to linker cell death.

757B

Specific small heat shock proteins regulate calcium homeostasis in the medial Golgi to 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. However, the molecular mechanisms underlying necrosis are not fully understood. We find that activation of the heat shock response pathway by means of heat preconditioning strongly suppresses necrotic cell death caused by extreme environmental conditions and hypoxia as well as excitotoxic neuronal death, in *C. elegans*. The heat shock response is a highly conserved gene expression program, which is engaged under conditions of stress and coordinates expression of specific genes that protect cells against various stressors. Removal of the heat shock factor 1 (HSF-1), the master transcription regulator which orchestrates the heat shock response, abolishes the protective effect of heat preconditioning. By contrast, overexpression of HSF-1 suppresses necrotic cell death triggered by various insults. While screening for potential mediators of the protective effect of heat preconditioning, we found that the small heat shock protein HSP-16.1 is both necessary and sufficient for protection against necrosis. HSP-16.1 exerts its protective effect by modulating calcium release from the Golgi apparatus. Interestingly, the Golgi specific Ca^{2+} pump PMR-1 is required for heat preconditioning to elicit its protective effect. Loss of PMR-1 function abolishes the capacity of *hsp-16.1* overexpression to protect against necrosis. Our findings suggest that intervention strategies based on selective manipulation of the heat shock response may effectively counter neurodegeneration.

758C

Starvation induces a global translational arrest that triggers germ cell apoptosis. **Laura Láscarez**[1], Carlos G. Silva-García[1], Tzvetanka D. Dinkova[2], Rosa E. Navarro[1]. 1) Instituto de Fisiología Celular- Universidad Nacional Autónoma de México, México; 2) Facultad de Química-Universidad Nacional Autónoma de México, México.

Starvation induces germ cell apoptosis in *C. elegans* through until now unknown mechanism. Furthermore during starvation, large granules similar to mammals stress granules (SGs) are formed in the core of the gonad. In some organisms like mammals the presence of SGs suggests a decrease in mRNA global translation due to aggregation of some of the translation initiation factors and specific mRNAs in these particles. Using a polysome profile analysis, we observed that starvation inhibits the global translational machinery, causing a reduction in the anti-apoptotic factor *ced-9* mRNA translation. Low levels of CED-9 protein were confirmed by Western blot analysis suggesting that this could be the mechanism for the induction of germ cell apoptosis. In a microarray analysis, we identified genes that alter their expression level when the animals are starved by 6 h. Interestingly several of these genes encode for RNA binding proteins that associate with RNA granules including stress granules, P-bodies and germ granules. We tested the role of these genes in granules formation and germ cell apoptosis by RNAi. Silencing of some of these genes, for example *cgh-1*, *car-1*, *hsp-1*, *pgl-1*, *cey-2*, *mex-5* and *gla-3* trigger physiological germ cell apoptosis. These same genes were required for starvation-induced apoptosis. Interestingly, silencing of genes that triggered physiological germ cell apoptosis also induced granules formation under normal growth conditions. On the contrary, silencing of *gla-3* (a member of the TTP family) inhibited the formation of granules during starvation and heat shock conditions suggesting that like in mammals, this protein is important for their assembly. We suggest that deregulation of granules formation observed during RNA binding proteins silencing correlates with changes in translational state of the organism explaining their role in stress induced apoptosis.

759A

Secreted VAPB/ALS8 major sperm protein domains regulate mitochondrial fusion and fission machinery localization in body wall muscle. **S. Lee**, M. Miller. University of Alabama School of Medicine Birmingham, AL.

Mitochondria are dynamic organelles that are critical for energy homeostasis and cell survival. Abnormalities in mitochondrial morphology and function are associated with diverse neurodegenerative diseases, but the mechanisms are not understood. C. elegans VPR-1 and human VAPB/ALS8 are homologous proteins with an N-terminal major sperm protein domain (MSP) and C-terminal transmembrane domain. A missense mutation (P56S) in the VAPB/ALS8 MSP domain is associated with amyotrophic lateral sclerosis and late-

onset spinal muscular atrophy, two neuropathies characterized by progressive muscle atrophy and motor neuron degeneration. Our lab has shown that the VPR-1 MSP domain is secreted by neurons and functions as a hormone that signals through Roundabout and Lar-like growth cone guidance receptors to modulate mitochondrial localization, division (fission), and function in body wall muscle (see abstract by Han et al.). MSPs signal to mitochondria by regulating actin-related protein 2/3 complex (Arp2/3) activity, stabilizing mitochondria at actin-rich I-bands. Here I show that VPR-1 regulates the localization of ARX-2, a component of the actin-related protein 2/3 complex, as well as multiple proteins that control mitochondrial fusion and fission in body wall muscle. In wild-type hermaphrodites, ARX-2 primarily localizes to the dense bodies, where it functions to promote mitochondrial elongation along I-bands. However, in vpr-1 null mutants, ARX-2 mislocalizes to the muscle belly along with mitochondria. Together with data presented by Han et al., my results support the model that secreted MSPs promote Arp2/3 complex localization at the dense bodies. In vpr-1 mutants, mislocalized Arp2/3 causes ectopic actin filaments in the muscle belly that displace mitochondria from the I-bands and modulate mitochondrial fission and fusion sites. These abnormal events promote mitochondrial mobility, elongation, and branching, and inhibit energy metabolism. My results reveal a critical role for the Arp2/3 complex in modulating the mitochondrial fission/fusion balance. I will also present preliminary data suggesting that the survival of motor neuron 1 protein SMN-1 functions to transport RNAs to the dense bodies that are critical for Arp2/3 activity and mitochondrial morphology.

760B
Controlling protease-driven lysosomal dependent "necrotic" cell death and beyond. **Cliff J. Luke**, Mark M. Miedel, Sangeeta R. Bhatia, Nathan J. Graf, Gary A. Silverman. Department of Pediatrics, Children's Hospital of Pittsburgh and Departments of Cell Biology and Physiology, University of Pittsburgh, Pittsburgh, PA.

Cell necrosis is a common pathologic feature of many disorders including ischemic injury, hemorrhagic shock and trauma. Necrotic cell death results from perturbations in the protease-inhibitor balance, which leads to excessive intracellular proteolysis, loss of membrane integrity and dissolution of sub-cellular architecture. The strong association between promiscuous intracellular proteolysis and cell death suggests that protease inhibitors serve as necrotic cell death regulators. Extensive searches within the largest family of protease inhibitors, the serpins (e.g., antithrombin and alpha-1-antitrypsin), failed to yield candidate regulatory genes. However, this result was not surprising as most serpins are secreted and unlikely to play a regulatory role intracellularly. We identified a subset of serpins that are abundantly expressed within the cytoplasm of metazoan epithelial cells. Since some of these intracellular serpins ($serpins_{IC}$) neutralize lysosomal cysteine and serine proteases, we hypothesized that they regulate intracellular proteolysis and enhance cell survival. This hypothesis was confirmed by showing that the *C. elegans* $serpin_{IC}$, SRP-6, exhibits a pro-survival function by blocking necrotic cell death. The newly defined necrotic cell death pathway was dependent upon calpains and lysosomal cysteine proteases, two in vitro targets of SRP-6. SRP-6 provided protection by blocking both the induction of, and the lethal effects from, lysosomal injury. Human intracellular serpins also play a role in cytoprotection as SERPINB3 (a human cysteine protease inhibitory serpin) could function in place of SRP-6, as well as protecting mammalian cell lines from cell death. We now hypothesize that multiple noxious stimuli converge upon an evolutionarily conserved, protease-driven core stress-response pathway that, in the absence of $serpin_{IC}$ regulation, leads to necrotic cell death rather than cell survival. The role of protease inhibitors in the necrosis pathway and the potential for therapeutic intervention will be discussed.

761C
The Role of Carbonic Anhydrase in Mediating Cell Stress Response following Neuronal Injury. **T. A. Matthews**[1], G.V.W. Johnson-Voll[2], K. W. Nehrke[1]. 1) Medicine, University of Rochester Medical Center, Rochester, NY; 2) Anesthesiology, University of Rochester Medical Center, Rochester, NY.

Carbonic anhydrases (CAs) comprise a family of enzymes, which catalyze the interconversion of carbon dioxide and water to bicarbonate and protons. CAs are involved in many physiologic and pathologic processes, ranging from respiration, electrolyte secretion and pH regulation to tumor metastasis and neurodegeneration. We have found that the nematode C. elegans expresses a CA that selectively localizes to the cell nucleus, is induced by hypoxia, and when lost results in neurodegeneration (i.e.; dysfunction followed by cell death). This is the first example of a classic alpha-CA that is targeted to the nucleus in any organism. Our results further suggest that this CA buffers nuclear pH. This is a heretofore unheralded and essentially unstudied physiologic occurrence with potentially significant implications for neuronal cell biology. We are currently using a cortical cell culture model to test whether the worm CA isoform protects mammalian neurons from various types of stress (e.g.; hypoxia, hypertonicity, acidosis and heat shock). We are also determining whether nuclear targeting is required for protection and whether mammalian neurons possess endogenous protective mechanisms that employ a nuclear CA ortholog. Our work has resulted in the tentative identification of a mammalian CA that appears to be stress-inducible and may be targeted to the nucleus. Finally, we will discuss preliminary data derived from a mouse model genetically engineered to lack this CA isoform, where primary cell culture and cortical lesions have been used to test neuronal susceptibility to stress.

762A
Improved Alzheimer's Disease Model of $A\beta_{1-42}$ toxicity. **Gawain McColl**[1,2], Blaine Roberts[1,2], Adam Gunn[1], Tara Pukala[3], Christine Roberts[4], Christopher Link[4], Robert Cherny[1,5], Ashley Bush[1,5]. 1) Mental Health Research Institute, University of Melbourne, Australia; 2) Center for Neuroscience, University of Melbourne, Australia; 3) School of Chemistry and Physics, University of Adelaide, Australia; 4) Institute for Behavioral Genetics, University of Colorado, USA; 5) Department of Pathology, University of Melbourne, Australia.

One of the hallmarks of Alzheimer's disease is the cerebral deposition of plaques composed of Amyloid-beta (Aβ) peptide. Human Aβ (e.g. in brain, CFS or plasma) is not found as a single species, but rather as diverse mixtures of various modified and truncated forms. *Caenorhabditis elegans* offers a simplified *in vivo* system in which to examine Aβ accumulation, including sub-cellular localization, and toxicity. We determined that an earlier model of Aβ expression in *C. elegans* accumulates $A\beta_{3-42}$ due to mis-cleavage of a synthetic signal peptide. To achieve correct signal peptide cleavage we inserted an extra Asp-Ala (DA) N-terminal to the hu-Aβ sequence in the transgene construct The molecular identity of the Aβ expressed was then determined via complimentary techniques. Using immunocapture and electrospray ionization mass spectrometry (ESI-MS) we determined the mono-isotopic molecular weight of the expressed Aβ as 4511.2586 Da with an error of 2.5 ppm. This mass is consistent with full length $A\beta_{1-42}$ (expected mass 4511.2697 Da). The $A\beta_{1-42}$ is expressed in body wall muscle cells, where it aggregates and results in severe, and fully penetrant, age progressive-paralysis. The *in vivo* accumulation of $A\beta_{1-42}$ also stains positive for amyloid dyes, consistent with *in vivo* fibril formation. This *C. elegans* model of full length Aβ expression can now be adopted to study underlying toxic mechanism(s) of $A\beta_{1-42}$.

763B
Utilization of alternative mRNA forms for CED-4/Apaf-1 during germ cell apoptosis. **J. Kaitlin Morrison**, Vince Contreras, Enhui Hao, Brett D. Keiper. Brody School of Medicine at ECU, Greenville, NC, 27834.

Germ cell apoptosis is the process by which superfluous oocyte progenetor cells are eliminated via the cell death (ced) signaling pathway. In the *C. elegans* gonad a large number of cells are fated for death before reaching oocyte maturity. These cells are believed to act as "nurse cells" providing cytoplasmic components needed by their sibling cells that reach maturity. Our previous studies suggest that protein synthesis regulation via the translation factor eIF4G (ifg-1) may contribute to apoptotic selection during germ cell development. RNA*i* knockdown of the long ifg-1 isoform (p170), but maintaining the shorter isoform (p130), induced extensive germline apoptosis and expression of CED-4 in the dying oocytes. IFG-1-dependent apoptotic events required not only the apoptosome protein CED-4, but also the apoptotic effector caspase, CED-3. These studies further demonstrated that IFG-1 p170 was a natural substrate for CED-3, creating a p130-like cleavage product, which may likewise increase the proportion of IFG-1 p130 in comparison to p170. Our project now focuses on the translation of the *ced-4* mRNAs as a result of an imbalance between the IFG-1 isoforms. Two previously known splice variants, *ced-4L* and *ced-4S*, differ in the splicing of exon 4 and have opposing activities on apoptosis. We are characterizing other mRNA variants of *ced-4* and their translational efficiency. Alternative forms of the mature, spliced message have been confirmed using both RTPCR and RNase Protection mapping. The distribution of *ced-4* mRNA variants and their translational efficiency was assayed in wildtype worms as well as strains temperature sensitive for *ifg-1* function or *ced-9* (Bcl-2) function. Our findings suggest a mechanistic link between translational control by IFG-1 and the expression of CED-4 that may trigger physiological germ cell apoptosis.

764C
Role of dynein light chain 1 in apoptosis: Using C. elegans as a model system. **Tine H Møller**, Anders Olsen. Molecular biology, University of Aarhus, Aarhus, Denmark.

In a screen for novel checkpoint proteins in C. elegans we have identified approximately 50 genes, that when inactivated cause resistance to stalled replication forks (hydroxyl urea). One of these genes encodes dynein light chain 1 (dlc-1), which we have investigated further. Consistent with a possible check point function of DLC-1 it has been shown that dlc-1 (RNAi) results in unpaired chromosomes in the oocytes and a timorous gremlin (1) We have also observed unpaired chromosomes and seen that inactivation of dlc-1 cause disorganization of the entire germline resulting in an altered distribution of germ cells. Furthermore, we have shown that dlc-1(RNAi) results in a significant increase in the number of apoptotic cells in the germline. DNA damage induces apoptosis in the germline of C. elegans in a cep-1 (p53 homolog) dependent manner (2). Due to the unpaired chromosomes we hypothesized that the increase in apoptotic cells in dlc-1(RNAi) worms could be due to DNA damage. However, we found that the increased number of apoptotic cells in dlc-1(RNAi) worms is forming independently of cep-1. We are now investigating whether the increase in the number of apoptotic cells is due to either a defect in engulfment of apoptotic cells or due to an upregulation of the apoptotic pathway. Also, induction of DNA damage by ionizing radiation does not further increase the number of apoptotic cells in dlc-1(RNAi) worms. These findings made us hypothesize that dlc-1 could be involved in transporting CEP-1 to the nucleus; however, we were unable to detect any difference in CEP-1 localization following dlc-1 inactivation. We are currently investigating whether CEP-1 is functioning properly after dlc-1(RNAi) by looking at the expression of CEP-1 target genes upon ionizing radiation. Our results implicate dlc-1 in the regulation of apoptosis, cell division and chromosomal crossover during meiosis. Even though the pathways for apoptosis, engulfment and cell division have been studied thoroughly in both C. elegans and mammalian cells, much can still be learned by looking deeper into these pathways and novel genes mediating these events may still be identified. (1)Dorsett and Schedl, 2009. Mol Cell Biol.(22):6128-39 (2)Gartner et al,2000. Mol Cell.(3):435-43.

765A

Identification and characterisation of genes essential for axonal maintenance. **Brent Neumann**, Massimo A. Hilliard. Queensland Brain Institute, The University of Queensland, Brisbane, Australia.

The long axonal process of a neuron can reach distances several orders of magnitude higher than the dimensions of the cell body. Active mechanisms are in place to ensure the integrity of this neuronal compartment is maintained over an animal's lifetime. However, the identities of the molecules needed to prevent the breakdown of axons largely remain unknown. Through mutagenesis screening in a sensitized genetic background, we have isolated a strain of *C. elegans* (*ky850*) in which axonal degeneration spontaneously occurs in the mechanosensory neurons. The *ky850* strain is insensitive to light mechanical stimuli and presents progressive degeneration of the PLM, ALM and AVM axons, with breaks first appearing in young adults and worsening as they age, leading to approximately half of all animals displaying a degenerative phenotype over their lifetime. Unlike animals defective for the *unc-70/β-spectrin* gene (Hammarlund *et al.* J Cell Biol. 2007), the axons of *ky850* animals do not show regeneration after breaks occur; instead the separated distal fragment/s undergo a stereotypical Wallerian-like degeneration pattern. In order to identify the causative mutation/s, we employed SNP mapping, followed by whole genome sequencing. These techniques have revealed a multigenic nature of the phenotype, highlighting the complexity of the axon maintenance machinery. One of the mutations lies within a poorly characterised, but highly conserved gene. Analysis of mutant strains, along with complementation and rescue experiments, has implicated this first gene as accounting for a proportion of the degeneration seen in *ky850* animals. We are currently investigating the nature of the second mutation in order to fully define the cause of the axonal degeneration phenotype of *ky850*.

766B

Effects of the *Wld*^{s} mutation on axonal degeneration and regeneration in *C. elegans* neurons. **Annika L.A. Nichols**, Brent Neumann, Massimo A. Hilliard. Queensland Brain Institute, Brisbane, Australia.

Distal axons of damaged neurons undergo a stereotypic degeneration process consisting of thinning, beading and fragmentation, which is commonly referred to as Wallerian degeneration. In mice, a dominant mutant strain (*Wld*^{s}) carrying a chimeric protein that includes part of the ubiquitin fusion degradation protein 2a (Ufd2a) and the complete sequence of nicotinamide mononucleotide adenylyltransferase 1 (Nmnat1), confers to the animal strongly delayed Wallerian degeneration. The Wld^{s} protective effect is not restricted to physically injured axons but extends to toxic insults and to different, but not all, neurodegenerative diseases. Several groups have used *Wld*^{s} mice, rats, *Drosophila* and Zebrafish to first, better understand how Wld^{s} confers protection to neurons and secondly as a tool to discover the molecular mechanisms of axonal degeneration. Surprisingly, a worm model expressing Wld^{s} in selective neurons is yet to be established. I will present my work on the development of transgenic *C. elegans* strains expressing the vertebrate Wld^{s} mutation in the six mechanosensory neurons ALMs, AVM, PVM and PLMs (*Pmec-4::Wld*^{s} and *Pmec-4::Wld*^{s}*::mCherry*) and the results of the Wld^{s} function in these neurons following laser-induced axotomy and genetically-driven axonal damage.

767C

Serotonergic blockade prevents age-related neurodegeneration in *C. elegans* model of Alzheimer's disease. **Jonathan T. Pierce-Shimomura**, Ashley Crisp. Section of Neurobiology, University of Texas at Austin, Austin, TX.

Alzheimer's disease (AD) is characterized by the selective degeneration of cholinergic neurons involved in memory. Although the precise etiology of AD remains unclear, the amyloid-precusor protein (APP) appears to be a causal agent for two main reasons. First, overexpression of *APP* or mutations in *APP* predict early-onset AD in humans. Second, dying neurons are surrounded by plaques composed of APP peptide fragments. With the simple architecture and ease of molecular manipulation of *C. elegans*, we explored the mechanisms of neurodegeneration caused by APP. The Li lab has previously found that overexpression of multiple copies of the APP-related gene called *apl-1* leads to lethality and vacuolization in *C. elegans* (Hornsten et al., PNAS 2007). We have now found that, as in human AD, overexpression of a single wild-type copy of *apl-1* or human *APP* result in age-related degeneration of a specific subset of cholinergic neurons in *C. elegans*. Deficits in two natural behaviors, swimming and egg-laying, correlate with the accumulation of APP protein specifically in these neurons and their eventual death. In addition, through genetic and pharmacological analyses, we have found that serotonergic signaling is both necessary and sufficient for degeneration of these neurons. Our results suggest that therapeutics designed to decrease serotonergic signaling may alleviate the pathology in mouse models of AD and perhaps even in patients with AD.

768A

The localization of CED-9/Bcl2 and CED-4/Apaf-1 is not consistent with the accepted model for C. elegans apoptosis induction. **Ehsan Pourkarimi**[1], Sebastian Greiss[1,2], Anton Gartner[1]. 1) Wellcome Trust Centre For Gene Regulation and Expression, University of Dundee, United Kingdom; 2) MRC Laboratory of Molecular Biology, University of Cambridge, United Kingdom.

C. elegans is an important model organism to study apoptosis. Seminal studies on developmental apoptosis uncovered a genetic pathway needed for nearly all developmental apoptotic cell deaths. EGL-1, the worm BH3-only family member, CED-9/Bcl-2, CED-4/Apaf1 and the CED-3 caspase are required for the majority of the apoptosis that occur in C. elegans. Genotoxic stress such as ionizing irradiation (IR) also triggers apoptosis in pachytene-stage germ cells. In contrast to their mammalian homologs CED-9 and CED-4 were reported to directly interact at the outer mitochondrial membrane and the decision between cellular live and death is mediated by the their direct interaction. In essence apoptosis is triggered by the transcriptional induction of egl-1. Subsequent EGL-1 binding to CED-9 is though to disrupt the CED-9/CED-4 complex. This leads to release of CED-4 from mitochondria and its trans-location to the nuclear periphery where its accumulation and oligomerization, is thought to be required for CED-3 activation. In this study we revisit the localization of CED-4 and CED-9 in the C. elegans germ line, somatic cells and during embryogenesis. We found that, in contrast to the current model, pro apoptotic CED-4 does not colocalize with mitochondrial CED-9, and CED-4 is constitutively localized at the nuclear membrane, even in healthy cells that do not undergo apoptosis. However, in the germ line the amount of perinuclear CED-4 does increase in response to IR but this accumulation is not directly correlated with apoptosis execution, as all the pachytene cells that pose CED-4 accumulation at their nuclear periphery do not die upon treatment with IR. This accumulation of CED-4 is dependent to cep-1/p53, egl-1 and ced-9. We also provide evidence that there is no overall colocalization of CED-4 and CED-9 during late stage embryogenesis as well as in somatic cells. In addition, we could not pull-down CED-9 by immuno precipitating the endogenous CED-4 from whole worm lysate. These results raise the question as how mitochondrial CED-9 prevents perinuclear CED-4 activation. We propose that anti apoptotic effect of mitochondrial CED-9 can-not be explained by its physical interaction with CED-4 therefore, novel factors are needed to transmit a pro-apoptotic signal from mitochondrially located CED-9 to perinuclear CED-4 to trigger apoptosis.

769B

Characterizing an uncharacterized protein: F16A11.2, a neuroprotective gene product with a putative role in RNA localization. **Arpita Ray**, Susan M. DeLeon, Kim A. Caldwell, Guy A. Caldwell. Biological Sciences, University of Alabama, Tuscaloosa, AL.

Our studies use *C. elegans* as an animal model for Parkinson's disease (PD) to identify genetic factors which may impact this condition. PD is characterized by the degeneration of dopaminergic (DA) neurons and the formation of protein inclusions that contain the α-synuclein (α-syn) protein. Overexpression of human α-syn specifically in the eight DA neurons of *C. elegans* causes neurodegeneration in an age- and dose-dependent manner. The F16A11.2 gene product was found as one of several significant modifiers in an RNAi screen for functional effectors of α-syn toxicity (Hamamichi *et al*., 2008 *PNAS*). F16A11.2 is the worm homolog of the human HSPC117 protein (also identified as C22orf28) and shares approximately 75% sequence identity to its human homolog. Interestingly, HSPC117 was determined to be a component of RNA trafficking granules in neurons, along with other proteins, such as CGI-99 (an mRNA transcriptional modulator) and DDX1 (an RNA binding protein). Accordingly, HSPC117 has the ability to bind to AU-rich elements within mRNA. The RtcB domain in F16A11.2 is highly conserved and, based on studies of its bacterial homolog, could represent an uncharacterized RNA modification enzyme. Here, we show that overexpression of the F16A11.2 cDNA in DA neurons not only significantly protected these cells from α-syn neurotoxicity but also that neuronal-specific RNAi knockdown of this target results in enhanced neurodegeneration. Similar results were obtained when worms were exposed to the DA neurotoxin, 6-hydroxydopamine (6-OHDA). Result of yeast two-hybrid (Y2H) screen point to a possible interaction of the F16A11.2 protein with several cytoskeletal factors including actin (ACT-1) and alpha-tubulin (TBA-1). We conducted double knockdown studies between F16A11.2 and those Y2H hits that have been shown to be expressed in neurons and/or have an RNA binding domain, using dopamine neuron-specific RNAi. Our preliminary findings indicate genetic interaction with F59G1.4, a worm homolog of the ceramide glucosyltransferase, DDX-1 and *mir-2*. As we continue to evaluate other positives from Y2H screening, we are also investigating the possible role of F16A11.2 in RNA trafficking by analyzing P granule localization. Collectively, these studies serve to illuminate the role of this evolutionarily conserved, but previously uncharacterized protein, coincident with the goal of uncovering new pathways associated with neuroprotection.

770C

Mild Frataxin suppression reduces fat accumulation, induces autophagy and protects from motility defects in *C. elegans*. **Alfonso Schiavi**[1], Alessandro Torgovnick[1], Evgenia Megalou[2], Nektarios Tavernarakis[2], Roberto Testi[1], Natascia Ventura[1]. 1) Department of Experimental Medicine and Biochemical Science, University of Rome Tor Vergata, Rome, Italy; 2) IMBB, Foundation for Research and Technology, Heraklion 71110, Crete, Greece.

In human, mutations in different nuclear-encoded mitochondrial proteins lead to a plethora of neurodegenerative disorders including Friedreich's ataxia (FRDA). FRDA, the most frequent inherited recessive ataxia, is ascribed to severely defective expression of frataxin. Symptoms become evident only when frataxin reduction and the consequent accumulation of mitochondrial damage are severe enough to cause cellular dysfunction and death. Notably, non-pathological levels of frataxin deficiency is associated with alteration in genes expression profiles, suggesting that cells attempt to cope with frataxin decrease by inducing compensatory adaptive pathways. These pathways, if characterized, may reveal novel potential strategies to prevent or postpone the established disease in human. Autophagy is a self-eating process responsible for the degradation and recycling of nonessential cellular components during periods of energy deprivation and can be either necessary or detrimental for tissue homeostasis and organismal survival. Accordingly, it has been associated both with prevention and causation of diseases in human, including neurodegenerative disorders. The role of autophagy in the pathogenesis of FRDA, and of other human mitochondrial-associated diseases (HMAD), is still largely unexplored. Using

C. elegans as model system for FRDA, we had previously shown that mild suppression of the frataxin *C. elegans* homolog, *frh-1*, reduces animal size and pigmentation, and extends lifespan in a p53/*cep-1* dependent. Complete *frh-1* KO arrests animal development. Ablation or functional alterations of specific sensory neurons extends C. elegans lifespan and we have now results showing that *frh-1* deficient animals, like in FRDA patients, display behavioral alterations ascribable to sensory neurons defects. We obtained very similar results suppressing the expression of several different HMAD related genes. Moreover, we found that *frh-1* RNAi induces autophagy and accumulate less fat in a p53 dependent manner. In support of autophagy being induced as a protective mechanism in response to *frh-1* decrease, animals extended lifespan requires the autophagic regulatory genes *bec-1*, *atg-1* and *pdr-1*. Importantly, *frh-1* RNAi reverts the motility defects not only in a wild-type background but also in *pink-1* KO animals. It will be interesting to test whether we can pharmacologically exploit the autophagic pathway to prevent or postpone neuronal defects in FRDA and other HMAD models.

771A

The RNA binding protein TIA-1.2 promotes stress-induced germ cell apoptosis and regulates granules assembly in the gonad core. **Carlos Silva**[1], Jorge Ramírez[2], Valerie Reinke[3], Rosa E. Navarro[1]. 1) Departamento de Biología Celular, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, México; 2) Unidad de Microarreglos, Universidad Nacional Autónoma de México, México; 3) Department of Genetics, Yale University of Medicine, USA.

When confronted with environmental stress, cells active different mechanisms to survive or die if necessary. Some of these mechanisms include stress granules (SGs) formation and apoptosis. SGs are cytoplasmic inclusions into which stalled translation initiation complexes are dynamically recruited in cells subjected to environmental stress and where mRNAs are stored and protected. Here we show that starvation, heat shock and UV light induce granules in the gonad core, and that these granules are similar in nature to mammalian stress granules because they dissociate in the presence of cycloheximide. TIA-1.2 is one of the three TIA-1/TIAR homologs in *C. elegans* whose role has not been described in nematode. We found that TIA-1.2 is required to induce granules formation during starvation and heat shock suggesting that, like in mammals, this protein is important for granules assembly. In normal conditions, germ cell apoptosis is a process important to maintain the homeostasis and quality of oocytes. However, environmental stress triggers this process. We found that TIA-1.2 is necessary to induce germ cell apoptosis in response to several stressful conditions like oxidative, osmotic and heat shock stress as well as starvation and DNA damage, suggesting that this protein could be acting at CED-9 level or downstream of it. Finally, the other TIA-1/TIAR related *C. elegans* genes, *tia-1* and *C07A4.1*, are not required to induce germ cell apoptosis under stress, but they play a redundant role in granules assembly under stress conditions.

772B

gex Complex Genes Are Involved in the Engulfment of Apoptotic Cells. **E Simionato**, M. E Hurwitz. Yale School of Medicine, New Haven, CT.

Abl is a conserved non-receptor tyrosine kinase involved in many cell biological processes, including cytoskeletal rearrangement, cell adhesion, cell migration and apoptosis. To understand the in vivo role of Abl, I am studying the Abl homolog, ABL-1. ABL-1 inhibits three processes in which cytoskeletal regulation is involved: engulfment of apoptotic cells, cell migration and apoptosis. The engulfment of the apoptotic cells is mediated by three parallel and partially redundant core signalling pathways (comprised of CED-1, CED-6, CED-7, DYN-1 and CED-2, CED-5, CED-10, CED-12 and ABI-1). CED-1, 6 and 7 activate the GTPase DYN-1 Dynamin, a regulator of membrane dynamics. CED-2, 5 and 12 activate the small GTPase CED-10 Rac, a regulator of the actin cytoskeleton. ABI-1, the target of ABL-1 in these processes, is also a regulator of the actin cytoskeleton. I aimed to identify the proteins through which ABI-1 acts in engulfment and migration to find potential downstream targets within Abl-dependent pathways. Abi proteins are found in several protein complexes in mammals. One such complex is the Scar/WAVE complex, called the GEX complex in worms, which mediates actin polymerization in response to activation by Rac signaling. Because all GEX genes are essential to the viability of the animal, I am using partial knockdown of gex complex genes using feeding RNAi to test their roles in engulfment. I have analyzed gex complex gene engulfment phenotypes using feeding RNAi in worms with mutations in dyn-1 and ced-10 pathway genes to look for enhancement of the engulfment defects. To date I have shown that partial knockdown of wve-1 and gex-2 enhance the engulfment defects of both ced-1/6/7 and ced-2/5/10/12 pathways. In addition, gex-2 enhances the distal tip cell migration defect of a ced-2 null mutation. To test if wve-1 and gex-2 affect the engulfment process independent of abl-1, I assayed the effect of wve-1 and gex-2 RNAi in strains doubly mutant for ced-10 pathway genes and abl-1. In these strains the loss of wve-1 and gex-2 enhances the engulfment defects to the same degree regardless of the presence of functional ABL-1, indicating that wve-1 and gex-2 act downstream of or in parallel to abl-1. These preliminary results indicate that WVE-1, GEX-2, like ABI-1, promote the engulfment of apoptotic cells and cell migration downstream of or in parallel to ABL-1. My studies are now focused on dissecting how these proteins act in relation to the other known proteins involved in these processes by the analysis of double mutant animals and studies of physical interactions among the SCAR/WAVE proteins, ABL-1 and other proteins involved in these processes.

773C

Establishing a RIP-seq method to study the role of RNA-binding proteins in germ cell apoptosis. **Deni Subasic**[1,3], Jochen Imig[2], Marko Jovanovic[1], Jonathan Hall[2], Andre Gerber[2], Michael Hengartner[1]. 1) Institute of Molecular Life Sciences, University of Zurich, CH-8057, Zurich, Switzerland; 2) ETHZ, Swiss Federal Institute of Technology, CH-8093 Zurich, Switzerland; 3) Molecular Life Sciences PhD program, Life Science Zurich Graduate School, ETHZ/UZH, Switzerland.

Post-transcriptional regulation of gene expression is essential for proper development and homeostasis. One of the most important ways cells achieve such regulation is through controlling mRNA stability, localization and translation via specific interactions with RNA-binding proteins (RBPs). Like microRNAs, RBPs have been shown to bind dozens to hundreds of target mRNAs, and have been implied in a number of human diseases. We are generally interested in understanding how the miRNA and RBP networks cooperate to regulate gene expression. Various RBPs have been shown to negatively regulate germ cell apoptosis in *C. elegans*. For example, GLD-1 negatively regulates translation of the p53 homologue CEP-1 by binding to its 3'UTR, thereby protecting the germ line from DNA damage-induced apoptosis. By contrast, the mechanism of action of most of the other apoptosis-affecting RBPs, including CPB-3, GLA-3, and DAZ-1, is unknown. To address this issue, we established a RIP-seq (ribonucleoprotein immunoprecipitation followed by deep sequencing) method to identify mRNA targets of RBPs that affect apoptosis. To optimize the method, we used GLD-1, as it is well characterized and has many known mRNA targets. In a first step, we generated rescuing RBP::STREP transgenic lines with microparticle bombardment. We next optimized the STREP coimmunoprecipitation of the RBPs together with their mRNA targets. RBP-associated mRNAs identified by RNA-seq on a SOLID 4 will be tested for their possible role in germ cell apoptosis using RNAi knock-down. The effect of RBP binding on the expression of the target mRNAs will be determined using targeted proteomics. Systematic analysis of the RBPs that affect germ cell death and of their target mRNAs might identify new pathways that regulate apoptosis and give a better insight into the RBP regulons that control development, differentiation and death in the *C. elegans* germ line.

774A

Modulation of stroke-like neurodegeneration by cell stress-resistance signaling pathways. . **Nazila Tehrani**[1,2], John Del Rosario[1], Moises Domingues[1], Itzhak Mano[1,2]. 1) Physiology, Pharmacology & Neuroscience, Sophie Davis Biomedical School, City College, NEW YORK, NY; 2) Program in Neuroscience, CUNY graduate school, The Graduate Center, City University of New York.

Stroke is a major health concern in the US, as it is the third leading cause of death and the leading cause of long-term hospitalization. The danger of brain damage caused by stroke disproportionally increases with age: when comparing people having a similar initial brain insult, young people have higher chances of recovery, while the condition of older people show greater tendency to worsen. The increased age-related sensitivity can be traced to the cellular level. Stroke-induced brain damage is triggered by excessive stimulation of nerve cells by the excitatory neurotransmitter Glutamate, causing necrotic neurodegeneration in a process termed excitotoxicity. We were recently able to successfully model excitotoxicity in the genetically-accessible nematode *C. elegans*, a system that offers a combination of particularly powerful research tools and high evolutionary conservation of cellular and molecular signaling pathways such as cell death and cellular aging. In the current project we focus on the Insulin/Insulin-like Signaling (IIS) pathway and its ability to regulate the susceptibility of cells to stress as a possible modulator of neuronal damage triggered by excitotoxicity. Having demonstrated that *age-1*/PI-3K and *daf-16*/Foxo3a can modulate the extent of neurodegeneration in our nematode model (with parallel findings obtained by our collaborator in mammalian cultures), we now move to investigate the involvement of *daf-16*/Foxo3a-regulated genes and interacting signaling pathways in neuronal damage. We combine analysis of candidate targets and an unbiased RNAi-based genetic screen to find regulators of cellular aging and stress that can also modulate susceptibility to excitotoxic neurodegeneration. Our study has the potential of providing new insights into age-related risk factors of brain damage in stroke, and suggesting new possible avenues for clinical intervention.

775B

Clathrin-mediated endocytosis and intracellular trafficking are required for necrotic cell death in *C. elegans*. **Kostoula Troulinaki**, Nektarios Tavernarakis. IMBB, FORTH, Heraklion, Crete, Greece.

In contrast to apoptotic cell death, necrosis is characterized by extensive loss of plasma membrane integrity. Damage to the plasma membrane leads to leakage of cytoplasmic material to the extracellular space, contributing both to the death of the cell itself and also to induction of inflammatory responses in the surrounding area. The mechanisms underlying plasma membrane damage are not well understood. We are investigating the possible role of clathrin-mediated endocytosis and intracellular trafficking in the execution of necrotic cell death inflicted by genetic and environmental insults. We find that depletion of the key endocytic machinery components suppresses neurodegeneration in *C. elegans*. Moreover, using genetically encoded fluorescent markers, we find that the number of early and recycling endosomes increases sharply and transiently upon initiation of necrosis. Interfering with kinesin-mediated endosome trafficking impedes cell death. Endocytosis synergizes with autophagy and lysosomal proteolytic mechanisms to facilitate necrotic cell death. Our findings demonstrate a prominent role of endocytosis in cellular destruction during necrotic cell death in the nematode.

776C

Investigation of the neuroprotective role of GIPC in a C. *elegans* model of Parkinson's Disease. **Michelle L. Tucci**, Laura A. Berkowitz, Kim A. Caldwell, Guy A. Caldwell.

University of Alabama, Dept Biological Sci, Tuscaloosa, AL. 35847.

Our work has focused on functional analyses designed to identify effectors of dopamine (DA) neurodegeneration using the model organism *Caenorhabditis elegans* (C. *elegans*). When human alpha-synuclein (α-syn), a protein found in Parkinson's Disease (PD)-associated aggregates, is overexpressed in the C. *elegans* DA neurons, age and dose-dependent neurodegeneration takes place. In a large-scale hypothesis-based RNAi screen for gene products that enhance α-syn aggregation and DA neuron degeneration, GAIP Interacting Protein C-terminus (GIPC) was identified as a neuroprotective factor (Hamamichi *et al*., 2008, PNAS). Evidence reveals a role for GIPC in transduction through recruitment of the G protein signal attenuating GAIP protein. The general association of GIPC with many receptors and transporters localized at the plasma membrane that undergo internalization, recycling and/or degradation provide a large spectrum to identify which of these functional roles GIPC may play in protecting the DA neurons from α-syn induced toxicity. We report that both the genetic loss and overexpression of C35D10.2, the C. *elegans* homolog of GIPC, is neuroprotective. Use of genetic knockouts, DA neuron-specific RNAi methods and DA neuron overexpression of candidate genes involved in the DA signaling cascade, endocytic trafficking and recycling/degradation allows us to determine GIPC-dependent functions and interactors involved are in neuroprotection. Preliminary analysis shows that loss of presynaptic D2 autoreceptor (DOP-2) has no effect on α-syn induced degeneration, nor does it alter the level of protection with the genetic loss of GIPC. However, the DA signal is required for protection with the GIPC overexpression. Additional neurotoxin (6-OHDA) and reporter assays suggest possible down regulation of the dopamine transporter protein, DAT-1, that may, in turn, influence cytosolic DA levels. These results indicate that components of DA neurotransmission, as well as the endocytic pathway may contribute to neuronal survival. Taken together, these studies further provide mechanistic insights into factors impacting DA neuron survival and Parkinson's Disease.

777A

Axonal Degeneration in *C. elegans* motor neurons: a model for Motor Neurone Disease. **Nicholas J. Valmas**[1], Ivan Cáceras[2], Hang Lu[2], Massimo A. Hilliard[1]. 1) Queensland Brain Institute, The University of Queensland, Brisbane, Australia; 2) Interdisciplinary Bioengineering Graduate Program, Georgia Institute of Technology, Atlanta, GA.

Motor Neurone Diseases (MNDs), such as amyotrophic lateral sclerosis (ALS), are neurodegenerative disorders specifically affecting the neurons that control muscle (motor neurons). Degeneration of the axonal domain of motor neurons is hypothesised to be causative of many MNDs. Only a handful of genes have been linked to MND pathology or to axonal degeneration, and therefore the genetic pathways responsible for these processes are largely uncharacterised.

We are focusing on the 19 GABAergic ventral nerve cord motor neurons of *C. elegans*, the DD and VD sub-classes. These neurons innervate the body wall muscle and control the locomotion of *C. elegans*, making them functional equivalents of the motor neurons targeted in MNDs. We aim to use these cells as a model to discover and characterise novel genes and mutations responsible for the axonal degeneration of motor neurons.

Using transgenic strains in which the DD and VD motor neurons are highlighted with GFP, we have conducted forward genetic screens and observed morphological changes indicative of axonal degeneration in these neurons. This observation suggests that genetic factors governing the axonal degeneration of motor neurons exist and are susceptible to mutagenesis. To complement the forward genetics, we are using a candidate-gene approach focusing on genes orthologous to human MND genes, such as *sod-1, tdp-1,* and *smn-1*. We are examining the motor neurons in *C. elegans* mutants of these genes to look for axonal degeneration phenotypes that may be linked to MNDs.

778B

Identification and characterization of molecular modulators of methylmercury-induced whole animal and dopamine neuron pathology in *C. elegans*. **Natalia VanDuyn**, Raja Settivari, Gary Sinclair, Richard Nass. Department of Pharmacology & Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Methylmercury (MeHg) exposure from occupational, environmental, and food sources is a significant threat to public health. MeHg poisonings in adults may result in severe psychological and neurological deficits, and *in utero* exposures can confer embryonic defects and developmental delays. Recent epidemiological and vertebrate studies suggest that MeHg exposure may also contribute to dopamine (DA) neuron vulnerability and the propensity to develop Parkinson's disease (PD). We have developed a novel *Caenorhabditis elegans* (*C. elegans*) model of MeHg toxicity that shows that low, chronic exposure confers embryonic defects, developmental delays, decreases in brood size and animal viability, and DA neuron degeneration. Toxicant exposure results in the robust induction of the glutathione-S-transferases (GSTs) *gst-4*, *gst-5*, *gst-12*, *gst-21*, and *gst-38*, with some GSTs largely dependent on the PD-associated phase II antioxidant transcription factor SKN-1/Nrf2. We also demonstrate that the expression of SKN-1, a protein previously localized to a small subset of chemosensory neurons and intestinal cells in the nematode, is also expressed in the DA neurons, and a reduction in SKN-1 gene expression increases MeHg-induced animal vulnerability and DA neuron degeneration. We will also present our initial studies from two genome-wide genetic screens to identify mediators and suppressors of MeHg-induced DA neuron degeneration, as well as our investigations using DA-associated mutants to elucidate the role that the neurotransmitter may play in the toxicity. Support contributed by: NIEHS ES014459, ES003299 and Vanderbilt University Toxicology Program Pilot Grant to RN; PhRMA Pre Doctoral Fellowship in Pharmacology/Toxicology and EPA STAR Graduate Fellowship to NVD.

779C

The role of plastin in Spinal Muscular Atrophy. **Melissa Walsh**[1], Anne Hart[2]. 1) Molecular Biology, Cellular Biology & Biochemistry Dept., Brown University, Providence, RI; 2) Neuroscience Dept. Brown University, Providence, RI.

Spinal Muscular Atrophy (SMA) is incurable, is the most common genetic cause of infant death in the United States, and is caused by low levels of the Survival Motor Neuron (SMN) protein. A previous study (Oprea *et al*., Science, 2008) reported that over-expression of plastin 3 (PLS3) ameliorates symptoms in less severe SMA cases and is found in a complex with SMN in neuronal tissues. PLS3 is an actin bundling protein and the mechanism of suppression remains unclear. Partial *smn-1* loss-of-function in *C. elegans* decreases locomotion assessed by body bends/minute (Sleigh *et al.*, Human Molecular Genetics, 2010) and causes resistance to immobilization by aldicarb, an acetylcholinesterase inhibitor. We find that PLS3 over-expression restores normal function in both assays, suggesting that the PLS3/SMN functional interaction is conserved in vertebrates and invertebrates. Future studies aim to elucidate the functional relationship between PLS3 and SMN1.

780A

Caenorhabditis elegans O-GlcNAc cycling mutants alter the proteotoxicity of models of human neurodegenerative disorders. **Peng Wang**, Brooke Lazarus, Michele Forsythe, Dona Love, Michael Krause, John Hanover. NIDDK, NIH, Bethesda, MD.

O-linked N-acetylglucosamine (O-GlcNAc) addition is an important post-translational modification that occurs on hundreds of proteins, including nuclear pore proteins, transcription factors, proteasome components and neuronal proteins. O-GlcNAc can be added onto and removed from serine or threonine residue by two evolutionally conserved enzymes: O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), respectively. O-GlcNAcylation is abundant in the brain and it has been linked to human neurodegenerative disease. We have exploited viable null alleles of the enzymes of O-GlcNAc cycling to examine the role of O-GlcNAcylation in well-characterized *C. elegans* models of neurodegenerative proteotoxicity. O-GlcNAc cycling dramatically modulated the severity of the proteotoxic phenotype in transgenic models of tauopathy, β-amyloid peptide and polyglutamine expansion. Intriguingly, loss-of-function of OGT alleviated, while loss of OGA enhanced these proteotoxicity phenotype. Consistent with these observations, the O-GlcNAc cycling mutants exhibit altered stress responses and changes in the protein degradation machinery. These findings suggest that modulators of O-GlcNAc cycling may prove useful for anti-neurodegenerative disease therapies.

781B

Analysis of cellular toxicity mechanisms of alpha-synuclein in *C. elegans*. **Nora Wender**[1], Jan Hegermann[1,2], Stefan Eimer[1,2]. 1) Molecular Neurogenetics, European Neuroscience Institute, Goettingen, Germany; 2) Center for Molecular Physiology of the Brain (CMPB), Goettingen, Germany.

Aggregation of α-synuclein (αS) in Lewy bodies is one of the pathological hallmarks of Parkinson's disease (PD). Mutations in αS as well as increased αS expression levels have been associated with PD. As the PD mutants of αS are more prone to forming fibrillar aggregates, these fibrils were initially considered to be causing toxicity. However, in different model systems no clear correlation between toxicity and fibril formation was found. This indicates that small oligomeric precursors rather than fibrils might be the toxic species. In order to investigate which form of αS is causing toxicity, we were making use of a designed variant of αS that stops at the stage of soluble oligomers. This variant is called TP αS (triple proline αS) as it was derived from wt αS by introduction of three proline residues. *In vitro* NMR studies as well as *in vivo* analysis of the aggregation of αS variants tagged with YFP in *C. elegans* muscle cells confirmed that the occurrence of αS fibrils was strongly reduced in TP αS. Next we were assessing toxicity of TP αS in comparison to wt αS as well as PD mutants of αS by investigation of neurite degeneration and impairment of dopamine-associated behavior upon expression of different αS variants in *C. elegans* dopaminergic neurons. We found that TP is the most toxic αS variant, exhibiting the most severe effects on dopaminergic neuron morphology and function. This suggests that indeed the small oligomers rather than αS fibrils are causing toxicity.

So far, the cellular function of αS and the mechanisms causing toxicity remain unclear. It is known that αS binds to intracellular membranes including the mitochondrial membrane. Furthermore, mitochondrial impairment upon expression of wt and mutant αS was reported. Recently it has been reported that several PD-associated genes play a role in mitochondrial dynamics and that loss of function or overexpression leads to morphological changes in the mitochondrial network. Therefore we aimed in understanding how expression of different variants of αS affects mitochondrial dynamics. Thus we were analyzing mitochondrial morphology and distribution by Electron Microscopy as well as Spinning Disk Microscopy. We found that expression of αS in *C. elegans* muscles and neurons leads to mitochondrial fragmentation and severe changes in mitochondrial morphology. Remarkably, similar changes could be observed in aged worms not expressing αS. It is therefore tempting to suggest that increased expression levels of αS accelerate mitochondrial aging.

782C

Transgenic *Caenorhabditis elegans* expressing Alzheimer's pathway proteins to produce β-amyloid toxicity. **Michael L Wood**, Anne Hart. Neuroscience, Brown, Providence, RI., RI.

Neuronal degeneration in Alzheimer's disease (AD) has been associated with intraneuronal aggregation of β-amyloid (Aβ). It has been previously shown that neuronal expression of Aβ in *Caenorhabditis elegans* results in intraneuronal accumulation of the

peptide along with phenotypes indicating toxicity including, defects in learning behaviors, chemotaxis deficits and hypersensitivity to serotonin (Link, 2006; Wu et al, 2006). Aβ is derived from a much larger precursor protein APP which undergoes two cleavage events in the amyloidogenic pathway. Our aim is to create transgenic *C. elegans* that generate Aβ precursor proteins within their neurons. This should lead to the aggregation of Aβ and neurodegeneration. We hope this approach will closely mimic the normal pathway of Aβ generation in an invertebrate model.

783A
Identification of genetic modifiers for amyloid-beta toxicity in a C. elegans Alzheimer's disease model. **Xiaohui Yan**, Adam Knight, Kim Caldwell, Guy Caldwell. Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL.

It is well known that insulin signaling pathways regulating longevity and healthspan are conserved from invertebrates to mammals. In humans, several age-related diseases are associated with protein misfolding or aggregation, including neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Others and we have shown that reduced insulin signaling attenuates the protein misfolding or cytotoxicity associated with poly-glutamine (polyQ) aggregation in HD, amyloid β (Aβ) aggregation in AD, and α-synuclein aggregation in PD C. elegans models. Based on the similarity of the effect of insulin signaling on the modulation of misfolded protein toxicity, we tested the hypothesis that a subset of the aging-associated genetic modifiers of PD-related phenotypes identified in our lab will also work for AD. By using a C. elegans AD model in which temperature-inducible muscle expression of human Aβ42 transgene leads to a reproducible paralysis phenotype upon temperature upshift, we identified seven candidate genes that accelerate Aβ-induced paralysis upon RNAi knockdown. These modifiers include components of ER associated protein degradation (ERAD) and select metabolic factors, including two genes encoding independent but functionally related enzymes, the serine hydroxymethyltransferase (SHMT) and the aminomethyltransferase of the glycine cleavage system (GCS). The reactions catalyzed by SHMT and GCS generate one-carbon units for folate one-carbon metabolism whose normal function supports a variety of cellular activities. This study provides the first evidence of a role for SHMT and GCS in modulating α-synuclein and Aβ related cellular toxicity. Interestingly, prior reports have shown that abnormal homocysteine level could affect epigenetic modulation of AD gene activity and that folate deficiency may also influence Aβ-induced neurotoxicity. Taken together, these findings expand our mechanistic understanding of the well established, but poorly understood, interface between metabolism and age-associated neurodegenerative diseases.

784B
Dysfunctional LRRK2/p38 signaling in response to ER stress leads to dopaminergic neurodegeneration. **Y. Yuan**[1], P. Cao[1], M. Smith[2], K. Kramp[1], Y. Huang[1], X. Zhu[2], S. Siedlak[2], N. Hisamoto[4], K. Matsumoto[4], M. Hatzoglou[3], H. Jin[1], Z. Feng[1]. 1) Dept Pharmacology, Case Western Reserve Univ, Cleveland, OH; 2) Department of Pathology, Case Western Reserve Univ, Cleveland, OH; 3) Department of Nutrition, Case Western Reserve Univ, Cleveland, OH; 4) Department of Molecular Biology, Institute for Advanced Research, Nagoya Solution-Oriented Research for Science and Technology, Chikusa-ku, Nagoya, Japan.

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, which is characterized pathologically by progressive degeneration of dopaminergic (DA) neurons in the substantia nigra (SN). Mutation of leucine-rich repeat kinase 2 (LRRK2) is the leading PD genetic cause, but the underlying molecular mechanisms remain unclear. Multiple roles of LRRK2 may contribute to dopaminergic neurodegeneration. Endoplasmic reticulum (ER) stress has also been linked to PD pathogenesis, but its interactive mechanism with PD genetic factors is largely unknown. Here, we used C. elegans, human neuroblastoma cells and murine cortical neurons to determine the role of LRRK2 in maintaining dopaminergic neuron viability. We found that LRRK2 acts through the p38 pathway to protect neuroblastoma cells and C. elegans dopaminergic neurons from the toxicity of 6-hydroxydopamine and/or human α-synuclein by supporting upregulation of GRP78, a key cell survival molecule during ER stress. A pathogenic LRRK2 mutant (G2019S), however, caused chronic p38 activation that led to death of murine neurons and age-related dopaminergic-specific neurodegeneration in worms. In the substantia nigra of human sporadic PD patients, we found that GRP78 and activated MAP kinases associated with PD pathology and co-localized with LRRK2. These observations establish a critical functional link between LRRK2 and ER stress, and suggest that dysfunctional LRRK2 signaling in response to ER stress is involved in PD pathogenesis.

785C
A genetic screen for modulators of apoptosis and engulfment in C. elegans. **Sheng Zeng**, Michael O. Hengartner. Institute of Molecular Life Sciences University of Zurich, Winterthurerstrasse 190, CH-8057, Zurich, Switzerland.

C. elegans has been used extensively for the study of the molecular mechanisms that control apoptosis and the clearance of apoptotic cells. We and others have previously shown that when cells are placed on the verge of apoptotic death, modulation of the engulfment signaling cascade that affect the extent of cell death: loss of engulfment signaling increases the fraction of cells that survive, whereas overactivation of engulfment signaling reduces cell survival. In order to identify new genes involved in the control of apoptosis, of cell corpse clearance, or in the cross-talk between these two pathways, we performed EMS screen for mutations that alter the number of surviving Pn.aap descendents in a ced-3(rf) background. We identified over 17 independent mutations with reduced Pn.aap cell numbers and over 6 independent mutations that with increased number of Pn.aap cells. Genome-wide sequencing identified candidate loci for several of these mutations. We are in the process of confirming the involvement of the identified genes in Pn.aap survival. We are particularly interested in the mutations with reduced Pn.aap numbers, as these might identify novel cell survival factors.

786A
Role of Nrf2/SKN-1 dependent and ER stress proteins in *C. elegans* models of Parkinson's disease and manganism. Raja Settivari, **Shaoyu Zhou**, Natalia VanDuyn, Jennifer LeVora, Gary Sinclair, Richard Nass. Department of Pharmacology & Toxicology, Indiana University School of Medicine, Indianapolis, IN.

Idiopathic Parkinson's disease (PD) and manganism are oxidative stress-related movement disorders that result in abnormal dopamine (DA) signaling and cell death. Both neurological disorders involve basal ganglia and mitochondria dysfunction, and suggest overlapping epidemiology, yet the origin of the pathogenesis and the molecular determinants common between the diseases are ill-defined. Nrf2/SKN-1 regulates the gene expression of phase II detoxification enzymes, and is upregulated following exposure to oxidative stress in mammals and the nematode *Caenorhabditis elegans* (*C. elegans*). Glutatione-S-transferases (GSTs) of the class pi (GST-π) are Phase II detoxification enzymes that conjugate both endogenous and exogenous compounds to glutathione to reduce cellular oxidative stress. GST-π's as well as ER stress associated proteins have also been implicated in PD-associated DA neurodegeneration. In this study we asked whether the GST-π orthologue GST-1 and ER associated proteins may modulate DA neuron vulnerability following exposure to different PD-associated xenobiotics. We utilized biochemical assays, immunofluorescence, transgenic *C. elegans*, RT-PCR, Western analysis, behavior and neuronal morphology analysis to characterize expression, localization and the role that SKN-1, GST-1 and ER stress-associated proteins play in manganese (Mn) and rotenone-induced DA neuronal death. In this study we show that SKN-1 and GST-1 are expressed in *C. elegans* DA neurons, exposure to either toxicant increases reactive oxygen species and induces GST-1 gene and protein expression, and the induction is largely dependent on SKN-1. We also show that GST-1 inhibits toxicant-induced DA neurodegeneration, as well as toxicant-induced movement deficits. Furthermore, we show that ER stress-associated proteins play a significant role in toxicant induced pathology. We will also describe our preliminary results from a novel genome-wide screen to identify mediators and suppressors of PD-associated DA neuron degeneration. This study provides the first *in vivo* linkage that a reduction in a xenobiotic metabolizing enzyme confers an increase in DA neuron vulnerability in models of PD and manganism. Support contributed by: NIH R011ES014459, NIH R01ES010563 (RN).

787B

How do lin-28 and lin-46 regulate hbl-1? **Jennifer Alaimo**, Kevin Kemper, Bhaskar Vadla, Eric Moss. Department of Molecular Biology, UMDNJ-SOM, Stratford, NJ.

We are investigating the relationship between the heterochronic genes *lin-28*, which controls larval seam cell fates, and *lin-46*, which acts immediately downstream of *lin-28* in an antagonistic manner. Specifically, we want to elucidate the effects of these two genes on the expression of *hbl-1*, a gene that is also involved in seam cell fates. Using the integrated *hbl-1::GFP::hbl-1 3'UTR* fusion construct as a reporter, we found that *lin-28* positively regulates *hbl-1* expression. In wildtype animals, this reporter is strongly expressed in the hypodermis from late embryogenesis and decreases until it is undetectable by the early L3. In a strain lacking the three *let-7* sisters (*let-7s*), *mir-48, mir-84* and *mir-124,* this *hbl-1* reporter was constitutively expressed (Abbott et al. 2005). However, when *lin-28* was removed in this *let-7s(0)* background, the *hbl-1* reporter was downregulated at the normal time. Our lab has also found that in the yeast two-hybrid system LIN-46 interacts with the conserved C-terminal zinc fingers of HBL-1. In fact, when these zinc fingers were expressed in wildtype animals, they displayed a retarded phenotype like *lin-46* null animals. These findings suggest that LIN-46 negatively regulates HBL-1 at the protein level. To further explore the relationships of *lin-28* and *lin-46* with *hbl-1*, we constructed a *lin-28; lin-46; let-7s* mutant strain and found the *hbl-1* reporter was constitutively expressed, consistent with its retarded phenotype (Abbott et al. 2005). Since the *hbl-1* reporter lacks the zinc fingers, it is not clear how LIN-46 effects the reporter's expression. Possibly, this constitutive expression resulted from a feedback loop involving HBL-1 protein. In parallel, we constructed a *lin-28; lin-46* mutant strain containing the same reporter. Surprisingly, the *hbl-1* reporter was expressed constitutively in this strain, and the GFP fluorescence was significantly brighter than wild type. This is in spite of the fact that *lin-28; lin-46* double mutants have wildtype development. These results are even more unexpected because we found that *lin-28; lin-46* mutants have precocious expression of mature let-7, a miRNA which probably targets the *hbl-1* 3'UTR. Furthermore, the *hbl-1* reporter has a dominant *rol-6* marker, whose rolling phenotype was suppressed in this strain. Future experiments aim to determine if the high reporter expression in the *lin-28; lin-46* strain reflects an increase in endogenous *hbl-1* expression. Additionally, a strain will be constructed of *lin-28; lin-46* carrying the *hbl-1* reporter as an extrachromosomal array without the *rol-6* marker. This will rule out any effects from the integrated transgene, as well as any from the *rol-6* marker.

788C

Deciphering the role of the tumour suppressor PAR-4/LKB-1 in cell cycle regulation and asymmetric cell division. **Laura Benkemoun**[1], Julien Burger[2], Nicolas Chartier[1], Lionel Pintard[2], Jean-Claude Labbé[1]. 1) Université de Montréal - IRIC, Montréal, Canada; 2) Institut Jacques Monod - CNRS, France.

We are interested in understanding how cell cycle progression is regulated during asymmetric cell division in developing organisms. The *C. elegans* embryo offers an ideal system to study this problem: the first division is asymmetric and the second division is asynchronous, the anterior cell dividing 2 minutes before the posterior one. Interestingly, in embryos mutant for the polarity gene *par-4*, the first division is asymmetric but the second division is synchronous. This suggests that PAR-4 is a major regulator of cell cycle timing in the early *C. elegans* embryo. PAR-4 is the *C. elegans* ortholog of the human tumor suppressor LKB1, a protein kinase that was shown to regulate cell polarity and cell growth. To elucidate how PAR-4 controls cell division asynchrony during *C. elegans* embryogenesis, we carried out a genome-wide RNAi screen to identify suppressors of *par-4*-mediated embryonic lethality. We found that depleting the gene *mus-101* suppresses *par-4* lethality and cell cycle timing defects at the 2-cell stage, supporting the notion that PAR-4 controls cell division asynchrony by negatively regulating a MUS-101-dependent activity. *mus-101* encodes the *C. elegans* ortholog of yeast Dpb11p and human TopBP1, a protein previously shown to regulate the initiation of DNA replication and the activation of the DNA replication checkpoint. This suggests that PAR-4 controls cell cycle timing through one of these MUS-101-dependent cellular processes. We are currently using various molecular and genetic approaches to identify which of these pathways functions downstream of PAR-4 during cell cycle progression and asymmetric cell division.

789A

Temporal regulation of *rnt-1* in seam stem cell divisions by heterochronic *let-7* family miRNAs. Rachael Nimmo[1], **Toby Braun**[2], Frank Slack[3], Alison Woollard[2]. 1) Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; 2) Department of Biochemistry, University of Oxford, Oxford, United Kingdom; 3) 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. 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, impacting on the cell cycle through negative regulation of the CDK inhibitor *cki-1*. In particular, increased expression of *rnt-1* in seam cells can drive additional symmetrical divisions. However, this effect appears to be restricted to over-expression at the L2 and L3 stages. Here we report the results of our studies into how RNT-1 levels are temporally regulated, partially in response to direct heterochronic signals, to ensure expression is restricted to license only the correct number of divisions.

We found that over the course of larval development, RNT-1 shows two layers of post-transcriptional regulation.

RNT-1 expression levels are decreased at later larval stages and in adulthood. We show here that *rnt-1* mRNA is a direct target of *let-7* family miRNAs, crucial components of heterochronic regulation at late larval stages. We have identified sites within the 3'-UTR of *rnt-1* mRNA that are critical for negative regulation through miRNAs and show that a *rnt-1* mutation can suppress the heterochronic seam cell proliferation phenotype observed in *let-7* family miRNA mutants.

In addition, RNT-1 expression in seam cells is oscillating in synchrony with the cell cycle. Expression is always highest before the seam cell divisions at the beginning of each larval stage and abolished immediately afterwards. It then accumulates again in time for the next division in the following larval stage. The rapid decline immediately after division is likely mediated through active protein degradation.

These results show that the RNT-1/BRO-1 complex acts as a direct link between heterochronic signals, providing stage-specific temporal identities to seam cells during larval development, and the regulation of the cell cycle. In addition, it acts as the target of a likely feedback mechanism by the cell cycle through the regulation of RNT-1 stability.

790B

The *mir-51* family of microRNAs antagonizes multiple microRNA-mediated developmental pathways. **John L. Brenner**, Benedict J. Kemp, Allison L. Abbott. Dept Biological Sciences, Marquette University, Milwaukee, WI.

microRNAs are essential for development. However, in C. elegans, the individual functions of most microRNAs remain largely unknown. We previously identified phenotypes associated with the loss of individual microRNAs in genetically sensitized backgrounds, including loss of *alg-1* activity[1]. Among these phenotypes, we found that loss of *mir-51* family members suppresses phenotypes observed in *alg-1* mutant worms. *alg-1* mutants display developmental timing defects that are similar to *let-7* family mutants. We therefore examined genetic interactions between members of the *mir-51* family and *let-7* family. We found that loss of *mir-51* family members suppressed the developmental timing defects of *let-7* family mutants. Further genetic analysis with other developmental timing genes suggests that the *mir-51* family functions upstream of *hbl-1*. In order to identify direct targets of the *mir-51* family whose up-regulation results in suppression of *let-7* family mutant phenotypes, we used RNAi to knockdown predicted *miR-51* family targets. Since we found that *hbl-1*, a microRNA target, was better regulated in the absence of *mir-51* family members, we examined the effect of loss of *mir-51* family members in other microRNA-mediated developmental pathways. We found that *mir-51* family members function antagonistically to other microRNAs, including *lsy-6* and *mir-240 mir-786*. Our analysis identifies a post-embryonic function for this family of microRNAs in the developmental timing pathway in addition to its function during embryogenesis[2,3]. This analysis also suggests a broader role for the *mir-51* family in microRNA-mediated developmental pathways.

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791C

Examining the kinetics of chromatin modifications associated with dosage compensation onset. **Laura Custer**, Györgyi Csankovszki. MCDB Department, University of Michigan, Ann Arbor, MI.

Dosage compensation (DC) is a specialized gene regulation process to equalize gene expression along the entire X chromosome between XX hermaphrodites and XO males. In hermaphrodites, expression from each X chromosome is down-regulated two-fold to equal the expression of the single male X chromosome. Dosage compensation is achieved by the dosage compensation complex, which localizes to the X chromosomes in hermaphrodites beginning around the time of gastrulation. Our lab has previously shown reduced levels of histone H4 lysine 16 acetylation (H4K16Ac), a mark associated with active transcription, on the adult hermaphrodite X chromosomes in a dosage compensation complex-dependent manner. We have also observed a dramatic increase in the levels of histone H4 lysine 20 monomethylation (H4K20me1) and stalled RNA Polymerase II associated with the X chromosomes in adult hermaphrodites. This study examines chromatin modifications on the X chromosome(s) during development, including time points before and after the onset of DC. We predicted that H4K16Ac levels on the X chromosomes would equal, or even exceed, autosomal levels in pre-gastrulation embryos. However, by quantification of immunofluorescent signals, H4K16Ac is reduced on the X chromosomes of hermaphrodite embryos even before the onset of DC. Male embryos also have reduced H4K16Ac levels on their single X chromosome. H4K16Ac levels remain reduced on the X chromosomes in dosage compensation complex mutants, suggesting that another mechanism is involved in X chromosome silencing in early embryos. Within the adult germline, the X chromosome(s) are silenced by MES-4 and the MES-2/3/6 complex, which resembles the Polycomb repressive complex. Mutations in the *mes* genes restore H4K16Ac levels on the X chromosomes in early embryos to the levels observed on autosomes. Dosage compensation onset coincides with the transition from plasticity to differentiation, a process regulated by the MES proteins. We are examining the relationship between Polycomb's function in X chromosome silencing, its role in differentiation, and the onset of DC. Together our results

describe a time course of H4K16Ac reduction and other chromatin modifications on the X chromosomes during development and suggest that the onset of differentiation coincides with a transition from MES protein-mediated X chromosome repression to DC.

792A

Function of AMPK during the L1 diapause in *C.elegans*. **Emilie Demoinet**, Julie Mantovani, Richard Roy. Biology, McGill University, Montreal, Qc, Canada.

During periods of prolonged nutrient stress, many organisms undergo developmental or reproductive diapause, which are reversible states of developmental dormancy. When starved, *Caenorhabditis elegans* can arrest at multiple points during development. The best characterized of these are the first larval stage (L1) and dauer diapauses. *C. elegans* L1 hatchlings can survive up to 2 weeks in the absence of food but do not initiate post-embryonic development- therefore this L1 arrest is a response to an insufficient level of nutrient to initiate postembryonic development, whereby development is suspended without obvious long-term morphological modification. We have shown that the maximal survival in the L1 diapause requires *aak-2*, one of the 2 homologues of the alpha subunit of AMP-activated protein kinase (AMPK).

AMPK is a metabolic master switch that is activated in response to various nutritional and stress signals. Its main function is to maintain cellular energy homeostasis by up-regulating pathways that produce ATP; while down-regulating energy-consuming anabolic processes. To better understand the role of AMPK in L1 diapause, we identified targets of these protein kinases using both genetic and biochemical strategies. Using differential 2D gel electrophoresis (DiGE) coupled with MS/MS we identified a number of endoplasmic reticulum (ER) proteins that were differentially expressed and phosphorylated between WT and *aak-2* mutant larvae.

The ER regulates calcium homeostasis and the synthesis of secretory proteins, while it is also the site for important maturation steps, including proper folding of nascent polypeptides. The efficiency of this folding depends on appropriate cellular conditions. Various stimuli collectively referred to as ER stress, such as ischemia, hypoxia, oxidative stress, and Ca2+ depletion, can lead to the accumulation of unfolded or misfolded proteins in the ER. This activates an adaptative signaling cascade known as the unfolded protein response (UPR). Our data suggest that AMPK regulates the UPR as a result of ER stress during the L1 diapause. We are currently investigating the molecular mechanisms that underlie this AMPK-mediated regulation.

793B

C. elegans body size is regulated by TGF-β signalling in multiple tissues. **Aidan Dineen**[1], Jeb Gaudet[2]. 1) Molecular and Medical Genetics, University of Calgary, Calgary, Alberta, Canada; 2) Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada.

Control of organ and body size is an interesting biological problem, particularly how growth of different tissues and organs is coordinated to ensure proportional size of all body parts. In *C. elegans*, body size is partially regulated by a TGF-β signaling pathway that also functions in male tail development and is therefore termed the Sma/Mab pathway (for Small and Male abnormal). Loss of function mutations in components of this pathway result in decreased post-embryonic growth, with adult *sma* mutants only achieving ~60-70% the body size of wild type animals. This small phenotype is due to a decrease in cell size, rather than cell number. Previous work has demonstrated that the Sma/Mab pathway can function cell-autonomously in the hypodermis to positively regulate body size. However, many of the components of this signalling pathway are expressed in additional organs such as the intestine and pharynx, raising the question of a possible role for this pathway in these organs. We find that TGF-β signalling is required for normal growth of the pharynx in addition to its previously described role in the hypodermis. We further show that contrary to previous models, the Sma/Mab pathway functions in multiple tissues to control body size. In particular, we find that pharyngeal expression of the R-Smad protein SMA-3 is sufficient to partially rescue both pharynx size and body size defects of *sma-3* mutants. These results suggest that the Sma/Mab signalling pathway can function non cell-autonomously to regulate cell size and therefore body size. In addition, we find that rescue of *sma-3* mutants improves as the number of *sma-3* expressing tissues is increased (e.g. expression of *sma-3* in pharyngeal muscles, pharyngeal marginal cells and the hypodermis provides better rescue than expression in any tissue alone). Overall, our results suggest a model where TGF-β signalling in multiple tissues activates one or more downstream secreted signals that act non cell-autonomously to regulate body size in *C. elegans*.

794C

A genome-wide RNAi screen for let-7 suppressors identifies novel players in the heterochronic pathway. **Matyas Ecsedi**, Magdalene Rausch, Helge Grosshans. Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland.

Timing of developmental decisions in the C. elegans hypodermis and vulva are coordinated by the heterochronic pathway consisting of microRNAs, transcription factors and signaling proteins conserved from worm to human. Accumulation of microRNAs and the following decrease in target levels act as a switch at all larval transitions and in multiple tissues e.g. in the hypodermis. The let-7 microRNA acts as a master regulator in this context by repressing other regulatory genes such as lin-41 and daf-12 at the appropriate developmental time. How the distinct processes required for proper differentiation in the seam cells such as cell-cycle exit, cell-cell fusion and alae secretion are orchestrated is not well defined. Moreover, the regulatory circuits timing proper let-7 expression and possible crosstalk to other heterochronic genes remain to be characterized. To address these questions, we performed a genome-wide RNAi screen in the let-7(n2853) hypomorphic strain showing decreased let-7 levels and function due to a point mutation. Our screen revealed 202 suppressors of the lethal vulval bursting phenotype. The list of these let-7 genetic interaction partners includes genes involved in cell cycle, cell division, signaling, nuclear organization, RNA metabolism, transcriptional and epigenetic regulation of gene expression. In addition to suppression of vulval bursting, 48 genes restored also proper expression of the adult specific col-19::GFP reporter in the let-7 mutant and thus corrected at least in part the heterochronic phenotype. Our results point toward a complex interplay between different layers of gene regulation, signaling and microRNAs in developmental timing. We are currently assessing the effect of the suppressors on let-7 function using GFP reporters expressed in the hypodermis. Our reporter faithfully recapitulates the 3'UTR dependent repression of targets by let-7 in its physiological context at the protein level. Preliminary results indicate that several novel let-7 suppressors restore repression of the target 3'UTR, presumably by directly or indirectly altering let-7 levels and/or activity. We will present the results of the ongoing reporter studies and characterization of endogenous miRNA and target levels in the candidate let-7 regulators.

795A

Identification of heterochronic genes that suppress over-expression of *mir-48*, a *let-7* family miRNA. **Theresa L B Edelman**, Tamar D Resnick, Sarah Malmquist, Ann E Rougvie. GCD, University of Minnesota, Minneapolis, MN.

Heterochronic genes regulate the timing of multiple developmental events in *C. elegans*. Disruption of heterochronic genes results in either the skipping or reiteration of certain events, causing precocious or retarded phenotypes, respectively. Analysis of heterochronic mutants led to the discovery of the first two miRNAs, *lin-4* and *let-7*. The subsequently identified *let-7* family members, including *mir-48*, *mir-241*, *mir-84*, share identity with *let-7* at their 5' ends, suggesting they target common mRNAs. Individual *let-7*-related miRNA mutants have little or no heterochronic phenotype. However, disruption of all three family members results in a retarded hypodermal seam cell defect, indicating these miRNAs function redundantly. In contrast, *mir-48* gain-of-function mutations, isolated from a *lin-4* suppressor screen, cause precocious defects in seam cells. To identify genes that interact with *mir-48*, we performed a screen to isolate suppressors of miR-48 over-expression. Worms that over-express miR-48 from a transgenic multicopy array have precocious defects in seam cells, as well as vulval precursor cells, which cause a failure to lay eggs. Suppressor strains were identified by isolating worms with restored egg-laying. Expected classes of suppressors include new heterochronic genes, miR-48 target genes, and genes that affect miRNA accumulation and function. 36 suppressors were identified from 48,000 haploid genomes screened. Precocious heterochronic phenotypes are suppressed to varying degrees within isolated strains, in both the vulva and the seam cells, suggesting that suppression is not limited to restoration of egg-laying, but rather affects developmental timing globally. Mutations identified so far include 5 alleles of the heterochronic gene *lin-66*, validating this approach for identifying heterochronic genes. Progress on molecular identification of the remaining suppressors will be reported.

796B

WNK-1 negatively regulates formation of diacylglycerol in *C. elegans*. **Takashi Fukuzono**, Kunihiro Matsumoto, Naoki Hisamoto. Grad. Sch. Sci., Nagoya Univ., Nagoya, Aichi, Japan.

WNK kinases are the causative genes of pseudohypoaldosteronism type II (PHA-II) disease, which is a familial autosomal-dominant illness featuring salt-dependent hypertension with hyperkalemia in human. We have previously shown that *wnk-1*, a WNK homolog in *C. elegans*, regulates excretory canal morphology and larval growth in worms. To identify players acting with WNK-1, we screened suppressor mutants for the larval arrest phenotype associated with the *wnk-1* deletion mutation. One of the suppressors, *sowl* (suppressor of *wnk-1* lethality)*-1* exhibits partial embryonic lethality (Emb) and polarity defect (Par) in the two-cell stage embryo. The *sowl-1* gene encodes an acylglycerol O-acyltransferase, which is required for triacylglycerol (TAG) synthesis. The TAG synthesis is important for storage and delivery of fatty acids, raising the possibility that the phenotypes observed in *sowl-1* mutants are caused by the defect in fatty acid metabolism. In fact, we found that addition of TAG or fatty acids was able to rescue the Par phenotype in *sowl-1* mutants. Furthermore, a diacylglycerol (DAG) analog PMA efficiently rescued both Emb and Par phenotypes in *sowl-1* mutants. On the other hand, addition of excess PMA to wild-type animals induced Larval arrest and the defect in the extension of excretory canals, whose phenotypes are similar to those observed in *wnk-1* mutants. These results suggest that WNK-1 negatively regulates DAG formation in *C. elegans*.

797C

Heterochronic genes and Wnt pathway components regulate asymmetric division and cell fate of epidermal stem cells in C. elegans. **Omid F. Harandi**, Victor Ambros. Moldecular Medicine, University of Massachusetts Medical School, Worcester, MA.

Aberrant regulation of spatio-temporal signals may cause the improper cell fate specification of stem/progenitor cells. Asymmetric cell division, and the underlying cell polarity, is a defining property of stem cell self-renewal. Loss of polarity leads to symmetric division, resulting in hyperproliferation of stem cells. Stem cells have been shown to switch between symmetric and asymmetric cell divisions in many systems and therefore the regulation of asymmetric and symmetric fates must be tightly regulated for normal development and tissue homeostasis. The lateral "seam" cells in *C. elegans* are multipotent epidermal stem-like cells aligned along each side of the worm body which produce seam cells, hypodermal cells and neuroblasts. Seam cells undergo an invariant developmental pattern of division, with a single asymmetric self-renewal division at each larval stage, and

specifically in the L2 stage, a single additional round of symmetric proliferative division. Heterochronic genes determine the relative timing of developmental events in *C. elegans* larvae. Mutants of heterochronic pathway display improper transitions between asymmetric and symmetric seam cell divisions, suggesting a role for this pathway in the temporal regulation of asymmetric and symmetric divisions. On the other hand, the Wnt pathway is implicated in cell polarity, self-renewal, somatic cell reprogramming, and tumorigenesis. A noncanonical Wnt pathway, known as "Wnt/β-catenin asymmetry" pathway regulates most of asymmetric cell divisions and polarity in *C. elegans* cell lineages including seam cells. Here, we report that the reduction of POP-1 (TCF) or LIT-1 (Nemo Like Kinase) by RNAi changes the asymmetric cell fates to symmetric cell fates, generating either two seam cells (in the case of *pop-1(RNAi)*) or two differentiating cells (in the case of *lit-1(RNAi)*). The nuclear level of POP-1 is asymmetrically distributed between the seam cell daughters and can be modulated negatively by LIT-1. Therefore LIT-1 has a crucial role in the transmission of Wnt-TCF signals to the asymmetric activity of POP-1. We examined whether the heterochronic genes may interact functionally with POP-1/LIT-1 in the control of asymmetric vs. symmetric seam cell fates. We found that high LIN-14 activity promotes asymmetric seam cell division, perhaps by potentiating the cell asymmetry activity of POP-1/LIT-1, whereas LIN-28 activity seems to promote symmetric seam cell division, perhaps by blunting the asymmetry activity of POP-1/LIT-1. We propose the heterochronic pathway may modulate thresholds for POP-1/LIT-1 activity in the stage-specific transitions between asymmetric to symmetric seam cell fates.

798A

The male tail tip heterochronic regulatory network. **R. Antonio Herrera**, Karin Kiontke, Matthew D. Nelson, Ji-Sup Yang, David H.A. Fitch. Center for Developmental Genetics, Biology, New York University, New York, NY.

The heterochronic pathway was discovered in *C. elegans* through mutations that cause temporal shifts in the development of the seam cells. The seam cells undergo stage-specific cellular divisions early in development followed by fusion and secretion of lateral alae, an adult structure. Examples for genes that regulate the early division patterns of seam cells are *lin-4, lin-14, daf-12*, and *lin-28*. The larval-to-adult transition is regulated by a different set of genes, *let-7, lin-41, hbl-1, lin-29, dre-1,* and the circadian rhythm orthologs *kin-20, lin-42* and *tim-1*.

Here we present evidence for new roles of classic heterochronic genes *lin-4, lin-14, lin-28, hbl-1, tim-1, kin-20,* and *lin-42* in regulating morphogenesis of the male tail tip (TTM). The tail tip is an epidermal tissue that fuses and retracts at L4 in males coincident with seam cell fusion and differentiation. In contrast to the seam cells, which undergo divisions at every larval stage, the tail tip cells are born during embryogenesis and remain seemingly inactive until L4. Previous work has demonstrated that morphogenesis in the tail tip is regulated by *let-7* and *lin-41*, but not *lin-29* (del Rio et al. 2006). *let-7(lf)* and *lin-41(gf)* mutants exhibit retarded phenotypes (un-fused and un-retracted tails), *lin-41(lf)* mutants show precocious phenotypes (early fusion and retraction). Additional late-acting heterochronic genes (*hbl-1, tim-1, kin-20*, and *lin-42*) also have precocious tail tip phenotypes. To our surprise, *lin14* and *lin-28*, genes that act early in seam cell development, also exhibit tail tip phenotypes (precocious), indicating that these genes are needed to prevent premature TTM.

In addition we observed male tail heterochronic defects but no effect on seam cell development in three genes, *lep-2, lep-5* and *blmp-1*. When mutated, *lep-2*, the sole *C. elegans* Makorin gene, and *lep-5*, a new non-coding RNA (see abstract by Vuong et al.), result in severely retarded TTM. Loss of the transcription factor *blmp-1* results in precocious tail tip phenotypes. *blmp-1* CLIP-Seq (modENCODE) showed binding of BLMP-1 2kb or less upstream of all tail tip heterochronic genes except *lep-2* and *lep-5*. This suggests that *blmp-1* plays an important role in the transcriptional regulation of the tail tip heterochronic network. We are currently using epistasis analyses to explore whether the genetic interactions of heterochronic genes known from seam cell development are also found in TTM.

799B

Mechanisms that maintain or re-establish VPC multipotency in dauer larvae. **Xantha Karp**, Iva Greenwald. Columbia University, New York, NY.

Cell fate decisions are made with exquisite temporal precision in *wild-type C. elegans* larvae. Remarkably, this precision is not impaired when development is interrupted by dauer diapause. Progenitor cells in dauer larvae are challenged to "remember" their place in the developmental sequence in order to resume development appropriately upon recovery. Vulval development is a useful system to study this problem. The six vulval precursor cells ("VPCs": P3.p-P8.p) are born in the L1 stage and remain multipotent until the early L3 stage when LET-60/Ras and LIN-12/Notch signaling specifies 1° and 2° vulval fates, followed by cell division. In dauer-interrupted development, specification and cell division occur during the post-dauer L3 stage, after feeding has resumed. Therefore, VPCs remain multipotent throughout dauer diapause.

The existence of a special mechanism that affects the developmental potential of VPCs and their descendants during dauer diapause was suggested by the work of Euling and Ambros (1996), who found that when VPCs divide prior to dauer formation in *lin-28(0)* larvae, their descendents appear to be reprogrammed back to multipotent VPC fate during dauer diapause, as assessed by lineage analysis of post-dauer L3 larvae. In the intervening time, many cell fate markers and other tools have become available allowing for further investigation of this potential phenomenon in *lin-28(0)* as well as in *wild-type*, where no lineage alterations occur.

Our analysis of VPC cell fate markers support the inference that VPCs are reprogrammed in *lin-28(0)* larvae. Furthermore, the dynamic expression pattern of one marker suggests that a comparable mechanism operates in *wild-type* dauers. *lag-2::YFP* is a marker for the 1° fate; in continuous development, it is expressed specifically in P6.p in the L3 stage in response to Ras signaling. We have found that in larvae undergoing the L2-dauer molt, YFP expression appears in P6.p, consistent with specification of the 1° fate, but that expression is lost in dauers, consistent with the re-establishment of multipotent VPC fate. We have examined the effect of mutations in dauer signaling pathways and our analysis suggests that *daf-16* coordinates the dauer formation decision with a the developmental need to prevent precocious VPC specification in dauer larvae.

800C

Comparative transcriptomics in five *Caenorhabditis* species reveals a unique embryonic milestone. **Michal Levin**, Tamar Hashimshony, Itai Yanai. Dept Biol, Technion - IIT, Haifa, Israel.

Embryonic development is broadly characterized by the presence of apparently distinct stages, however their actual distinctiveness and significance remain poorly understood. To address this question, we examined the expression levels of all genes throughout the embryology of five *Caenorhabditis* species. These nematodes are phylogenetically distant and undergo development at different rates, allowing us to probe which developmental stages exhibit transcriptomes independent of these differences. We report evidence that development is punctuated with non-gradual transcriptomic changes. In particular, the difference in time it takes to arrive at the epithelial morphogenesis (ventral enclosure) stage across the species is independent of the transcriptomic difference among them. Thus we provide evidence for the existence of a developmental milestone that is independent of time. The ventral enclosure stage is enriched with the expression onset of Homeodomain and Zn-finger transcription factors and genes involved in muscle and neuronal functions, suggesting that this specific stage is essential for the progression of embryonic development by integrating diverse signals to orchestrate morphogenesis. In addition, genes whose onset of expression occurs in this stage are enriched with RNAi derived phenotypes such as slow development and late developmental arrest, further supporting the coherence of this stage. Our work provides a molecular definition of a developmental stage in terms of its time independent, non-gradualistic and integrative nature.

801A

The *C. elegans period* homolog *lin-42* regulates developmental timing and molting. **Katherine A. McCulloch**, Angela Barr, Ann E. Rougvie. GCD, Univ Minnesota, Minneapolis, MN.

The heterochronic genes in *C. elegans* regulate the timing of developmental events, and the study of developmental timing in worms has led to the discovery of genes and pathways that play conserved roles in timing. The *C. elegans period* homolog *lin-42* is a key player in the heterochronic pathway. In other organisms, period is a critical part of the circadian clock, which synchronizes gene expression and physiology to the light-dark cycle. LIN-42 shares multiple domains of homology with this family of proteins, in particular the PAS domain, which mediates protein interactions. Also like *period*, *lin-42* mRNA and protein levels cycle. In *C. elegans*, *lin-42* expression oscillates with the larval cycle rather than the light-dark cycle, as *period* expression does. The *lin-42* genomic locus is complex, and produces four isoforms. Pre-existing alleles of *lin-42* all left one isoform intact; therefore, the phenotype of *lin-42(0)* animals was unknown. Using Mos-Tc1 technology, we engineered a *lin-42* allele with a 10.2 kb deletion that removes the coding region of each isoform. We have found that *lin-42(0)* mutants have similar phenotypes as previously characterized *lin-42(lf)* alleles; however, phenotypes are more severe in *lin-42(0)*. *lin-42(lf)* and *lin-42(0)* animals have precocious heterochronic defects; for example, the hypodermal seam cells terminally differentiate one stage too early in these mutants. Also like *lin-42(lf)*, null mutants have a molting defect. In *lin-42(lf)* mutants, entry into the molt is delayed and the duration of molts is often extended. In *lin-42(0)*, this phenotype is more severe. These animals not only delay but often fail to complete ecdysis, which results in early larval lethality and marked developmental delay in animals that survive. Our analyses have revealed some new clues of the role of *lin-42* in larval development. From genetic studies, *lin-42* acts in parallel with *lin-28* and in opposition to *daf-12* and let-7-family miRNAs to prevent the L2-to-L3 transition from occurring too early. Furthermore, qRT-PCR analyses indicate that *lin-42* is required to ensure that the miRNA *let-7* is not expressed too early. Recently, several reports have shown that *daf-12* and *lin-28* act in opposition to regulate the accumulation of *let-7*. Interestingly, while *daf-12* regulates *let-7* paralogous miRNAs as well, *lin-28* and *lin-42* specifically regulate *let-7*. Also, reports illustrate that *let-7*, like *lin-42*, regulates molting. Further work is needed to elucidate how *lin-42* regulates *let-7* accumulation, and how this network controls developmental timing and molting. We are pursuing a combination of biochemical, molecular and genetic analyses to further this goal.

802B

ECM Dynamics in the Molting Cycle. **Vijaykumar Meli**, Alison Frand. Biological Chemistry, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA.

The molting cycle involves the periodic synthesis and removal of the collagen-rich exoskeleton, but the underlying molecular mechanisms are not understood. We recently discovered and characterized the novel MLT-10 family of secreted proteins, which together may act as both structural and instructive components of the exoskeleton. Here, we describe the progressive formation of extracellular MLT-10 fibers during the molts, as detected using tagged fusion proteins and quantitative fluorescence microscopy. We also characterize the effects of various *mlt-10* mutations on the organization of cuticle collagens and

development of the stem-cell like lateral seam cells. We have now expanded the scope of our studies on ECM dynamics to include the *fbn-1* gene, which emerged from our previous RNAi screen for molting-defective larvae. The annotated FBN-1 protein is distantly related to human fibrillins, which are the major components of elastic fibrils defective in Marfan Syndrome and related disorders of connective tissue. We have identified four alternatively spliced isoforms of the *fbn-1* gene. Further, we find that *fbn-1* is transiently and reiteratively expressed in the hypodermis during each of the four molts, using a transcriptional gfp-pest fusion gene. Together, these findings suggest that FBN-1 may be incorporated into newly-synthesized matrices during larval development. Consistent with that view, the inactivation of *fbn-1* appears to disrupt the deposition of certain cuticle collagens. Our ongoing research will examine the function and regulation of both MLT-10 and FBN-1 during the molting cycle.

803C
Regulation and function of the *let-7*-related miRNA miR-48 in developmental timing. **Tamar D. Resnick**, Brittany Werre, Ann E. Rougvie. Genetics, Cell Biology, and Development, University of Minnesota, Minneapolis, MN.

The heterochronic genes of *C. elegans* regulate temporal development, ensuring that developmental events proceed in correct sequence and at appropriate times. These genes, along with spatial and sexual regulators, are essential for proper patterning of the adult animal and include the first-identified miRNA genes, *lin-4* and *let-7*. Loss of function of these miRNAs leads to reiteration of certain developmental events and delay of adult tissue differentiation, referred to as a "retarded" phenotype. *let-7* miRNA shares identity at its 5' end with several other miRNAs including miR-48, miR-241, and miR-84, suggesting that they may target an overlapping set of mRNAs. These *let-7*-related miRNAs are expressed earlier in development than is *let-7*. Individual deletion of these genes results in only mild defects, however a triple deletion leads to a strong retarded phenotype, indicating that these miRNAs function redundantly. Inappropriately early and elevated expression of *mir-48* caused by regulatory mutations leads to "precocious" phenotypes in which certain developmental patterns are skipped and subsequent events occur too early, underscoring the importance of proper temporal control of these miRNAs. We have taken advantage of the heterochronic gene pathway as a robust model for understanding regulation of miRNA expression and the relationships of related miRNAs. We are analyzing the *cis*-regulatory elements required for proper expression of miR-48 and miR-241, which is located approximately 1.8 kb upstream. Using mosSCI methods, we have generated lines with single copy transgenes inserted into a consistent, characterized genomic site. We are examining the effects of *cis*-regulatory mutations on the mir-241/mir-48 locus using qPCR to quantify mature miRNA levels directly and phenotypic analysis to determine consequences for developmental timing. To identify additional genes involved in developmental timing and miRNA expression or function, we screened for suppressors of precocious phenotypes arising from *mir-48* over-expression from multicopy transgenic arrays. Among the suppressors recovered were 5 alleles of the heterochronic gene *lin-66*, which has been suggested to regulate LIN-28 in parallel with the *let-7*-family miRNAs. We are investigating the mechanism of this suppression by *lin-66*, including direct testing of whether it is mediated by LIN-28 modulation.

804A
mir-237 is regulated by *lin-14* and functions in early developmental timing. **Mitchell A. Romens**, Benedict J. Kemp, Allison L. Abbott. Biological Sciences, Marquette University, Milwaukee, WI.

In *C. elegans*, appropriate control of two key developmental timing regulators, *lin-14* and *lin-28*, involves the activities of the *lin-4* microRNA and a *lin-4* independent positive feedback loop between *lin-14* and *lin-28*. This *lin-4* independent feedback loop is likely mediated by microRNA activity. However, the regulators of the positive feedback loop have not been identified. We are testing the hypothesis that the *lin-4* family member, *mir-237*, functions in the positive feedback loop. We found that loss of *mir-237* enhances the *lin-46* retarded extra seam cell phenotype, indicating a reiteration of the L2 stage program. This enhancement is dependent on *lin-28* activity. In addition, we found that loss of *mir-237* activity in worms lacking *lin-4* and with reduced activity of *lin-14* results in extra seam cells. We also showed that loss of *mir-237* is able to partially suppress the precocious phenotype of a *lin-14* reduced function allele, indicating that *mir-237* may function downstream of *lin-14*. Interestingly, *mir-237* expression is greatly reduced in *lin-4* mutant worms (Esquela-Kerscher et al., Developmental Dynamics 234:868-877, 2005) in which LIN-14 and LIN-28 protein levels are elevated. Using qRT-PCR we found that in *lin-14* null worms, *mir-237* expression is increased more than 4-fold relative to wild-type controls at the L1 stage. We also show that in *lin-14* gain-of-function worms, *mir-237* levels are substantially reduced relative to wild-type. Similar results are observed using a *mir-237* transcriptional reporter, indicating that *mir-237* transcription is repressed by *lin-14* activity. Our current model is that LIN-14 acts, directly or indirectly, to repress *mir-237* expression during the L1 stage and as LIN-14 levels drop in the L2 stage, *mir-237* begins to be expressed and can then act to repress *lin-28* to control the L2-to-L3 transition.

805B
A recessive gene controls adaptation to a chemically defined medium in C. elegans. **Liusuo Zhang**, L. Rene Garcia. Howard Hughes Medical Institute, Department of Biology, Texas A&M University.

A chemically defined axenic medium, C. elegans maintenance medium (CeMM), has been established for culturing of C. elegans (Lu & Goetsch, 1993). Worms grown on CeMM plates take longer to develop and exhibit a prolonged reproductive period with a decreased brood size (Szewczyk et al., 2006). For N2 animals, about 10% of them develop into adults at day 8 after hatching, whereas the others develop into adults within the following 10 days. Compared to the lab-bred N2 strain, CB4856, a wild Hawaiian strain, takes greater than 16 days to reach adults. We are interested in determining genetic and molecular basis of developmental evolution that promotes animal cells to adapt to alternative nutritional environments. By screening known mutants which are possibly involved in feeding and growth, we observed that hermaphrodites contain mutations in fat-3, lev-11, ser-7, tph-1 and unc-73 grow much faster than N2 worms in CeMM (30-80% mutants reach adults between 5 and 7 days), while daf-2, daf-19, che-2, mdt-15, osm-9, unc-26, unc-32 and unc-42 mutants grow much slower or developmentally arrest in L1 (the majority could not develop into adults by two weeks). Through screening for EMS generated N2 mutant hermaphrodites that grow faster in CeMM, we identified the rg1402, rg1003 and rg804 alleles, which cause 90%, 90% and 75% of the worms to develop into adults on the synthetic medium by day 5, respectively. In addition, we have screened another 10 alleles which could develop to adults between day 5 and 6. Two alleles which cause the worms develop into adults at day 4 were identified through EMS mutagenesis of rg1003. We will serially select and then EMS-mutate hermaphrodites to gradually breed lines that become more adapted and fit for growth in CeMM. Outcrossing experiments demonstrated that enhanced adaptation to CeMM caused by rg1402, rg1003 and rg804 are inherited as a single-locus recessive trait. We are using classical mapping together with whole genome sequence of rg1003 to determine the molecular identity of the recessive allele. The characterization of the fast growth mutants in CeMM will greatly facilitate to systematically analyze the profound impact of nutrition on animal physiology, energy homeostasis and metabolism diseases. Lu N.C. & Goetsch K.M., 1993. Carbohydrate requirement of Caenorhabditis elegans and the final development of a chemically defined medium. Nematologica 39:303-311. Szewczyk N.J. et al., 2006. Delayed development and lifespan extension as features of metabolic lifestyle alteration in C. elegans under dietary restriction. J Exp Biol 209:4129-4139.

806C

Translational control of cyclin B by PUF-8 and GLD-1 is essential for germ cell development. **Priti Agarwal**, Rana Mainpal, Kuppuswamy Subramaniam. Biological Sciences and Bioengineering, Indian Institute of Technology, KANPUR, Uttar Pradesh, India.

Proteins of the PUF family play an important role in germ cell development in diverse organisms. These are RNA-binding proteins that function as translational regulators of their mRNA targets. Earlier results from our laboratory have shown that the C. elegans PUF protein, PUF-8, is essential for the maintenance of germline stem cells (GSCs). PUF-8, along with another RNA-binding protein called MEX-3, directly promotes GSC mitosis. To investigate the underlying mechanism, we disrupted cell cycle-related genes by RNAi on puf-8(-) background and screened for suppression of any of the puf-8(-) phenotypes. One of the phenotypes of puf-8(-) is excessive production of sperm. This phenotype is suppressed by the RNAi-mediated disruption of cyb-2.1, which encodes a cyclin B ortholog. As evidenced by the phenotypes of both cyb-2.1(RNAi) and cyb-2.1(tm2027), a null allele, CYB-2.1 is required for neither GSC mitosis nor spermatogenesis. Consistently, CYB-2.1 does not appear to be produced in both mitotic and meiotic germ cells: transgenic worms carrying the GFP:cyb-2.1 3'UTR transgene, do not express GFP in these cells. In contrast, removal of PUF-8 misexpresses GFP:cyb-2.1 3'UTR in the mitotic germ cells, indicating that PUF-8 suppresses cyb-2.1 translation in these cells via cyb-2.1 3'UTR. Our gel shift assays reveal that the PUF-8 protein is capable of directly binding to cyb-2.1 3'UTR. Further, mutations that abolish this binding misexpress GFP:cyb-2.1 3'UTR in mitotic germ cells. These results show that PUF-8 suppresses cyb-2.1 translation by interacting with its 3'UTR. GFP:cyb-2.1 3'UTR transgene bearing 3'UTR mutations that abolish PUF-8 binding continue to suppress GFP expression in meiotic germ cells. We find that a different RNA-binding protein, namely GLD-1, suppresses cyb-2.1 translation in the meiotic germ cells, again acting via cyb-2.1 3'UTR, but through a sequence that is distinct from the PUF-8-binding sequence. Thus, our results show that both PUF-8 and GLD-1 control cyb-2.1 translation sequentially - PUF-8 in the mitotic region and GLD-1 in the subsequent meiotic region - to prevent premature expression of CYB-2.1. These results support the model that the translation suppression is one of the mechanisms by which germ cells protect themselves from the influence of factors required later during embryogenesis.

807A

Investigating the role of WEE-1.3 in *C. elegans* oocyte maturation. **Anna K. Allen**, Jessica E. Nesmith, Andy Golden. LBG, NIDDK, National Institutes of Health, Bethesda, MD.

Meiosis is a specialized cell cycle essential in all species for the generation of functional gametes. The female gamete, or oocyte, becomes arrested early in development typically during meiosis I, and must be reactivated at a later time point in order to ensure fertilization. This reactivation is termed oocyte meiotic maturation and is required in all female species. We are using *C. elegans* to investigate the mechanisms behind oocyte meiotic maturation. Our lab has previously shown that an inhibitory kinase of the cell cycle, WEE-1.3, is required for proper timing of oocyte meiotic maturation. Upon depletion of WEE-1.3 via RNA interference oocytes mature precociously and although the oocytes encounter sperm, they are not fertilized and the worm becomes sterile. The mechanism by which *wee-1.3* RNAi results in precocious oocyte maturation, potential genetic interactors with *wee-1.3* and the subcellular localization of wild type WEE-1.3 remain unknown. To begin to address these questions we generated transgenic worms containing a translational fusion of WEE-1.3 to GFP. We found that WEE-1.3 exhibits perinuclear localization throughout the germline and distinct punctae in many of the cells comprising the multicellular embryo. We are currently performing colocalization studies in attempt to describe the WEE-1.3 expression pattern in more detail. In order to determine genes that interact with *wee-1.3*, we performed an RNAi suppressor screen utilizing 1874 embryonic lethal clones from the OpenBiosystems RNAi library. From this screen we identified 150 genes that when depleted in conjunction with depletion of WEE-1.3 result in a restoration of fertility. We have begun to confirm the identified suppressors and to characterize the mechanisms through which they suppress the sterility of *wee-1.3* RNAi, which includes analyzing the germlines of suppressed worms to determine the state of oocyte maturation. We will also report on whether any suppressors are affecting the localization of CDK-1, a substrate of WEE-1.3. The strength of all these approaches is its potentiality for identifying novel components of both the cell cycle and oocyte meiotic maturation.

808B

Phenotypic differences between deletion alleles of the histone demethylase *rbr-2*. **Stacy Alvares**[1,2], Ebony Joyner[3], Shawn Ahmed[2,4]. 1) SPIRE Postdoctoral Fellowship Program, UNC Chapel Hill, NC; 2) Department of Biol, UNC, Chapel Hill, NC; 3) Department of Natural Sciences, Fayetteville State University, Fayetteville, NC; 4) Department of Genetics, UNC Chapel Hill, NC.

RBR-2 is a histone 3 lysine 4 demethylase whose mammalian homolog is expressed in regenerative tissues and exhibits dynamic changes at gene promoters during stem cell differentiation [Christensen et al. 2007; Dey et al. 2008]. The *C.elegans* deletion allele *rbr-2(tm1231)* results in increased H3K4me3 and H3K4me2, but inconsistent *rbr-2* phenotypes have been reported: RNAi knockdown of *rbr-2* can extend lifespan [Lee et al. 2003], as can overexpression of *rbr-2* in germ cells, whereas *rbr-2(tm1231)* results in decreased lifespan [Greer et al. 2010]. Moreover, *rbr-2(tm1231)* has low brood size and weakly penetrant multivulval phenotypes, the latter possibly being consistent with a role for *rbr-2* in the SynMuv histone modification pathway [Christensen et al. 2007]. Based on the role of mammalian RBR-2 in stem cell maintenance, *rbr-2(tm1231)* was examined for germ cell maintenance phenotypes. *rbr-2(tm1231)* animals appear smaller than wild-type and show a progressively sterile (Mortal Germline) phenotype when reared at 25°C. Outcrosses confirmed that these phenotypes are tightly linked to *rbr-2(tm1231)*. However, a second allele of *rbr-2*, *ok2544*, did not become progressively sterile at 25°C and did not confer small or multivulval phenotypes. Thus, some phenotypes observed for *tm1231* are either allele specific or due to a tightly linked additional mutation(s).

809C

The role of BEC-1 in germ line development in C. elegans. **Kristina Ames**[1,2], Alicia Meléndez[1,2]. 1) QC CUNY, New York, NY; 2) Graduate Center, New York, NY.

Autophagy is a cytoprotective mechanism defective in numerous pathological conditions including cancer. Autophagy is characterized by the formation of autophagosomes, double membrane vesicles that engulf cytoplasmic components and long lived proteins. Autophagosomes fuse with lysosomes, where sequestered material is degraded. Autophagy is a survival pathway that is involved in adaptation to stress, including oxidative stress and nutrient deprivation. Additionally, its regulation is important in various physiological processes such as cell growth, proliferation and death. *C. elegans bec-1* is the ortholog of *beclin 1/ATG6/VPS30* in mammals and yeast, an important regulator of autophagy. Beclin 1 is a conserved autophagy protein that has been shown to have a role in tumor suppression in mammalian cells. Moreover, its expression is frequently decreased in tumorigenic tissues. *C. elegans* BEC-1 is crucial for viability, development, normal movement, longevity, dauer morphogenesis, and dauer larval survival. In addition, we have recently reported that BEC-1 has a role in endocytosis, specifically in the retrograde transport of MIG-14/Wntless from endosomes to the Golgi network. In the *C. elegans* germ line, the decision for stem cell proliferation vs. meiotic entry is regulated by the GLP-1/Notch signaling pathway. *glp-1* gain of function mutations *(gf)* result in a tumorous phenotype with constant proliferation; thus, this mutant serves as a model to study tumorigenesis in *C. elegans*. We hypothesize that BEC-1 acts in a tumor-related pathway in *C. elegans*. To determine if BEC-1 plays a role in germline proliferation and/or meiotic entry in *C. elegans*, we constructed double mutants with a *bec-1* null mutation and various loss of function and gain of function *glp-1* mutations. We have found that loss of *bec-1* activity suppresses the *glp-1(lf)* phenotype and enhances the *glp-1(gf)* tumorous phenotype. Thus, BEC-1 may act as a negative regulator of GLP-1/Notch activity. BEC-1 may function through its autophagic or endocytic function. We are currently performing additional experiments to better understand the nature of the BEC-1 involvement in germ line proliferation and/or differentiation.

810A

A comparative evolutionary model for the study of meiotic mechanisms. **Joshua Bayes**[1,2], Abby Dernburg[1,2,3]. 1) Howard Hughes Medical Institute; 2) Dept of Molecular and Cell Biology, Univ of California, Berkeley, Berkeley, CA; 3) Life Sciences Division, Lawrence Berkeley National Lab, Berkeley, CA.

Meiosis is a specialized cell division process that reduces a diploid cell to haploid gametes. This evolutionary innovation underlies sexual reproduction and genetic diversity. Conserved features of the meiotic program include homologous chromosome recognition (pairing), establishment of the synaptonemal complex (SC) between paired homologs (synapsis), and formation of physical linkages, or chiasmata, between paired chromosomes through crossover recombination. Despite its important function and wide conservation, many meiotic mechanisms and genes diverge rapidly.

To address why these differences exist and how evolution has tailored the mechanistic details of meiosis among different species, we have initiated efforts to develop the satellite species *Pristionchus pacificus* as a comparative model of meiosis. Cytologically, meiosis in *P. pacificus* closely resembles the progression observed in *C. elegans*, but these nematodes are separated by a large evolutionary distance. *In silico* searches of the *P. pacificus* genome using known meiotic protein sequences from *C. elegans* and other genera have revealed interesting avenues for investigation. The *P. pacificus* genome lacks apparent orthologs of many genes that mediate homolog pairing and synapsis in *C. elegans*, including the pairing center zinc finger proteins characterized by our laboratory. More surprisingly, meiotic recombination mechanisms in *P. pacificus* may also contrast with the pathways elucidated in *C. elegans*. *P. pacificus* appears to express two members of the RecA recombinase superfamily: RAD-51, which is required for somatic DNA repair and meiotic recombination, and the meiosis-specific DMC-1 protein, which is absent in *C. elegans*. Moreover, *P. pacificus* contains genes encoding members of the Hop2 and Mnd1 protein families, which appear to have been lost in *C. elegans*.

To identify additional genes involved in pairing, synapsis, and recombination, a "High incidence of males" screen was carried out. Mutants identified in this screen are currently being genetically mapped and characterized using cytological tools I have developed in *P. pacificus*. Ongoing work will provide insight into the evolution of meiotic mechanisms.

811B

Identification and characterization of direct targets of the *Caenorhabditis elegans* global sexual regulator TRA-1 by chromatin immunoprecipitation. **Matthew R. Berkseth**[1], Kohta Ikegami[2], Jason D. Lieb[2], David Zarkower[1]. 1) Dept. of Genetics, Cell Biology and Development, University of Minnesota, Minneapolis, MN USA; 2) Dept. of Biology and Carolina Center for Genome Science, University of North Carolina at Chapel Hill, Chapel Hill, NC USA.

Caenorhabditis elegans naturally occurs as two highly dimorphic sexes, the XX hermaphrodite and the XO male. Sex is determined by a genetic pathway culminating in the transcription factor TRA-1, the worm homologue of vertebrate GLI proteins. Null mutations in *tra-1* result in hermaphrodite-to-male sex reversal, indicating that TRA-1 and its downstream targets are responsible for generating all sexual dimorphism in the worm.

However, only a few direct TRA-1 targets have been described, and additional biologically important targets likely remain to be identified. To identify TRA-1 target genes throughout the *C. elegans* genome, we have performed chromatin immunoprecipitations using an affinity-purified rabbit polyclonal TRA-1 antibody followed by deep sequencing (ChIP-seq) at several developmental timepoints. We have successfully identified the majority of previously described TRA-1 binding sites with this approach, and have also identified many novel TRA-1 binding sites, implicating nearby genes as putative TRA-1 targets. To examine what role these putative TRA-1 targets may play in sexual development, we have examined their loss-of-function phenotypes using genetic mutants and RNAi. We have also examined the expression patterns of putative TRA-1 targets in both sexes using both simple enhancer-fusion reporters and translational fusion reporters generated by fosmid recombineering. Several putative TRA-1 targets exhibit male-specific expression, consistent with direct transcriptional repression by TRA-1. We have ablated the TRA-1 binding sites in these reporters to determine how TRA-1 binding site ablation affects the expression patterns of these reporters, and in the case of translational fusion reporters whether this altered expression causes sexual differentiation phenotypes.

812C

The eIF4e-binding protein PQN-45 is required for P granule formation and sperm/oocyte switch. Madhu Sharma[1], Gregory M. Davis[1], Hyun-Min Kim[2], Monica P. Colaiacovo[2], **Peter R. Boag[1]**. 1) Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Victoria, Australia; 2) Department of Genetics, Harvard Medical School.

Translational regulation of mRNAs is a key mechanism for regulating gene expression in the gonad. A major mechanism for regulating translation is to prevent the formation of the preinitiation complex by inhibiting the binding of eIF4G to eIF4e by other eIF4e-binding proteins. We have investigated the function of the eIF4e-binding protein PQN-45 and identified that it interacts genetically and physically with a conserved complex of translational regulators including the DEAD box RNA helicase CGH-1 and the RNA-binding protein CAR-1. Strikingly, male *pqn-45(tm2944)* are fertile, while *pqn-45(tm2944)* hermaphrodites are sterile and display a range of abnormalities including gonad bifurcation, precocious oocyte maturation and defects in P granule formation. Immunostaining indicates that CGH-1 and CAR-1 fail to localise to P granules and are diffusely found throughout the goand, while other P granule markers (PGL-1, GLH-2, -4) appear to be less affected in their P granule localisation.

A surprising result was that PQN-45 and CGH-1 are required for normal regulation of the germ cell sex-determination. *pqn-45(tm2944)* mutant hermaphrodites display a temperature sensitive Masculisation of the germline phenotype (Mog). This phenotype is synthetically enhanced in *pqn-45(tm2944):cgh-1(RNAi)* and *pqn-45(tm2944):larp-1(RNAi)* hermaphrodites, but not in *pqn-45(tm2944):car-1(RNAi)*. We have identified the mRNA of the key sex-determination factor *fem-3* is associated with the CGH-1 RNA-protein (RNP) complex. We have also identified the RNA-binding protein and *fem-3* mRNA translational regulator, LARP-1, is associated with the CGH-1 RNP complex. Our hypothesis is that the *fem-3* mRNA is translationally repressed during the adult hermaphrodite stage through the co-ordinate activity of the LARP-1/CGH-1/PQN-45 and FBF/NOS-3 RNP complexes.

We propose a model in which PQN-45 and CGH-1 function as broad-scale cap-dependant translation inhibitors and are targeted to specific mRNAs via their interaction with individual RNA-binding proteins.

813A

Implication of microRNA pathway in C. elegans germline biogenesis. **Syed Irfan Ahmad Bukhari[1]**, Alejandro Vasquez-Rifo[1], Monique Zetka[2], Jean-Yves Masson[1], Martin J Simard[1]. 1) Laval University Cancer Research Centre, Quebec City, Quebec, Canada; 2) Department of Biology, McGill University, Montreal, Quebec, Canada.

Small non-coding RNA pathways have been reported to assume important roles in the regulation of nearly all developmental and cellular processes. However, the contribution of the microRNA pathway in the germline remains poorly characterized. We therefore decided to address whether this small non-coding RNA pathway plays an important role in germ cell formation using C. elegans as animal model. We first observed that the loss-of-function of key players in the microRNA pathway lead to severe defects in animal fertility. Animals carrying null alleles of Drosha and Dicer are sterile while animals lacking the Argonaute alg-1 and alg-2 genes display various germline defects. An extensive analysis of the germline of these argonaute mutants reveals defects in the gamete formation resulting in reduced brood size. We observed that ALG-1 and ALG-2 proteins are localized to the distal tip cell or DTC, a specialized cell located at the tip of both gonadal arms that regulates mitosis-meiosis transition. Re-establishing the expression of these proteins in the DTC partially rescue the germline defects observed in mutant animals. Our data suggests the paramount importance of the microRNA pathway in the C. elegans germline biogenesis. Using germline-specific microarrays, genetic and in silico analysis, we are currently attempting to identify the specific regulatory pathway in germ line which is targeted by microRNAs. This work is supported by the Natural Sciences and Engineering Research Council of Canada (MJS and JYM).

814B

Mutations that Interact with the Tip60 HAT Complex Can Suppress SHE-1. **Xiangmei Chen[1]**, Yiqing Guo[2], Ronald Ellis[2]. 1) Graduate School of Biomedical Sciences, UMDNJ-SOM, Stratford, NJ; 2) Dept Molec Biol, Univ Med & Dentistry NJ, Stratford, NJ.

Comparing evolutionary changes that occurred in several independent lineages can help reveal their underlying mechanisms. Phylogenetic studies suggest that hermaphroditism evolved independently in the nematodes *C. elegans* and *C. briggsae*. In *C. briggsae*, *she-1* is a novel gene that promotes hermaphrodite spermatogenesis, since *she-1* mutants develop as male/female strains. To learn what genes interact with *she-1* to control hermaphroditic development, we isolated 5 recessive and 7 dominant suppressors.

Two of these suppressors might act with the Tip60/NuA4 Histone Acetyl-Transferase complex to regulate germ cell fates. First, the recessive mutation *v92* caused 81% synthetic lethality with *trr-1(RNAi)*, which targets the Tip60/NuA4 HAT complex. By contrast, we see almost no lethality in the single mutants. Furthermore, *v92* suppressed the feminization normally caused by *trr-1* in 7% of the surviving animals. We saw an even stronger interaction using RNAi against *mys-1*, another component of the Tip60/NuA4 HAT complex. Although neither *trr-1* nor *mys-1* RNAi cause lethality on their own, completely inactivating this complex is lethal. Thus, we suspect that *v92* works with the Tip60 complex to carry out an essential function.

Second, the dominant mutation *v99* suppressed the feminization caused by *trr-1* in 3% of the animals, and suppressed that caused by *mys-1* in 32% of the animals. Although *v99* caused a low level of lethality on its own, it did not show synthetic lethality with *trr-1* or *mys-1*. Thus, we suspect its activity might be limited to controlling germ cell fates.

To identify the genes these suppressors define, we are now cloning *v92* and *v99*. By using SNPs, we have mapped *v92* to a 600 kb region on LG*II*, and are testing candidate genes. The *v99* mutation maps to a larger region on LG*III*.

815C

Loss of ALS-associated protein VAPB/ALS8 increases apoptosis in the germ line. **P. Cottee**, J. Vibbert, S. Han, M. Miller. Dept Cell Biol, Univ Alabama at Birmingham, Birmingham, AL.

Sperm motility and oocyte maturation in C. elegans are dependent on the Major Sperm Protein (MSP). MSP is secreted from sperm and binds to the Eph receptor VAB-1 and other unknown receptors expressed on oocytes and sheath cells. The MSP domain is an evolutionarily conserved motif found in a number of proteins. A point mutation (P56S) in the MSP domain of human VAP/ALS8 has been linked to the neurodegenerative disease amyotrophic lateral sclerosis (ALS) and late-onset spinal muscular atrophy (Nishimura et al., 2004). ALS patients and ALS mouse models exhibit reduced VAPB expression, suggesting that VAPB plays a widespread role in pathogenesis. Previous work has shown that the VAPB MSP domain is cleaved, secreted, and acts as a ligand for Eph receptors. The P56S mutation causes protein aggregation and failed secretion (Tsuda et al., 2008). VPR-1 is the worm VAPB homologue. Loss of this protein in the worm recapitulates many of the pathologies observed in ALS patients, including mitochondrial dysfunction and lipid metabolism defects. Further, vpr-1 null mutant animals are sterile due to germ cells failing to proliferate and differentiate. Pan neuronal expression of VPR-1 in null mutant animals partially rescues the metabolic defects, and partially rescues the germ-line defect in some animals while causing tumors in others. Examination of the sterile phenotype has shown the distal tip cells of VPR-1 null mutant animals are enlarged and have shorter processes compared to those of wild-type animals. The morphology of sheath cells in null mutants is also severely disrupted. Using apoptosis markers, including a sheath cell driven CED-1::GFP transgene, we have observed an increase in germ line apoptosis in vpr-1 mutant hermaphrodites. Silencing of selected genes involved in the physiological and DNA-damaged induced apoptosis pathways suggests that the increase in apoptosis is of a physiological basis rather than from DNA damage. Given the sheath cell defect, we are currently examining if these cells are capable of clearing apoptotic cells. My current goal is to delineate the basis of the apoptosis defect and to identify genes that modify the apoptosis phenotype.

816A

The sexually dimorphic germline stem cell niche. **Sarah Crittenden[1]**, Ipsita Mohanti[2], Dana Byrd[2], Karla Knobel[2], Judith Kimble[1,2]. 1) Howard Hughes Medical Institute; 2) Department of Biochemistry University of Wisconsin-Madison, Madison, WI.

Germline stem cells (GSCs) maintain the adult germline tissue in both sexes. Distal tip cells (DTCs) contribute to the niche for GSCs in both sexes, but the DTCs are sexually dimorphic with respect to cell number (1 per gonadal arm in hermaphrodites, 2 in males) and expression of the Notch ligand, LAG-2 (high in hermaphrodites, low in males (Chesney et al., 2009)). Moreover germ cells within the niche are sexually dimorphic: the spermatogenic male germ cells divide two times faster than oogenic hermaphrodite germ cells throughout the mitotic zone (D. Morgan et al., 2010). The male GSC niche therefore provides a distinct but related mode of GSC control.

In adult hermaphrodite germlines, which are oogenic, the distal tip cell (DTC) and Notch signaling maintain an immature, stem-cell-like state in germ cells that reside within 6-8 cell diameters from the distal end (Cinquin et al., 2010). We have now identified a similar distal pool of stem-cell-like germ cells in males that reside within 11-13 cell diameters of the distal end. The distal pool, which extends further from the distal end in males, also contains more germ cells (hermaphrodite: 45-70 gc, male: 60-91 gc). Therefore, the distal pool of males has distinct properties and may have distinct modes of regulation.

Characteristics of DTC cellular architecture correlate with the distal pool. The hermaphrodite DTC cell body forms a cap covering the first 4 rows of germ cells; this cap extends both short intercalating processes to embrace germ cells within the first 9 rows from the distal end as well as longer processes along the surface nearly to the point of meiotic entry. We have found that male DTCs also have a cap and short intercalating processes as well as longer superficial processes. Our model is that the cap defines the region of highest Notch signaling and that intercalating processes may also anchor the GSCs distally.

Knowledge of the Notch ligands expressed by the DTC in each sex is essential for

understanding the niche. We are currently analyzing the battery of DSL ligands for both expression and function in the DTCs of both sexes.

817B

Caenorhabditis elegans' GLB-12 regulates germline apoptosis levels and vulval development. **S. De Henau**[1], L. Tilleman[2], D. Hoogewijs[3], L. Moens[2], S. Dewilde[2], J.R. Vanfleteren[1], B.P. Braeckman[1]. 1) Center for Molecular Phylogeny and Evolution, Ghent University, Ghent, Belgium; 2) Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium; 3) Institute of Physiology and Zürich Center for Integrative Human Physiology ZIHP, University of Zürich UZH, Zürich, Switzerland.

Caenorhabditis elegans has the surprisingly high number of 33 globins. These globins are diverse in both gene and protein structure and are localized in a variety of cells, mostly neurons. Here, for the first time, we show that a globin, GLB-12, is actively involved in the regulation of developmental processes, namely in the regulation of germline apoptosis levels and vulval formation. This is demonstrated by the following results. Firstly, RNAi against *glb-12* in RNAi-hypersensitive strains, such as *nre-1(hd20) lin-15B(hd126)*, causes severely reduced fecundity and, in accordance with this observation, increased levels of apoptosis in the germline. This indicates that GLB-12 is an active inhibitor of germline apoptosis. Furthermore, *glb-12*(RNAi) also induces a protruding vulva (58% of adult worms) and developmental abnormalities (15% of adult worms). Secondly, using a translational reporter, we observed expression of GLB-12 in several head and tail neurons as well as in the nerve cord. Interestingly, this globin is also expressed in the developing vulva and in the gonadal sheath cells of the adult hermaphrodite, which may be related to the protruding vulva phenotype and the increased levels of apoptosis of the *glb-12*(RNAi) worms respectively. Thirdly, both major *glb-12*(RNAi) phenotypes, the reduced fecundity and the protruding vulva, seem to be regulated through a Ras/MAPK signaling pathway, as worms defective in this pathway do not show these phenotypes. Lastly, we have found that applying *glb-12*(RNAi) to the N2 bristol strain - in which neurons are less sensitive to RNAi - causes neither a protruding vulva nor developmental abnormalities. However, the RNAi *does* cause a reduction in the fecundity of the worm, though not to the same extent as seen in the RNAi-hypersensitive strain. In addition, applying *glb-12*(RNAi) to the *glb-12* translational reporter, which can be considered a wild type, showed that GLB-12 is only absent in the gonadal sheath; it is still present in the nerve cells and in the developing vulva. These results indicate that GLB-12 is active in both neuronal and non-neuronal tissues, and that its effect in both tissue types seems to be cumulative. GLB-12, thus, functions as a repressor of germline apoptosis and a regulator of vulval development and seems to act through the Ras/MAPK pathway.

818C

PCH-2's Role in Regulating the Synapsis Checkpoint of Meiosis I in Caenorhabditis elegans. **Alison J. Deshong**, Needhi Bhalla. University of California Santa Cruz, Santa Cruz, CA.

Meiosis occurs when diploid cells undergo one round of DNA duplication and two rounds of chromosome segregation. In Caenorhabditis elegans, the resulting haploid gametes go on to combine, creating the next generation. Proper chromosome segregation requires pairing of homologous chromosomes; this pairing is then stabilized by the formation of the Synaptonemal Complex (SC), a protein structure that facilitates homologous recombination. Failure of chromosomes to segregate properly may lead to aneuploidy and birth defects. Two distinct checkpoints can be activated to cull aberrant meiotic nuclei. The DNA damage checkpoint and the synapsis checkpoint monitor successful completion of recombination and synapsis respectively. The pch-2 gene was identified in C. elegans as a homolog of the pch-2 gene in yeast. Both genes appear to regulate meiotic progression in response to defects. In C. elegans, PCH-2 localizes to the SC and is required for the synapsis checkpoint; however, little is known of what specific role PCH-2 plays. Sequence analysis indicates that PCH-2 belongs to the diverse AAA protein family. This protein family is known to contain ATPase domains, the catalytic function of which is used to disrupt protein-protein or protein-DNA interactions. We wish to ask the question 'What proteins are PCH-2 interacting with, in vivo, to accomplish its role in the synapsis checkpoint?' To answer this question we will perform immunoprecipitations on whole worm lysates using a PCH-2 antibody and identify interacting proteins by mass spectrometry analysis. These results will allow us to further elucidate PCH-2's function at the synapsis checkpoint in C. elegans.

819A

Characterization of new sex determination alleles in *C. briggsae*. **Jill A. Dewar**[1], Carlos Carvalho[2], Paul Stothard[1], David Pilgrim[1]. 1) University of Alberta, Edmonton, Canada; 2) University of Saskatchewan, Saskatoon, Canada.

The *Caenorhabditis* genus of nematodes uses a male-female (XX/XO) system of reproduction, with the exception of *C. elegans* and *C. briggsae*, which have independently evolved into hermaphrodite-male species. Since the 'basic' sex determination molecular signaling pathway is apparently intact among the genus, these two species provide us with the opportunity to understand the effects of evolutionary pressures on essential signaling pathways. Although this process is relatively well understood in *C. elegans*, we aim to better understand the mechanisms of homologous genes in *C. briggsae*, and to identify points of divergence between the species. To identify new alleles of genes involved in this process, we performed a suppressor screen on the allele *tra-2(ed23ts)*, and have isolated a number of mutations which map to *fem-1, fem-2* and *fem-3*. A number of these lesions have already been characterized, and we are currently working to determine whether molecular interactions or enzymatic activity are disrupted in the missense alleles. This information will allow for a comparison of structure/function between species, and will be essential to our understanding of signaling pathway evolution.

820B

Suppressor of *fog-1(q253)* rescues fertility in hermaphrodites but not in males. **Kristin R. Douglas**, Kimberly L. Butnik. Dept Biol, Augustana Col, Rock Island, IL.

Germ cell sex determination in *C. elegans* is determined by a complex regulatory network including a number of proteins involved in translational regulation. One such protein, FOG-1, acts at the end of the genetic hierarchy and is required for spermatogenesis. All germ cells in *fog-1* mutants become oocytes, even in males. FOG-1 is a member of the Cytoplasmic Polyadenylation Element Binding (CPEB) protein family of RNA binding proteins, which, in other model systems, have been shown to regulate translation of target messages. FOG-1 regulators and target messages are currently unknown.

To better understand how FOG-1 promotes spermatogenesis, we identified dominant suppressors of *fog-1(q253)*. We expect to identify proteins that interact with or regulate FOG-1 with this screen. One suppressor allele, *aug2*, rescues the spermatogenesis defect and restores fertility in hermaphrodites. However, *aug2* males make sperm but are infertile. Additionally, some males produce a small number of oocyte-like cells. Identification of the gene mutated in *aug2* animals will further elucidate the mechanism of FOG-1 action in germ cell sex determination.

821C

Analysis of HIS-73, a Sperm Specific Histone H3 Variant, Uncovers a Link between Sperm Signaling and TGF-β Signaling. **T Matthew Edwards**, Christopher Bean, William G Kelly. Department of Biology, Emory University, Atlanta, GA.

Histone H3 variants have been shown to be important in numerous biological processes, including development. Examples of such are the testis specific histone variants found in mammals. We have focused on one such histone variant in *C. elegans*, HIS-73, which is a highly divergent histone H3 in its N-terminal sequence. We have shown that HIS-73 expression is limited to sperm at both the mRNA and protein level. Interestingly, the in situ pattern suggests that his-73 expression is confined to post-meiotic sperm. A deletion allele, *his-73(tm1643)*, exhibits a reduction in brood size as well as an egg-laying and a ovulation rate defect. We have demonstrated that *his-73* mutant sperm alone are capable of causing these phenotypes. Additionally, we also observed a genetic interaction between *his-73* and *htas-1*, a sperm specific H2A variant, supporting the idea that HIS-73's functional role is in sperm chromatin.

While investigating the egg-laying rate defect, we uncovered a genetic link between HIS-73 and the TGF-β (DAF-7) signaling pathway. Canonically, the DAF-7 pathway has been shown to play two roles: first during larval stages in the dauer decision and subsequently in the adult sensing of environmental cues to limit egg-laying. A mutation in a downstream target of DAF-7, *daf-3(e1376)*, is able to suppress the brood size, egg-laying rate, and ovulation rate defects seen in *his-73(tm1643)*. We went on to show that this suppression is not occurring within the sperm itself, but rather within the hermaphrodite, suggesting an interesting interplay between environmental and sperm signals.

Our working model for HIS-73's role in sperm is that it may replace canonical H3 and prevent compaction in specific regions, and thus allow efficient expression of genes that are necessary for normal sperm function. A likely candidate is the major sperm protein (MSP) family, which plays a role in regulating the rate of ovulation. We are currently working to directly test this model. We hope this data will provide interesting insight into a role for chromatin in sperm development and fertility as well as insights into links between sperm signaling and TGF-β signaling.

822A

mel-28 Genetic Interaction Screen Reveals Networks Underlying Germ-line Development in *C. elegans*. **Anita G. Fernandez**[1], Emily Mis[2], Neha Kaushik[1], Matthew Fasullo[1], Fabio Piano[2]. 1) Dept Biol, Fairfield Univ, Fairfield, CT; 2) NYU Center for Genomics and Systems Biology, New York, NY.

Many developmental processes require the collaboration of multiple gene products. MEL-28/ELYS is a large AT-hook protein required for nuclear envelope integrity and chromosome segregation in metazoans. Although *mel-28* is a strict maternal-effect lethal gene, it is expressed at high levels throughout the germ line as well as in the early embryo, yet *mel-28* animals have no defects in germ-line function and produce wild-type numbers of embryos. We predicted that other molecules might buffer the germ-line function of MEL-28. To identify additional proteins working with MEL-28 we performed a high-throughput genetic interaction screen seeking synthetic sterile phenotypes in *mel-28* homozygous animals using RNAi. We used RNAi to test ~92% of the genes in *C. elegans* genome in *mel-28* mutants and in wild-type animals. We found multiple genes that cause synthetic sterility when RNAi-depleted in a *mel-28* mutant background. These include genes that encode factors required for translation, nuclear envelope components, and chromatin organization factors, among others. Our results genetically dissect the pleiotropic functions of a maternal-effect gene and show the interplay between *mel-28* and several critical processes required during germ-line development.

823B

IFE-1: A Key Regulator of Germ Cell Protein Synthesis. **Andrew J. Friday**, Jacob Subash, Melissa A. Henderson, Brett D. Keiper. Biochemistry, Brody School of Med at ECU, Greenville, NC, 27834.

Gamete development is governed largely by regulated translation initiation on stored

mRNAs. The rate limiting step is their recruitment by translation initiation factors (eIF's) to polyribosomes. eIF4E isoforms are the first translation factors to interact with mRNAs, specifically recognizing their methylated guanosine cap structures. Our lab has shown that three of the five *C. elegans* eIF4E isoforms (IFE-1-5) recruit unique subsets of mRNAs. Consequently, individual IFE gene knockouts result in unique phenotypes in the soma and/or germ line. IFE-1 is the only isoform found in P-granules and associated with PGL-1 in the germ line. Loss of IFE-1 causes temperature-sensitive sterility due to defective cytokinesis in secondary spermatocytes as well as diminished oogenesis and reduced viability of oocytes. Reintroduction of flag-tagged IFE-1 into the *ife-1*(bn127) null mutants caused a partial recovery of fertility and gametogenesis phenotypes. In order to further understand the mechanistic role of IFE-1 in mRNA translation during oocyte and spermatocyte development, polysomal microarray analyses were performed to identify the unique set of mRNAs recruited by IFE-1. Current evidence suggests that key post-meiotic steps in gametogenesis are regulated by IFE-1 action on a specific subset of mRNAs.

824C

PUF-8 is an Inhibitor of Germ Line Stem Cell Proliferation. Hilary E Racher, **Laura R Gauthier**, Dave D Hansen. Biological Sciences, University of Calgary, Calgary, Alberta, Canada.

Understanding how stem cell populations are maintained is an important biological problem. Using the germline stem cells (GSCs) in the distal *C. elegans* germ line as a model, this work contributes to the understanding of stem cell maintenance by characterizing the role of PUF-8 in regulating the balance between self-renewal and differentiation. PUF-8 is a member of the conserved PUF family of RNA-binding proteins that regulate the expression of target mRNAs by binding to their 3' UTRs. PUF-8 is one of twelve *C. elegans* Pumilio homologues and has been shown to regulate spermatogenesis (Subramaniam and Seydoux, 2003), germline sex determination in hermaphrodites (Bachorik and Kimble, 2005), vulva development (Walser *et al.*, 2006), and to promote mitotic cell cycle procession (Ariz *et al.*, 2009; Bachorik and Kimble, 2005). We have found that *puf-8* also inhibits stem cell proliferation. *puf-8* mutants do not have excess stem cell proliferation on their own; however, *puf-8(0)* strongly enhances germline over-proliferation in a *glp-1(gf)* genetic background, resulting in a very robust germline tumour. Genetic analysis reveals that *puf-8* does not function in either of the *gld-1* or *gld-2* pathways that function downstream of GLP-1/Notch signaling to control the balance between self-renewal and differentiation. Rather *puf-8* likely functions genetically upstream of *gld-1* and *gld-2* as a negative regulator of mitosis-promoting components. The tumours formed in *puf-8(0)*; *glp-1(gf)* mutant animals are due to a failure of proliferating germ cells to enter meiotic prophase, rather than meiotic cells dedifferentiating into proliferative cells. The PUF-8 protein is most highly expressed in the distal germ line, with highest expression mid-way through the mitotic zone. mRNAs bound by PUF-8 are currently being characterized via co-immunoprecipitation in order to identify PUF-8's important translational targets. This work identifies PUF-8 as a key regulator in controlling stem cell proliferation in the *C. elegans* germ line. Understanding PUF-8's role in this process will assist in understanding how stem cell populations are controlled in general.

825A

The MES proteins cooperate to influence gene expression patterns in the *C. elegans* germ line. **Laura Gaydos**[1], Andreas Rechtsteiner[1], Coleen Carroll[2,3], Wenchao Wang[2,4], Susan Strome[1,2]. 1) MCD Biology, UC Santa Cruz, Santa Cruz, CA; 2) Department of Biology, Indiana University, Bloomington, IN; 3) AIT Laboratories, Indianapolis, IN; 4) Dana-Farber Cancer Institute, Boston, MA.

In *C. elegans*, the MES proteins are required for germline viability, which is necessary for producing the next generation and for the propagation of species. The MES proteins modify histone tails on nucleosomes and influence gene expression. MES-2, MES-3 and MES-6 form a trimeric complex and are responsible for methylation of histone H3 on lysine 27 (H3K27) in the germ line. MES-2 and MES-6 have homologs in *Drosophila* and mammals, which are part of the Polycomb Repressive Complex 2. MES-4 also has homologs in other species and is responsible for H3K36 methylation in the germ line of *C. elegans*. We want to figure out the gene targets of MES regulation and whether MES-2/3/6 and MES-4 have the same or different gene targets. To do this, we performed microarray analysis of mes mutant germ lines along with genetic tests. We found that *mes-2; mes-4* double mutants are sterile a generation earlier than either single mutant and that MES-2 and MES-4 influence expression of some of the same genes. These results suggest that MES-2/3/6 and MES-4 cooperate to influence gene expression. Genes down-regulated by the MES proteins in the germ line include ubiquitously expressed genes on the X and somatically expressed genes on the autosomes. Genes up-regulated by the MES proteins in the germ line include germline-specific genes on the autosomes. These findings suggest that the MES proteins are important for reducing expression of genes not tolerated at high levels during germline development, and enhancing expression of germline genes. Interestingly, we found that the fertility of *mes-2*, *mes-3*, and *mes-6* males depends on the gamete source of the X chromosome, which underscores the important role that MES-2/3/6 serve in regulation of genes on the X chromosome.

826B

Phenotypic characterization of the tropical hermaphroditic species *Caenorhabditis* sp. 11. **Clotilde Gimond**, Nausicaa Poullet, Alessandra Mauri, Nicolas Callemeyn-Torre, Stephanie Grimbert, Paul Vigne, Celine Ferrari, Christian Braendle. Institute of Developmental Biology and Cancer, CNRS- University Nice Sophia-Antipolis, Nice, France.

The majority of *Caenorhabditis* species exhibits a male-female mode of reproduction, and hermaphroditism has evolved only twice, in *C. elegans* and *C. briggsae.* Recently, a third hermaphroditic species, *C.* sp. 11, has been identified by Marie-Anne Félix (strain JU1373, La Réunion). Since then, the isolation of more than 30 additional *C.* sp. 11 wild isolates from La Réunion, Cap Verde, Puerto Rico, Hawaii, Guadeloupe and mainland South America (French Guiana, Brazil) indicates that this species occurs primarily, if not exclusively, in tropical regions. Here we present the results of our current phenotypic characterization of recently collected wild isolates of *C.* sp. 11 with the aims (i) to quantify the degree of intraspecific variation and its correlation with geographic origin, (ii) to test for plasticity and genotype-by-environment interactions in reproductive traits at different temperatures and (iii) to ask how *C.* sp. 11 phenotypic characteristics differ from the ones observed in *C. elegans* and *C. briggsae*. This survey focuses mainly on germline and reproductive features (e. g. germ cell number, sperm number and size, offspring number) and basic life history traits (e.g. male production, propensity to enter dauer, longevity). Our initial observations confirm that *C.* sp. 11 isolates are overall much more heat-tolerant (and less cold-resistant) than *C. elegans* isolates, e.g. they maintain their reproductive output at 27°C similar to tropical *C. briggsae* strains. Interestingly, there is also substantial variation in reproductive output among different *C.* sp. 11 isolates, and many isolates have a much reduced offspring number relative to N2 and AF16. This reduction in offspring number correlates with a much reduced sperm and germ cell number, and we are currently testing to what extent germline size and reproductive output are modulated by temperature.

827C

The role of microtubules in regulating dynamic RNP granules in aging or stressed oocytes. **Kevin T Gorman**, Tiffany Makowski, Jennifer Schisa. Central Michigan University, Mt. Pleasant, MI.

When sperm become depleted in *C. elegans* hermaphrodites, ovulation arrests and oocytes accumulate in the gonad arm. During meiotic arrest, the distribution of RNA and RNA-binding proteins changes dramatically in oocytes. Several of these molecules transition from being uniformally distributed throughout the cytoplasm, to forming large cytoplasmic foci known as ribonucleoprotein (RNP) granules during arrested ovulation or environmental stresses. The function of RNP granules has yet to be elucidated, but they are hypothesized to protect maternal mRNA during periods of arrest or stress. Our lab has been conducting an RNAi screen to identify genes that are required for the assembly of RNP granules in meiotically-arrested oocytes, and we have identified over 50 genes to date that are necessary for the RNA-binding protein MEX-3 to assemble into large granules. We are currently characterizing these genes and exploring the gene families identified in the screen. One interesting finding was the beta-tubulin genes, ben-1 and tbb-1. Previous work by the Greenstein lab has shown that microtubules become cortically enriched in meiotically-arrested oocytes[1], and this localization is similar to that of RNP granules. Interestingly, in yeast and mammalian cells, disruption of microtubules stimulates the formation of P bodies (processing bodies), while such disruption results in a failure of stress granules to form. Taken together, these findings suggest the possibility that microtubules have a role in regulating the assembly of oocyte RNP granules. We are currently taking a pharmacological approach to complement the RNAi studies and assessing the role of microtubules in the assembly and dissociation of RNP granules in both meiotically-arrested and stressed oocytes. Our overall goal is to better understand the role of the cytoskeleton in the dynamics of RNPs in oocytes. [1]Harris, J.E.,et al., 2006. Major sperm protein signaling promotes oocyte microtubule reorganization prior to fertilization in Caenorhabditis elegans. Dev. Biol. 299, 105-121.

828A

Loss of a nuclear hormone receptor unravels diet-dependent germline sterility. **Xicotencatl Gracida**, Christian R. Eckmann. Max Planck Institute for Cell Biology and Genetics, Dresden, Germany.

The development of a tissue is tightly coordinated with the availability of nutrients. In *C. elegans*, the connection of germline development and nutrition starts in the first larval stage, when initial mitotic activity of the primordial germ cells, Z2 and Z3, depends on the nutritional status of the larvae. Further dependence on nutritional inputs for germ cell proliferation[1] is also evident in later larval stages, and in the adult under starvation conditions[2]. In metazoans, coordination of homeostasis, metabolism and development is largely achieved by nuclear hormone receptors NHRs, which are transcription factors typically regulated by lipid molecules. Despite the influential role of nutritional inputs on germline development, germline intrinsic mechanisms that survey nutritional conditions are poorly described. Moreover, there is little understanding on how diet impacts germline development.

We have found that hermaphroditic germline development requires the nuclear hormone receptor *nhr-114* to cope with different diets. Although *nhr-114* is expressed in both sexes, we found that its loss leads to hermaphrodite but not male sterility. Sterile *nhr-114* hermaphrodites develop under-proliferated germ lines that lack oocytes, consistent with our observed germ cell-specific *nhr-114* function. In addition to its germline role, we found evidence that *nhr-114* activity in the soma strongly affects germ line development. Interestingly, the penetrance of sterility among *nhr-114* hermaphrodite batches is not constant, suggesting that the *nhr-114* phenotype is influenced by environmental factors. Consistent with this observation, we discovered that feeding *nhr-114* mutants with a specific *E. coli* strain different from OP50, completely abolishes sterility. Our observations suggest that loss of *nhr-114* sensitizes germline development to food sources. Hence, the discovery of *nhr-114* provides a molecular link to how *C. elegans* hermaphrodites could

cope with changing dietary sources in a natural environment. We continue to explore this avenue by investigating a likely connection with germline post-transcriptional control mechanisms.

[1]Michaelson et al. Development 137, 671-680 (2010); [2]Angelo et al. Science 326, 954 (2009).

829B

Translational regulation and the control of meiotic maturation. Caroline Spike, Donna Coetzee, **David Greenstein**. GCD Dept, University of Minnesota, Minneapolis, MN USA.

C. elegans oocytes undergo meiotic maturation and ovulation in response to major sperm proteins (MSPs), hormonal signals released from sperm. MSPs also function as cytoskeletal elements in the actin-independent motility of nematode sperm. The follicle-like gonadal sheath cells, which form gap junctions with oocytes and contract to drive ovulation, function as the initial MSP sensors. G protein-stimulated cAMP-dependent protein kinase A activity in the gonadal sheath cells is required for all germline responses to MSPs. In the germ line, the meiotic maturation response requires the combined activities of two Tis11-like CCCH zinc-finger proteins, OMA-1 and OMA-2 [Detwiler et al. (2001) Dev. Cell 1: 187]. To elucidate the mechanisms by which the OMA proteins promote meiotic maturation, we developed methods for purifying OMA ribonucleoprotein particles (OMA RNPs) from oocytes. OMA RNPs were purified in both the presence and absence of MSP signaling and proteins were identified using mass spectrometry. OMA RNPs contain a large number of germline-expressed RNA-binding proteins including translational activators and repressors. To distinguish core OMA RNP components from those tethered by RNA, we purified OMA complexes after treatment with RNase A. Core OMA RNP components include multiple subunits of the GLD-2 poly(A) polymerase and CCR4/NOT1 deadenylase complexes and many RNA-binding proteins, including MEX-3, MEX-1, and LIN-41. OMA RNP components that appear to be tethered primarily via RNA associations include the P-body proteins CGH-1/p54 and CAR-1/Rap55. OMA RNPs are cytoplasmically dispersed in oocytes when MSP is present but are found in foci when MSP is absent, as has been observed for a number of germline-expressed RNA-binding proteins [Schisa et al. (2001) Development 128: 1287; Jud et al. (2008) Dev. Biol 318: 38]. These data are consistent with the hypothesis that OMA-1/2 might regulate the translation of mRNAs encoding proteins involved in meiotic resumption or the oocyte-to-embryo transition. To test the role of OMA RNP components in translational regulation, we used microarrays to define mRNA components of OMA RNPs. This analysis identified a set of approximately 400 germline-expressed mRNAs that are enriched in OMA RNPs. We are testing the 3'UTRs of candidate OMA target genes and have found several that appear to mediate OMA-1/2-dependent translational repression in oocytes. We will investigate whether OMA-1/2 targets are also regulated by OMA-associated proteins, potentially in response to the MSP signal.

830C

A set of spermiogenesis inhibition genes identified by a suppressor screen of *spe-27*. **Gizelle Hacopian**, Ubaydah Nasri, Craig LaMunyon. Biological Sciences, Cal Poly Pomona, Pomona, CA.

Spermiogenesis is the process by which spermatids differentiate to become mature spermatozoa. During spermiogenesis in *C. elegans*, pseudopods extend from the spermatids and enable the sperm to crawl to the fertilization site within the hermaphrodite reproductive tract. A signal transduction pathway that activates spermiogenesis involves genes in the *spe-8* group (*spe-8, spe-12, spe-19, spe-27,* and *spe-29*). Mutations in any of the *spe-8* group genes disrupt spermiogenesis. In order to identify additional genes involved in spermatid activation, a suppressor screen was performed on *spe-27(it132ts)I* mutants. Numerous suppressor mutants were recovered; most were discovered to harbor *spe-6* mutations. Here we report on several non-*spe-6* suppressor mutants and their phenotypes. The suppressor mutations described here are in separate genes and include *spe-4(hc196)I, hc197, hc198, hc201, hc202, zq9,* and *zq10*. None of these suppressors are allele specific: they suppress other *spe-8* group genes such as *spe-19* and *spe-29*. These suppressor mutations do not restore wild type fertility to *spe-27(it132ts)*, resulting instead in lifetime fecundity ranging from only four progeny to nearly 20 progeny. However, the suppressor mutations in a wild-type background vary significantly from one another. *hc196* and *hc197* mutants produce fewer than 20 progeny at 25C, whereas *hc198* and *hc201* exhibit near wild type fecundity at 25C. Male suppressor mutants all harbored activated sperm, suggesting that these mutations bypass the spermiogenesis activation pathway resulting in premature spermatid activation. SNP mapping revealed that *spe-4(hc196), hc197, hc198*, and *hc202* are on Chromosome I, and *hc201* is on Chromosome V. Illumina sequencing achieving 25x coverage was performed on all but the spe-4 mutation, and the data were analyzed using the Genious Pro™ software package. Single candidate mutations were identified for *hc197* and *hc198*, and two candidates were found for *hc201*. Resequencing confirmed the mutations. Transformation rescue is ongoing, and we will discuss rescue of phenotypes such as these where the mutant phenotype is difficult to identify on an individual basis. Taken together, the suppressor mutations define a set of genes whose products inhibit spermatid activation until relived by the *spe-8* group gene products. These inhibitory gene products appear to involve the membranous organelles and MSP packaging. We hypothesize that prevention of MSP polymerization is crucial to maintaining the spermatid stage.

831A

Identifying mechanisms critical for sperm guidance to oocytes. **Hieu D. Hoang**, Johnathan W. Edmonds, J. Prasain, Michael A. Miller. Cell Biology Dept, University of Alabama at Birmingham, Birmingham, AL.

Fertilization is dependent upon fusion of sperm and oocyte. The mechanism(s) by which motile sperm find oocytes within the female reproductive tract is not well understood. Our published data provide strong evidence that C. elegans oocytes secrete F-series prostaglandins that guide sperm to the spermatheca (Kubagawa et al., 2006; Edmonds et al., 2010). Prostaglandins (PGs) are lipid hormones that are targets of nonsteroidal anti-inflammatory drugs, such as aspirin and ibuprofen. Although PG synthesis pathways in mammals have been largely delineated, these pathways in worms are unexplored. The C. elegans genome lacks PG G/H synthase homologs, which catalyze the 1st step in PG synthesis, yet encodes a wide array of predicted PG D, E, and F synthases that act downstream. Our model is that PG synthesis in oocytes initiates by a novel mechanism that generates substrates for conserved PG synthases that in turn generate F-series PG analogs. Consistent with this model, we have identified several predicted PG synthases that are required non-autonomously for sperm guidance (Edmonds et al., 2010). We are using electrospray ionization tandem mass spectrometry, genetic analyses, and in vitro assays to identify roles for these enzymes in PG synthesis. Preliminary data suggest that worms express numerous PG synthases that are essential for synthesizing specific F-series PG types. These enzymes tend to be broadly expressed, raising the possibility that PGs have unknown functions outside of the reproductive system. Another important unanswered question is the mechanism(s) that transduces PG signals in C. elegans sperm, as the genome does not encode obvious homologs of mammalian PG receptors. We have identified mutations in multiple G-protein coupled receptors (GPCRs) that disrupt sperm guidance to the spermatheca. Our data support the hypothesis that these GPCRs function autonomously in sperm. The GPCRs are members of a multi-gene family that is clustered within a chromosomal region enriched in genes important for fertilization (Miller et al., 2004). Mutations in individual GPCRs cause incompletely penetrant defects in sperm guidance, suggesting that their functions partially overlap. We are testing this hypothesis by using the MosDEL transposon-mediated deletion strategy to knock out multiple GPCRs within the cluster. The results may help identify PG receptors in C. elegans.

832B

Protein synthesis factor isoforms are selective for mRNAs that promote meiosis and differentiation in germ cells. Melissa A. Henderson[1], Vince Contreras[1], Anren Song[2], Sara Labella[3], Nadejda Korneeva[2], J. Kaitlin Morrison[1], Andrew J. Friday[1], Monique Zetka[3], Robert E. Rhoads[2], **Brett D. Keiper[1]**. 1) Biochemistry and Molecular Biology, Brody School of Medicine at East Carolina University, Greenville, NC 27834; 2) Biochemistry and Molecular Biology, LSU Health Sciences Center, Shreveport, LA 71130; 3) Department of Biology, McGill University, Montreal, Quebec H3A 1B1, Canada.

Translational control of mRNAs is a vital mode of gene regulation in animal germ cells. Only recently has the role of translation initiation factors (eIFs) in such translational control been appreciated, in that they exercise selectivity among mRNAs. We have identified mRNAs specifically favored by individual isoforms of eIF4E and eIF4G, two subunits of the cap-binding initiation complex, in *C. elegans* germ cells. Deficiency in a single isoform of eIF4E (e.g. IFE-1 or IFE-2) prevents efficient translation of small subsets of mRNAs unique to that isoform. Some encode proteins of critical function in oocyte and/or spermatocyte differentiation. As a consequence, loss of a single eIF4E isoform causes a phenotype unique to that isoform. Loss of IFE-1 led to inefficient translation of select mRNAs (e.g. *mex-1* and *oma-1*), a cytokinetic defect in spermatocytes, and limited oocyte viability. Loss of IFE-2 caused aberrant meiotic chromosome segregation due to poor translation of different mRNAs (*msh-4* and *-5*). By contrast, depletion of the cap-dependent eIF4G isoform (p170 IFG-1), binding partner to both IFEs, promoted germ cell apoptosis by inducing CED-4 expression and apoptosome assembly in the ill-fated oocytes. Our findings suggest that eIF4Es play function-specific roles in meiosis, late oogenesis and spermatogenesis, whereas eIF4G isoforms promote a balance of translation mechanisms that governs survival and cell death. The evidence points to a positive regulatory network of eIF4E-eIF4G-mediated translational control directing developmental competence during gametogenesis.

833C

Elucidating genetic mechanisms by which somatic cAMP signaling regulates MSP-dependent oocyte meiotic maturation. **Seongseop Kim**, J. Amaranath Govindan, David Greenstein. GCD, University of Minnesota, Minneapolis, MN USA.

Oocyte meiotic maturation is a conserved developmental transition, defects in which are the major cause of human birth defects and infertility. C. elegans provides a genetic model for studying the control of oocyte meiotic maturation by hormonal signaling and soma-germline interactions. The meiotic maturation processes in C. elegans and mammals share a number of similarities. Major sperm protein (MSP) and luteinizing hormone, though unrelated in sequence, both trigger meiotic resumption using somatic $G\alpha_s$-adenylate cyclase pathways and soma-germline gap-junctional communication. Shared responses include cortical cytoskeletal rearrangement, nuclear envelope breakdown, meiotic spindle assembly, and changes important for the oocyte-to-embryo transition. Here we describe progress in elucidating the genetic mechanisms by which the somatic gonad receives the MSP signal and transduces the oocyte response. $G\alpha_s$-adenylate cyclase signaling in the follicle-like gonadal sheath cells is required for all described MSP responses in the germline. Because the gonadal sheath cells form gap junctions with oocytes, we considered whether cAMP generated in sheath cells might trigger cAMP-dependent protein kinase A (PKA) activation in the oocyte. We used genetic mosaic analysis to test this possibility. We found that kin-1, which encodes the PKA catalytic subunit, is required for meiotic maturation in the sheath cells, but not oocytes. To identify downstream effectors of MSP signaling, we conducted a genetic screen for mutations that suppress the sterility caused by a null mutation in the

adenylate cyclase (acy-4) required in sheath cells for meiotic maturation. We recovered 63 suppressor of adenylate cyclase (sacy) mutations. We are in the process of mapping and cloning sacy mutations using whole genome sequencing. Preliminary analysis of sacy mutations has identified components of the CoREST repressor complex and a DEAD-box RNA helicase. Genetic mosaic analysis of kin-1 in an spr-5 mutant background indicates that spr-5 is epistatic. Therefore, spr-5, which encodes the LSD1 histone demethylase component of the CoREST complex, might participate in setting the germline context by repressing the transcription of genes that interfere with the normal signaling mechanism. Three allelic sacy mutations appear to result from missense mutations in a highly conserved DEAD-box helicase. Interestingly, this helicase was biochemically characterized as a component of OMA-1 ribonucleoprotein particles (see a poster by C. Spike). These data provide insight into how germline context and translational control ensure that meiotic maturation is coupled to sperm availability.

834A
VHA-19 is essential for trafficking of RME-2 in *Caenorhabditis elegans* oocytes and is important for the osmotic integrity of the embryo. **Alison J. Knight**, Lisa McEwan, Nicholas Johnson, Carolyn Behm. Research School of Biology, Australian National University, Canberra, Australia.

VHA-19 is an essential *Caenorhabditis elegans* protein of unknown function that has been predicted to associate with the vacuolar ATPase enzyme complex (V-ATPase). Although an association between VHA-19 and the V-ATPase has yet to be shown, a small region of the C-terminus of VHA-19 is similar to a sequence in the mammalian protein Ac45. This is of interest because Ac45 has been shown to bind to the V0 domain of the V-ATPase (1). Thus, it is possible that VHA-19 may also bind to the V-ATPase V0 domain and may have similar functions to Ac45.

We have used RNA interference (RNAi) to show that VHA-19 is essential for *C. elegans* larval development and embryogenesis (2). We now show that VHA-19 is important for protein trafficking in the *C. elegans* oocyte and for early embryogenesis. This is based on four key observations. First, silencing *vha-19* expression exclusively in the germ line of *C. elegans* elicits a similar phenotype to that observed when *vha-19* expression is silenced systemically in wild type *C. elegans*: embryos that fail during embryogenesis or die as larvae before the third larval stage. Second, mating normal *fog-2* male *C. elegans* with *fog-2* females fed on *vha-19* dsRNA does not rescue the phenotype. Third, defects in the structure and number of oocytes produced are observed in the oviducts of *vha-19(RNAi) C. elegans*, while the spermatozoa appear normal. Fourth, oocytes in the oviduct of *vha-19(RNAi)* adults are unable to endocytose VIT-2::GFP and VHA-19 is essential for trafficking of RME-2, the receptor for this process, to the oocyte plasma membrane. Interestingly, we have also observed that VHA-19 is important for the maintenance of osmotic integrity of the egg: *vha-19(RNAi)* embryos are osmotically sensitive and in the uterus are permeable to the dye acridine orange. VHA-19 is expressed in the excretory cell (among other tissues), which is the key tissue controlling osmoregulation. This is the first analysis of the role of VHA-19 in the oocytes and embryonic development.

1. Feng et al., 2008. J Biol Chem 283, 13194-13204.
2. Knight et al, 2007. International Worm Meeting Abstract.

835B
LET-363/TOR and RSKS-1/S6K promote proper germline development in C. elegans. **Dorota Z. Korta**[1], Simon Tuck[2], E. Jane Albert Hubbard[1]. 1) Skirball Institute of Biomolecular Medicine, Dept of Pathology, NYU School of Medicine, New York, NY; 2) Umeå Center for Molecular Pathogenesis, Umeå University, Umeå, Sweden.

It is well established that nutritional status affects many aspects of physiology, including reproduction and cell proliferation. However, the molecular mechanisms by which nutritionally sensitive molecular pathways control cell proliferation are not well understood. Recently, our lab has shown that insulin/IGF-like receptor (IIR) signaling is required for robust larval germline proliferation. The TOR (Target of Rapamycin)-S6K(p70 ribosomal S6 kinase) pathway is responsive to insulin signaling and nutrition in mammals. However, in *C. elegans* the pathway is not well defined. We have found that *let-363*/TOR, *daf-15*/Raptor, and downstream target *rsks-1*/S6K are required for proper proliferative germ cell accumulation during larval stages, and this effect is germ line-autonomous. We investigated the mechanism by which germ cell numbers were affected in *rsks-1* null mutants. We found that loss of *rsks-1* leads to general slowing of larval germ cell proliferation and a distal shift in germ cell meiotic entry, suggesting it affects both proliferation and differentiation. Furthermore, *rsks-1* likely acts in parallel to both *glp-1*/Notch and the *daf-16*/FOXO branch of the insulin pathway. The *rsks-1* germline defect is not suppressible by mutations that mediate the role of *rsks-1* in longevity, namely *pha-4*/FOXA and *egl-9*. Thus the reason *rsks-1* animals are long lived cannot simply be due to decreased numbers of proliferating germ cells. We are actively investigating effectors of the *rsks-1* germline phenotype.

836C
Sex-specific regulation of gonadal development in *C. elegans*. **Mary B. Kroetz**[1], W. Clay Spencer[2], Rebecca D. McWhirter[2], Matthew R. Berkseth[1], David M. Miller, III[2], David Zarkower[1]. 1) Department of Genetics, Cell Biology & Development, University of Minnesota, Minneapolis, MN; 2) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, TN.

The gonad of *C. elegans* originates as a four-cell primordium in the embryo. Initially it is morphologically identical in both sexes, but soon after the animal completes embryogenesis the gonad begins to develop into one of two distinct sex-specific organ structures. Despite the extensive sexual dimorphism and previously defined cell lineages of the gonad, the genetic pathways that direct the sex-specific development of this organ remain largely unknown. All sexual dimorphism is under the control of the TRA-1 transcriptional regulator, but no gonadal direct targets of TRA-1 are known. The overall aim of this work is to define the genetic network of sex-specific gonadal development, focusing on the direct targets of TRA-1. To identify the sex-specific gonadal regulators, cell-specific microarray-based mRNA expression profiling was conducted. Single sex populations of animals were generated by employing sex determination pathway mutants. The somatic gonadal precursor cells (Z1/Z4) expressed a gfp reporter allowing for their identification by FACS from dissociated embryonic cells. Z1/Z4-enriched transcripts were identified for both sexes. The majority of proteins with known Z1/Z4-enriched expression were identified by this method, and a number of novel Z1/Z4-enriched transcripts have subsequently been validated by reporter analysis, confirming the effectiveness of this approach. Work is currently underway to validate several of the sex-enriched transcripts. Recently our laboratory has conducted TRA-1 chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-seq). Comparison of the sex-specific gonadal transcriptome with the direct targets of TRA-1 as identified by ChIP-seq should identify which sex-specific transcripts are directly regulated by TRA-1 and presumably at the top of the hierarchy of gonadal regulators.

837A
A model of germline population dynamics: in-silico analysis and in-vivo validation. Yaki Setty[1], Diana Dalfó[2], Dorota Z. Korta[2], E. Jane Albert Hubbard[2], **Hillel Kugler**[1]. 1) Microsoft Research Cambridge; 2) New York University School of Medicine, Skirball Institute, Kimmel Stem Cell Center.

Populations of cells undergo division and differentiation during the formation and maintenance of tissues and organs. Yet the dynamics of such cell populations over time is often difficult to understand. Self-renewing stem cell populations pose a particularly interesting case. The difficulty in understanding stem cell dynamics is not only due to an incomplete picture of cellular and molecular processes involved, but also due to the integration of multiple influences such as anatomical constraints, cell-cell communication, and cell division itself. To begin to address this problem, we developed a general computational model of an anatomically restricted cell population that responds to a localized signal from a "niche" but for which the precise cellular basis for renewal is unknown. Specifically, we constructed a dynamic model of the *C. elegans* germ cell population and analyzed simulations from this model to examine the effects of and interplay between Notch signaling, cell cycle control, and anatomical constraints. The model, based on a subset of available data, produced simulations that recapitulate basic behaviors of the system, including proper responses to genetic manipulations. Moreover, model simulations generated predictions related to maintenance of the proliferative zone. We subsequently validated these predictions in laboratory experiments. Our analyses of model simulations and laboratory results suggest that (1) when ligand-receptor interaction occurs over a relatively short distance (that is, reaching only the distal-most germ cells), small differences in this distance introduce unexpected variability that likely accounts for incomplete penetrance and “all-or-nothing” phenotypes, (2) alternative explanations may exist for previously-observed complex differentiation dynamics upon withdrawal of receptor activity, (3) population dynamics and anatomical constraints influence niche-residence and (4) germ cell proliferation rate during larval stages influences the differentiation pattern in the adult.

838B
Comparative functional analysis of hSTIM1 and *C. elegans* STIM-1. **Rashmi P Kulkarni**, Raphael Courjaret, Khaled Machaca. Weill Cornell Medical College in Qatar.

STIM1, the ER Calcium sensor couples to Orai1, the Calcium channel to mediate Store-Operated Calcium Entry (SOCE). hSTIM1 is localized to the ER membrane when Ca^{++} stores are full. On Ca^{++} store depletion, hSTIM1 forms puncta that localize to the cortical ER and bind hOrai1 to allow Ca^{++} influx. hSTIM1 when expressed in *Xenopus* oocytes can gate XOrai1 to induce SOCE on store-depletion. The *C. elegans* STIM1 (CeSTIM-1a) when expressed in HEK293 cells shows puncta formation without store-depletion (*Gao et. al*. 2009). These puncta however can only gate CeORAI-1 on store-depletion. We expressed CeSTIM-1a in *Xenopus* oocytes and found it to be localized in a similar punctate pattern. These puncta failed to recruit XOrai1 either under store-full or store-depleted conditions similar to the reported results (*Gao et. al.* 2009). We are conducting comparative structure-function analysis of the hSTIM1 and CeSTIM-1a to better define the structural features within the STIM1 molecule required for its clustering into puncta and for its ability to interact with and gate Orai1. CeSITM-1a has two other isoforms (CeSTIM-1b and c) that remain uncharacterized. We have detected these isoforms by RT-PCR using total RNA from a mixed-stage hermaphrodite population and cloned them. Currently we are characterizing the localization patterns of these isoforms and studying their function in Ca^{++} signaling.

839C
RFP-1, an E3 ubiquitin ligase, is involved in regulating the proliferation vs. differentiation decision in the *C. elegans* germline. **Lindsay Leahul**, Brendan Bakos, Dave Hansen. Department of Biology, University of Calgary, Calgary, Alberta, Canada.

The *C. elegans* germ line provides a good model to study the behavior of stem cell populations in relation to the signaling cues that maintain the essential balance between proliferation and differentiation. In the germ line, the GLP-1/Notch signaling pathway is responsible for maintaining the pool of germline stem cells. We identified the proteasome as playing a role in regulating the balance between proliferation and differentiation through the identification of a weak loss of function allele in a proteasome alpha subunit that enhances over-proliferation. In the proteasome mutant, proteins that would normally be degraded by the proteasome are not. Our current model is that the over-proliferation phenotype is due to

proteins, that normally promote proliferation and are degraded as cells differentiate are not being properly degraded. As a first step to identify the proteins that are not being degraded properly in this genetic background we performed an RNAi screen of all E3 ligase substrate recognition adaptors in the *C. elegans* genome. If we identify the E3 ligase that is targeting specific proteins for degradation by the proteasome, we can then use biochemical techniques to identify the proliferation promoting proteins that the E3 ligase is targeting for degradation. From this screen we identified *rfp-1*, a single subunit RING finger E3 ubiquitin ligase. RNAi knockdown of *rfp-1* enhances the over-proliferation phenotype of a weak *glp-1* gain of function allele. Consistent with the RNAi results, an *rfp-1* deletion allele (*ok572*) also enhances the *glp-1(gf)* over-proliferation phenotype. Double mutant analysis of *rfp-1(ok572)* with downstream pathway genes *gld-1, gld-2*, and *gld-3* shows no enhancement of the over-proliferation phenotype. *rfp-1(ok572)* does, however, appear to enhance the more sensitive genetic background over-proliferation phenotype of *gld-2(0); nos-3(0)*. In order to discover the proliferation promoting proteins that RFP-1 is targeting for degradation to the proteasome, we have created a RFP-1::TAP tagged protein and integrated it into the *C. elegans* genome using Mos Single Copy Insertion. This integrated strain will be used for immunoprecipitation to identify the degradation targets of RFP-1.

840A
Development of a C. elegans Spermiogenesis and Sperm Function Protein Interaction Network. **Matthew R Marcello**[1,2,3], Marina Druzhinina[2,3], Gunasekaran Singaravelu[2,3], Andrew Singson[2,3]. 1) Department of Molecular Genetics, Microbiology and Immunology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ; 2) Waksman Institute, Rutgers University, Piscataway, NJ; 3) Department of Genetics, Rutgers University, Piscataway, NJ.

The proper temporal and spatial organization of proteins during the spermatid to spermatozoa transition is necessary for the spermatozoa to function properly during fertilization. However, the protein-protein interactions necessary for ensuring spermiogenesis and sperm function during fertilization are not well understood. Sperm-enriched genes necessary for these processes are underrepresented in high throughput protein-protein interaction studies because of the low abundance of these proteins relative to the entire worm proteome. The purpose of this study was to generate a protein-protein interaction network for the proteins that are necessary for spermiogenesis and sperm function in C. elegans. The development of this protein interaction network will allow for the identification of novel interactions between proteins necessary for these processes and will also further our understanding of how spermiogenesis and sperm function are coordinated. To identify interacting proteins, we performed pair-wise split-ubiquitin yeast two-hybrid analysis of the full-length gene products of genes that have a known function in spermiogenesis (fer-1, spe-4, spe-6, spe-8, spe-12, spe-19, spe-27, and spe-29) or sperm function during fertilization (fer-14, spe-9, spe-13, spe-38, spe-41, and spe-42). Our results indicate that for some genes that that interact genetically there are physical interactions between their gene products. For example, spe-6 is known to interact with spe-19 and spe-29 genetically; our results indicate that SPE-6 also physically interacts with SPE-19 and SPE-29. We also identified novel protein-protein interactions, including interactions between SPE-8 and SPE-19 and SPE-9 and SPE-19. We represented the network topology using Power Graphs analysis rather than standard representations so as to remove redundancy and simplify network visualization. A future goal is to develop an integrated network model that incorporates additional datasets, including genetic interactions, expression profiles, localization data, and additional protein interaction data, along with the protein-protein interaction network we have generated. The integrated network of spermiogenesis and sperm function proteins will provide a robust source for generating hypotheses about how to assemble functional spermatozoa capable of fertilization.

841B
Insulin, Notch, Cyclins and the Regulation of Germline Development. **David Michaelson**, E. Jane Albert Hubbard. Developmental Genetics, Skirball Institute, Kimmell Center for Stem Cell Biology, Department of Pathology, NYU School of Medicine.

In general, differentiated cells do not proliferate. However, the precise relationships between cell cycle control and proliferation/differentiation decisions are not well understood.

The C. elegans germ line is a useful model system to study the relationship between cell fate and cell cycle. GLP-1/Notch signaling is a critical regulatory pathway in the proliferation versus differentiation (mitotic versus meiotic) decision. Recently we showed that insulin/IGF-like receptor (IIR) signaling mediated by DAF-2 regulates cell cycle progression in the larval germ line, leading to the proper number of proliferative cells in the adult (Michaelson et al., 2010 *Development*). In *daf-2* mutant larvae, relative to wild type, both mitotic index and S phase index are reduced and a greater proportion of cells are found in G2. In contrast, animals with reduced glp-1 activity also display a reduced number of proliferative germ cells but display a normal mitotic index. Rather, the reduction in proliferative germ cell number is due to distally displaced differentiation. Thus IIR and Notch signaling affect the germ line differently with respect to cell cycle and the proliferation/differentiation decision. We also identified a soma-autonomous role for the insulin-like ligands INS-3 and INS-33 in promoting larval germ cell cycle progression. Further, we demonstrated that this role for DAF-2/IIR is DAF-18/PTEN and DAF-16/FOXO-dependent and is autonomous to the germ line.

More recently, we have begun investigating genetic interactions between the insulin pathway and cell cycle components with respect to germ cell cycle. We found interactions with a subset of G2/M and G1 cyclins. This suggests that insulin signaling may influence germ line cell cycle both through G1 and G2 mechanisms.

842C
Role of METT-10, a putative methyltransferase, in the proliferation vs meiosis decision. **Ariz Mohd**, Maia Dorsett, Tim Schedl. Genetics, Washington University School of Medicine, St. Louis, MO.

S-adenosyl methionine (SAM) dependent methyltransferases are conserved in both prokaryotes and eukaryotes and carry out methylation of DNA, RNA, proteins and metabolites. SAM-dependent methytransferases have been implicated in various important functions in numerous biological systems, such as chromatin regulation, signal transduction etc. and aberrations lead to a number of disorders including cancer. In the C. elegans gonad *mett-10*, the ortholog of human METT10D, has been shown to function to inhibit the germ cell proliferative fate (Dorsett et al. 2009). Loss of function alleles as well as a novel tumorous allele (*oz36*) of *mett-10* enhance the tumorous phenotype of a *glp-1* weak gain-of-function allele (*wgf*) at the permissive temperature. To better understand the role of *mett-10* in the germ line, we examined genes found to be in a physically interacting network with METT-10 from a large-scale yeast two-hybrid screen (Li et al. 2004). A number of these genes were found as enhancers of *glp-1*(*wgf*) in an RNAi screen. Of these, dynein light chain 1 (*dlc-1*) has already been reported to enhance the tumorous phenotype of both *glp-1*(*wgf*) as well as *mett-10*(*oz36*) (Dorsett and Schedl 2009). RNAi of another gene *cks-1*, which encodes Cyclin-dependent kinase regulatory subunit 1, also enhances the tumorous phenotype of both *glp-1*(*wgf*) and *mett-10*(*oz36*). CKS-1 is a small and highly conserved CDK interacting protein that functions with CDK-1 in cell cycle progression and gene transcription. *cks-1*(*ne549*), a temperature sensitive allele, also enhances the tumorous phenotype of *glp-1*(*wgf*) as well as *mett-10*(*oz36*). The later case results in late onset tumors indicating that the balance between proliferation and differentiation has been compromised. Additionally, *cks-1*(*ne549*) in a glp(+) background shows delayed meiotic progression. Preliminary data suggests that the unusual function of *cks-1* in inhibiting the proliferative fate may be independent of *cdk-1*. In other systems, CKS-1 has been shown to recruit the proteasome for its function. We are currently testing if this is also the case for its role in the germ line.

843A
FOG-1 activates *fog-3* expression. Dyan Morgan[2], Johan Jeong[2], **Daniel Noble**[1], Judith Kimble[1,2,3]. 1) Integrated Program in Biochemistry, University of Wisconsin-Madison, Madison, WI; 2) Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 3) Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI.

FOG-1 promotes the sperm fate at the expense of oogenesis in both sexes: XO *fog-1* mutants are somatically male but make only oocytes; similarly XX *fog-1* mutants are somatically hermaphrodite but make only oocytes (1). FOG-1 is a divergent Cytoplasmic Polyadenylation Element Binding (CPEB) protein (2, 3). *Xenopus* CPEB binds CPEB binding elements (CPEs) in the 3'untranslated region (3'UTR) of target mRNAs to regulate translation via polyadenylation or deadenylation depending on associated cofactors. Genetically, FOG-1 acts at the end of the germline sex determination pathway as does another key regulator of sperm fate specification, FOG-3 (4). Our work provides three lines of evidence suggesting that FOG-1 controls the *fog-3* mRNA directly. First, purified recombinant FOG-1 binds the *fog-3* 3'UTR in a CPE-dependent manner *in vitro*. Second, FOG-1 antibodies immunoprecipitate *fog-3* mRNA from L3 hermaphrodite extracts, whereas control IPs do not. Third, the abundance of an epitope-tagged and rescuing FOG-3 protein drops precipitously in *fog-1* mutants, while *fog-3* mRNA is not reduced. We suggest that FOG-1 promotes sperm fate specification by translationally activating *fog-3* expression. Experiments are in progress to test this idea with transgenes carrying either a wild-type *fog-3* 3'UTR or a mutant *fog-3* 3'UTR that lacks CPEs.

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844B
RNA localization in the germline. **Alexandre PAIX**, Geraldine SEYDOUX. Molecular Biology and Genetics , Johns Hopkins University School of Medicine , Baltimore, MD.

Many genes in the C. elegans germline are regulated by postrancriptional mechanisms acting through 3' UTR sequences. We are interested in how this regulation manifests itself at the level of RNA localization. To address this question, we are developing an in situ hybridization protocol to localize mRNAs in the germline with subcellular resolution. We will present our initial results comparing mRNAs that are ubiquitously expressed and mRNAs that are post-transcriptionally regulated in the mitotic zone.

845C
Robustness and evolvability of sex determination pathway. **Manish Parihar**, Andre Pires

da Silva. Biology, University of Texas at Arlington, Arlington, TX.

Robustness is a ubiquitous property of living systems that enables persistence of traits in the face of genetic, epigenetic, and environmental perturbations. Phenotypes in higher organisms are buffered and maintained consistent in spite of the fluctuation in the internal and external environments. Robustness plays a very significant role in evolution of traits but the question whether it increases or decreases the ability of the system to evolve still remains unanswered and the underlying buffering mechanisms are largely unknown. We are taking advantage of the well studied sex determination (SD) pathway in *C. elegans* to study the robustness of this genetic network. Interestingly, it seems that the rate of evolution of sex determination is different between the soma and the germline. In this study, we are using sensitized background to test the differences in robustness that may explain the differences in rate of evolution between the two types of tissues. Mutants of the SD pathway *her-1, tra-2 and tra-1* were introgressed into five genetic backgrounds to make sensitized strains. We will present results on the phenotypic characterization of these lines.

846A

RNP granules: insights into their regulation and function. **Joseph R Patterson**, Megan P Wood, Jennifer A Schisa. Central Michigan University, Mount Pleasant, MI.

As sperm is depleted in *Caenorhabditis elegans* hermaphrodites, ovulation arrests and ribonucleoprotein (RNP) granules assemble in the arrested oocytes. RNP granules are also induced in non-arrested oocytes by environmental stresses such as heat shock, osmotic shock, and anoxia. The large RNP granules in oocytes are hypothesized to maintain mRNA stability and prevent precocious translation when fertilization is delayed or a stress is present. The assembly of RNP granules in response to extended meiotic arrest and stress is conserved in a related species, *Caenorhabditis remanei*. We have focused on two broad questions: 1) how is the assembly of RNP granules regulated? and 2) what is the consequence to an arrested oocyte if RNP granules fail to assemble normally? To gain insights into the mechanism for RNP granule assembly we first performed an ultrastructural analysis of the *C. remanei* germline. We were very surprised to see dramatic nuclear blebbing along the nuclear envelope of oocytes of unmated and heat stressed females. A combination of TEM, confocal analyses, and live imaging studies indicate the blebs appear to detach from the nucleus and may traffick to the cell cortex and assemble into annulate lamellae, in close proximity to RNP granules. Using RNAi, we further show that several nucleoporins are required for the assembly of RNP granules, and a disruption in RNP granule assembly coupled with a low frequency of nuclear blebbing in arrested oocytes negatively impacts embryonic viability. Our observations support a model where nuclear membrane blebbing is required to increase the trafficking of nucleoporins to the cell cortex in stressed or meiotically-arrested cells and to facilitate the recruitment of RNA and protein components of RNPs into large complexes. To further address the question of the function of RNP granules, we are performing additional fertility tests after preventing normal RNP granule assembly. We are using RNAi to knockdown genes that have recently been identified in our lab as required for normal RNP granule assembly, and performing mating assays to assess any consequences to embryonic viability. We expect these experiments to address part of our hypothesis for the function of RNP granules in meiotically arrested oocytes.

847B

A novel role of the RNA helicase VBH-1 during stress. **Daniel Paz-Gomez**, Rosa E. Navarro. Departamento de Biol Celular y del Desarrollo, Instituto de Fisiologia Celular, Universidad Nacional Autonoma de Mexico, Mexico, D.F., Mexico.

VBH-1 is a DEAD-box RNA helicase closely related to Vasa and Belle from *D. melanogaster*. DEAD-box RNA helicases resolve misfolded RNA regulating in this way RNA-RNA and 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 constitutively to P granules, but is also found in the cytoplasm of all blastomeres during embryogenesis, and in the male and hermaphrodite gonad. In this work we found that following different kinds of stress (heat-shock, starvation, sperm depletion) VBH-1 associates to granules in the gonad core, oocytes, and in both somatic and germline blastomeres during early embryogenesis. In every case, VBH-1 granules localized with or near CGH-1-positive granules. CGH-1 is a DEAD-box helicase usually found in small cytoplasmic foci that might resemble processing bodies, which under stress conditions aggregates into bigger foci. Intriguingly VBH-1 association to these granules does not depend on a single sequence since three different non-overlapping fragments that comprise the entire sequence are able to localize into foci after heat shock. To further understand the possible role of VBH-1, and its closest homolog in worms (LAF-1) in stress response, we made a lifespan assay at 37°C and found that *vbh-1(RNAi)* and *laf-1(RNAi)* animals were more sensitive to heat-shock than control animals. We found that VBH-1 is also expressed at low levels in the soma by Western Blot analysis using extracts from the germline proliferation defective mutant *glp-4(bn2)*. Seemingly, this somatic expression is responsible for VBH-1 mediated response to stress since germline-specific silencing of *vbh-1* had no effect on animals survival at 37°C. Our work points towards a previously unknown role of VBH-1 during different kinds of stress. We are currently working on finding of mRNA targets of VBH-1 during stress; so far we have found the down-regulation of a small heat-shock protein in *vbh-1(RNAi)* animals.

848C

Evolution and phenotypic plasticity of the germline and reproductive system in *Caenorhabditis* nematodes. **Nausicaa Poullet**, Christian Braendle. Institute of Developmental Biology and Cancer, CNRS - University of Nice Sophia-Antipolis, Nice, France.

To better understand the role of gene-environment interactions in development and evolution, we study quantitative natural genetic variation and plasticity of the *Caenorhabditis* germline and reproductive system in different environments. Although the basic germline organization and processes are conserved among *Caenorhabditis* species, reproductive output and schedules vary both within and between species. We are interested in understanding how differences in offspring number and quality are coupled to the underlying properties and processes of the germline (e.g. sperm number, germ cell number, proliferation, apoptosis) and to what extent they are plastic, i.e. vary across different (ecologically relevant) environments. In an initial analysis, we have quantified germline and reproductive phenotypes in 15 isolates of the three hermaphroditic species (*C. elegans*, *C. briggsae* and *C.* sp. 11). Overall, sperm number, germ cell number and offspring number are positively correlated; however, sperm number does not always closely match offspring number, indicating that isolates and species may differ in sperm fertility or efficiency of sperm use. The most striking observation is that many *C.* sp. 11 isolates show a highly reduced offspring, sperm, germ cell number and mitotic zone relative to *C. elegans*. To carry out an integrative analysis of germline and reproductive plasticity, we have characterized how e.g. germ cell proliferation, entry into meiosis and apoptosis are modified in N2 animals exposed to diverse conditions (such as liquid, starvation, different bacterial food sources, ethanol, acetic acid, temperature shifts, osmotic or hypoxia). Our results confirm that diverse germline processes are highly environmentally sensitive. We also show that stressful conditions may reduce offspring number through reduction of either sperm fertility or number, as well as defects in germline progression. The plastic responses in reproductive features of *C. elegans* N2 may differ greatly from the ones observed in other wild isolates of *C. elegans*, *C. briggsae* or *C.* sp. 11, revealing considerable genotype-by-environment interactions. We will discuss these and other results in the context of how such differential plasticity of the reproductive system contributes to germline integrity and reproductive success in variable environments.

849A

Maintenance of adult proliferative germ cells. **Zhao Qin**, E. Jane Albert Hubbard. Developmental Genetics Program, Helen and Martin Kimmel Center for Stem Cell Biology, Skirball Institute of Biomolecular Medicine, NYU Cancer Institute, Department of Pathology NYU School of Medicine, 540 First Ave, New York, NY.

Failure to maintain stem cells with age is associated with conditions such as tissue degeneration and increased susceptibility to tissue damage in many organisms, including humans. We are using the *C. elegans* germ line as a general model for stem cell aging because it combines a well-established genetic model for aging studies with a well-defined and accessible stem cell system, providing a unique opportunity to dissect the effects of aging on stem cell dynamics. We hypothesize that aging affects stem cells through regulating interactions between stem cells and their microenvironment, the niche, which has been shown to be required for stem cell maintenance during normal tissue homeostasis.

850B

Mapping and Characterization of *Caenorhabditis elegans* Mutant Defective in Sperm Function. **Sina Rahimi**, Gunasekaran Singarevelu, Andrew Singson. Waksman Institute of Microbiology, Piscataway, NJ.

The molecular identities of the proteins participating in sperm and egg interactions during fertilization are unraveled by the isolation and mapping of mutants defective in the process. Here we report our preliminary results on mapping and characterization of *as38*, a non-conditional sperm function defective mutant that we isolated from a recent screen induced by Ethyl Methyl Sulfonate (EMS). Sperm function is defined as the ability of a normal, developed sperm to bind and enter the oocyte to execute fertilization. We have placed *as38* in "*spe-9* class" of mutants which are a group of mutants known for defective sperm function. Gonad morphology and the other processes of gamete development and differentiation occur normally in *as38*. Although leaky, hermaphrodites of *as38* show significantly lower brood size in comparison to wild type. *As38* shows temperature dependent decline in brood size. Examination of the oocytes that exit spermatheca indicates the lack of sperm entry and hence the unfertilized oocytes become endomitotic. The sperm from *as38* male can successfully migrate into the spermatheca of recipient hermaphrodite. However, the fertility is significantly compromised in *as38* males. Sperms from dissected males show normal morphology and undergo normal in vitro activation in response to Pronase E treatment. Using two factor mapping, we have placed *as38* on the right arm of the Linkage Group IV near *unc-26*. We are currently performing three factor mapping to clone the *as38* allele.

851C

glp-4 encodes the valyl amino-acyl tRNA synthetase VARS-2. **Suchita Rastogi**[1], Ben Borgo[1], Paul Fox[1], Nannette Pazdernik[1], Elaine Mardis[1], Yuji Kohara[2], Jim Havranek[1], Tim Schedl[1]. 1) Genetics, Washington University School of Medicine, St. Louis, MO; 2) National Institute of Genetics, Mishima, Japan.

glp-4 is defined by the mutation bn2, which at a restrictive temperature (25C) results in adult hermaphrodites that are apparently normal somatically but with few germ cells. *glp-4(bn2)* has been widely used to generate germline-deficient animals for studies of aging, pathogenesis, stress resistance and to assess germline versus somatic gene expression.

To identify the encoded gene product, *glp-4(bn2)* was sequenced to >30x coverage using the Illumina Genome Analyzer. Within the ~2Mb genetically mapped region containing *bn2*, coding changes were identified in 5 genes. RNAi of these genes identified only one that gave a sterile phenotype, Y87G2A.5 (*vars-2*). *glp-4(bn2)* failed to complement the

vars-2 deletion *tm3947* for fertility at 25C. Tightly linked suppressors (*bn2bn39, bn2bn40 & bn2oz283*) were isolated and sequencing identified missense mutations within *vars-2*.

vars-2 encodes a cytoplasmic class I valyl amino-acyl tRNA synthetase that catalyzes attachment of valine to its cognate tRNA. *bn2* Gly296Asp and the intragenic revertants are in different parts of the CP-1 editing domain. Homology modeling of VARS-2 with the crystal structure of valRS from *T. thermophilus* suggests a molecular mechanism for the *bn2* mutation (inappropriate deacylation) and suppression by the revertants. The sterile phenotype of *glp-4(bn2)* at 25C likely results from a reduced pool of charged valyl-tRNA leading to reduced protein synthesis. *vars-2* likely also functions in the soma: 1) *vars-2(tm3947)* is larval lethal; 2) *in situ* hybridization shows somatic expression; and 3) structural analysis suggests that *vars-2* has unique functions that can not be compensated by paralog *vars-1* - VARS-2 is predicted to charge tRNAs with all four Val anti-codons while VARS-1, which has a bulky Trp residue in the anti-codon recognition pocket, is likely unable to bind anti-codons with third position purines. Thus *glp-4(bn2)* may also result in a reduction in protein synthesis in somatic tissues even though there is no obvious somatic phenotype; this occurs with the *rrt-1(gc47)* mutation in arginyl-amino-acyl tRNA synthetase, which results in reduced protein synthesis yet does not display strong somatic phenotypes. Since reduced translation can lead to alterations in lifespan and stress resistance, this property, rather than germline depletion, may be the cause of the *glp-4(bn2)* organismal aging and stress resistance phenotypes.

852A

A role for cardiolipin in the gonad development of *Caenorhabditis elegans*. **Taro Sakamoto[1]**, Nagaharu Yokomori[1], Yukae Otomo[1], Motoki Ohno[1], Takao Ioue[2,3], Hiroyuki Arai[2,3], Yasuhito Nakagawa[1]. 1) Kitasato University, Tokyo, Japan; 2) The University of Tokyo, Tokyo, Japan; 3) CREST, JST, Saitama, Japan.

Cardiolipin (CL) is a major membrane phospholipid that localizes to the mitochondrial inner membrane. Recently, CL has been paid attention as a functional phospholipid. At the cellular level, CL has been shown to have a role in the mitochondrial energy production, mitochondrial membrane dynamics, and apoptosis triggering. Although the biochemical features or cellular function of CL are well investigated, its in vivo physiological functions are poorly understood. In this study, by generating deletion mutants of CL synthase gene (*crls-1*), we show that CL is required for the gonad development in *C. elegans*.

To ensure that the CRLS-1 protein actually has a CL synthase activity, we determined the CL content in worms whose *crls-1* gene was knocked down by RNAi. In *crls-1(RNAi)* worms, the CL content was decreased to 39% that of mock RNAi worms. Concurrently, PG content, which is the precursor of CL, was increased to about eight-fold that of mock RNAi. Knock down of *crls-1* did not substantially affect on other phospholipid contents. These data indicate CRLS-1 is a functional homolog of CL synthase in *C. elegans*.

crls-1 mutants, *crls-1(tm2542)*, showed sterile phenotype in hermaphrodites with impaired oogenesis and reduced germ cell number. *crls-1* mutants produced 6-8 eggs per worm, and all of these eggs failed to hatch. The reduced brood size of *crls-1* mutants were restored by introduction of *crls-1* gene under the control of heat shock promoter, indicating the deletion of *crls-1* gene is responsible for the sterility of *crls-1* mutants. To further examine the reduction of germ cell number in *crls-1* mutants, we generated a double mutants *crls-1(tm2542) glp-1(ar202)*. *glp-1(ar202)* is a gain-of-function mutant allele, in which the GLP-1/Notch signaling pathway in gonad is constitutively activated, and the germ cells divide mitotically throughout the gonad arm. In *crls-1(tm2542) glp-1(ar202)*, the size of gonad and the number of germ cells were remained to be reduced as compared with *glp-1(ar202)* single mutants, indicating that the reduction of germ cell number in *crls-1* mutants is not caused by down-regulation of the GLP-1/Notch signaling.

Therefore, CL could have an important role for *C. elegans* gonad development through the maintenance of proper germ cell proliferation.

853B

The DEAD box RNA helicase Cr-VBH-1 is a constitutively P granule component in *C. remanei* germ cells and plays an important role on its germline function. **Laura Silvia Salinas Velázquez**, Ari Franco-Cea, Laura Lascarez-Lagunas, Ernesto Maldonado, Rosa E. Navarro. Instituto de Fisiología Celular, UNAM. Circuito Exterior s/n Ciudad Universitaria, C.P. 04510 Mexico, D.F. Mexico.

In *C. elegans*, P granules are important to regulate translation of specific mRNA and store proteins that are important in the development of the gonad and embryogenesis. P granules contain several DEAD box RNA helicases whose function is unwind specific mRNAs and/or facilitate RNA-protein interactions. One member of this family of RNA helicase is VBH-1 which is related to Vasa/Belle from *Drosophila*. We previously demonstrated that VBH-1 is necessary for function and development of the *C. elegans* germline. To understand better VBH-1 function, we are studying its homolog in *C. remanei*. Using an antibody against this protein, we found that like other members of the Vasa family, Cr-VBH-1 is expressed in the cytoplasm of germ cells and is constitutively associated to P granules. By RNAi, we found that Cr-VBH-1 is required for proper sperm activation as well as for maintaining spermatogenesis. Additionally, we found that Cr-VBH-1 is important for oogenesis and embryo development. Our data shows that Cr-VBH-1 might play a role in germline apoptosis as well as in cell death that occurs during embryogenesis. These results highlight that despite the different mating systems in these two species, VBH-1 function has only partially diverged and that extra layers of regulation are likely to have emerged in *C. elegans* to accommodate its androdioecious mating system. We believe that the role of Cr-VBH-1 is highly conserved and whose function is related to the global sex determination pathway, which is responsible of the sexual fate somatic and germline. This work shows the importance of this family of proteins during evolution.

854C

The tumour suppressor ING-3 regulates the DNA damage-induce apoptosis pathway in C. elegans. **Sitar Shah**, Jingjing Luo, JB Rattner, Karl Riabowol, Paul Mains. Biochemisty and Molecular Biology, Hlth Sci, Univ Calgary, Calgary, AB, Canada.

The INhibitor of Growth (ING) family of proteins are involved in multiple cellular processes including apoptosis. Caenorhabditis elegans ING-3 shows the highest homology to mammalian ING proteins, which have been shown to function in apoptosis by an unknown mechanism. The objectives of this project were to investigate the role of ING-3 in germline apoptosis and to explore potential new phenotypes arising from the lack of ing-3 function in C. elegans. Recent experiments in C. elegans have implied that the ing-3 gene likely functions in concert with the C. elegans p53 homolog, cep-1, to induce germ cell apoptosis in response to ionizing radiation. However, ing-3 does not influence the transcription of the cep-1 target egl-1 in the DNA damage-induced apoptosis pathway, therefore, excluding regulation of cep-1 transcriptional activity as a function of ing-3 in germline apoptosis. Experiments established that ing-3 feeds into the pathway downstream of ced-9, at the level of ced-4 and that this may involve a mechanism linked to the activity of ceramide, a known component of the germline apoptotic pathway. Additionally, the effects on lifespan, chromatin structure and nuclear architecture were examined in ing-3 mutant worms. ing-3 animals showed a 25% increase in average lifespan and an overall reduction in histone H3 acetylation with a small proportion of neurons showing highly condensed heterochromatic regions. This proportion of heterochromatic cells may account for the weak kinker, uncoordinated movement phenotype displayed by ing-3 animals. The experiments in this study will aid in our understanding of the role of ING-3 in apoptosis, chromatin remodelling, lifespan and nuclear architecture.

855A

SPE-44 functions as an early transcription factor in spermatogenesis. Madhura Kulkarni[1], Katherine E. Guevel[2], **Diane C. Shakes[2]**, Harold E. Smith[3]. 1) Cell Biology and Molecular Genetics, University of Maryland, College Park, MD; 2) Department of Biology, College of William and Mary, Williamsburg, VA; 3) Genomics Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD.

Although the factors which regulate the C. elegans sperm-oocyte decision are well characterized, the transcription factors that direct the subsequent differentiation of oocytes and spermatocytes remain elusive. We report the identification of spe-44 as a critical early regulator of sperm-specific transcription. Loss of spe-44 results in sperm-specific sterility. In spe-44 spermatocytes, the onset of spermatogenesis appears largely normal with the notable exception that the major sperm protein (MSP) fails to assembly into fibrous bodies. The spermatocytes nevertheless continue developing as they detach from the rachis and initiate the meiotic divisions. However they subsequently fail to undergo cleavage and instead exhibit a striking cell cycle arrest with stable, supernumerary asters. The spe-44 gene is expressed in the pachytene region of the germ line at the onset of spermatogenesis, but it is not detectable in oocyte-producing germ lines. Microarray screening demonstrates that spe-44 is required for transcriptional activation of several hundred sperm-enriched genes, including a number with known functions in sperm development. The spe-44 gene product is highly conserved throughout the phylum and exhibits male-enriched expression in other Caenorhabditis species, suggestive of a conserved functional role in nematode spermatogenesis. Our results constitute the first identification of a transcriptional regulator whose primary role is the activation of cell-type-specific transcription in the germ line.

856B

Regulation of Hermaphrodite Development by the F-box Protein SHE-1. **Y Shen**, Ronald E Ellis. Molecular Biology, UMDNJ-SOM, Stratford, NJ 08084.

In two androdioecious species of *Caenorhabditis*, F-box proteins play critical roles in hermaphrodite development. In *C. elegans*, FOG-2 interacts with GLD-1 to repress the translation of *tra-2* mRNAs. Although *C. briggsae* lacks an ortholog of FOG-2, the F-box protein SHE-1 acts independently of GLD-1 to down-regulate TRA-2.

Because a missense mutation in the F-box domain inactivates SHE-1, it might function as a classical F-box protein, bringing a target to the E3 ubiquitin ligase complex, where it is marked for degradation. To identify possible SHE-1 targets, we are using the yeast two-hybrid system. From a screen of about 340,000 cDNAs, we identified three genes that gave multiple, independent clones. One of these clones passed two further tests. First, PQN-94 interacts with SHE-1 when used as bait in the yeast two-hybrid system. Second, using RNA interference to knock down *pqn-94* partially suppresses the she-1 phenotype. At 25°C, *she-1*(*v49*); control(RNAi) mothers never produced hermaphrodites, but *she-1*(*v49*); *pqn-94*(RNAi) mothers produced 7% hermaphrodites. Additional tests with *she-1*(*v35*) showed the same pattern of suppression. However, knocking down *pqn-94* on its own had no phenotype.

To see if SHE-1 controls germ cell fates by targeting PQN-94 for degradation, we are carrying out three sets of experiments. (1) We are testing both proteins for interaction when expressed in HEK 293 cells. (2) We are preparing antibodies to each protein, to test their *in vivo* activities and distributions. And (3) we are looking for other proteins that interact with PQN-94, to learn how it might influence germ cell fates.

857C

Two likely GLP-1/Notch targets are essential for germline stem sell maintenance in both larvae and adults. Aaron M. Kershner[1], **Heaji J. Shin[2]**, Judith Kimble[1,2,3]. 1) Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 2) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI; 3) Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI.

C. elegans germline stem cells (GSCs) are maintained by GLP-1/Notch signaling and two redundant PUF family mRNA binding proteins, FBF-1 and FBF-2 (collectively, FBF). Notch signaling is essential for GSC maintenance in both larvae and adults, while FBF is essential only in late L4s and adults. The *fbf-2* gene is the only Notch target gene known to date with a key role in GSC maintenance (1). Therefore, we sought additional Notch targets crucial for germline self-renewal. Two genes, *lst-1* and T27F6.4, were identified as likely FBF targets (2) and also putative Notch targets (3). To ask whether *lst-1* and T27F6.4 control GSC maintenance, we analyzed an *lst-1* deletion mutant and T27F6.4 (RNAi) animals (progeny of L4s fed T27F6.4 RNAi bacteria). No effect was seen when either gene was removed singly, but depletion of the two together had a phenotype indistinguishable from that of a null *glp-1* mutant: germ cell number was reduced from ~2000 to 8-10 total in L3 stage and these few germ cells differentiated as sperm. We conclude that *lst-1* and T27F6.4 are redundant in their control of larval GSC proliferation and therefore dub T27F6.4 *sygl-1*, for synthetic germline proliferation defective. A different RNAi regimen, which depleted *lst-1* and *sygl-1* specifically in adults, revealed that these two regulators also control adult germline self-renewal. We suggest that the *lst-1* and *sygl-1* genes are pivotal for GSC maintenance throughout postembryonic germline development.

References: (1) Lamont and Kimble (2004) Dev Cell 7(5):697-707; (2) Kershner and Kimble (2010) PNAS 107(8):3936-41; (3) Yoo and Greenwald (2004) Science 303(5658):637-8.

858A

Regulation of the trafficking of TRP-3 channel by a novel protein during spermiogenesis in *Caenorhabditis elegans*. **Gunasekaran Singaravelu**, Indrani Chatterjee, Sina Rahimi, Marina Druzhinina, Andrew Singson. Waksman Inst Microbiol, Piscataway, NJ.

Despite undergoing normal development and acquiring normal morphology, mutation in *spe-38* or *trp-3/spe-41* causes identical phenotype in *C. elegans* - the mutant sperm fails to fertilize oocyte. SPE-38 is a nematode-specific novel protein and TRP-3/SPE-41 is a Ca^{2+} channel. Localization of both of these proteins is confined to the membranous organelle in the undifferentiated spermatids. In mature spermatozoa, SPE-38 is localized to psedopod and TRP-3/SPE-41 is localized to the plasma membrane. Dynamic redistribution of TRP-3/SPE-41 from membranous organelle to plasma membrane is dependent on SPE-38, since in *spe-38* mutant spermatozoa, TRP-3/SPE-41 is trapped within the membranous organelle and fails to reach cell surface. Split-ubiquitin yeast two hybrid analyses revealed the segments of SPE-38 and TRP-3/SPE-41 required for the regulation of the dynamic physical interaction between these proteins. The correlation between the ability or inability of the various forms of SPE-38 to rescue *spe-38* mutant and the required segments of SPE-38 for its physical interaction with TRP-3/SPE-41 suggest the *in vivo* relevance of the interaction between SPE-38 and TRP-3/SPE-41.

859B

Chemically reprogramming the adult sperm/oocyte fate decision. **Elena P. Sorokin**[1,2], Clinton T. Morgan[3,4], Judith Kimble[1,2,3,4,5,6]. 1) Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI; 2) Biotechnology Training Program, University of Wisconsin-Madison, Madison, WI; 3) Integrated Program in Biochemistry, University of Wisconsin-Madison, Madison, WI; 4) Medical Scientist Training Program, University of Wisconsin-Madison, Madison, WI; 5) Department of Biochemistry, University of Wisconsin-Madison, Madison, WI; 6) Howard Hughes Medical Institute, University of Wisconsin-Madison, Madison, WI.

The molecular basis of germ cell specification as sperm or oocyte remains poorly understood. In most animals (nematodes, fruitflies and mice), somatic signaling controls germline sexual fate, but the regulators in germ cells that direct the sperm or oocyte fate are best understood in *C. elegans*. Based on that knowledge, we recently devised a method to chemically reprogram germline sex: U0126, a MEK kinase inhibitor, transforms a *puf-8; lip-1* masculinized germline to produce functional oocytes instead of sperm (Morgan et al., 2010). Here we report the use of this chemical method to analyze two critical aspects of germ cell fate reprogramming. First, we mapped the reprogramming to cells at the boundary of the mitotic and transition zone, where virtually all germ cells have entered the meiotic cell cycle. Consistent with this map, active MAP kinase and the terminal sperm fate regulator FOG-1 rapidly decrease in the transition zone after U0126 treatment. Therefore, the U0126-mediated effect on the sperm/oocyte decision occurs as germ cells enter meiotic prophase. Second, we used RNA-seq to profile changes in mRNA expression after chemical reprogramming. Specifically we compared RNAs from U0126-treated *puf-8; lip-1* vs U0126-treated N2s and from U0126-treated vs DMSO vehicle-treated *puf-8; lip-1* over an 18-hour time course. Preliminary results reveal a highly-enriched binding motif in the promoters of mRNAs differentially expressed upon reprogramming. Moreover, RNAi against the likely transcription factor affects germline sex. This approach therefore promises to extend our molecular understanding of germ cell fate determination to a transcriptional level. We conclude that chemical reprogramming provides an invaluable tool for analyzing the sperm/oocyte decision.

Morgan, C. T., Lee, M. H., Kimble, J., 2010. Chemical reprogramming of *Caenorhabditis elegans* germ cell fate. Nat Chem Biol 6, 102-4.

860C

A peroxiredoxin antioxidant enzyme regulates the timing of hermaphrodite spermatogenesis. **Joanne Stamford**[1], Christian Eckmann[2], Elizabeth Veal[1]. 1) Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle, United Kingdom; 2) Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

Peroxiredoxins (Prdx) are highly conserved, abundant antioxidant enzymes with multiple, diverse roles in responses to reactive oxygen species (ROS). ROS, such as hydrogen peroxide, are produced in cells during normal aerobic metabolism and in response to various stimuli and have the potential to cause oxidative damage. The peroxidase activity of peroxiredoxins has an important role in detoxifying hydrogen peroxide to prevent this damage. However, it is now widely accepted that hydrogen peroxide also has signaling functions and, in addition to their ROS-protective roles, peroxiredoxins are also important regulators of hydrogen peroxide signaling. Previous studies in *C. elegans* have shown that the thioredoxin peroxidase activity of the cytosolic 2-Cys Prdx, PRDX-2, is required in the intestine to protect against the toxic effects of exogenous hydrogen peroxide [1]. However, PRDX-2, is also required in other tissues for normal *C. elegans* growth and fecundity [1, 2]. Here, we investigated the basis for the reproductive defect of the *prdx-2* mutant. We have established that a shortened period of spermatogenesis contributes to the lower fecundity of *prdx-2* mutant and *prdx-2* RNAi-treated hermaphrodites. We find that *prdx-2* mutants exhibit a slight delay in larval development including the onset of hermaphrodite spermatogenesis. However, despite this developmental delay, *prdx-2* mutants switch prematurely from spermatogenesis to oogenesis resulting in the production of fewer sperm. Data will be presented from genetic studies to determine the underlying mechanism responsible for the premature switch to oogenesis in PRDX-2-deficient animals. There is a growing appreciation of the important role that ROS, such as hydrogen peroxide, play in regulating a variety of biological processes, including cell growth, differentiation and migration. Our data revealing that a peroxidase, PRDX-2, is required for the appropriate timing of the sperm/oocyte switch raises the intriguing possibility that ROS may also be intimately involved in controlling this cell fate decision. [1] Olahova M, Taylor SR, Khazaipoul S, Wang J, Morgan BA, Matsumoto K, Blackwell TK, Veal EA. (2008) PNAS 105(50):19839-19844. [2] Isermann K, Liebau E, Roeder T, Bruchhaus I. (2004) J Mol Biol. 338:745-55.

861A

A sperm competition mutant with defects in sperm motility. Daniela Chavez, **Gillian Stanfield**. Dept of Human Genetics, University of Utah, Salt Lake City, UT.

Although males are not necessary for reproduction in *C. elegans*, if mating occurs then male sperm win: cross progeny are produced to the exclusion of self progeny. We would like to understand the mechanistic basis for male sperm precedence. It has been shown previously that precedence is due to an intrinsic characteristic of male sperm cells, that it requires motility, and that it correlates with the larger size of male sperm. Using a genetic screen for males with reduced precedence, we identified a mutant, *me69*, that has defects in sperm usage under conditions of competition with wild-type sperm. *me69* male sperm show reduced precedence when they compete with wild-type hermaphrodite sperm. However, *me69* hermaphrodites have normal self fertility, arguing against a generalized spermatogenesis defect. Interestingly, *me69* sperm show a normal size distribution. *me69* male sperm show motility defects; after transfer to the hermaphrodite, sperm accumulate poorly in the spermatheca. We are analyzing their migration in more detail to determine whether this difference arises from poor targeting, poor retention, or some other alteration in motility. We have identified a candidate gene encoding a divergent kinase that is expressed in sperm. A deletion mutant shows migration and precedence defects similar to those of *me69*. We are determining the localization of this kinase and investigating the possibility that either its levels or localization may be different in males vs. hermaphrodites.

862B

Sperm-egg fusion generates a biphasic traveling calcium wave in *C. elegans*. **J. Takayama**, S. Onami. Lab. for Developmental dynamics, RIKEN QBiC, Japan.

Fertilization starts with a dynamical change of intracellular calcium concentration in a pattern characteristic to each species. In *C. elegans*, Samuel et al. (2001) revealed the occurrence of a single calcium elevation, but the detailed spatiotemporal pattern and its underlying mechanism was unknown. Here we investigated the spatiotemporal pattern of the calcium dynamics, and analyzed the mechanism for the generation of the observed pattern both experimentally and theoretically.

First, a detailed spatiotemporal calcium dynamics was visualized by a fluorescent calcium indicator and time-lapse spinning-disk confocal microscopy. The quantification of the movies by computational image processing methods revealed that the fertilization calcium dynamics forms a biphasic traveling wave unique to *C. elegans*. The wave composed of a fast local wave that arises from the sperm entry point at the time of the sperm entry, followed by a slow global wave that propagates throughout the cell. To understand the physical mechanism by which the biphasic wave pattern is generated, we analyzed a mathematical model known as the bistable reaction-diffusion equation. This model assumes (1) simple diffusion of calcium ions and (2) a uniformly distributed calcium releasing machinery whose releasing rate depends on calcium concentration itself. This releasing rate reflects a simplified reaction mode of a calcium-induced calcium-release channel (CICR channel), such as IP_3 receptor, which is known to be involved in the propagation of fertilization calcium waves. We found that, under these assumptions, a transient calcium elevation at the one end of the oocyte can generate a biphasic traveling wave similar to the observed pattern. Next, we asked what process in the fertilization is required for the generation of the calcium wave by imaging *spe-9*, *spe-41* and *spe-11* mutants, which are defective in sperm-egg recognition, sperm-egg fusion and a signal transduction after fusion, respectively. Calcium signal hardly changed in *spe-9(hc88)* or *spe-41(sy693)* mutant oocytes passing through the spermatheca, whereas a wild type-like wave was observed in *spe-11(hc77)* mutant. These results suggest that the sperm-egg fusion

is the key process for the generation of the calcium wave.

Taken together, it is suggested that sperm-egg fusion transiently elevates the calcium concentration at the sperm entry point and the local rise is propagated by CICR machinery thereby forming a biphasic traveling wave pattern characteristic to *C. elegans*.

863C

The conserved PAM-1 aminopeptidase somatically regulates pachytene progression. **Chris Trzepacz**[1], Craig Mello[2]. 1) Department of Biological Sciences, Murray State University, Murray, KY; 2) Program in Molecular Medicine and Howard Hughes Medical Institute, University of Massachusetts Medical School, Worcester, MA.

Mutations in *pam-1*, the *C. elegans* ortholog of the puromycin sensitive aminopeptidase, result in reduced brood sizes and a highly penetrant embryonic lethal phenotype. Phenotypically, *pam-1* embryos display defects in polar body extrusion, sister chromatid segregation, and zygotic cell-fate specification. In addition these functions, we have identified a novel role for PAM-1 in facilitating the transition of the *C. elegans* germline through meiotic prophase. Germ cells utilize the length of the gonad to systematically transition through the stages of meiotic prophase I. Exit from pachytene and entry into diplotene occurs at the bend between the distal and proximal regions of the gonad, after which the nascent oocytes begin to cellularize, increase in volume, and queue into a single file in preparation for fertilization. However, the gonads of *pam-1* animals display an expanded region of pachytene-stage germ cells that frequently extend past the gonad curvature and into the proximal arm of the gonad. Because the transition from early to late pachytene is triggered by the temporal activation of the conserved MPK-1 signaling pathway, we examined the relationship between PAM-1 and these signaling components. Culture of *let-60*, *mek-2*, and *mpk-1* reduced-function alleles on *pam-1* RNAi food, or genetically combining *pam-1* with these alleles, produces synergistic increases in the penetrance of the expanded pachytene and fecundity phenotypes. Direct visualization of MPK-1 activity by immunostaining reveals that MPK-1 activation is delayed in the *pam-1* germline. This delay in MPK-1 activation leads to an expansion of the early pachytene region and the extension of the late pachytene region into the proximal gonad. We examined the expression of PAM-1 by creating strains rescued by a *pam-1p:gfp:pam-1* transgene. GFP:PAM-1 expression was detected in the somatic gonadal tissues, gut cells, and robustly in neurons. GFP:PAM-1 colocalizes with the expression of a gonadal sheath cell-specific transgene (*lim-7p:mCherry*), and somatic RNAi deficient *rrf-1(pk1417)* animals cultured on *pam-1*(RNAi) food fail to develop the expanded pachytene phenotype. We conclude that in addition to its embryonic responsibilities, PAM-1 functions in the somatic gonad to govern neighboring germline meiotic transitions by regulating the germinal Ras/MAPK pathway, perhaps by facilitating the production and delivery of indeterminate, inductive signaling molecules.

864A

Regulation of RAS/MAPK signaling by PUF-8 and GAP-3 is essential for meiotic progression. **Samir Vaid**, Mohd Ariz, Kuppuswamy Subramaniam. Biological Sciences and Bioengineering, Indian Institute of Technology, KANPUR, India.

Mitosis- meiosis balance in germline stem cells (GSCs) is a useful paradigm to investigate how proliferation vs differentiation decision is controlled in tissue homeostasis. In Caenorhabditis elegans, PUF-8, a member of the well conserved family of RNA-binding proteins, contribute to GSC maintenance by directly promoting GSC mitosis. However, it functions in primary spermatocytes to promote meiotic progression. At 25 °C, primary spermatocytes in about 40 per cent of puf-8(-) worms exit meiosis and dedifferentiate into germ cell tumor. In contrast, they are largely normal at 20 °C. In a synthetic screen to identify genes that enhance puf-8(-) phenotype, we have identified gap-3, a GTPase activating protein of the RasGAP family, as functionally redundant with puf-8 in ensuring proper progression through meiosis. In the puf-8(-) gap-3(-) double mutant, both types of gametes exit meiosis and proliferate in an uncontrolled manner forming germ cell tumor even at 20 °C. In contrast, either single mutant is normal. Since RasGAP proteins are known to regulate RAS signaling pathway, we examined the levels of various components of this pathway in the double mutant. We find that the level of phosphorylated MPK-1, the active form of MAP kinase, is significantly elevated in the double mutant, but not in either single mutant, germ cells. Since GAP-3 is likely to inactivate RAS signaling by activating the GTPase activity of RAS, we wanted to investigate how PUF-8 contributes to RAS signaling. We find that PUF-8 directly interacts with the 3'UTR of let-60, the worm ortholog of RAS, and suppresses its translation in germ cells progressing through meiosis. Mutations in let-60 3'UTR that affect interaction with PUF-8 in vitro, increase the levels of GFP reporter in transgenic worms that express GFP under the control of let-60 3'UTR. Consistent with this, partial suppression of let-60 activity through RNAi rescues puf-8(-) gap-3(-) to wild-type. Thus, our results indicate both GAP-3 and PUF-8 negatively regulate RAS/ MAP kinase pathway in meiotic germ cells, and either one of them is sufficient for MAPK signaling required for meiotic progression. Earlier, another worm PUF protein, FBF, has been shown to control RAS/MAP kinase pathway in mitotic germ cells suppressing the expression of MPK-1 (Lee at al., PLOS Genetics, 3; e233). Our results described here shows that PUF-8 controls this pathway in the later meiotic stage by suppressing the translation of let-60 mRNA. Taken together, these observations highlight the importance of continued modulation of RAS/MAP kinase signaling in germ cell development.

865B

Detachment of the pharynx in *lim-7(tm674)* mutants occurs embryonically. **Laura G. Vallier**. Dept Biol, Hofstra Univ, Hempstead, NY.

LIM-7 is one of the seven *C. elegans* proteins in the LIM-homeodomain (LIM-HD) family of transcription factors, whose members are conserved in developmental processes throughout evolution in a wide-range of tissues. There are six subclasses of LIM-HD proteins and *C. elegans* has a representative in each group. LIM-7 belongs to the Islet subclass, whose mouse ortholog is the earliest marker for cardiac lineages and whose rat ortholog binds to the insulin gene enhancer. In *C. elegans*, three reporter constructs with differing parts of the *lim-7* genomic region each give different expression patterns. Expression of transgenes fused to LIM-7 has been observed in the gonadal sheath cells, the URA neurons, the labial neurons, unidentified cells in the area of the nerve ring, and body wall, head and rectal muscles and other cells as well. A deletion of *lim-7* results in L1 lethality with pleiotropic defects before death including uncoordinated movement and pharynx unattached (Pun) phenotypes, among others. Mutations in genes encoding other transcription factors such as *ast-1*, *cdh-4*, *die-1*, *unc-39* and *elt-5* are also associated with Pun phenotypes. Close inspection of the Pun phenotype caused by *lim-7* revealed that its origin was embryonic. With this in mind, a 4D analysis was undertaken to determine the timing and manner of detachment of the pharynx. Early results indicate that the primordial pharynx either begins to attach or attaches transiently and then detaches and then retracts. Results of the analysis will be presented. Determining the mechanism of pharynx detachment will give a better understanding of the role that LIM-7 plays in embryonic development.

Voutev et al 2009 Characterization of the *Caenorhabditis elegans* Islet LIM-homeodomain ortholog, *lim-7* FEBS Letters **583**:456-464.

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866C

Centriole elimination during C. elegans oogenesis. **Lukas von Tobel**[1], Tamara Mikeladze-Dvali[1,2], Pierre Gönczy[1]. 1) Ecole Polytechnique Fédérale de Lausanne, Lausanne; 2) Ludwig-Maximillians-Universität München, München.

Centrosomes, the primary microtubule organizing centers of animal cells, consist of a pair of centrioles surrounded by pericentriolar material (PCM). Centrosome number must be strictly regulated both in the soma and the germline for genome integrity. The single centrosome present at the onset of the cell cycle in somatic tissues duplicates to give rise to two entities, one of which is ultimately inherited by each daughter cell. Gametogenesis poses a unique challenge to this general pattern of centrosome inheritance because one of the parental centrosomes must be eliminated so that only one centrosome is inherited by the zygote. In most metazoans, including humans and C. elegans, centrioles are eliminated in the female germline, whereas they are maintained during spermatogenesis and are thus contributed strictly paternally to the zygote. The molecular basis underlying centriole elimination in the female germline is not understood. To begin addressing this question, we examined the distribution of centriolar proteins such as SAS-4, SAS-5 and SAS-6, as well as PCM components such as SPD-5 and TBG-1 during oogenesis. We established that centrioles and the surrounding PCM disappear at the onset of oocyte cellularization after the exit from pachytene. The disappearance of centrioles at this stage is supported by serial section electron microscopy whereby centrioles were present in the pachytene stage but absent in the loop region thereafter. Furthermore, by using mutants where the germline sex can be manipulated, we show that the somatic cells surrounding the gonad most likely do not contribute to centriole elimination. To investigate the dynamics of centriole elimination, we developed methods for live imaging of centriolar proteins tagged with GFP to monitor the process in the intact animal. Our preliminary data suggests that centriole elimination occurs within 15 minutes. This descriptive analysis sets the stage for the identification of the mechanisms underlying the evolutionarily conserved process of centriole elimination.

867A

Identifying TEG-1 interacting proteins that are involved in germline development. **Chris Wang**, Dave Hansen. Department of Biological Sciences, University of Calgary, Calgary, AB, Canada.

In the C. elegans germline, mitotically dividing cells are found at the distal end of each gonad arm, while the more proximal cells enter meiosis and differentiate into gametes. The GLP-1/Notch signaling pathway is the major player in promoting distal germ cell proliferation by suppressing two redundant pathways; one containing GLD-1 and the other GLD-2, which each promote meiotic entry. Genetic enhancer screens using a *glp-1* weak gain-of-function background had identified *teg-1* (tumours enhancer of glp-1(gf)) as a gene in maintaining the balance between proliferation and meiotic entry. We have identified UAF-1 (the large subunit of the U2 small nuclear ribonucleoparitcle auxiliary factor) and VIG-1 (Drosophila Vasa Intronic Gene ortholog) as potential TEG-1 binding proteins through proteomics. Results from co-immunoprecipitation and bacterial pull-down experiments suggest that TEG-1 directly interacts with UAF-1. Moreover, a complex containing CD2BP2 (human TEG-1 homolog) and U2AF65 human UAF-1 homolog) is detected in HeLa extracts. The human CD2BP2 also physically binds to U2AF65 *in vitro*. These data, together, indicate that the direct interaction between TEG-1 and UAF-1 is conserved from worms to humans in RNA independent manner. Antibodies raised against VIG-1 detect the protein in the cytoplasm of the germ cells by indirect immunofluorescence. Like *teg-1* mutations, *vig-1* null mutants also enhance the over-proliferation phenotype in a weak *glp-1* gain-of-function background. In addition, *vig-1; teg-1* double mutants show pleiotropic germline defective phenotypes. As VIG-1 has been shown to be a component of the miRNA-induced silencing complex, we are currently investigating possible genetic interactions between genes in the miRNA pathway and genes in the genetic pathway controlling the balance between proliferation and differentiation. In

addition, we are determining the genetic epistasis of *vig-1* in the proliferation/differentiation pathway. A series of biochemical experiments are being performed to verify the interaction between VIG-1 and TEG-1. Results from both genetic and biochemical experiments would provide a better understand on the mechanism of VIG-1in regulating the proliferation vs. meiotic entry decision in the germ line.

868B
The PP2A phosphatase subunit PPTR-1 is required to stabilize embryonic P granules during mitosis. **Jennifer T. Wang**, Christopher M. Gallo, Geraldine Seydoux. Molecular Biology & Genetics, Johns Hopkins Univ / HHMI, Baltimore, MD.

P granules, conserved ribonucleoprotein complexes, are important for germ cell development. In embryos, maternally inherited P granules are preferentially maintained in the germ (P) lineage. Imaging and mutant analysis revealed that P granule segregation occurs in two steps. First, granules in the anterior cytoplasm are disassembled by the RNA binding proteins MEX-5/MEX-6. Second, during mitosis, granules in the posterior cytoplasm grow in size and fuse. This growth concentrates P granule components in the posterior cytoplasm, ensuring their preferential inheritance by P blastomeres. To identify the molecules involved, we performed an RNAi screen in a GFP::PGL-1 strain and identified pptr-1, a regulatory 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. In a pptr-1 mutant, P granules disassemble during mitosis and equal levels of P granule RNAs and proteins are inherited by the P and somatic blastomeres. Surprisingly, segregation of other asymmetric proteins, such as PIE-1, is unaffected in pptr-1 mutants, indicating that 1) PPTR-1 is required specifically for P granule asymmetry and 2) segregation of P granules can be uncoupled from the segregation of other germ plasm components. We have found that PPTR-1 localizes to P granules, raising the possibility that PPTR-1 may interact directly with P granule components. Current efforts to identify the mechanism by which PPTR-1 promotes P granule segregation will be discussed, as well as the role of P granule segregation in germ cell development.

869C
Assembly of a Membrane-associated Complex Required for the Oocyte-to-embryo Transition. **Yuemeng Wang**, Geraldine Seydoux. Molecular Biology & Genetics, Johns Hopkins University /HHMI, Baltimore, MD.

The transition from oocyte to embryo occurs in the absence of transcription and therefore requires changes in the activity, level and localization of many oocyte proteins. A complex composed of a kinase (MBK-2) and three pseudo phosphatases (EGG-3, EGG-4 and EGG-5) is required to modify oocyte proteins at fertilization. The MBK-2 complex is anchored at the oocyte membrane and is released into the cytosol upon fertilization. Release of the complex is accompanied by degradation of the three pseudophosphatases and activation of the MBK-2 kinase. The goal of my project is to understand how the MBK-2 complex is assembled and disassembled at the correct time during development. By examining the localization of each component, I have found that MBK-2, EGG-3, EGG-4 and EGG-5 show dynamic localization patterns during early oogenesis and co-localize at the oocyte membrane only towards the end of oogenesis when the oocyte is getting ready for fertilization. I am now determining what signals are required to localize the complex at the membrane.

870A
The role of the protein kinase CHK-1 in early germ line development in *C. elegans*. **Ashley B. Williams**, W. Matthew Michael. Molecular and Computational Biology, University of Southern California, Los Angeles, CA.

The germ line precursor cells Z2 and Z3 arise at approximately the 100-cell stage during embryogenesis and arrest in the G2 phase of the cell cycle. Z2 and Z3 remain quiescent until newly hatched L1 larvae begin feeding, and only then do they enter mitosis. Several signaling pathways control the release of the G2 arrest; however, the molecular mechanisms are not yet fully understood. The essential serine/threonine protein kinase CHK-1 is best known as an effector protein in the DNA damage response, but it also has essential functions during normal development. High-efficacy RNAi against *chk-1* causes embryonic lethality, while milder *chk-1* RNAi allows normal development to adulthood, although the F1 offspring of P_0-treated worms fail to develop a germ line. Using a genetic approach, supported by live imaging and confocal immunofluorescence microscopy, we have explored the function of CHK-1 in germ line development.

Our data show that CHK-1 is activated in Z2 and Z3 by a temporally controlled mechanism in response to larval feeding. Activated CHK-1 then regulates the timing of the entry of Z2 and Z3 into mitosis. Attenuation of CHK-1 activity hastens entry of Z2 and Z3 into mitosis and, as a result, DNA damage accumulates and germ line development fails. Because CHK-1 activation normally requires DNA replication, these findings reveal a novel function for CHK-1 in controlling the timing of mitosis after release of a G2 arrest. Progress towards determining the molecular basis for this novel function of CHK-1 will be reported.

The present results reveal a new function for CHK-1 in G2-arrested cells that preserves genome integrity and promotes germ line development in *C. elegans*. This new function for CHK-1 may have important broader implications for understanding the biological processes required to ensure proper cell cycle re-entry by quiescent cells.

871B
GLD-1 binding marks specific mRNA targets for accumulation in oocytes. Claudia Scheckel, Dimos Gaidatzis, **Jane E. Wright**, Rafal Ciosk. Friedrich Miescher Institute, Basel, Switzerland.

Maternal mRNAs loaded into transcriptionally quiescent oocytes are stored in an inactive but stable form, in case of human oocytes for several decades, to support the oocyte-to-embryo transition (OET). These mRNAs are thought to be stable by 'default', due to a global repression of mRNA decay pathways. However, we find that a large group of mRNAs encoding factors driving OET is specifically stabilized in the C. elegans germ line by the combined function of two conserved RNA regulators. One of them, the sequence-specific RNA binding protein of the STAR family, GLD-1, represses translation of associated mRNAs. This appears to mark them for stabilization that depends on CGH-1, a DDX6-like RNA helicase, which is a component of germline RNA/protein granules and somatic processing (P) bodies. Our findings suggest that the GLD-1 and CGH-1-dependent pathway for mRNA storage ensures efficient accumulation, and consequently function, of OET regulators.

872C
Regulation of P granule stability via PGL proteins. **M. Yonetani**[1], M. Hanazawa[2], A. Sugimoto[2,3]. 1) Osaka University, Osaka, Japan; 2) RIKEN, Center for Developmental Biology, Kobe, Japan; 3) Tohoku University, Sendai, Japan.

P granules are large ribonucleoprotein (RNP) complexes specifically segregated into the germ line cells. We have previously reported that PGL proteins (PGL-1 and PGL-3) function as the scaffold to form P granules and require two functional domains; an RGG box for recruiting RNA and RNA-binding proteins, and a self-association domain for assembling globular granules (Hanazawa, Yonetani, and Sugimoto, 2011). In the fertilized egg P granules are present throughout cytoplasm, and as the cell is polarized, P granules become localized to the posterior sides, and through each of cell divisions, they are specifically segregated to the germ line cells. As previously reported (Brangwynne, et al. 2009), this asymmetric localization of P granules in early embryos is dependent on the differences of their stability along the anterior-posterior axis, which is regulated downstream of the PAR proteins: P granules are unstable at the anterior, while they are more stable at the posterior. We found that PGL-3 was phosphorylated in vivo, thus hypothesized that the stability of P granules may be regulated by the phosphorylation of PGL proteins. To test this hypothesis, we constructed GFP-PGL-3 variants having mutations in potential phosphorylation sites and examined their ability to form granules in early embryos. We found that a PGL-3 variant having a Serine-to-Alanine mutation failed to form granules and dispersed in the cytoplasm. The Serine-to-Glutamate mutation of the same residue did not affect the ability to form granules. We are further testing whether this Serine is indeed phosphorylated. As an alternative possibility, protein-protein interaction of PGL-3 with other proteins could affect the P granule stability. To identify interactors of PGL-3, co-immunoprecipitates with PGL-3 were analyzed by mass-spectrometry. The candidate interactors included several known P granule components, P body components and ubiquitin-proteasome (UPS)-related factors. We are analyzing whether the UPS system is involved in the regulation of P granule stability.

873A
Exploring the role of RNP granules in regulating RNA stability. **Mark T Zeigler**, Breanna Rice, Jennifer Schisa. Biology, Central Michigan University, Mt. Pleasant, MI.

RNA-binding proteins function in a diversity of ways to regulate RNA metabolism, including RNA translation, stability, and subcellular localization. Increasingly, it has become clear that many of these regulatory processes occur in compartmentalized regions of the cytoplasm, including RNP (ribonucleoprotein) structures such as stress granules and P bodies (processing bodies). We have been investigating RNPs that are induced in *C. elegans* oocytes in response to extended meiotic arrest or stress. Based on their composition, the hypothesis for the function of the RNP granules is to regulate mRNA stability and/or translation during periods of extended meiotic arrest or stress. The Schisa lab has identified 59 genes as required for RNP granule assembly in an RNAi screen. To investigate the role of RNP granules in regulating mRNA stability, qRT-PCR is being performed. We are currently comparing levels of pos-1 mRNA, an mRNA at high levels in RNP granules, in arrested *fog-2* oocytes with levels in arrested *fog-2* oocytes after RNAi inhibits assembly of RNP granules. The goal is to determine whether failure to assemble RNP granules affects RNA stability. We are using pgk-1 as our control RNA. We are planning to assay several mRNAs known to localize to RNP granules in arrested oocytes, and have a large number of RNAi targets to use to interfere with RNP granule assembly. We hope that this approach will address part of our hypothesis for the function of RNP granules in meiotically-arrested oocytes.

874B

N2-specific differences in behavior, growth, and physiology are explained by a laboratory-derived allele of *npr-1*. **Erik C. Andersen**[1], Raj Ghosh[1], Josh S. Bloom[1], Justin P. Gerke[1], Leonid Kruglyak[1,2]. 1) Lewis-Sigler Inst, Princeton Univ, Princeton, NJ; 2) HHMI.

Over the past forty years, an amazing amount of research has been performed on a single *C. elegans* strain: N2. This strain may have gone through as many as 1000 generations (over 16 years) before long-term storage was discovered (1). During that time, N2 likely accumulated many mutations through selection in the laboratory. We know of at least two genes that are affected by laboratory-derived mutations, *npr-1* and *glb-5*, which regulate an oxygen avoidance behavior in the presence of food (1). Not only is the N2 strain easier to pick and propagate, as clumping, bordering, and burrowing are highly reduced, but we will present evidence that it grows larger, has more offspring, crawls faster, and is more resistant to pathogen infection than wild *C. elegans* strains because of the lab-derived allele of *npr-1*. We mapped quantitative trait loci (QTL) for each of these traits using an advanced intercross recombinant inbred line (RIAIL) collection between N2 and the wild isolate from Hawaii CB4856. Additionally, we mapped thousands of gene expression differences between these two strains to QTL overlapping *npr-1*. We will present data that suggest *npr-1* is the causal gene for many of these traits, using nearly isogenic lines (NILs) and loss-of-function alleles, and the growth and physiological differences are caused by the *npr-1*-mediated oxygen avoidance behaviors.

We are interested in identifying natural variants between *C. elegans* strains for a variety of phenotypic traits. Nevertheless, *npr-1* makes complex trait mapping more difficult, as it explains the majority of the genetic trait variance for crosses involving N2. To circumvent the laboratory-derived *npr-1* allele, we constructed a collection of 359 RIAILs using CB4856 and a nearly isogenic line that has the normal form of *npr-1* in an otherwise N2 background. However, this NIL with corrected *npr-1* has other N2-specific laboratory-derived mutations, so using high-throughput selective sequencing, we have identified the most divergent *C. elegans* strains for use in future quantitative genetic studies. Because assays on agar plates lead to differences in food exposure, oxygen concentration, and humidity, we have optimized high-throughput liquid assays of growth and fecundity using robotic liquid handling, worm sorting, and image analysis.

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

875C

Exploring the mutational landscape of Caenorhabditis. Dee Denver[1], Larry Wilhelm[1], Peter Dolan[2], Dana Howe[1], K Gafner[1], **Charles Baer**[3]. 1) Dept. Zoology, Oregon State University, Corvallis, OR; 2) Division of Science and Math, University of Minnesota at Morris, Morris, MN; 3) Dept. of Biology, University of Florida, Gainesville, FL.

Mutation is often metaphorically referred to as "the fuel of evolution" because without mutation, evolution would grind to a halt. Different groups (species, higher taxa) evolve at different rates, but the extent to which variation in mutation underlies variation in the rate of evolution is unknown. The rate and molecular spectrum of mutations are known to vary at all taxonomic levels, including between individuals in the same population. However, the causes of variation in genome-wide mutational properties are difficult to disentangle, because variation in mutational properties could result from the effects of genotype, environment or both. We report single-nucleotide mutation (SNM) rates and spectra for two genotypes of C. briggsae (HK104, PB800) and C. elegans (N2, PB306) in which mutations were allowed to accumulate under relaxed selection for 250 generations under uniform conditions. Seven mutation accumulation ("MA") lines from each genotype were sequenced with Illumina technology at ~6X average coverage. As found previously in the N2 strain of C. elegans, the average SNM rate is ~2 x 10^{-9}/gen. Overall SNM rate varied between the four genotypes, mainly due to the low rate of the PB306 strain of C. elegans. Of the six possible paired mutation types, two (A:T->T:A and A:T->G:C) varied between genotypes. G:C->T:A transversions were more common than in the standing within-species genetic variation, as found in previously in N2. Also as found previously in N2, mutations from G and C to T and A are much more common than from A and T to G and C. These findings generalize the conclusion that mutational bias alone cannot explain the observed genome-wide base composition or the transition/transversion ratio.

876A

Proximate and ultimate cumulative effects of temperature on spontaneous mutation in C. briggsae and C. elegans. Dejerianne Ostrow, Suzanna Lewis, Chikako Matsuba, Matthew Salomon, Laurence Sylvestre, Kerry Regan, Brandon Tabman, Ambuj Upadhyay, **Charles F. Baer**. Dept Biol, Univ Florida, Gainesville, FL.

A long-standing hypothesis in the field of molecular evolution is that the rate of molecular evolution is positively correlated with metabolic rate. Implicit in this idea (sometimes made explicit) is that the mutation rate is positively correlated with metabolic rate. Unfortunately, several factors are confounded with metabolic rate, including generation time, population size and temperature. Further, to the extent that metabolic rate is correlated with temperature (strongly in ectotherms, less strongly in endotherms), direct mutagenic effects of temperature are often further confounded with environmental "stress". Here we report results from an experiment designed to de-confound the effects of temperature, generation time, evolutionary history, and environmental stress to investigate the relationship between temperature and mutation rate. We allowed spontaneous mutations to accumulate under relaxed selection in C. briggsae (PB800 strain) and C. elegans (N2 strain) at low temperature (18° C) for 100 generations and high temperature (26° C) for 170 generations. The high temperature environment is markedly stressful for C. elegans by the objective criteria of reduced fecundity and survivorship whereas it is not for C. briggsae. We assayed a demographic measure of fitness relative to the unmutated common ancestor ("control") in both sets of mutation accumulation (MA) lines at both temperatures. The results are both interesting and complicated. In all four cases (both sets of MA lines in both species), the cumulative decline in fitness was greater when assayed at high temperature, although the effect was small in the C. briggsae high temperature MA lines. This result suggests that there is a significant class of mutations with (high) temperature-dependent effects. In C. briggsae, high-temperature MA lines decline in fitness faster than low-temperature MA lines, and that result does not depend (much) on the assay temperature. Thus, it appears that temperature itself is mutagenic in C. briggsae. Conversely, in C. elegans, the cumulative effects of mutations are greater when assayed at high temperature than at low temperature, but there is little difference between high and low-temperature MA lines. This result suggests that in C. elegans, on average, there is no relationship between temperature and mutation rate. Direct characterization of the molecular mutational spectrum is underway and will help resolve the relative contributions of mutation rate and allelic effects to the cumulative mutational decay in fitness.

877B

Ray Pattern Variation in *C. elegans*: Mapping a Major-Effect QTL on LGV. **Scott E. Baird**, Daniel Bailey. Dept Biological Sci, Wright State Univ, Dayton, OH.

Cryptic variation of ray pattern has been observed in recombinant-inbred lines (RIL) derived from crosses of N2 and CB4856 strains of *C. elegans* (Guess et al., 2007). The variant pattern consists of the anterior displacement of ray 3 into a position immediately adjacent to ray 2. QTL analyses of these RIL identified a major-effect QTL on the left arm of chromosome V (QTL-V). One RIL, QX34, possessed a recombinant chromosome V that contained CB4856 alleles from -12.72 to -7.93 cM, a region that precisely corresponded to QTL-V. When this chromosome (QX34-V) was crossed into an otherwise N2 background (strain PB2034), the variant ray pattern was observed at a frequency of 0.50. Based on genotypes of other RIL with recombination breakpoints in this region, it appeared that QTL-V resulted from allelic variation at two or more loci. To confirm this result, we are mapping the gene(s) in this region responsible for the observed variation in ray pattern. Initial experiments demonstrated that the CB4856 alleles responsible for QTL-V were recessive to the corresponding N2 alleles. From deletion heterozygotes, it was determined that at least one gene responsible for QTL-V lies to the right of -9.00 cM. However, deletion of a region from -12.06 to -8.15 did not uncover the ray pattern variant. Therefore, either allelic variation at two or more genes is required for QTL-V or the gene responsible for QTL-V resides between -8.15 and -7.93 cM. Attempts at recombination mapping of QTL-V are ongoing but have been hindered by an apparent suppression of recombination in PB2034/N2 heterozygotes.

878C

A Bias Caused by Ectopic Development Creates Sexually Dimorphic Sperm In Nematodes. **Christopher C. Baldi**, Jeffrey Viviano, Ronald E. Ellis. Dept Molecular Biology, UMDNJ-SOM, Stratford, NJ.

LaMunyon and Ward identified an intriguing sexual dimorphism in androdioecious nematodes — the hermaphrodites produce smaller sperm than the males. In addition, they showed that larger male sperm compete better for fertilization. These results suggested that selection had created this dimorphism in sperm size.

When we used RNA interference to create *C. remanei XX* animals that develop as hermaphrodites (Baldi and Ellis 2009), their sperm were much smaller than those of *C. remanei* males. Since these animals do not produce sperm in the wild, this sexual dimorphism cannot have been created by selection. Thus, we propose that it occurs in nematodes as the result of a developmental bias.

To identify the cause of this developmental bias, we are studying mutations that affect sex determination in *C. elegans.* First, *tra-2; xol 1 XX* males make large sperm like normal *XO* males, so sperm size is not determined by the ratio of *X* chromosomes to autosomes. Second, *fem-3(q96) XX* animals, which do not make oocytes, produce sperm that are about 25% larger than those of normal *XX* hermaphrodites. This result and additional studies imply that the production of oocytes by the germ line slightly decreases sperm size, perhaps because they compete for resources. Third, mutations that completely transform the somatic tissues of *XX* animals into male fates cause a large increase in sperm size, but mutations that cause only a partial transformation have weaker effects. Thus, we propose that several somatic tissues cooperate to nurture larger sperm in males than in hermaphrodites.

These results imply that hermaphrodites produce smaller sperm than males because their bodies are not adapted for nurturing larger sperm. Hence the ectopic production of sperm in female bodies can account for this sexual dimorphism in size. We suspect that the ectopic expression of other developmental programs also alters traits during evolution.

Finally, phylogenetic studies show that males from gonochoristic species make larger sperm than either males or hermaphrodites from androdioecious ones. Thus, we believe that incipient hermaphroditic species make sexually dimorphic sperm because of a developmental bias. Over time, selection further reduces sperm size in both sexes.

879A

Cryptic evolution of transcriptional regulation through changes in *cis* and *trans*. **Antoine Barriere**[1], Kacy Gordon[2], Ilya Ruvinsky[1,2]. 1) Dept Ecology & Evolution and Institute for Genomics and Systems Biology,The University of Chicago, Chicago, IL; 2) Dept Organismal Biology and Anatomy, The University of Chicago, Chicago, IL.

Although expression patterns of *unc-47* are indistinguishable between *C. elegans* and *C. briggsae*, previous experiments suggested that the mechanisms regulating expression of this gene have diverged between the two species. For example, in addition to recapitulating the endogenous expression pattern, the *C. briggsae* promoter of *unc-47* drives ectopic

expression in several neurons when placed in *C. elegans*. We experimentally demonstrated that the *C. briggsae* promoter evolved to drive a stronger expression, but this was compensated by a weaker trans-regulatory environment. We will report on the identification of specific *cis*- and *trans*-regulatory differences responsible for functional divergence and compensatory changes. These results document the details of compensatory evolution in which subtle lineage-specific modifications of interacting *cis*- and *trans*-regulators together maintain a conserved expression pattern of a target gene.

880B

Evolution in viral sensitivity to the Orsay virus in natural *C. elegans* isolates. **Tony Bélicard**, Marie-Anne Félix. IJM, Paris, France.

We isolated the first natural viruses infecting *Caenorhabditis* nematodes: the Orsay virus in *C. elegans* isolate JU1580 and the Santeuil virus in *C. briggsae* JU1264 (Félix & al., 2011). We more recently found a third virus in *C. briggsae* JU1498 (Le Blanc virus). These viruses cause disorders in intestinal cells of their host and are horizontally transmitted.

Their genomes are composed of two single-stranded positive RNA segments carrying 3 ORFs. One of them, the ORF δ, has no homology with any known ORF (Félix & al., 2011). We aim to identify its role during infection. We thus cloned it and are currently expressing it in a JU1580 background in order to know whether it affects the anti-viral response of the worm.

In order to evaluate natural variation in sensitivity to these viruses, we scored the susceptibility of natural isolates and standard laboratory strains of *C. elegans* and *C. briggsae*. The results reveal i) a species specificity of infection by each virus and ii) intraspecific variation in sensitivity within both species for their respective viruses.

First, we found a species specificity of each virus for a specific *Caenorhabditis* host species. Indeed, the Santeuil and Le Blanc viruses do not infect JU1580, while the Orsay virus does not infect JU1264 and JU1498

Second, we evaluated the geographic and genetic distribution of Orsay virus susceptibility in a worldwide set of 25 *C. elegans* isolates representing wild genetic diversity. We measured the viral load by RT-qPCR. Preliminary results suggest that only a subset of isolates from the « Old world » are sensitive to the virus and none of the "New World". This diversity seems to be partially linked with their ability to perform a small RNA response that acts in anti-viral defense (Félix & al., 2011; poster by Nuez & Félix).

We plan to determine the genetic architecture and identify the molecular basis for this intraspecific variation in Orsay virus susceptibility. One approach is to cross closely related sensitive and resistant strains to obtain Recombinant Inbred Lines. We will test the susceptibility to the virus in these lines in order to find loci involved in the last evolutionary event causing resistance/sensitivity to the virus.

By identifying these loci, we will be able to describe the last step in the "arms race" between *C. elegans* and its natural virus.

881C

Stochastic influences on physiology and the outcomes of mutation in C. elegans. **Maria O. Casanueva[1]**, Ben Lehner[1,2]. 1) EMBL-CRG Systems Biology Unit; 2) ICREA, CRG, UPF, Barcelona, Spain.

Most 'disease causing mutations' in humans do not actually cause disease in all of people who carry them. The reasons why mutations are detrimental to one individual but of no consequence to another are likely diverse and include epistatic interactions with other sequence variants as well as interactions with environmental risk factors. However, in clonal model organisms such as C. elegans incomplete penetrance still occurs even in the absence of genetic or environmental variation. We are dissecting how inter-individual variation in gene expression alters the outcome of mutations among individuals. Further, how the environment can influence mutation buffering, and what the consequences of this variation in buffering is for wild-type individuals. We will present data that implicate a variety of highly stochastic genes as components of penetrance, predictors of mutation outcome, and determinants of physiological diversity. Sponsors: European Research Council (ERC) Starting Grant, ICREA, MICINN Plan Nacional, AGAUR, ERASysBio to B.L. and Beatriu de Pinos postdoctoral fellowship to O.C. .

882A

Removal of selection pressure leads to convergent male behavior in Caenorhabditis. **Audrey S Chang**, Matthew Rockman. Center for Genomics and Systems Biology, New York University, New York, NY.

Caenorhabditis males typically deposit copulatory plugs over the vulvae of their mates upon completion of sperm transfer. However, in some strains of *C. elegans*, males deposit copulatory plugs on other males in the absence of hermaphrodites. For example, in AB2, an Australian strain, plugs are deposited over the excretory pores of other males; in CB4856, a Hawaiian strain, plugs are frequently deposited on various parts of the male body. The cause of this anomalous plugging behavior is largely unknown. Because hermaphroditism has arisen independently multiple times in the *C. elegans* species group, a survey of plugging behavior in multiple strains and species of Caenorhabditis may shed light on why this trait has evolved. Our results suggest that this breakdown of male behavior is possibly a consequence of relaxed selection on male function. Additionally, we localize the genomic regions responsible for head plugging and body plugging in *C. elegans*. We show that two distinct loci are responsible for these aberrant male behaviors. Using these results and other techniques, we will characterize the genes underlying anomalous plugging and, furthermore, determine whether breakdown in the same genetic pathways has generated convergent behavioral phenotypes in multiple lineages.

883B

Adaptation and effective recombination in *C. elegans* populations with standing variation. **I. Chelo**, H. Teotónio. Instituto Gulbenkian de Ciência, Oeiras, Portugal.

The way genetic variation is structured within populations has long been recognized to affect rates of adaptation. With inbreeding and variable heterozygosity among individuals, linkage disequilibrium among loci is maintained or augmented and meiotic recombination might not be sufficient to create novel beneficial genotypes. Empirical tests of how the structure of variation affects adaptation are scarce. Here, we describe the molecular population genetics of adaptation in *Caenorhabditis elegans* populations under alternative mating systems and which differ in the amount of inbreeding among individuals. By using an hierarchical crossing scheme, the variation present in 14 different wild isolate strains was mixed into a single hybrid population that breeds in the usual mixed-mating androdioecious system with both males and hermaphrodites. By introgression of a *fog-2* null mutant into this hybrid population an obligatory outcrossing dioecious population was created. Upon these populations experimental evolution was performed with discrete and non-overlapping generations, and at high population sizes, for 100 generations. 351 SNPs were tracked during the course of the experiment at both IV and X chromosomes. Androdioecious populations start with higher inbreeding than the dioecious population as expected, but quickly evolve to a random mating condition. Surprisingly, higher heterozygosity and less linkage disequilibrium is maintained under androdioecy than what would be expected under neutrality, as estimated from forward simulations of experimental evolution. Directional selection is ubiquitous and mostly homogeneous under both mating systems, with initial generations showing a greater number of divergent allele frequencies. However, many more markers diverge under androdioecy than dioecy, a pattern which is correlated with the rapid attainment of an equilibrium of differentiation with dioecy. At a focal region of about ±10cM, we test the hypothesis that assortative mating determines effective recombination during adaptation. Using a model for balancing selection on heterozygosity, we show that under androdioecy ancestral linkage disequilibrium can be maintained by selection at a single locus, while under dioecy the higher haplotype diversity observed might be explained with overdominant selection at multiple loci.

884C

Identification of ascr#1 as the female sex pheromone in the free-living nematode Panagrellus redivivus. **A. Choe[1]**, A.T. Dossey[2], T. Chuman[2], R. Ajredini[2], D. Kogan[1], H. Alborn[3], F. Kaplan[3], H. Von Reuss[4], F. Schroeder[4], A.S. Edison[2], P.W.Identification of ascr#1 a Sternberg[1]. 1) HHMI & Dept Biol, Caltech, Pasadena, CA; 2) Dept of Biochemistry & Molecular Biology, University of Florida; 3) USDA Laboratory; 4) Boyce Thomson Institute, Cornell University.

Nematodes produce and respond to pheromones in order to help them form an appropriate behavioral or developmental response to their environment. Pheromones have been studied in the free-living nematode Caenorhabditis elegans (Clade 9A) , helping us to understand how small-molecules convey information about population density and mate availability. Panagrellus redivivus is a free-living gonochoristic nematode (Clade 10B) that serves as a useful comparative model to the hermaphroditic species C. elegans. We have developed a robust, semi-automated bioassay to study the attraction and repulsion of P. redivivus males and females to purified worm-secretions. We have performed activity-guided fractionation of P. redivivus liquid cultures, in combination with NMR and LCMS analysis, to identify gender specific sex pheromones. Here we report the isolation of the natural ascaroside, ascr#1, from the free-living nematode P. redivivus by large-scale purification. This is the same pheromone that plays a small role in dauer-formation in C. elegans, but does not play any role in C. elegans male or hermaphrodite attraction. We have evidence that ascr#1 not only attracts P. redivivus males, but also repels P. redivivus females, indicating that the same pheromone can be used across multiple genera for different purposes both between species and within species. *The first two authors contributed equally to this work.

885A

The evolution and genetics of speciation between C. briggsae and C. sp. 9. **Asher D. Cutter**, Joanna L. Kozlowska. Dept Ecology/Evolutionary Biol, Univ Toronto, Toronto, ON, Canada.

The recent discovery that the new species *C.* sp. 9 could hybridize with *C. briggsae* to generate viable and fertile F1 female progeny opens the door, for the first time, to applying the power of the *Caenorhabditis* model system to what Darwin called the "mystery of mysteries": speciation (Woodruff et al. 2010). We have begun exploiting this system to investigate the evolution and genetics of reproductive isolation between these species in several dimensions: pre-mating isolation, post-mating pre-zygotic isolation, and post-zygotic isolation. At the pre-mating level, we are using chemotaxis assays to determine the extent of species-specificity in attraction to female mating pheromone produced by *C.* sp. 9. While we find that male attraction appears to attenuate as a function of evolutionary divergence across the phylogeny, this "love potion no. 9" may not provide a species-specific mating signal. Post-zygotically, we confirm that inter-species crosses conform to Haldane's rule in that males are disproportionately detrimentally affected in hybrids and that parent-of-origin asymmetries are rampant, in accord with Darwin's corollary to Haldane's rule (Woodruff et al. 2010). In addition, we document significant within-species heritable variation for between-species hybrid incompatibility. This phenomenon of "variable reproductive isolation" is emerging as a general pattern in many organisms, and *C. briggsae* provides an exceptional system to dissect its genetic causes. We are now applying QTL mapping approaches with recombinant inbred lines (RILs) to determine the genetic basis to this intra-specific variation in hybrid incompatibility. I will also discuss a potentially new incipient speciation system involving *C. remanei* and strains from China.

Woodruff GC, Eke O, Baird SE, Felix MA, Haag ES (2010). Insights into species divergence and the evolution of hermaphroditism from fertile interspecies hybrids of *Caenorhabditis* nematodes. Genetics 186: 997-1012.

886B

Natural ecology of *C. elegans* in Germany. Philipp Dirksen, Fabian Peters, Wei Chen, Carola Petersen, **Katja Dierking**, Hinrich Schulenburg. Department of Evolutionary Ecology and Genetics, University Kiel, Kiel, Germany.

Taking a step outside the laboratory and exploring *C. elegans* in its natural habitat is part of our attempt to combine ecological, evolutionary, and molecular approaches to study host-microbe interactions under more realistic conditions. Here we report our recent findings on natural *C. elegans* populations from Germany. In 2010, we isolated nematodes from two different locations, Kiel at the Baltic Sea and Münster (Roxel) in northwest Germany. These samples are currently used to assess the diversity of associated microbial organisms, including pathogens as well as possible mutualists. We furthermore evaluate natural variation in ecologically relevant traits such as pathogen resistance, microbe-related choice behaviors and also mating incompatibilities. These analyses serve to identify the selective constraints that act on natural *C. elegans* populations and that are thus likely important determinants of nematode life-history characteristics as well as the underlying molecular signaling cascades.

887C

Ecology of *Caenorhabditis*: sharing of natural habitats by different *Caenorhabditis* species. **Marie-Anne Félix**[1], Christian Braendle[2]. 1) CNRS - Institut Jacques Monod, Paris, France; 2) CNRS - IBDC, Nice, France.

Many *Caenorhabditis* species proliferate in a variety of rotting vegetal substrates: fruits, flowers, plant stems. We will report on wide sampling of such substrates and possible carrier invertebrates, focusing on 1) French Guiana; 2) mainland France.

In French Guiana, we sampled mostly in wild tropical forest around the Nouragues CNRS Biological Station. Besides finding three new species, *C.* sp. 12, 17 and 18 (see abstract by Kiontke et al.), we determined the spatial distribution of six *Caenorhabditis* species at different scales and constituted a frozen isolate collection. *Caenorhabditis* species often co-occur in the same individual fruit or flower. Their spatial distribution over the location at one timepoint appears inhomogeneous.

In mainland France, rotting fruit and stem habitats were found to be shared by *C. elegans* and *C. briggsae*. Both species were isolated throughout most of France. In addition, *C. remanei* or *C.* sp. 13 were found each in one location. We sampled a systematic spatio-temporally structured set of rotting apples in an apple variety orchard in Orsay. We scored the prevalence of each species in different parts of the same apple, in different apples below a given tree, throughout the orchard and at 19 timepoints over three years. *C. elegans* and *C. briggsae* were abundantly found and may co-occur in the same apples. However, their temporal distribution did not coincide. *C. briggsae* was found alone in summer; both species co-occurred in early fall and *C. elegans* remained alone in late fall. This temporal sharing of the habitat coincides with their temperature preference in the lab. In relatively natural habitats of France (wood, heath), rotting stems of several plant species yielded *Caenorhabditis*. Both *C. elegans* and *C. briggsae* were found in the same plant species and sometimes in the same individual stem.

Most populations were analyzed on the day of sampling, which enabled us to determine the census and the stage of worms. Population sizes spanned a range of 1 to over 10,000 Caenorhabditis individuals in one fruit, flower or stem. Some populations of intermediate size contained all non-dauer stages expected from a proliferating population. Samples with high census always contained some L2d and dauers; some did not contain L3 and L4s at all, indicative of a population entering the dauer stage at the end of a proliferative stage.

888A

Comparison of early embryogenesis in eight species of *Protorhabditis*. **Hélène Fradin**[1,2], Karin Kiontke[1], David H.A. Fitch[1], Fabio Piano[1,2]. 1) Department of Biology, New York University, USA; 2) Center for Genomics and Systems Biology, New York University, USA.

Previous studies have found that species in the sister group of *Caenorhabditis*, the *Protorhabditis* group (1), show early embryonic cellular patterns strikingly different from that in *Caenorhabditis*. In embryos at the four-cell stage, all four blastomeres are arranged in a row instead of in the rhomboid pattern (2). To better characterize these differences, we have undertaken a systematic study that combines phylogenetic analysis with time-lapse microscopy. First, we reconstructed a molecular phylogeny for nine species of the *Protorhabditis* group. We then compared early cellular events and cell lineage division timing in eight species from the *Protorhabditis* group, using *C. elegans* as a reference. Our molecular phylogeny confirms that the monophyletic *Protorhabditis* group contains species of the genera *Protorhabditis*, *Prodontorhabditis* and *Diploscapter*, the latter of which has traditionally been treated as a separate Family Diploscapteridae. We find two clades within the *Protorhabditis* group: clade A includes *Diploscapter* species as well as some *Protorhabditis* species, and clade B includes *Prodontorhabditis* species and some *Protorhabditis* species. Analysis of the time-lapse movies confirmed that early embryogenesis in the *Protorhabditis* group is quite different from that in *Caenorhabditis*. In both clades at the two-cell stage, the posterior blastomere P1 divides first, and the axis of division of the anterior blastomere AB is parallel to the antero-posterior axis (3,4). This is in contrast to C. elegans where AB divides first and its axis of division is transverse. However, we found distinct differences between the two clades within *Protorhabditis* at the four-cell stage. Clade A species show the "four-cell-in-a-row" phenotype that has been described previously (3,4). For clade B, we observed a novel cellular phenotype. In these species, AB divides much later than P1, giving rise to at least three descendants before AB begins to divide. This difference in timing prevents the four descendants of AB and P1 from being positioned in a row. Early development in the *Protorhabditis* group is much slower than in *C. elegans*, with clade B displaying an even slower development than clade A. In both clades, the germline divides faster relative to other lineages. 1. W. Sudhaus, D. Fitch, *J Nematol* 33, 1 (2001). 2. C. Dolinski, J. G. Baldwin, W. K. Thomas, *Can J Zool* 79, 82 (Jan, 2001). 3. V. Lahl, J. Schulze, E. Schierenberg, *Int J Dev Biol* 53, 507 (2009). 4. M. Brauchle, K. Kiontke, P. MacMenamin, D. H. Fitch, F. Piano, *Dev Biol* 335, 253 (Nov 1, 2009).

889B

Functional genomics of two Antarctic nematodes, *Panagrolaimus davidi* and *Plectus murrayi*. **Hiroshi Kagoshima**[1,2], Yuji Kohara[2]. 1) Transdisciplinary Research Integration Center, Japan; 2) National Institute of Genetics.

Antarctica is an extreme environment for life. Antarctic animals must remain either frozen or dry for much of the year, and they can grow only during short period when liquid water is available from melting snow in the summer. Even then, they are exposed to periodical freeze-thaw. We aim to elucidate how the Antarctic animals adapt to the extreme environment, using two Antarctic nematodes. (1) *Panagrolaimus davidi*: We analyzed 25,000 cDNAs prepared from *P. davidi* cultivated at 20°C with water and food (good condition for therir growth). We found *P. davidi* constitutively expressed high level of stress response genes, such as heat resistant gene, *lea-1*, even in the good condition. We are currently performing sequence analysis of another cDNA library generated from *P. davidi* cultivated at 4°C (cold-stressed condition), to screen candidate genes for cold tolerance, by the comparison of expression profiles between two libraries. (2) *Plectus murrayi*: We were able to recover nematodes from a frozen moss sample collected 25.5 years ago, near Showa station, eastern Antarctica. We identified this nematodes as *Plectus murrayi* using morphological keys and confirmed it by their 18S and 28S rRNA sequences. They can grow on water agar plates and feed bacteria grew from the original isolates (most probably, *Pseudomonas* sp. derived from Antarctica). They tolerated freezing stress (-20°C and -80°C for 24 hrs) and desiccation stress (dried at 98% and 76.5% relative humidity for 24 hrs).

890C

Natural variation of gonad independent vulva induction among *Pristionchus pacificus* isolates. **Simone Kienle**, Ralf J. Sommer. Max Planck Institute for Developmental Biology, Department for Evolutionary Biology, Tuebingen.

Pristionchus pacificus has been established as a model system in evolutionary developmental biology and evolutionary ecology. Previous studies have focused on macroevolutionary differences between the reference strain of *P. pacificus* (PS312) and *Caenorhabditis elegans* (N2). These studies have shown that, while the vulva in PS312 and N2 is formed from homologous precursor cells, different signaling pathways are required for vulva induction. In contrast to EGF/Ras signaling in N2, PS312 relies on Wnt signaling with an atypical wiring of this signaling system. To add a microevolutionary perspective to the evo-devo studies in *P. pacificus*, we isolated more than 150 different strains from around the world and examined natural variation, with particular emphasis on vulva induction. These strains showed high diversity, both at the molecular and the phenotypic level. We chose 19 genetically and geographically highly diverse strains and analysed vulva induction by gonad ablation experiments. In wild type PS312 animals, differentiation of the vulva precursor cells (VPCs) is completely abolished after Z(1,4) ablation, suggesting that vulva differentiation in this strain depends on the precursor cells of the somatic gonad. However, in most of the analysed *P. pacificus* strains, VPCs were still able to differentiate after ablation of the gonad. To identify the loci involved, we crossed the two isolates showing the most extreme phenotypes, PS312 and an isolate from Bolivia (RS5275). We obtained recombinant inbred lines (RILs) by selfing individual F2 and near isogenic lines (NILs) by continuously backcrossing the RILs to RS5275 animals. Gonad ablation experiments and genotyping of more than 200 RILs revealed a 430kb region on Chromosome I, corresponding to *C. elegans* Chromosome V, associated with the VPC differentiation trait. This interval contains 20 genes of which one is a known developmental control gene. Sequencing this gene for both strains revealed no substantial differences in the coding but high amount of variation in the non-coding regions. We used DNA-mediated transformation experiments to study the concentration dependence of this natural variation and to show how natural variation interacts with redundant cell fate specification mechanisms. Together, the microevolutionary perspective of *P. pacificus* vulva development is an effort to integrate evo-devo studies in evolutionary theory.

891A

DPY-1 plays a conserved role in nematode cuticle formation. Sandrine Jacob[1], Christian Olendrowitz[2], Delphine Bernard[1], Joachim M. Muriel[3], Charlotte Kenning[4], Olivier Poupel[1], Jan Hagermann[2], Danny Tuckwell[5], Ralf Sommer[4], Stefan Eimer[2], **Bernard Lakowski**[1]. 1) Nematode Genetics Group, Department of Neuroscience, Pasteur Institute, Paris France; 2) European Neurosciences Institute, Göttingen, Germany; 3) Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland; 4) Max Plank Institute of Developmental Biology, Tübingen, Germany; 5) University of Manchester, Manchester United Kingdom.

The cuticle of nematodes is a complex structure of highly cross-linked proteins and is mainly composed of collagens and cuticulins. By taking advantage of the very specific spectrum of mutations induced by the absence of the *dog-1* DNA helicase, we were able to

identify the *Caenorhabditis elegans dpy-1* gene. We also report the cloning of the orthologous gene from the satellite model system *Pristionchus pacificus*. *dpy-1* genes encode large unusual cuticulin proteins most similar to the CUT-6 protein. DPY-1 proteins have a signal sequence, a von Willebrand Factor A (vWFA), a Zona Pellucida (ZP), a transmembrane domain and several other regions of conservation. Using bioinformatic searches and phylogenetic analysis, we have identified fragments of *dpy-1* genes in several parasitic nematode species. We provide evidence that *dpy-1* genes have an ancient origin in the Nematode phylum. Mutations in the *C. elegans* and *P. pacificus dpy-1* genes both cause a similar strong Dpy phenotype and are required for the proper structure of the cuticle, indicating an evolutionary conservation of DPY-1 function. In the absence of *dpy-1*, animals display defects in the maintenance of the annuli and annular furrows of the cuticle that help to radially constrict the body and help to give the cuticle its flexibility and deformability. This may explain the movement problems and the defects in maintaining a fully elongated form seen in *dpy-1* mutants.

892B

Spontaneous and UV-induced mutations in a small region of the C. elegans genome, the ben-1 locus. **Cecilia C. Mello**, Andrew Fire. Pathology Department, Stanford University School of Medicine, Stanford, CA.

What is the spectrum of spontaneous mutations? How are mutation spectra altered in the presence of strong mutagens? To answer these questions, I used a powerful screen for isolating both spontaneous and induced mutations using the ben-1 gene, a non-essential beta-tubulin. Wild-type animals are sensitive to paralysis upon benomyl treatment, ben-1 mutants are resistant. This work of spontaneous and UV-induced mutations gives us a baseline for further studies. The strength of this approach is that it focuses mutations in a small region of the genome, allowing for a detailed analysis. This one locus does not offer a complete view of the genome, and I intend to expand this analysis into larger regions of the genome.

893C

Comparative proteome analysis of *Caenorhabditis elegans* cultured at 2 different temperatures—20°C and 25°C. **K. Monobe[1]**, Y. Ishido[1], A. Terasawa[1], Y. Tohsato[1], T. Hayano[2], M. Ito[1]. 1) Dept. of Bioinfo, College of Life Sci. Ritsumeikan University, Kusatsu, Shiga, Japan; 2) Dept. of Biomed. Sci., College of Life Sci. Ritsumeikan University, Kusatsu, Shiga, Japan.

Caenorhabditis elegans is a model multicellular organism. The project involving the sequencing of its entire genome was completed in 1998. The genome is the blueprint of the life system, but directly elucidating the life system from the genome is difficult even if recently high biotechnologies are used. Therefore, the proteome, which is directly related to function, has become important for understanding the life system of multicellular organisms. Therefore, we initiated the nematode proteome project for comparing and analyzing protein expression at different developmental stages and culture temperatures for two nematodes—*C. elegans* and *C. briggsae*.

In this study, for comparative proteome analysis, we focused on the young adult stage and on two culture temperatures—20°C and 25°C—for *C. elegans*. Initially, age-synchronized adults were collected three times by using the alkali-bleach method. The collected samples were analyzed using 2-dimensional difference gel electrophoresis (2D DIGE). The protein spots were detected and matched among gels by using image analysis software. A total of 1,755 protein spots were detected with high reproducibility on at least two of the three gels run for the two different culture temperatures. In addition, spots with protein expression levels that differed between the two culture temperatures were analyzed using the Student's *t*-test ($p \leq 0.05$). From the 1,755 spots, 202 spots at 20°C and 169 spots at 25°C had high protein expression levels. We focused on these spots, and several spots were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

894A

Variations in sensitivity to external RNA interference in the Caenorhabditis genus. **Isabelle Nuez**, Marie-Anne Félix. CNRS, Inst J Monod, Paris cedex 13, France.

The introduction of dsRNAs 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 the worms in a solution containing bacteria expressing dsRNAs or by feeding them with such bacteria. In contrast to *C. elegans*, *C. briggsae* and *C. remanei* are naturally insensitive to external RNAi. In the Hunter lab, a *C. elegans* mutant, *sid-2*, was isolated, which is defective in RNAi through externally administered dsRNAs. *sid-2* encodes a transmembrane protein, localized at the luminal membrane of intestinal cells and required for the uptake of dsRNAs. They were also able to show that the *C. briggsae* insensitivity to external RNAi can be rescued by transgenesis with *Cel-sid-2* (Winston et al, 2007), but it is unclear whether this transgenic approach can be applicable to other species. We and others have recently isolated new *Caenorhabditis* species from rotting fruits and flowers, so we tested their sensitivity upon external RNAi. We targeted *actin*, and found that some species are naturally sensitive to RNAi by feeding, whereas others are not. We mapped our results onto the phylogenetic tree of the *Caenorhabditis* genus (see abstract by Kiontke et al.) and found that loss and/or gain of this feature in the *Caenorhabditis* genus is complex. Interestingly, we identified one case, *C. angaria*, which is insensitive to RNAi targeting *actin* but sensitive to external RNAi targeting *C. ang.* RNA polymerase II gene. We also found germ line-restricted sensitivity to RNAi in the *C. el.* JU1580 strain, naturally infected by a nodavirus, perhaps due to the defect of somatic RNAi pathway in this wild isolate (Félix et al, 2011). Finally, we succeeded in rendering *C. remanei* sensitive to external RNAi after transgenesis with *Cel-sid-2*, similarly to the experiment performed on *C. briggsae* by Winston et al, indicating that this approach can be used for sensitizing species to external RNAi. Overall, our results provide the first broad investigation of sensitivity to external RNAi within the *Caenorhabditis* genus, and can be valuable for designing reverse genetic experiments in these new species. We propose that the sensitivity to external RNAi is evolving fast within the *Caenorhabditis* genus, and this may be relevant to adaptation of nematode defense against viruses.

895B

Effect of Mutation Accumulation Environment on Environmental Variance in Fitness in Caenorhabitis. **Rayshard Rogers**, Charles Baer. Department of Biology, University of Florida Genetics Institute, P.O. Box 118525, Gainesville, Florida 32611.

It has been known for a long time that environmental stress and mutations of large effect increase sensitivity to random environmental noise ("microenvironmental variance", VE). We recently quantified the effects of spontaneous mutation on VE in two species of nematodes in the genus Caenorhabditis, C. briggsae and C. elegans and found that the increase in VE for lifetime productivity is on the same order as that of the change in the trait mean, which is consistently greater in C. briggsae than in C. elegans. Here we report results from a new experiment in which mutations were allowed to accumulate at high (26°C) and low (18° C) temperatures in the same two species and fitness subsequently assayed at high and low temperatures. In C. briggsae, VE increased more with MA at 26 than at 18; there was little effect of MA temperature in C. elegans. Similarly, in C. briggsae the increase in VE was greater when assayed at high temperature; there was little effect of assay temperature in C. elegans. These results suggest that there is an important class of temperature-sensitive mutations in C. briggsae which in turn affect sensitivity to microenvironmental variation that is not present in C. elegans.

896C

BACK TO NATURE: DEFINING THE MICROBIOTA OF WILD C. ELEGANS. **Buck S. Samuel[1]**, Christian Braendle[2], Marie-Anne Félix[3], Gary Ruvkun[1]. 1) Dept. of Molecular Biology, Massachusetts General Hospital and Dept. of Genetics, Harvard Medical School, Boston, MA; 2) Institute of Developmental Biology and Cancer, CNRS, University of Nice Sophia-Antipolis, Nice, France; 3) Institut Jacques Monod, CNRS, Universities of Paris 7 and 6, Paris, France.

Like all of us, C. elegans lives in a microbially dominated world. They naturally proliferate habitats rich in microbes, like rotting fruits and decaying vegetation. Interactions with these as yet unnamed microbes undoubtedly span a spectrum from constant confrontation (pathogens) to relative indifference (food) and perhaps even mutual benefit (symbionts). Interestingly, in contrast to N2, wild C. elegans harbor intestinal microbes. In addition to food, it is tantalizing to speculate that these worms might enlist microbes to improve its fitness (e.g., resistance to pathogens, harmful chemicals, etc.), just as we and many other animals have done. Within a habitat, C. elegans expansion is affected by a host of environmental and intrinsic (genetic) factors. Specifically, similar habitats in close proximity can harbor anywhere from no worms to dauers to actively proliferating populations; our hypothesis is that the mixture of microbes present is a key determinant of C. elegans' success. To examine this question, we performed culture-independent sequencing of microbial small subunit rDNA from habitats with wild C. elegans populations collected during several field seasons and different locations. In addition, we isolated worms to directly examine their more closely associated microbes. Using this dataset, we are able to address: (a) what are the commonly encountered microbes; (b) do groups of microbes correlate with population success; and (c) does C. elegans maintain a microbiota? Initial results from habitats indicate that bacteria belonging to four divisions (phyla), Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria are common in C. elegans' habitats, and occasionally seven more rare divisions are observed. However, there are also great variations in the bacterial diversity and richness among habitats (10-100+ species); we are testing bacterial species found in many habitats for their close association with worms. Notably, cultured microbial mimics of these habitats dramatically alter C. elegans' growth: e.g., proliferation on a Proteobacteria-rich mimic occurs faster than on a Bacteroidetes-rich mimic. Comparisons of responses of wild and lab-raised worms are also in progress. Determination of the microbes in C. elegans natural habitats is a first step in expanding our understanding of how microbes can influence host fitness and resistance to ecological pressures.

897A

Experimental test of the consequences of host-parasite coevolution. **Hinrich Schulenburg[1,2]**, Leila El Masri[1,2], David Laehnemann[2], Patrick Guenther[2], Nicolaas K. Michiels[2]. 1) Evolutionary Ecology and Genetics, University of Kiel, Kiel, Germany; 2) Animal Evolutionary Ecology, University of Tuebingen, Germany.

The coevolution between host and parasite is believed to be associated with high evolutionary dynamics affecting various life-history characteristics and the underlying genetics. We use experimental evolution between the nematode host *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis* to explore the consequences of coevolution. Our current results demonstrate that *B. thuringiensis* evolves into two very distinct phenotypes in response to either coevolution with the host (e.g., maintenance of virulence, no biofilm production) or evolution in the absence of an antagonist (e.g., loss of virulence, pronounced biofilm production). The host *C. elegans* shows similar although less pronounced adaptations (e.g., increased resistance, reduced body size). Based on genomic analyses, we are currently exploring the underlying molecular genetics. Our study provides experimental evidence for the high selective dynamics that result from host-parasite coevolution.

898B

Evolution of Sex-biased Expression in the Caenorhabditis genus. **Cristel G Thomas**[1], Renhua Li[2], Ian Korf[3], Harold E Smith[2], Brian Oliver[2], Eric S Haag[1]. 1) Department of Biology, University of Maryland, College Park, MD, USA; 2) National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD 20892, USA; 3) UC Davis Genome Center, University of California Davis, Davis, CA, USA.

Mating systems shape genome structures, both indirectly through their effects on population genetics and directly due to the genetic control of reproductive traits. Most extant Caenorhabditis species are gonochoristic (male/female), while the most studied species, *C. elegans* and *C. briggsae*, are androdioecious (self-fertile hermaphrodite/male). Both selfing species display an overall reduced ability to mate, suggesting that the selective pressure on maintaining efficient mating was weakened as selfing arose. The genes underlying these traits were likely to have been expressed in a sex-biased fashion in the gonochoristic ancestor, and we hypothesized that as selfing emerged their regulation was modified or they were lost altogether. This hypothesis is especially interesting given that selfing species have consistently smaller genome sizes than their gonochoristic relatives. We sought to address whether a disproportionate loss of genes with sex-biased expression accompanies the loss of mating-related traits in Caenorhabditis hermaphrodites. One approach uses RNA-seq to perform a comprehensive survey of adult sex-biased transcripts in *C. japonica*, *C. brenneri*, *C. remanei* and *C. elegans fog-2(q71)*. This allows us to determine the overall number and fraction of sex-biased genes for each species, and hence test the above hypothesis genome-wide. Another approach involves identification and characterization of specific male genes lost in one or more hermaphrodites. Taken together, our results indicate a substantial erosion of reproductive genes has occurred in selfing lineages. We suggest that, with respect to reproductive biology, *C. elegans* and *C. briggsae* are actually rather poor models for the rest of Caenorhabditis.

899C

Quantitative Proteomic Analysis of 3 Developmental Stages of *Caenorhabditis briggsae* by Using 2D DIGE and iTRAQ. A. Terasawa[1], **Y. Tohsato**[1], Y. Ishido[1], T. Hayano[2], M. Ito[1]. 1) Dept. of Bioinfo., College of Life Sci., Ritsumeikan University, Kusatsu, Shiga, Japan; 2) Dept. of Biomed. Sci., College of Life Sci., Ritsumeikan University, Kusatsu, Shiga, Japan.

Caenorhabditis briggsae is closely related to *C. elegans*. These two species diverged from a common ancestor about 100 million years ago but appear to have almost identical morphology. Comparative analysis of these two species is expected to provide new evolutionary knowledge and reveal gene functions. Although *C. elegans* has been well studied, very few proteomic studies have been performed on *C. briggsae*.

In this study, we identified and quantitatively analyzed the protein expression profiles for 3 different developmental stages of *C. briggsae*-embryo, larval-1, and adult stages—by using 2-dimensional difference gel electrophoresis (2D DIGE) and isobaric tag for relative and absolute quantitation (iTRAQ). A total of 1,385 spots were detected for these 3 developmental stages by using 2D DIGE; of these, 104 were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Shotgun analysis with iTRAQ was used to identify 2,272 proteins. The protein expression patterns were analyzed using cluster analysis and multiple testing, and the correlation between these cellular functions was assessed. The results showed that the expression of proteins with cellular components tended to be higher in the larval-1 stage than in the embryo and adult stages.

900A

Constructing and screening an RNAi Library in *C.briggsae* reveals evolution in phylogenetically young proteins and transcription factors. **Adrian Verster**[1], Arun Ramani[1], Sheldon Mckay[2], Felix Marie-Anne[3], Andew Fraser[1]. 1) Department of Molecular Genetics, University of Toronto, Toronto, Canada.Molec Gen, Univ Toronto, Toronto, ON, Canada; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, USA; 3) Institut Jacques Monod, CNRS - University Denis Diderot, Paris, France.

Understanding how much of genome evolution corresponds to functional change is critical to our understanding of evolution. Identifying orthologues which have evolved different functions between closely related organisms would allow us to assess the type of genomic changes that drives functional evolution, the central goal of this project. In order to identify a relatively unbiased set of these genes we constructed an RNAi library in *C.briggsae* consisting of 1333 genes, which corresponds to 93% of one-to-one orthologs of the genes in *C.elegans* that have easily observable RNAi phenotypes. We screened this library to identify genes which show different loss of function phenotypes between the species. To confirm these data, we repeated positive genes with a second RNAi clone targeting a different portion of the *C.briggsae* gene, and verified that this RNAi library shows similar knockdowns to those that we observe in the orthologous *C.elegans* by qPCR. After validation we found that 9% of genes show a different loss of function phenotype between *C.elegans* and *C.briggsae*, implying that a significant number of orthologous genes have different functions in related species.

These genes with different loss of function phenotypes form an excellent set for studying genome evolution. They are highly enriched for transcription factors as well as proteins found only in nematode genomes. Molecularly these genes are more divergent in protein sequence than genes with the same phenotype, as well as more divergent in expression levels. These data argue that genomic functional change in this model is driven by the evolution of protein sequence and expression and that transcription factors and phylogenetically young proteins undergo the most change.

901B

Independent evolution of PP1 phosphatases for regulation of diverse sperm motility structures. **Jui-ching Wu**, Aiza Go, Susan Mirosoian, Mark Samson, Thais Cintra, Tammy Wu, Margaret Jow, Rodrigo Estrada, Eric Routman, Diana Chu. Dept Biol, San Francisco State Univ, San Francisco, CA.

The rapid evolution of male reproductive proteins has generated distinctive sperm motility structures, including tubulin-based flagella in mammals and Major Sperm Protein (MSP)-based pseudopods in nematodes. Despite such molecular divergence, we have found that sperm-specific PP1 phosphatases, which function in mouse sperm motility, are required for male fertility in *C. elegans*. We were thus interested to determine how GSP-3/4 can function in motility in species with morphologically distinct sperm.

First, using genetic, molecular, and cell biological approaches in *C. elegans*, we find the sperm-specific PP1 phosphatases GSP-3 and GSP-4 (GSP-3/4) are functionally redundant and required for male fertility in worms. During meiosis, GSP-3/4 are required for proper sperm meiotic chromosome segregation and localize to sperm chromatin. Post-meiosis, GSP-3/4 shift localization from chromatin to pseudopods, suggesting GSP-3/4 are also required for motility. Consistent with this, *gsp-3/4* mutant spermatids develop shorter pseudopods with greatly reduced pseudopod treadmilling activity and are thus immotile. GSP-3/4 spatially regulate MSP: they concentrate at the pseuodpodial base and regulate MSP disassembly during morphogenesis. Thus, GSP-3/4 regulate sperm motility through modulating MSP cytoskeleton dynamics during sperm activation.

How have sperm-specific PP1 phosphatases come to play a central role in sperm motility function in mouse and nematodes given that GSP-3/4 regulate aspects of motility specific to nematode sperm? To determine if sperm-specific PP1 phosphatases in nematodes and mammals arose from a common ancestor that adapted sperm-specificity, we conducted a phylogenetic analysis of PP1 proteins in species from yeast to humans. We determined that sperm-specific PP1 phosphatases evolved in separate lineages and not from a common ancestor. Therefore, animals from a broad range of species, regardless of their sperm morphology, have separately adapted PP1 phosphatases as critical regulators of male reproduction.

902C

Establishing *C. briggsae* as a Model System for Speciation Research. **Zhongying Zhao**[1], Stephane Flibotte[2], Donald Moerman[3], Robert Waterston[4]. 1) Department of Biology, Hong Kong Baptist University, Hong Kong, China; 2) Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC, Canada; 3) Department of Zoology, University of British Columbia, Vancouver, BC, Canada; 4) Department of Genome Sciences, University of Washington, Seattle, WA, USA.

Speciation is a fundamental biological process that has fascinated biologists for well over a century. Mechanistic research into speciation lies at the very heart of evolutionary biology. Genetic approaches play a central role in dissecting the incompatible loci in species hybrids. Molecular characterization of speciation has recently become possible largely due to the development of rapid high resolution genomic mapping approaches. To take advantage of this new methodology certain genetic tools must first be in place and most importantly, a pair of closely related species, called sister species, that can mate with each other and produce viable progeny must be available. While most model organisms have been used for speciation research, it has not been possible to use *C. elegans*, the best studied nematode species, as no sister species has been identified. Recent identification of a *C. briggsae* sister species, *sp. 9* opens the door for the study of speciation research in nematodes. However, lack of sufficient genetic tools in both species limits their use in speciation research. Here we report on multiple genetic tools we have built to facilitate this type of research in *C. briggsae*.

First, we made approximately seventy integrated *C. briggsae* strains that brightly express either myo-2::GFP or other fluorescent reporters by bombardment. We are presently mapping the random transgene integration loci to produce a dense genetic map consisting of dominant visible markers. These markers will facilitate the introgression steps normally required for mapping of hybrid incompatible loci. Second, we developed a 12X SNP based microarray and are using it to map the independent stable GFP expressing strains (12 mapped to date). We achieved a mapping resolution up to 200 kb with this chip. Third, we crossed five of the 12 mapped transgenic lines repeatedly into *sp. 9* to produce an introgression line and found 2 of the transgene loci cannot be rendered homozygous in an otherwise *sp.9* background, indicating incompatibilities conferred by the *C. briggsae* genomic fragments closely linked with the transgenes. We are using a refined mapping strategy to clone the incompatible loci. This study outlines a framework for using the nematode to study the mechanisms of speciation.

903A

Screening for novel regulators of *rnt-1* in stress response. **Soungyub Ahn**[1,2], Kiho Lee[1,2], Junho Lee[1,2]. 1) Institue of Molecular Biology and Genetics; 2) School of Biological Sciences, Seoul National University, Seoul, Korea.

RUNX family transcription factors have various essential roles in mammalian development and carcinogenesis. Alternative splicing, post-transcriptional regulation and interaction with other nuclear components offer various levels of regulation and essential functions of RUNX proteins. In *Caenorhabditis elegans*, *rnt-1* is the sole homolog of the RUNX family, and expressed in the intestine and hypodermal seam cells. In addition, RNT-1 represses its own transcription with BRO-1 but DBL-1 acts to activate *rnt-1* expression at the post-embryonic stages. We focused on a novel *rnt-1* regulation mechanism in *C. elegans*. We previously identified that the stability of RNT-1 in the intestine is regulated by various stresses. The p38 MAPK pathway, which is important to stress response, mediates the increase of RNT-1 stability in the intestine. *pmk-1*, MAPK in the p38 MAPK pathway, phosphorylates RNT-1 and this phosphorylation of RNT-1 stabilizes RNT-1 by inhibiting ubiquitination on RNT-1. We are trying to find regulators which involved in this *rnt-1* regulation by using puromycin selection and EMS mutagenesis.

904B

Identifying genes that interact with APL-1, a Protein Related to Human Amyloid Precursor Protein. **Adanna G Alexander**, Chris Li. Biology, City College CUNY, The Graduate Center CUNY, NEW YORK, NY.

One of the characteristic pathologies of Alzheimer's Disease (AD) is the presence of beta-amyloid plaques in the brains of patients. In mammals, the beta-amyloid peptide is a cleavage product of amyloid precursor protein (APP), which belongs to a family of proteins that all possess high sequence similarities. For instance, mammals have three APP and related proteins with overlapping functions, making it difficult to understand the role of APP in the pathogenesis of AD. The nematode *Caenorhabditis elegans* contains only one APP related gene, *apl-1*. APL-1 is has a small cytoplasmic domain, a single transmembrane domain, and an extracellular domain (APL-1EXT). Loss of *apl-1* results in larval lethality due to impaired molting during larval transitions and abnormal morphogenesis. Surprisingly, animals carrying the *apl-1(yn5)* mutation produce only APL-1EXT and are viable. Futhermore, APL-1EXT expression from neurons is sufficient for *C. elegans* viability. The precise function and interacting partners of APL-1 and APL-1EXT, however, remain a mystery. To address this problem, we are taking a multi-pronged approach. First, we are performing RNAi and mutagenesis screens to identify genes that bypass the need for APL-1 activity. Second, we are performing non-denaturing polyacrylamide gel electrophoresis (nativePAGE) to identify proteins that interact with APL-1EXT in its native state. Protein extracts from each *C. elegans* developmental stage, including eggs, larval stages 1 through 4 and adults, were analyzed. By using extracts from each developmental stage, were be able to track APL-1EXT interacting partners as the animal matures. Interacting partners that co-immunoprecipitated with APL-1 will be subjected to mass spectroscopy to determine their molecular identities. These experiments will allow for the identification of genes in the *apl-1* pathway, taking us a step closer towards understanding the role of APP in mammals and the pathogenesis of AD.

905C

Microtubules and fertilization: The MEI-1/katanin mediated cytoskeletal transition from meiosis to mitosis in the developing embryo. **Sarah M Beard**, Paul Mains. Medical Genetics, University of Calgary, Calgary, AB, Canada.

During embryonic development, dramatic changes of the C. elegans cytoskeleton occur in the transition from the meiosis to mitosis requiring precise regulation of molecules specific to each type of spindle. Defects in this specific microtubule organization during development can result in tissue pathologies, aneuploidy or even cancer. The microtubule severing complex, MEI-1, is responsible for the differences in spindle, being required in meiosis to keep the spindle small but is inactivated prior to mitosis. This inhibition of MEI-1 during mitosis is dependent on MEL-26/CUL-3 E3 ubiquitin ligase complex targeting MEI-1 for degradation during mitosis. The first aim of the project is to measure anti-MEI-1 staining levels in several mutant strains to determine how known genes function relative to one another. Another pathway, involving the anaphase promoting complex (APC) and the MBK-2/DYRK kinase, has been found to promote mitotic MEI-1 degradation in parallel to MEL-26 mediated degradation of MEI-1. We wish to decipher whether APC and MBK-2 act in parallel or sequentially relative to one other in this process. We are also interested in deciphering he exact role of CUL-2, another E3 ubiquitin ligase, that is previously known to prevent MEL-26 from accumulating during meiosis. Making double mutants should resolve whether CUL-2 is the missing ligase for MBK-2 mediated MEI-1 degradation functioning in parallel to the MEL-26/CUL-3 pathway, or acting sequentially as an upstream activator of MEL-26/CUL-3. We expect that APC acts sequentially with MBK-2 to degrade MEI-1, and that CUL-2 acts with MBK-2 in parallel to MEL-26/CUL-3 mediated MEI-1 degradation. The second aim of the project is to continue investigating potential regulatory components of the cytoskeleton in the transition from meiosis to mitosis. We will conduct targeted RNAi screens for missing components of the pathway such as kinases, ubiquitin ligases and substrate adaptors. For example, Fem-1, a substrate adaptor for CUL-2 E3 ubiquitin ligase involved in sex determination, could be potential hit for MEI-1 regulation during embryonic development. This project will assist in decoding the key regulatory molecules of the developmental remodeling of the cytoskeleton and progressively work our way back to the initial triggers of the pathway at fertilization.

906A

Identification of transcription start sites and novel transcripts in *C. elegans*. **Ron Chen**, Thomas Down, Julie Ahringer. The Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

Transcription start sites (TSSs) for most *C. elegans* genes are not known because the majority (>70 %) are trans-spliced. The 5' end of primary transcripts are spliced off and degraded, being replaced by a 22nt spliced-leader RNA. This lack of knowledge of transcription initiation sites has hampered research in defining promoters and mapping regulatory elements in *C. elegans*.

In theory, mRNAs that have initiated but not elongated fully should not yet have undergone trans-splicing and so would retain the original 5' end with a methyl cap. To identify such nascent transcripts, we have isolated and sequenced 5' capped short nuclear RNAs (20-100 nt). We find that many of these sequences indeed mark transcription initiation sites for not only non-trans-spliced, but also trans-spliced transcripts. For trans-spliced genes, TSSs usually lie 30-150nt upstream of the trans-splice sites.

Transcription initiation sites fall into three major classes: single prominent starts, major starts with a cluster of weaker starts, and clusters of weak starts. In addition, we detect shared sequence motifs around TSSs allowing an analysis of promoter architecture in *C. elegans*. Combining data from the identified TSSs and sequences from long capped RNA (>200 nt), we also discovered novel transcripts. The functions of these novel transcripts in gene expression regulation are being investigated.

The identification of transcript start sites should facilitate analyses of genomic regulatory features in *C. elegans*.

907B

Cis-regulating network in the ALA neuron. **Elly S Chow**, Cheryl Van Buskirk, Paul Sternberg. HHMI, Division of Biology, California Institute of Technology, Pasadena, CA.

The ALA neuron is so far the only neuron known to be involved in regulating the EGF-induced sleep-like state in C. elegans. We have previously discovered a network of three homeobox-containing transcriptional regulatory proteins that regulate expression of let-23/EGFR and other ALA-expressed genes. By comparing multiple nematode genomic sequences, we have identified highly conserved regions in the vicinity of coding sequence of two transcription factors (ceh-14 and ceh-17) and three of the functional genes (plc-3, ida-1, ver-3). These elements contain a MEME motif and a cluster of putative homeobox transcription factor binding sites. We analyzed 150 bp genomic elements for their ability to confer cell-type specific gene expression on the pes-10 basal promoter using a reporter gene assay. In each case, the element directed expression in the ALA neuron specifically, except in the case of the plc-3 element, expression is also found in two vulva cells. These elements thus contain ALA-specific regulatory sequences. We believe these elements may serve as probes for finding ALA -expressing genes in the genome. To verify our hypothesis, we continue to identify ALA-specific elements in all the known genes expressed in the ALA. To identify additional ALA-expressed genes, we also seek to profile enriched transcripts in the ALA neuron. We hope to identify crucial cis-regulatory elements and the logic of gene expression regulation in the ALA neuron.

908C

Dissecting the role of *ncbp-1/ncbp-2* in determining ray patterns in *C. elegans* males. JHT Wong, **KL Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, ----, Hong Kong.

In *C. elegans* males, organogenesis of sensory rays requires proper cell lineage, assembly and patterning. A number of genes are acting in the ray patterning process, including the *dbl-1* TGFβ pathway and a novel protein *mab-21*. A genome-wide RNA interference screen was performed to identify additional components, and two novel genes - nuclear cap-binding proteins 1 and 2 (*ncbp-1 and ncbp-2*) were identified. Loss-of-function of either one of them displayed ray 6,4 fusion resembling that of *mab-21* mutant. *ncbp-1* and *ncbp-2* have been reported to be involved in the splicing process of primary-micro-RNA, pri-*let-7*, in *C. elegans*. Orthologs of *ncbp-1* and *ncbp-2* also form complex and bind to capped-RNA, assisting the splicing of both pre-mature RNAs and primary micro-RNAs. If this activity is conserved, they are required to complex with the target transcripts, the products of which are needed for ray patterning after proper splicing. At the beginning, we confirmed NCBP-1 and NCBP-2 have complex formation ability using a yeast-2-hybrid assay. These two genes have overlapping temporal and spatial expression pattern in male tail rays, suggesting a common locale of action. Its RNA binding ability would be confirmed using RNA immunoprecipitation(RIP). We will study the interactions between NCBP-1/NCBP-2 and the patterning genes. Mutations of patterning genes in the TGFβ pathway appeared to be epistatic to *ncbp-1/ncbp-2* mutants. Identification of these targets genes regulated by the NCBPs will be achieved by RNA immune-pull down assays. The relative amounts of pre-mRNAs and the identity of the candidate would be determined and quantified using a *Drosophila* cells and *C. elegans* without NCPBs as hosts. Also, overexpression of *mab-21* or *mab-18* cDNAs in the absence of *ncbp-1/ncbp-2* would be performed in an attempt to rescue the ray defects, the results of which will be discussed. (This study is supported by Research Grants Council, Hong Kong.).

909A

The nature of *tbx-2* expression pattern in *C. elegans* male tail. KK Ip, **KL Chow**. Division of Life Science, Hong Kong Univ Sci & Technol, Hong Kong, Hong Kong.

Development of *C. elegans* male tail sensory rays involves multiple processes such as ray lineage, ray assembly, patterning and morphogenesis. Previously we identified *tbx-2* as a gene responsible for ray assembly during male tail development. Ray assembly defect can

be found in all rays in *tbx-2* mutants, but the expression pattern of *tbx-2* in adult male rays was restricted to the structural cells of rays 1, 5, 7 and occasionally in R4B neurons, as revealed by GFP transcriptional reporter. *tbx-2* is under its own negative regulation in all rays via direct binding of the protein to a negative regulatory element on its locus region; disruption of which results in up-regulation of *tbx-2* expression in all ray structural cells in male tail. We are interested in how *tbx-2* expression pattern is only subjected to tight negative feedback in rays other than ray 1, 5, 7 and what its significance is. Several candidates of modifiers of *tbx-2* expression, including ray patterning genes such as the SMADS, and *ceh-43*, a homeobox gene, were evaluated. tbx-2 reporter activity was altered in SMADS mutants. *tbx-2* expression is reduced in ray 5 in general. Site specific mutation of a ceh-43 putative binding site - CAATTA - on promoter of *tbx-2* transcriptional GFP reporter results in loss of GFP signal in rays, hinting a direct regulation of *tbx-2* by *ceh-43*. Protein-DNA binding assays will be carried out to confirm this direct association. Meanwhile, additional upstream components, e.g., *lin-32*, and its interaction with ray assembly genes has been examined. Our final goal is to delineate the developmental program leading to ray assembly and patterning. The expression pattern of the *tbx-2* also hinted ray-specific function of *tbx-2* on ray identity control. We have investigated this feature using neurotransmitter (NT) production as a ray identity marker in *tbx-2* mutants, where changes in ray specific NT marker expression was noted. For example, *flp-5*, originally expressed in neuron B of rays 1, 5, 7 is also expressed in ray 4 of *tbx-2* mutant males. Subsequent temperature shift experiment showed that *tbx-2* is required for the determination step, but not for the maintenance step. Whether such changes are a result of ray identity alteration executed by *tbx-2* expression remains to be resolved. (This study is supported by Research Grants Council, Hong Kong).

910B

Comparative genomics reveals novel regulatory mechanism for the transcription factor RFX/DAF-19 in C. elegans. **Jeffrey S C Chu**[1,2], Maja Tarailo-Graovac[1], Jun Wang[1], Bora Uyar[1], Domena Tu[1,2], Joanne Trinh[1], Bob Johnsen[1], David Baillie[1], Nansheng Chen[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) Medical Genetics, University of British Columbia, Vancouver, BC, Canada.

RFX transcription factors play important roles in cilia biogenesis and maintenance by transcriptionally regulate ciliary genes. Their target genes have been associated with a number of disease conditions collectively called ciliopathies. *daf-19* is the only known member of the RFX family in *C. elegans*. RFX regulation of ciliary genes is conserved between humans and C. elegans. The DNA binding site, known as the X-box motif, is also conserved. Thus, *C. elegans* has been effectively used as a model organism to identify RFX target genes. Past studies have identified target genes by searching for X-box motifs in *C. elegans* using bioinformatics, functional genomics, and comparative genomics methods. All of the DAF-19 target genes studied to date possess a single consensus X-box motif. Some genes (*bbs-2* and *osm-5*) were found to possess two X-box motifs. We hypothesize that tandem X-box motifs could have cooperative roles. To test this hypothesis, we compared the gene sets between 4 *Caenorhabditis* species to find *C. elegans* genes with multiple X-box motifs within 500-bp promoter region. The *C. elegans* gene set is an extensively curated set, but this is not the case for the remaining *Caenorhabditis* species. In order to employ comparative genomics for X-box motif searches, we improved the gene sets for 3 *Caenorhabditis* species using genBlastG, a homology based gene predictor that we recently developed. Using comparative genomics with the improved gene set, we identified 15 genes that have conserved X-box motifs in all species and have multiple X-box motifs in *C. elegans*. We examined one gene, F25B4.2, in detail. Using singly integrated reporter constructs, we have shown that F25B4.2 is expressed in ciliated neurons. This expression is dependent on the two 15-bp X-box motifs as well as DAF-19 indicating that F25B4.2 is a DAF-19 target gene. When the proximal motif is removed, expression in ciliated neurons is ablated. When the distal motif is removed, we observed an elevated expression suggesting the distal motif has a repressive role. This is the first to report a putative repressive X-box motif in *C. elegans*. Our data suggest that two X-box motifs cooperate together to regulate specific expression level of F25B4.2. We model that having multiple X-box motifs in the promoter could achieve specific expression level. Our identifications of X-box motifs will improve our understanding on RFX mediated regulation in *C. elegans* and in other organisms including humans.

911C

The dynamics of gene expression in early larval development. **Shu Yi Chua**[1], Hee Sun Shin[1], Jean Thierry-Mieg[2], Danielle Thierry-Mieg[2], David L. Baillie[1]. 1) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 2) NCBI, NIH, Bethesda, MD20894, USA.

Understanding early development in an organism is an on-going effort. The nematode Caenorhabditis elegans grows from an egg to an adult through multiple developmental processes, such as gonadal development and larval molts. These processes involve concerted gene transcription at precise times to ensure the successful maturation. Specifically, we are interested in studying the transcriptome of the nematode as it develops from L1 larvae to L2 larvae. The development of massively-parallel sequencing technologies has made it possible for us to study the dynamics of gene expression in a population of synchronized worms using RNA-Seq methods. To construct samples for our study, we synchronized the nematodes via sodium hypochlorite treatment and allowed the purified eggs to hatch overnight. Poly-adenylated mRNA messages extracted from nematodes cultured on plates was reverse-transcribed into cDNA samples, of which 200bp fragments were then sequenced using an Illumina Genome Analyzer II.

In a preliminary study, we compared the transcriptomes of post-hatching L1 nematodes to those fed an additional 8hrs. Illumina sequencing produced ~15million and ~22million 42-bp paired-end reads for the respective samples. These reads were mapped back to the virtual transcriptome via MAQ. As expected, the effects of the additional development time was observable in the sequencing reads. For example, genes involved in biological processes in the late L1 stage of development, such as cell divisions, gonadal development, biosynthesis, metabolism and the larval molt, displayed some of the highest fold increases. In contrast, genes involved in cell-cell signaling displayed the largest fold decrease. We also looked at ribosomal genes and found that whilst most appeared to be transcribed in a stoichiometrical ratio, a subset did not to follow the trend.

In addition, as compared to data available during the inception of our project (Wormbase WS170), our data also provided additional experimental support for existing gene models. Our RNA-Seq data improved suggested many gene models should be improved with 3' and 5' extensions, novel exons, splice sites, poly-adenylation sites, and in some cases, gene merges.

912A

Regulation of intestinal fasting induced transcription of nhr-206 is DAF-16/FOXO dependent. **Ahmed Chughtai**, Jan Novotny, Frantisek Behensky, Zdenek Kostrouch, Marta Kostrouchova. Laboratory of Model Systems, Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague, Czech Republic.

In C. elegans, the tissue specific expression of four out of seven nuclear receptors that are clustered on chromosome V (nhr-206, nhr-208, nhr-207, and nhr-154) is induced by fasting. This regulatory response can be seen also in the case of some nuclear hormone receptors that are present in the corresponding chromosomal region in genome of C. briggsae. Here, we studied the fasting dependent transcription regulation of nhr-206, which arouse by the most recent gene duplication in this gene cluster and shows the strongest fasting induced intestinal expression. Promoter analysis indicated a presence of DAF-16/FOXO binding site in the vicinity of the start of transcription of nhr-206. We have created transgenic lines containing the promoter of nhr-206 fused to gfp in daf-16 null mutants - CF1038 (mu86) strain and analyzed the expression pattern in comparison with lines prepared on N2 genetic background. In contrary to N2 based lines, the daf-16 null mutants did not show fasting induced expression in intestinal cells. Quantitative PCR detected up-regulation of nhr-206 in the cDNA prepared from total RNA of complete larvae indicating that strong expression of nhr-206 that is seen in both fasted as well as fed larvae in pharynx is DAF-16/FOXO independent and can be effectively up-regulated in fasted animals on the daf-16/FOXO null background. This shows that the newly multiplied nhrs adopted DAF-16/FOXO for fasting dependent gene expression regulation in the intestine. Acknowledgement: We thank Dr. A. Fire for vectors. The work was supported by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

913B

In vivo Regulation of the Alternative Splicing of the Pro- and Anti-Apoptotic Gene *ced-4*. **Anna Corrionero**, Bob Horvitz. HHMI, Dept. Biology, MIT, Cambridge, MA 02139 USA.

The processing of pre-mRNAs by alternative splicing provides a cell with the ability to generate multiple mRNAs from a single gene, thus enormously expanding proteome diversity. Despite efforts to study this process, the regulation of alternative splicing *in vivo* and in a tissue- or developmental stage-specific manner as well as the functional implications of alternative splicing are not well understood. The *C. elegans* CED-4 protein promotes the activation of the caspase CED-3 and is essential for canonical programmed cell death. However, *ced-4* is alternatively spliced, giving rise to two different isoforms with antagonistic functions: the main isoform, CED-4S, is pro-apoptotic, while CED-4L is anti-apoptotic. *ced-4* is the only apoptotic gene known to be alternatively spliced in *C. elegans*. How ced-4 alternative splicing is regulated is largely unknown. To study the regulation of ced-4 alternative splicing *in vivo* we are generating reporters in which the alternatively spliced region of *ced-4* is followed by a fluorescent-protein cDNA specifically in-frame with one or the other isoform, so that expression of CED-4L will give rise to GFP while generation of CED-4S will give rise to RFP. This approach should allow us to determine the isoform ratio during development at a single-cell level and to correlate this ratio with a specific cell's fate. We then will characterize regulators of this alternative splicing event by performing a genetic screen for mutants with an altered fluorescent protein ratio (e.g., decreasing the ratio of CED-4S to CED-4L). We will determine the expression patterns of new spicing regulatory factors and establish the mechanisms by which they modulate *ced-4* alternative splicing using biochemical and molecular approaches. We hope that by studying *ced-4* alternative splicing using a fluorescent reporter system we will characterize not only factors generally involved in alternative splicing but also modulators of the apoptotic pathway itself.

914C

Regulation of X chromosome transcription in Caenorhabditis species. Sarah Albritton, **Sevinc Ercan**. Department of Biology, New York University, New York, NY.

Animals with different numbers of X chromosomes in males and females possess mechanisms to compensate for the difference in the X-linked gene dose between the two sexes. In addition to the X chromosome dose difference between the sexes, the presence of a single-copy X chromosome per two-copy diploid autosomes creates an important problem for males, because all X-linked genes are haploinsufficient compared to the autosomal genes. In C. elegans, hermaphrodites (XX) contain two Xes, whereas males (XO) contain a single X, therefore facing X haploinsufficiency. By performing microarray analysis of RNA abundance in XX and XO worms, we observed that the overall transcript levels from the X chromosome in both XX and XO animals is similar to that of overall expression from

autosomes. This suggests that transcription from the single X in XO L3 hermaphrodites (TY2205, her-1(e1520) sdc-3(y126) V; xol-1(y9) X) is increased approximately two-fold. The mechanism of this upregulation is unclear. We had shown that the X chromosome promoters have higher GC content compared to the autosomes (Ercan et al 2010), suggesting a DNA-encoded mechanism of transcriptional regulation. We will study X upregulation by comparing transcription of orthologus genes that are on the X versus autosomes in four Caenorhabditis species. Ercan S, Lubling Y, Segal E, Lieb JD. High nucleosome occupancy is encoded at X-linked gene promoters in C. elegans. Genome Res. 2011 Feb;21(2):237-44. PMID:21177966.

915A

Chromosome-wide gene regulation and higher-order chromosome structure in C. elegans dosage compensation. **C.A. Fassio**, E. Crane, A. Michel, S. Uzawa, B.J. Meyer. HHMI and Dept. of Molecular and Cell Biology, U. C. Berkeley, Berkeley, California 94720, USA.

Dosage compensation, the essential process that equalizes X-linked gene expression between sexes, provides an excellent system to examine the complex mechanisms that control gene expression across large chromosomal domains. In C. elegans, the condensin-like dosage compensation complex (DCC) reduces transcriptional activity of both hermaphrodite X chromosomes by half to equal expression from the single male X1. The DCC binds two classes of sites on X: rex sites (recruitment element on X), which recruit the DCC autonomously2.3 and dox sites (dependent on X), which robustly bind the DCC only when attached to X3. Long-range interactions between rex and dox sites appear important for full DCC occupancy at dox sites3. The homology of DCC components to condensin suggests that the DCC facilitates long-range regulation of X-linked gene expression by restructuring the X chromosome. Interactions between rex sites may facilitate loading of the DCC and/or targeting of the complex to dox sites. Consistent with this hypothesis, pairs of rex sites interact more frequently in XX and mutant XO embryos in which the DCC is loaded onto X than in XO embryos, in which the dosage compensation does not occur. Additionally, I have shown that the DCC binds an ectopic rex site integrated on the X chromosome. This ectopic binding site is in close proximity to an endogenous rex site that is located over 2 Mb away more often than a control integration sequence. These results further suggest that DCC occupancy imposes structural changes on the X chromosome. Using 5C, I am asking how altered DCC occupancy at specific loci affects the frequencies of long-range interactions among rex sites and between rex and dox sites. These data will show whether increased DCC occupancy corresponds to increased interaction frequencies between binding sites. I am also performing RNA-seq to to ask how localized changes in DCC occupancy affect transcriptional activity of X-linked genes and determine how transcription is influenced by DCC binding. These experiments address the transcriptional consequences of DCC occupancy at defined sites and determine the role of DCC-mediated changes in chromosome structure in this process. 1 Meyer BJ. Curr Opin in Genet & Dev (2010); 2 Jans J. et al. Genes & Dev. (2009); 3 Pferdehirt R. el al. Genes & Dev. (2011).

916B

HLH-17 affects dopamine signaling in C. elegans through the DOP-1, DOP-2 and DOP-3 receptors. **Chaquettea M Felton**, Casonya Johnson. Georgia State University, Atlanta, GA.

Dopamine (DA) signaling regulates many behavioral and functional responses to external and internal stimuli in vertebrate and invertebrate systems. Defects in DA signaling cause a wide range of debilitating neurophyschiatric disorders. To study DA signaling, researchers use model organisms like the soil nematode Caenorhabditis elegans, to mimic these disease states. The effects of dopamine in these systems are mediated by G-coupled protein receptors (GPCRs), which are grouped into D1-like and D2-like classes. C. elegans express the D1-like receptor DOP-1 and the D2-like receptors DOP-2 and DOP-3. In our lab we work with HLH-17, a member of the bHLH transcription factor family. Since HLH-17 is highly expressed in the glia-like cells surrounding the CEP dopaminergic neurons, we wanted to know if HLH-17 was required for dopamine signaling. Our current data suggests that HLH-17 may be a master regulator upstream of the dopamine receptor genes: dop-1, dop-2 and dop-3. Firstly, microarray and RT-qPCR analyses show that HLH-17 affects the expression of dop-1, dop-2 and dop-3; mRNA levels are decreased by at least two fold in hlh-17 animals. Secondly, we determined that hlh-17 animals do not respond to endogenous nor exogenous DA. Egg-laying occurs as the result of the antagonistic effects of serotonin and dopamine; serotonin induces egg-laying while dopamine inhibits it in wild-type (WT) animals. hlh-17 mutants are able to respond to exogenous serotonin but not to exogenous dopamine. Basal slowing is a measurement of endogenous DA. WT animals slow down when they encounter a bacterial lawn; however, hlh-17 mutants move at about the same speed both in the presence and absence of food. Locomotion was also tested with an immobilization assay. In this assay, a moderate concentration of DA cause paralysis in WT animals as a result of increased DA signaling, and requires dop-3 expression. In this assay hlh-17 mutants, like dop-3 mutants, become paralyzed at a much slower rate than WT. Together, these data suggest that HLH-17 may be a master regulator upstream of dop-1, dop-2 and dop-3.

917C

rsr-2, the ortholog of the human spliceosome component SRm300/SRRM2, regulates diverse developmental processes including germline sex determination. **Laura Fontrodona**[1], Tomás Morán[1,3], Montserrat Porta-de-la-Riva[1], Mònica Díaz[2,4], David Aristizábal[2], Alberto Villanueva[1], Simó Schwartz Jr.[2], Julián Cerón[1]. 1) Genetics and Functional Genomics in C. elegans, Bellvitge Biomedical Research Institute - IDIBELL, L'Hospitalet de Llobregat, Barcelona 08908, Spain; 2) Drug Delivery and Targeting, CIBBIM, Vall d'Hebron Hospital, Barcelona 08035, Spain; 3) present address: Institute of Molecular Biology of Barcelona, IBMB - CSIC, Parc Científic de Barcelona, Barcelona 08028, Spain; 4) present address: Omnia Molecular. Parc Científic de Barcelona - UB, Barcelona 08028, Spain.

Protein components of the splicing machinery are highly conserved in eukaryotes and regulate developmental processes at multiple levels. Despite the macromolecular complex nature of the spliceosome, there is a vast amount of evidence supporting the specific functions of its individual components. RSR-2, the *Caenorhabditis elegans* ortholog of the human spliceosomal protein SRm300/SRRM2, in contrast to the yeast ortholog Cwc21p, is essential for viability. Since strong inactivation of *rsr-2* produces severe phenotypes like embryonic and larval lethality, we took advantage of the mild effect of RNAi by feeding to study functions of *rsr-2* during development. We found that partial inhibition of *rsr-2* causes masculinization of the hermaphrodite germline due to the failure to switch the germ cell fate from sperm to oocytes. We have characterized such function through genetic epistasis analysis, in situ hybridizations, and transgenic reporter strains that evidence a ubiquitous presence of *rsr-2* in the soma but a specific expression pattern in the germline. One of these reporters showed RSR-2 nuclear and located at dynamic nuclear speckles. Moreover, total RNA was extracted from synchronized *rsr-2(RNAi)* animals and hybridized to tiling arrays. Intriguingly, while general splicing seems not to be affected, we observed a global reduction of transcript levels except for those transcripts of genes located at chromosome X. Taking into account the functional coupling between transcription and splicing, we suggest that RSR-2 could be a spliceosome component non-essential for splicing but required to regulate transcription depending on the favorable or harmful status of the spliceosome. Next, we intend to decipher the implication of *lin-35* and *prp-8* in *rsr-2* functions since they are genetic and protein interactors respectively. Finally, we are generating tools (transgenics and antibodies) to co-immunoprecipitate RSR-2 along with DNA and RNA to deeper explore its molecular mechanisms of action.

918A

The identification of the functional components of the male-specific CEM neurons. **Lan FU**, Gus C. M. CHAN, King L. CHOW. Division of Life Science, HKUST, Hong Kong.

CEMs are the only four male-specific cephalic neurons located in the head region of *Caenorhabditis elegans*. They are required for sex pheromone perception possibly for sensory input (Chasnov *et al.,* 2007). However, their specific role and molecular function in this process is largely unknown. In order to define the cellular identity of CEM and active elements conferring its function, we are analyzing the CEMs' transcriptome using Illumina sequencing technique. Although *ced-4* mutant hermaphrodites have non-functional CEMs preserved, these animals are not able to respond to sex pheromone. Ectopic *fem-3* expression in the nervous system could masculinize CEMs and significantly raised the sensitivity of *ced-4* hermaphrodites to sex pheromone. Some cellular properties of CEM could also be partially restored with this *fem-3* expression. PolyA RNA from the functional CEMs was obtained from such masculinized *ced-4* mutant hermaphrodites and was compared with the polyA RNA from *ced-4* mutant hermaphrodites with feminized CEMs. FLAG-tagged poly(A)-binding protein (PABP) was expressed under the CEM-specific *pkd-2* promoter and the protein product crosslinked with the CEM-specific polyA RNA. The uniquely expressed or highly enriched RNA species in these functional CEMs are recovered. These unique components reflecting the molecular properties of CEM will be presented, where their potential involvement of the biological activity in the CEMs will be discussed. (The study is supported by Research Grants Council, Hong Kong.).

919B

Transcriptional regulation of pharyngeal gland sub-type expressed genes. **Vikas Ghai**, Jeb Gaudet. Department of Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada.

Organ development is a complex process, and a simple model to study this is the *C. elegans* pharynx. We have previously identified a core sub-set pharyngeal gland expressed genes under the control of the gland specific bHLH transcription factor, HLH-6. *hlh-6* mutants are feeding defective (as are animals were the gland are ablated) suggesting a role in for the glands in feeding. *hlh-6* is under control of several *cis*-regulatory elements, including the gland expressed element HRL3 (Hlh-6 Regulatory element 3). In addition to regulating *hlh-6*, HRL3 directly regulates several other HLH-6 independent gland expressed genes. Interestingly, while both HLH-6 and HRL3 and most of their targets are expressed in all gland cells, there are several targets whose expression is restricted to specific gland cell sub-types. Here we show that several of these targets undergo negative regulation by unique *cis*-elements in their promoters to achieve these patterns. Here we will discuss one of these genes, *Y8A9A.2*

Y8A9A.2 is a Thrombospondin-like protein expressed in the g1p and g2 gland sub-types. present in the promoter is a consensus HRL3 site, which when mutated results in a complete loss of expression. Also present is a highly conserved 20 bp region, which is necessary and sufficient to repress expression in the g1a glands. In this region is a highly conserved NHR (Nuclear Hormone Receptor) binding site, and when mutated results in loss of repression in the g1a gland cells. We tested several NHR genes expressed in the glands, and found a that when *Y8A9A.2* is examined in a *nhr-48(-)* mutant the result is a similar loss of repression in the g1a gland cells, suggesting that NHR-48 may be the trans-factor acting to repress expression of *Y8A9A.2* in the g1a cells.

We know gland fates are specified differently from other pharyngeal cell types due to the combinatorial action of various transcriptional repressor and activators. We don't observe a g1a to g1p/g2 sub-type fate transformation in *nhr-48(-)*, suggesting the differences in gland sub-types are the level of differential expression of some of these functional genes, and the

understanding how genes like *Y8A9A.2* function will ultimately lead to the understanding of the exact role of these subtypes.

920C

Characterization of specific gene programs regulated by Mediator subunit CDK-8. **Jennifer M. Grants**, Stefan Taubert. Department of Medical Genetics, University of British Columbia, Centre for Molecular Medicine and Therapeutics, and Child & Family Research Institute, Vancouver, BC.

Regulation of transcription is crucial for organismal development and homeostasis. The Mediator is an evolutionarily conserved multi-protein complex that acts as a general or specific coregulator of transcription in eukaryotes. Cyclin dependent kinase 8 (CDK-8) is one Mediator subunit that influences the specificity of the complex. As part of a four-subunit "kinase module", CDK-8 associates with the Mediator complex to exert transcriptional repression or activation at specific loci. We are interested in dissecting the specific gene programs regulated by CDK-8.

By microarray gene expression profiling, we have found that in *cdk-8(tm1238)* null mutant nematodes, 167 genes are downregulated and 149 genes are upregulated compared to wild type (N2). Among these deregulated genes certain gene programs are overrepresented, including over 100 genes that respond to pathogen infection in wild type nematodes. Thus, we hypothesize that CDK-8 may be an important coregulator of the innate immune response in *C. elegans*.

We are further investigating the ability of *cdk-8(tm1238)* null mutants to mount an appropriate immune response to the pathogenic bacterial strain *Pseudomonas aeruginosa* PA14. We are using real-time PCR to quantify the expression of specific innate immunity genes after exposure of N2, *cdk-8(tm1238)*, *cic-1(tm3740)* (*cic-1* encodes cyclin C, the critical dimerization partner of CDK-8) and *cdk-8 cic-1* double mutants to PA14 (vs. OP50). In addition, we will test whether these mutants exhibit compromised survival in a PA14 slow killing assay. These experiments will contribute to our understanding of the specialized roles that CDK-8 plays in regulation of nematode physiology.

921A

A new model of C. elegans embryogenesis with cell contacts and spatio-temporal gene expressions. **Johan Henriksson**[1], Jürgen Hench[1,2], Martin Lüppert[1], Akram Abou-Zied[1,3], David Baillie[4], Thomas R. Bürglin[1]. 1) Dept Biosciences & Nutrition, Karolinska Institutet, Stockholm, Sweden; 2) Department of Pathology, University Hospital Basel, Switzerland; 3) Faculty of Applied Medical Sciences, University of Tabuk, Saudi Arabia; 4) Dept. of Molecular Biology and Biochemistry, Simon Fraser University, Canada.

The events that specify cell fates during embryogenesis are highly dynamic. To understand them, one would ideally like to obtain precise expression levels for each developmental control gene in each type of cell. We have developed a multi-channel spatio-temporal (4D) microscopy framework (poster: J.Henriksson "Endrov - an open source framework for image processing and analysis", www.endrov.net) 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::His timelapse microscopy. One of these, ceh-5, we selected for in depth analysis (abstract: Lois Tang et al.).

Analysis of expression patterns shows that they are very reproducible. We have mapped the expression patterns onto a model of normal *C. elegans* development previously made in our lab (Hench et al. PMID:19527702), and have compared patterns in terms of similarity, and shown when and where genes are turned on. We have developed this technique both on a simplified view which is the most reproducible, and on an approximate single-cell level. Our model allows quantitative comparison of many expression patterns simultaneously, and may elucidate how transcription factors regulate development and cell differentiation.

922B

Microarray analysis on laser-dissected tail tips identifies genes regulated by *dmd-3* during male tail tip morphogenesis. **R. Antonio Herrera**[1], Karin Kiontke[1], D. Adam Mason[2], Ji-Sup Yang[1], David H. A. Fitch[1]. 1) Center for Developmental Genetics, Biology Dept, New York University, New York, NY; 2) Biology Dept, Siena College, Loudonville, NY.

We study tail tip morphogenesis (TTM) in *C. elegans* males to understand how cell-fusion and changes in cell shape and position are regulated. The four cells in the male (but not hermaphrodite) tail tip fuse during the fourth larval stage and retract anteriorly. The resulting adult tissue is dome-shaped instead of whip-like as in larvae and hermaphrodites. The DMRT transcription factor *dmd-3* is necessary and sufficient for TTM and therefore plays a central role in this process (Mason et al. 2008). Males mutant for *dmd-3* show no tail tip retraction, and ectopic expression of *dmd-3* causes tail tip retraction in hermaphrodites. *dmd-3* is partially redundant with another DMRT transcription factor *mab-3* (Mason et al. 2008). Forward and reverse genetic screens identified 216 tail tip genes, representing 24 molecular pathways (Nelson et al. 2011). A network analysis of these genes and epistasis experiments positioned *dmd-3* at the center of the network (see abstract by Nelson, Kiontke et al.).

Our objective here is to find all genes downstream of *dmd-3* during TTM. To this end, we are performing tail tip-specific gene expression analyses. Tail tip tissue is obtained by laser capture micro-dissection (LCM). Linearly amplified transcripts from this tissue are hybridized onto Affymetrix *C. elegans* GeneChips. We are comparing gene expression profiles from worms that undergo TTM (wild-type males and hermaphrodites ectopically expressing *dmd-3*), and from worms that do not (wild-type hermaphrodites and *dmd-3;mab-3* double mutant males). Preliminary analysis on tail tips from wild-type and *dmd-3;mab-3* males confirms that *dmd-3* and *mab-3* are transcriptional regulators during TTM. We found that 134 genes are differentially-expressed greater than 2-fold in double mutant males, with a majority of genes showing an increase of expression relative to wild-type. This finding is consistent with the prediction that *dmd-3* and *mab-3* are transcriptional repressors. We are currently obtaining additional tissue for further analyses to separate the effect of *dmd-3* from that of *mab-3*. Candidate TTM genes identified in this analysis are validated by quantitative PCR on tail tip RNA and analyzed for tail tip expression via fluorescent reporters.

923C

Operon transcriptional complexity: dissection via recombineered fosmid-based reporters. **Nisha Hirani**, Stephen Sturzenbaum, Colin Dolphin. Kings College London, Institute of Pharmaceutical Science, London, SE1 9NH.

Operons are common in prokaryotic genomes but, except for Nematoda, are uncommon in eukaryotes. Nematoda "operons" comprise gene clusters with short, approx. 100bp, intergenic regions. A discrete nascent transcript, transcribed from a major upstream promoter, undergoes *trans*-splicing and 5' addition of one of two possible spliced-leader sequences, followed by *cis*-splicing and finally translation of mature mRNA. Operons are common in *C. elegans* and approx. 15% of genes are clustered into operons. The significance of Nematoda operons is unclear although evidence suggests they may have arisen as a consequence of genome evolution and compaction - the "easy come, slow go" scenario. The classical view of operon transcription is of an upstream promoter generating a single "operon" transcript, however evidence suggests operon gene transcription maybe more complex. For example, microarray data indicates that correlated operon gene expression is weaker with increasing intergenic distance suggesting expression is influenced by intergenic sequence-located elements. Further evidence comes from comparison of expression data between fluorescent protein (FP) transcriptional reporters driven either by the upstream "promoter" or intergenic "internal promoter" sequences. These two reporter types generated different expression patterns indicating the presence of regulatory elements within the operon. As an alternative to these transcriptional reporters dissection of operon-clustered gene expression could be investigated using translational reporters generated directly from genomic clones. As they contain 5' and 3' flanking and intronic sequences such genomic clone-based reporters are more likely to contain all, or many, of the regulatory elements associated with the gene of interest (GOI). Furthermore, these reporters are ideal for analyzing operon transcriptional complexity as the size of the fosmid insert (approx. 40kb) easily accommodates an intact operon. Tagging each operon gene with a different FP would permit operon gene expression analyses within the genomic context of that operon. We have used counter-selection recombineering to insert seamlessly commercially-synthesized, *C. elegans*-codon-optimized FP genes into the 3' ends of genes within a number of different 3-gene operons each contained within a different fosmid clone. The suitability of these FP genes, as reporters for multiple tagging has been validated by both *in vivo* and *in vitro* experiments. Operon targets, with confirmed gene structures and preliminary expression pattern data available from published literature, have been identified and the corresponding, multi-gene-tagged fosmid-based reporters created in readiness for expression pattern analyses.

924A

Temporal control of organogenesis by PHA-4/FoxA. **Hui-Ting Hsu**[1,2], Susan Mango[2]. 1) Dept Oncological Sci, Univ Utah, Salt Lake City, UT; 2) Department of Molecular and Cellular Biology, Harvard University, Cambridge MA.

The PHA-4/FoxA forkhead transcription factor in nematodes plays an integral role in all aspects of pharyngeal development, from the earliest specification of pharyngeal precursors to the determination of definitive cell types (1-3). Previous studies in the Mango lab have suggested that PHA-4 activates the expression of many pharyngeal genes directly (4). Our previous studies revealed that binding site affinity for PHA-4 contributes to target diversity in vivo. In fact, the affinity between PHA-4 and its target promoters contributes to the timing of gene expression (4). Mutation of a high affinity PHA-4 binding site to a lower affinity site results in a later onset of expression; conversely, switching a low affinity site to a higher affinity advances gene activation (4). However, the exact mechanism by which PHA-4 temporally regulates all of its targets through binding site affinity remains unclear. To address this question, we employed the NSA (Nuclear Spot Assay) which uses LacO/LacI::CFP to mark pseudochromosome arrays and PHA-4::YFP to track *pha-4* (5-7). By tracking both YFP and CFP signals and their distributed domains in the nucleus, we can follow patterns of association between PHA-4 and its target promoters in living embryos with precise spatial and temporal resolution. Our data indicate that PHA-4::YFP binds to target promoters hours before the transcription firing and that the level of PHA-4::YFP association is significantly different between high versus low affinity sites at early embryonic stages. In addition, manipulation of PHA-4 levels at early embryonic stages (2E to 8E) by heatshock (over expression) or RNAi (reduced expression) can alter the onset of pharyngeal expression. These data suggest that PHA-4 binds to pharyngeal target promoters through different affinity sites at early embryonic stages and this binding may prime the timing of gene expression at later stages. We will further investigate the PHA-4 occupancy at endogenous loci during development by chromatin immunoprecipitation. Together, this study will help clarify how binding site affinity influences temporal regulation of transcription. Reference 1.Mango S.E., Lambie E.J., and Kimble J. Development 120 : 3019. (1994) 2.Horner M., Quintin S., Domeier M.E., Kimble J., Labouesse M. and Mango S.E. Gene & Development 12:1947. (1998). 3.Gaudet J., Muttumu S., Horner M., and Mango S.E. PLoS Biol 2 : e352. (2004) 4.Gaudet J., and Mango S.E. Science 295 : 821. (2002) 5.Carmi I., JKopczynski J.B. and Meyer B.J. Nature 396:168. (1998) 6.Belmont

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925B

RNA polymerase III transcribes a defined subset of tRNAs and snoRNAs at the nuclear pore in *C. elegans*. **Kohta Ikegami**, Jason Lieb. Department of Biology and Carolina Center for Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, NC.

The "gene gating" hypothesis proposes that highly transcribed genes are localized near nuclear pore complexes (NPCs) for efficient mRNA export and translation. While studies in yeast have widely supported this idea, the situation in metazoans is unclear. Using genome-wide chromatin immunoprecipitation (ChIP) with antibodies specific to Nup93/NPP-13, a core component of NPCs, we identified genomic regions associated with NPCs in *C. elegans* embryos. NPP-13 associates almost exclusively with genes encoding transfer RNAs (tRNAs) and small nucleolar RNAs (snoRNAs). NPP-13 is associated with 27% (163) of all annotated tRNA genes, and 38% (46) of annotated snoRNA genes. ChIP of the RNA polymerase III (Pol III) machinery revealed that when tRNA or snoRNA genes are associated with NPP-13, they are strongly bound by TFIIIB, TFIIIC and Pol III itself. RNA-seq data allowed us to assess transcript levels of individual snoRNA genes and confirmed that almost all (96%) NPP-13-associated snoRNA genes are transcribed. Of snoRNA genes that are not associated with NPP-13, only about half are transcribed, and they are transcribed by Pol II and not Pol III. Pol II-transcribed genes surrounding sites of NPP-13 association are not particularly highly transcribed. Our data suggest that metazoans utilize a gene gating system that involves Pol III-mediated transcription at the nuclear pore, possibly to facilitate export of tRNAs or snoRNAs, ultimately facilitating protein synthesis.

926C

Possible regulation of *bed-3* by *blmp-1*. **Takao Inoue**. Biochem, National Univ Singapore, Singapore, Singapore.

blmp-1 encodes the *C. elegans* ortholog of Blimp1, a SET-domain containing transcription factor. The modENCODE project identified a binding site for BLMP-1 in the intron 3 of the *bed-3* gene, within a region where a vulva and hypodermis specific enhancer element was previously identified. We found that RNAi against *blmp-1* causes down-regulation of a *bed-3::gfp* reporter containing intron 3. Moreover RNAi against *blmp-1* caused a molting defect similar to a strong *bed-3* mutant. These results suggest that *blmp-1* regulates *bed-3*.

927A

Searching for transcriptional regulators of C. elegans proteasome subunits. **CongYu Jin**[1], YueHong Yang[1], Akihiro Mori[2], Yuji Kohara[2], Xuan Li[3], Johan Dethlefsen[4], Johan Henriksson[4], Keith Blackwell[3], Thomas Bürglin[4], Carina I. Holmberg[1]. 1) Molec Cancer Biol Program, Univ Helsinki, Helsinki, Finland; 2) National Institute of Genetics, Japan; 3) Joslin Diabetes Center, Harvard Stem Cell Institute, and Dept of Pathology, Harvard Medical School, Boston, MA, USA; 4) Dept of Biosciences and Nutrition, Karolinska Institutet, Stockholm, Sweden.

The 26S proteasome is a highly conserved multi-subunit protease that controls protein stability in the cell. It degrades damaged and misfolded proteins, as well as redundant key regulatory proteins, and is therefore involved in multiple cellular processes such as cell cycle control, signal transduction, stress response, and apoptosis. So far, the transcriptional regulation of the proteasome complex has not been well characterized, especially in multi-cellular organisms. By using transcriptional GFP reporters for several proteasome subunit genes and RNAi assays, we have established the existence of a compensatory "bounce-back" response in C. elegans, namely the up-regulation of various proteasome subunit genes in response to proteasome inhibition. We found that this feed-back response is mostly dependent on the transcription factor SKN-1, the C. elegans ortholog of mammalian Nrf1/2/3 proteins that have been recently found to mediate a similar response in mammalian cell cultures. However, RNAi targeting skn-1 alone did not have a major effect on expression levels of various proteasome subunits, suggesting that SKN-1 is mainly serving as a transcriptional regulator of proteasome subunit genes under stress conditions. In order to identify transcription factors that contribute to proteasome subunit expression under normal conditions, we used in silico approaches to search for binding sites of known transcription factors and novel cis-regulatory modules in promoter regions of various proteasome subunits. We have identified an 11-bp module that exists in promoter regions of multiple proteasome subunits. Deletion and/or substitution of the candidate module in several proteasome subunit transcriptional GFP reporters caused alterations in fluorescence intensity and distribution. Our data indicate that the predicted module serves as a cis-regulatory element of various proteasome subunit genes. We are currently looking for the transcription factor that binds to this element. Taken together, out data suggest that multiple transcription factors contribute to transcriptional regulation of proteasome gene expression, both under normal and stress conditions.

928B

The homeobox transcription factors, CEH-14 and TTX-1, regulate expression of *gcy-8* and *gcy-18* in *C. elegans*. **Hiroshi Kagoshima**[1,2], Yuji Kohara[1]. 1) National Institute of Genetics, Japan; 2) Transdisciplinary Research Integration Center.

To investigate mechanism of cell-specific transcription, we chose AFD thermosensory neuron for our target cell and have analyzed nine AFD-specific promoters. Among these promoters, we focused on analysis of *gcy-8* and *gcy-18* promoters which have arisen from recent gene duplication, suggesting they may conserve upstream regulatory mechanisms. Indeed, reporter expressions from both promoters were downregulated in either mutant of the homeobox genes, *ttx-1* or *ceh-14*, and the expressions of *gcy-8* and *gcy-18* were completely abolished under *ttx-1; ceh-14* double mutant background. Moreover, forced expression of both the transcription factors in AWB chemosensory neuron induced ectopic expression of *gcy-18* in AWB. These results suggest that *ttx-1* and *ceh-14* transcription factors play pivotal roles in *gcy-8* and *gcy-18* expressions. We also showed direct interaction of TTX-1 and CEH-14 proteins with gcy-8 and gcy-18 promoters by gel shift assay using recombinant GST fusion proteins, and identified the binding sites of the transcription factors. We are currently analyzing mutated promoter constructs to confirm if these sites determined by in vitro analysis are required for in vivo function.

929C

Novel fat-reducing plant extracts as therapeutics in the treatment of obesity using a C. elegans model. **Shail Kaushik**[1,2], Harsh Bais[1]. 1) Plant and Soil, University of Delaware, Newark, DE-19716; 2) Delaware Biotechnology Institute 15 Innovation Way, #145 Newark, DE-19711.

Abstract: Obesity is a leading preventable cause of death in developed countries around the world. As reported in a 2007 report of the World Health Organization, there are more than 1.6 billion obese and overweight individuals worldwide; and this number is twice the number of individuals who suffer from starvation and malnutrition. The major reason for this is the imbalance between the amount of food intake and the energy expenditure in the average person. However, diet and exercise alone are not always sufficient as obesity is a complex issue governed by a multitude of factors. In the past two decades, there has been a surge in marketing of drugs, which regulate body fat. However, the use of these products is accompanied by a plethora of side effects. Plants extracts and plant based products are rapidly gaining popularity in the treatment of various physiological and metabolic disorders. In this study we screened several plant extracts and root exudates on fat biosynthesis and storage in nematode model C. elegans. Although genomic screening for fat regulatory genes has been studied in several single and multi-cellular organisms, C. elegans was our model organism of choice due its small size, a well-developed genomic database, and ease of handling. Nile red staining of fixed worms fed on target plants species extract (Momordica charantia) revealed considerable differences in the number of fat bodies in the treated worms. We also showed that the significant reduction in fat content increased the overall lifespan in the treated worms. We will dissect the fat regulating pathway and study its implications on insulin signaling and lifespan events in worms by exploiting the whole transcriptomic and metabolomics approach in C. elegans. Results obtained from our studies will help us understand processes that may involve synergism between longevity and fat metabolism in higher animals.

930A

The affects of DNA sequence on chromatin architecture and gene expression. **Colton E Kempton**, Elliot Winters, Benjamin Jorgensen, Steven M Johnson. Department of Microbiology and Molecular Biology, Brigham Young University, Provo, UT.

We seek to understand the effects of chromatin architecture (specifically nucleosome positioning) on gene expression. Within the nucleus, all of the requisite information for an organism's survival, growth and development is encapsulated. The majority of this information is most likely contained in the DNA and chromatin. Eukaryotes have large genomes that must be carefully packaged and compacted to fit within the relatively small nuclei of their individual cells and still be functional. DNA wrapped around a histone octamer comprises a single nucleosome and represents the primary level of DNA packaging and compaction. The physical positions of nucleosomes affect gene expression by controlling access to regulatory elements in a genome's sequence. To study the effects of nucleosome positioning on gene expression, we are attempting to position and/or reposition nucleosomes that surround genic regulatory elements by manipulating non-coding and non-regulatory flanking DNA sequences. This DNA manipulation is done on constructs harboring reporter genes that we inject into *C. elegans* forming transgenic lines of animals. Currently we are constructing and analyzing the effects of various combinations of putative nucleosome repelling and attracting sequences in combination with various enhancer and promoter combinations. Our preliminary observations indicate that in certain promoter/enhancer combinations reporter expression decreases over multiple generations. In the future we hope to maintain strong reporter expression over multiple generations by using these putative nucleosome repelling and attracting sequences to reposition nucleosomes and or influence their formation. Long term we expect that these data and observations will contribute to general understanding of how chromatin architecture influences gene expression and the role of DNA sequence on that architecture.

931B

The overlapping roles of *hlh-1* and *unc-120* in bodywall muscle differentiation. **Steven G. Kuntz**[1], Brian Williams[1], Paul Sternberg[1,2], Barbara Wold[1]. 1) Div Biol, California Inst Tech, Pasadena, CA; 2) Howard Hughes Medical Institute.

Bodywall muscle differentiation in *Caenorhabditis elegans* is resistant to individual mutations of key transcription factors, including the major regulators *hlh-1* and *unc-120*. Though over-expression of either factor is sufficient to induce muscle differentiation, neither is necessary. How the regulatory network is structured to allow this has not been described. To address this, we sought to both identify what these transcription factors regulate in bodywall muscle and what role HLH-1 binding has in this regulation. Through loss of function analysis, we identified genome-wide regulatory targets of *hlh-1* and *unc-120* using RNA-seq. 1445 targets of *hlh-1* and 3674 targets of *unc-120* were identified, with 760 of these genes being targets of both. Crosstalk was identified between the networks of

hlh-1 and *hlh-8*, which is involved in non-bodywall muscle development. We then mapped the relationship between the regulatory targets and HLH-1 binding, as measured by ChIP-seq. In the process, we quantified bodywall muscle RNA. While regulatory targets are enriched for HLH-1 binding, the majority of HLH-1 binding actually occurs away from genes that are affected by *hlh-1* loss of function. Nevertheless, HLH-1 binding is associated with both the expected E-box and, near genes belonging to specific regulatory subgroups, novel motifs that may serve as binding sites for cooperative transcription factors. Our data suggest that shared target genes and overlapping regulation buffer the *hlh-1* and *unc-120* mutant phenotypes.

932C

FOX-1 family and UNC-75 regulate neuron-specific alternative splicing of the *unc-32* gene. **Hidehito Kuroyanagi**[1], Masatoshi Hagiwara[2]. 1) Grad Sch Biomed Sci, Tokyo Med & Dental Univ, Chiyoda-ku, Tokyo, Japan; 2) Dept Anatomy & Developmental Biology, Grad Sch Med, Kyoto Univ, Kyoto, Japan.

The *C. elegans unc-32* gene, encoding the a subunit of V_0 complex of vacuolar-type H^+-ATPases, has two sets of mutually exclusive alternative exons. To analyze the selection specificity of the exons, we generated *in vivo* alternative splicing reporters for exon 4 and exon 7 separately, and found that these exons were regulated in tissue-specific manners; neurons predominantly expressed exon 4b and exon 7a. As UGCAUG stretch in the intron downstream from exon 7b was conserved among several nematode species, we introduced the exon 7 reporter into the FOX-1 family mutant background, and confirmed that FOX-1 family genes *asd-1* and *fox-1* redundantly regulate neuron-specific selection of exon 7a of the exon 7 reporter as well as the endogenous *unc-32* gene. In search for further mutants defective in the expression of the exon 7 reporter in neurons, we isolated several *unc-75* mutant alleles. *unc-75* encodes a neurons-specific CELF family RNA-binding protein, function of which has not yet been characterized. We analyzed the splicing pattern of the endogenous *unc-32* mRNA in the *unc-75* mutants and found that the amounts of neuron-specific exon 7a isoform as well as exon 4b isoform were reduced in the mutant. Furthermore, mutation in *unc-75* fully suppressed the Uncoordinated (Unc) phenotype of the *unc-32 (e189)*mutant, which has a base substitution in the acceptor site of exon 4b, confirming that UNC-75 regulates exon 4b of the endogenous *unc-32* gene.

933A

SMG-1 may possess dual mRNA surveillance functions . **Luciana Leopold**[1], Matt Eckler[1,2], Yan Liu[1], Channing Der[3], David Reiner[3], 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; 3) Department of Pharmacology and Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC, USA.

Nonsense-mediated RNA decay (NMD) is an mRNA surveillance mechanism conserved throughout eukaryotic species. Premature termination codons (PTCs) and resulting abnormal 3'UTRs activate NMD to trigger precocious degradation of aberrant mRNAs. SMG-1 is a phosphatidylinositol kinase-related protein kinase known to function as a key component of the NMD pathway in both humans and *C. elegans*. We isolated a phenotypically novel allele of *smg-1*, *yp3*, from a *mortal germline* mutant strain and are characterizing its effects on NMD. The phenotype of animals harboring the *unc-30(e596)* mutation, which generates a canonical PTC target of NMD, was robustly suppressed by the null *smg-1* alleles *r904* and *e1228*, the temperature-sensitive *smg-1* alleles *cc545* and *cc546*, as well as by *yp3*. The *reIs9*[$P_{lin\text{-}26}$*::gfp::Smg-S* 3'UTR + *rol-6(d)*] reporter strain expresses hypodermal-specific *gfp* transcript with an abnormally long 3'UTR, and consequently GFP expression is minimal in wild-type animals where the message is degraded by the SMG machinery. GFP expression from *reIs9* was enhanced by all tested *smg-1* alleles, including *yp3*, consistent with these alleles compromising degradation of aberrant mRNA. In contrast, the Smg-sensitive paralyzed Unc phenotype of *unc-54(r293)* was suppressed by *smg-1* alleles *r904*, *e1228*, *cc545* and *cc546*, but surprisingly *unc-54(r293)* was not suppressed by our novel allele, *yp3*. Thus, *smg-1(yp3)* is a separation-of-function mutation that is proficient in degrading a subset of aberrant mRNA species that is targeted for degradation by the SMG machinery.

934B

Regulation of calreticulin gene expression by sumoylation in *C. elegance*. **Yun-Ki Lim**[1], Sunkyung Lee[2], Joohong Ahnn[2], Do Han Kim[1]. 1) School of Life Sciences, GIST, Gwangju, Korea; 2) Department of Life Science, College of Natural Sciences, Hanyang University, Seoul, Korea.

Sumoylation is a reversible post-translational modification which is involved in diverse biological processes including protein stability, nuclear-cytosolic transport, DNA binding activity, and gene expression. In *C.elegans*, sumoylation has been shown to be critical in gonadal development by modulating chromatin integrity and transcription (Broday et al., Genes & Dev. 18, 2380 2004). Calreticulin is a calcium binding protein residing in endoplasmic reticulum that plays pivotal roles in calcium homeostasis, chaperone activity, and glycosylation and transcriptionally up-regulated by unfolded protein response (UPR) in *C.elegans*. In order to understand the role of sumoylation on the UPR response in nematode, we attempted to study the function of SUMO conjugating enzyme UBC-9 on calreticulin gene regulation. *ubc-9* mutant worms showed defective vulval development similar to *smo-1*, lack of SUMO protein. We found that, upon abolishing sumoylation, GFP fluorescence driven by calreticulin promoter was increased in *C. elegans*. Furthermore, the increased GFP fluorescence was attenuated by knockdown of *xbp-1*, indicating that sumoylation is required for transcriptional repression of calreticulin in *C. elegans*. We are currently investigating underlying mechanism to elucidate how sumoylation controls gene expression of calreticulin in *C.elegans*.

935C

Identification of transcription factors regulating *lin-39* expression. **Wan-Ju Liu**, David Eisenmann. Biological Sci, UMBC, Baltimore, MD.

Our lab studies the development of the vulva in *C. elegans*. 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 we wish to identify the transcription factors that regulate *lin-39* expression in the embryo and larva.

Previous work by our lab and others has identified several transcription factors and chromatin regulatory proteins that regulate *lin-39* expression. To 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 regions from *C. elegans*, *C. briggsae* and *C. remanei* were aligned and 31 conserved regions were found (referred to as triple conserved regions (TCRs)). We grouped the 31 TCRs into 12 fragments (RF1-RF12) that were used as 'baits' in yeast One Hybrid (Y1H) screens. From three different library screens (in collaboration with the Walhout lab), we found several transcription factors that might bind to *lin-39* promoter regions, and are validating the results with further experiments. First, to determine if the protein regulates *lin-39* in worms, we performed RNAi against the transcription factor genes in worms carrying *lin-39::GFP* constructs to look for changes in GFP expression. Second, to validate the interaction between the transcription factors and the *lin-39* promoter regions, we performed gel shift assays. Third, we performed RNAi against the transcription factor genes on wild type worms to observe whether there are any phenotypes in *lin-39*-dependent processes. The transcription factors we are currently working on are *nhr-43*, *alr-1*, *ztf-17*, *lin-26*, *tbx-9*, and *bed-3*. The results and progress for each gene will be described in more detail.

Finally we are also trying to identify factors that bind to a 338 bp fragment of the *lin-39* promoter that may be required for initiation of *lin-39* expression in the embryo. We identified a GATA factor, ELT-6, which binds to a GATA site in this fragment of *lin-39* and positively regulates *lin-39* expression in the embryo. An update on these results will also be described.

936A

Analysis of cell fate from single-cell gene expression profiles in C. elegans. **Xiao Liu**[1], Sarah Aerni[2], Fuhui Long[3], Hanchuan Peng[3], John Murray[4], Serafim Batzoglou[2], Robert Waterston[4], Gene Myers[3], Stuart Kim[1]. 1) Dept Developmental Biology, Stanford Univ, Stanford, CA; 2) Department of Computer Science, Stanford University, Stanford, CA 94305; 3) Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, VA 20147; 4) Department of Genome Sciences, University of Washington, Seattle, WA 98195, USA.

We developed an automatic image analysis system to measure the expression patterns of fluorescent reporters at the resolution of single cells in C. elegans. We generated expression profiles of 93 genes in 363 specific cells from L1 stage larvae. Among various developmental features, cell fate specificity and organ specificity dominate gene expression programs. Nevertheless, cell lineage can play a significant role in gene regulation among cells of the same fate. For example, the 23 nuclei in the hyp7 syncytium express different sets of transcription factors based on whether they are derived from AB lineage or C lineage. The quantitative gene expression profile in cells of invariable cell lineage also allows computation analysis of development process. For each cell division, we calculated difference in gene expression between daughter cells to identify molecular differentiation in the worm cell lineage. These examples illustrate how quantitative gene expression signatures for single cells can provide a new way to visualize underlying molecular mechanisms that define cell fate.

937B

Identification of evolutionary conserved regulators of dietary restriction using the "Ortho2ExpressMatrix". **Andreas Ludewig**[1], Thomas Meinel[2], Frank Doering[1]. 1) Institute of Human Nutrition and Food Science, Christian-Albrechts-University of Kiel, Heinrich-Hecht-Platz 10, 24118 Kiel, Germany; 2) Structural Bioinformatics Group, Institute for Physiology, Charité-University Medicine Berlin, Thielallee 71, 14195 Berlin, Germany.

During the past decades, increasing amounts of data derived from high throughput approaches assaying all kinds of species, tissues and diseases have been accumulating into gigantic pools of data. However, the usability and the accessibility of these data for the generation of new scientific information is sometimes difficult. Here, we apply a novel developed bioinformatic tool - the Ortho2ExpressMatrix (Meinel et al., 2011) to summarize, compare and interpret gene expression data from various dietary restriction (DR) experiments performed in the model organisms mouse and C. elegans. We use the Ortho2ExpressMatrix to integrate complex gene family information, computed from sequence similarity with gene expression profiles of the two species exposed to DR and ad libidum feeding conditions. We come up with a comprehensive list of genes that are co-regulated in both species under DR. All in all, huge cohorts of data have been condensed to a list of 18 mouse genes and 24 assigned putative functional C. elegans orthologs, that can be directly used for experimental set ups further elucidating the molecular mechanisms that control DR related phenomena.

938C

In vivo analysis of splicing factor genes in regulating alternative splicing in *C. elegans*. Yanling Teng, Xiaoyang Gao, **Long Ma**. State Key Laboratory of Medical Genetics, Central South University, Changsha, Hunan, China.

RNA splicing is carried out by the spliceosomal machinery involving over one hundred proteins and numerous non-coding RNAs. Alternative splicing generates multiple mRNA isoforms from a single pre-mRNA and is a major driving force for proteomic complexity. The *in vivo* analysis of splicing factors in regulating alternative splicing in animals remains a challenge due to a shortage of sensitive endogenous splicing reporters and viable mutants of critical splicing factors. Previously we have isolated multiple *C. elegans* mutations affecting the U2AF large subunit and SF1/BBP, two critical splicing factors. Using a microarray-based screen, we identified a *C. elegans* gene, *tos-1*, that exhibited three of the four major types of alternative splicing: intron retention, exon skipping and, in the presence of U2AF large subunit mutations, the use of alternative 3' splice sites. Mutations in the splicing factors U2AF large subunit and SF1/BBP altered the splicing of *tos-1*. 3' splice sites of the retained intron or before the skipped exon regulate the splicing pattern of *tos-1*. Using *tos-1* splicing as a reporter, we examined how *smn-1(ok355)*, a deletion mutation of the survival of motor neuron protein homologous gene, affects RNA splicing. We found that *smn-1(ok355)* caused increased intron 1 retention of *tos-1* but did not affect exon 3 skipping or the choice of alternative 3' splice sites. Our genetic system, which includes *tos-1* as an endogenous splicing reporter and a series of mutations affecting the splicing factors U2AF large subunit and SF1/BBP, could be used for studying the genetics of novel splicing factor genes and for analyzing *in vivo* functions of splicing factor genes related to human diseases.

939A

Analysis of temporal expression data of C.elegans early embryo. **T. Maeshiro**[1], S. Nakayama[1], K. Monobe[2], M. Ito[2]. 1) SLIS, Univ Tsukuba, Tsukuba, Japan; 2) Ritsumeikan Univ., Kusatsu, Japan.

We have measured and analyzed the whole genome microarray data of C.elegans early embryo, starting from the single cell stage up to forty minutes, measured with ten minutes interval. The measurement was repeated three times, and the distribution of cell stages on each measurement point was verified with optical microscope to ensure the synchronization accuracy of the experiment. The most frequent cell stages at each measurement points are: one and two cells of approximately equal frequency at zero minute; cell division stage from two to four cells at 10 minutes; four cell stage at 20 minutes; eight cell stage at 30 minutes; cell division stage from eight to twelve cells at 40 minutes. The number of expressed genes varies, starting with 47.03% of all genes, decreasing to 46.10% at 10 minutes, then increasing to reach a peak of 48.72% at 30 minutes and decreasing again to 47.71% at 40 minutes. Since the cell stages at 10 minutes and 40 minutes are the middle of cell division process, respectively two to four and eight to twelve, smaller number of genes function during cell division than the steady cell stage, where the cell division process is not visually identified. Furthermore, the number of expressed genes in stable cell stages increases monotonically with the increase of number of cells. It is evident that the total number of genes increases in later development stage when the cell specialization is more diversified, but our data indicate that the similar phenomena is already occurring at the early development stage.

940B

Structure-function analysis of MDT-15, a conserved transcriptional co-regulator required for metabolic homeostasis. **Allan Mah**, Ada Kwong, Stefan Taubert. Medical Genetics, University of British Columbia, CMMT, Vancouver, British Columbia, Canada.

The Mediator is a conserved transcriptional coregulator complex that functionally links transcription factors and RNA polymerase II. MDT-15 is a subunit of the Mediator that is required for metabolic adaptation in *Caenorhabditis elegans*. Specifically, MDT-15 regulates the basal and stimulated expression of many genes in response to physiological stimuli including fasting, and exposure to toxins and heavy metals. Consistent with these functions, depleting or mutating *mdt-15* leads to defects in fat storage, toxin sensitivity, life span, lethargy, fecundity, and target gene expression. To regulate gene expression, MDT-15 interacts with transcription factors. Some of these interactions occur via the conserved KIX domain of MDT-15, which binds many nuclear hormone receptors. However, no other functional regions have been defined in MDT-15. To dissect how MDT-15 functions molecularly we are performing structure-function analyses. Like the N-terminal KIX domain, the C-terminal region of MDT-15 is also well conserved. We discovered that the C-terminus activates transcription in yeast, suggesting that it has a role in recruiting the transcriptional machinery. Another functional region is defined by the *mdt-15* mutant allele *tm2182*, as loss of this region causes the phenotypes described above. The *mdt-15 (tm2182)* allele appears to be a hypomorph because we expect that a null mutation cause lethality like depletion of *mdt-15* by RNAi. By sequencing the *mdt-15* mutant cDNA, we discovered that instead of the predicted premature stop, the mutation results in an in-frame deletion (MDT-15del). As MDT-15del might contain protein binding regions, we are identifying protein-protein interactions by yeast two-hybrid screening. We have found several potential interactors for MDT-15del, including homeobox transcription factors. Thus, our preliminary data suggests the MDT-15del region interacts with different types of transcription factors than the KIX domain. We will confirm our yeast two-hybrid results with GST-pull down and co-immunoprecipitation experiments. Furthermore, we are investigating whether the MDT-15del region is important for intracellular localization and/or protein stability by expressing full-length MDT-15, the mutant protein, and MDT-15del fused to GFP and studying the consequences to intracellular localization and expression levels. From these experiments, we are characterizing functional regions in MDT-15, an important regulator of metabolism, and identifying novel interacting transcription factors that might participate with MDT-15 to regulate metabolic and stress response.

941C

A 3'UTR clone library of the nematode *C. elegans*: a resource for 3'UTR biology. **M. Mangone**, J.M. Lucas, M.R. Gutwein, D. Mecenas, K.C. Gunsalus, F. Piano. Dept Biol, New York University, New York, NY.

Metazoan messenger RNAs contain poorly defined cis-acting elements within their 3' untranslated regions (3'UTRs) that modulate gene expression at the post-transcriptional level. These elements play important roles in development, metabolism, and their dysfunction are associated with disease states such as autism, depression, diabetes, Alzheimer's and cancer. Using a combination of deep sequencing, high-throughput 3'RACE, and manual curation of public datasets, we have recently reported a 3'UTR encyclopedia defining ~26,000 3'UTRs for ~75% of protein-coding genes in the *C. elegans* WS190 genome. This study identified 3'end-processing elements, evolutionarily conserved blocks, predicted miRNA target binding sites, and alternative termination sites within thousands of 3'UTRs. The surprising complexity revealed by these data suggests that 3'UTR-mediated post-transcriptional gene regulation is prevalent on a genome-wide scale *(1)*. To build a comprehensive resource for 3'UTR biology we have been constructing a library of 3'UTR clones using modular vectors that facilitate downstream *in vivo* functional analyses. We have completed our first pass of directed 3'UTR cloning obtaining at least one 3'UTR isoform for ~15,000 genes annotated in WS190, and we are currently isolating and deep-sequencing 3'UTR isoforms from our last batch of ~6,000 3'UTR minipools. These clones will be made available to the community through distributors and through our website UTRome.org *(2)*. In addition, we have developed a suite of destination vectors to adapt the 3'UTRome to perform large-scale RNAi screens by feeding, to study the contribution to gene expression provided by different 3'UTR isoforms *in vivo* using dual-colors reporters, and to facilitate the introduction of 3'UTRs into worms either by injection or ballistic transformation. Moreover, we are now preparing entry vectors that will allow the studying of the contribution of 3'UTR isoforms to mRNAs localization using a MS2-derived system compatible with MosSCI technology. In conclusion, we have provided the first whole transcriptome-level description of the 3'UTRome in any organism with single-nucleotide resolution. Furthermore, our 3'UTR clone library provides a powerful tool to probe 3'UTR biology at a systems-level scale. Our work will help to better understand 3'UTR biology and push forward the study of important cis-regulatory elements in the genome.

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942A

Factors Affecting the Mean, Variance and Predictive Power of a Lifespan-Predicting Biomarker. **Alexander R. Mendenhall**[1], Pat Tedesco[1], James R. Cypser[1], Larry Taylor[1], Anita Lowe[1], Roger Brent[2], Thomas E. Johnson[1]. 1) Institute for Behavioral Genetics, Department of Integrative Physiology, University of Colorado, Boulder, Colorado; 2) Division of Basic Sciences, Fred Hutchison Cancer Research Center, Seattle, Washington.

Variation in gene expression among genetically identical individuals grown together in a uniform environment has been largely unexplored in *C. elegans*. In 2005, Rea et al. showed that the induced expression level of a transcriptional reporter of a small heatshock protein (*Phsp-16.2::gfp*) in young adult *C. elegans* could predict lifespan and thermotolerance. However, these studies used a single transgenic insertion (*gpIs1*), which contains approximately 450 copies of the transgene in a tandem array integrated into chromosome IV. To determine if different transgenic reporter alleles of the same gene show similar predictive capabilities and variance in expression, we generated several new transgenic lines expressing green and red fluorescent proteins under control of the *hsp-16.2* promoter; these lines include three new single-copy reporters. Worm-to-worm variance in transgene expression is independent of locus, copy number, and fluor. Variance in GFP signal, measured either as worm-to-worm variance among individual worms or as cell-to-cell variance among matched intestine cells, is not significantly different between strains. The average expression profiles of the single-copy and tandem-array reporters display the most and least GFP in the exact same intestine cells; however, cell-to-cell measures are still preliminary and ongoing. Growth temperature affects the mean and variance of constitutively expressed reporters (*Pvit-2::gfp* and *Pgpd-2::gfp*). In contrast, mean and variance of the induced expression of *Phsp-16.2::gfp* are independent of growth temperature. Interestingly, we observed spontaneous, constitutive *Phsp-16.2::gfp* expression at 25º C. Elimination or attenuation of specific signaling systems results in reduced worm-to-worm variance in *Phsp-16.2::gfp* expression. Thus, worm-to-worm variance in reporter expression is actively increased by intrinsic individual differences in and/or stochastic fluctuation of signal transduction between individuals. Finally, both single copy and tandem array transgenes predict thermotolerance and lifespan. Our studies demonstrate that the predictive capacity of the *Phsp-16.2::gfp* biomarker is not dependent on any specific transgene configuration. This abstract marks the beginning of a systems biology approach to dissect metazoan gene expression variance at the level of individual animals and cells to determine how single reporters can predict complex phenotype outcomes such as lifespan.

943B

GEI-8 is a transcription co-repressor orthologous to NCoR/SMRT with developmental functions in C. elegans. **Pavol Mikolas**[1], Frantisek Behensky[1], Vladimir Saudek[1], Michael Krause[2], Zdenek Kostrouch[1], Marta Kostrouchova[1]. 1) Laboratory of Model Systems,

Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague, Czech Republic; 2) Laboratory of Molecular Biology, NIDDK/National Institutes of health, Bethesda, MD.

Nuclear receptor corepressors regulate transcription through the assembly of the 'corepressor complex' with histone-deacetylase activity. Two paralog corepressors, N-CoR and SMRT with partially overlapping but distinct functions have been characterized in vertebrates. By sequence similarity, we identified GEI-8 as the closest homologue of N-CoR/SMRT in C. elegans and found experimental evidence for its corepressor function. gei-8 is expressed in neurons, muscle and intestinal cells in all developmental stages with a peak in the L4 stage. Although the inhibition of gei-8 did not induce a visible developmental phenotype, a homozygous deletion in gei-8 coding region in the mutant VC1213 that was kindly provided by the C. elegans consortium resulted in severe developmental phenotype with a prominent neuromuscular impairment, slow growth, infertility and larval arrest. Aldicarb and levamisole assays indicated a defective cholinergic signaling. Whole genome microarray analysis of mutant line revealed sets of derepressed genes with potential developmental functions as well as genes with abnormally lowered expression. Inhibition of derepressed genes by RNAi in mutant larvae induced further developmental changes indicating that the transcription repressive function of GEI-8 constitutes its development regulating network. Our results indicate, that gei-8 is an ortholog of vertebrate corepressors N-CoR and SMRT and plays crucial roles in larval development and neuronal functions. These results support the general role of NCoR/SMRT correpressors in metazoan transcription and development regulation. The work was supported by the Grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

944C

Biochemical characterization of canonical and variant C.elegans nucleosomes. **Ahmad N Nabhan[1]**, Margaret Jow[1], Diana Chu[1], Geeta Narlikar[2]. 1) 1.Department of Biology, 1600 Holloway Avenue, San Francisco State University, San Francisco, CA; 2) 2.Department of Biochemistry and Biophysics, University of California, 600 16th Street, San Francisco, CA.

The compaction level of chromatin is a key factor in regulating gene expression. One mechanism to change gene accessibility is the modulation of DNA-nucleosome interactions through introduction of histone variants. For example, deposition of the H2A variant H2A.Z in X. laevis inhibits the formation of highly compacted chromatin and causes gene activation (Fan 2002). Despite their importance, how histone variants modulate the interactions of nucleosomes with DNA is not well characterized, particularly in C. elegans. To understand this, we are focusing on the histone H2A subtype in C. elegans, which consists of canonical H2A, HIS-35, HTZ-1, and HTAS-1. H2A subtypes vary considerably across species: C. elegans H2A is 87% identical to its X. laevis counterpart, while HTZ-1 is 20 amino acids longer. In addition, HTAS-1 is a sperm-specific H2A variant unique to nematodes. These differences suggest C. elegans canonical and variant-containing nucleosomes may have unique biochemical properties for regulating gene expression in C. elegans. To understand this, we are reconstituting C. elegans nucleosomes containing each of the histone H2A subtypes and canonical versions of C. elegans H2B, H3, and H4. Each protein in these nucleosomes has been expressed in E.coli. The purified histones will be used to reconstitute the nucleosome in vitro and will serve as a platform to analyze the biochemical characteristics of canonical and variant-containing nucleosomes. We will analyze how the variation in the tails of histone variants affects the mobility and spatial arrangement of nucleosomes. This will be done be measuring the electron paramagnetic resonance of tagged histone arrays. Furthermore we will assess the stability of variant and canonical containing nucleosomes. Previous studies show Saccharomyces cerevisiae HTZ-1 nucleosomes and sperm-specific human H3.3 containing nucleosomes are both less stable than their canonical counterparts (Zhang 2005, Tachiwana). Therefore we expect variant containing nucleosomes to be less stable than canonical nucleosomes. These studies will also allow us to determine how variation between C. elegans and Xenopus histones may influence nucleosome stability and chromatin structure. Zhang et al. Genome-Wide Dynamics of Htz1, a Histone H2A Variant that Poises Repressed/Basal Promoters for Activation through Histone Loss. Cell, Volume 123, Issue 2, 21 October 2005, Pages 219-231. Tachiwana et al. Nucleosome formation with the testis-specific histone H3 variant, H3t, by human nucleosome assembly proteins in vitro Nucleic Acids Res. 2008 April.

945A

AlkB8 (C14B1.10) regulates metabolism in C. elegans. **Johana Nakielna**, Petr Yilma, Vladimir Saudek, Marta Kostrouchova, Zdenek Kostrouch. Laboratory of Model Systems, Inst inherited metab disorders, First Faculty of Medicine, Charles University in Prague, Czech Republic.

Homologues of the bacterial DNA/RNA repair enzymes AlkB are found in many metazoan species. There are nine mammalian AlkB homologues formed by the Fe(II) and 2-oxoglutarate dependent dioxygenases including AlkB1 to AlkB8 and an obesity and diabetes related protein FTO. In the C. elegans genome, six clear homologues of AlkB proteins are found by homology searches. AlkB8 is more complex then the other members of the AlkB family. Three distinct functional domains can be recognized in its structure. In addition to its AlkB like demethylase domain, it contains an RNA binding motif at the N-terminus, and an S-adenosyl methionine - dependent methyl transferase at the C-terminus. AlkB8 homologues were shown to be involved in methylation of Wobble base in several tRNAs but no phenotype was found in AlkB8 null mice. In this work, we show that C. elegans AlkB8 is expressed stably during all developmental stages. We show that inhibition of AlkB8 leads to enhanced metabolic rate and faster larval development compared to the control larvae. Keeping with a general metabolic function, the overexpression of AlkB8 from transgenes regulated by heat shock responsive promoters revert the effects observed by RNAi. Our results show that C. elegans is a suitable model for functional analysis of metabolic functions of AlkB8. Acknowledgements: The work was supported by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

946B

Defining target genes of the transcriptional repressor protein CTBP-1. **Hannah R. Nicholas[1]**, Surya Setiyaputra[1], Melinda S-Y Tan[1], Chu-Kong Liu[2], Aaron Lun[1], Sashi Kant[1], Merlin Crossley[3], Joel Mackay[1]. 1) School of Molecular Bioscience, Univ Sydney, Sydney, Australia; 2) Victor Chang Cardiac Research Institute, Sydney, Australia; 3) School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, Australia.

Mammalian members of the C-terminal binding protein family of transcriptional repressors are recruited to promoters through interactions with DNA-bound transcription factors that contain amino acid motifs of the form PXDLS. Although similarly able to interact with PXDLS-containing transcription factors, we have found that the sole *C. elegans* member of this protein family, CTBP-1, also contains intrinsic DNA binding capacity in the form of a THAP domain. We have identified additional THAP domain-containing CtBPs in the nematode, echinoderm and cephalochordate lineages. The distribution of these lineages within the animal kingdom suggests that the ancestral form of the animal CtBPs may have contained a THAP domain that was subsequently lost in the vertebrate lineage.

Since determining the structure of the THAP domain of CTBP-1 by nuclear magnetic resonance spectroscopy, we have used a variety of biophysical approaches to define the DNA contact surface of this domain and to assess the affinity of binding to an 11 bp consensus binding site derived from site selection experiments. Using the CisOrtho program[1] we have identified promoters that contain putative CTBP-1-THAP binding sites, representing candidate CTBP-1 target genes. With reference to both our own and published[2] microarray datasets comparing transcripts from wild type animals with those from *ctbp-1* mutants, and to expression pattern data, we have defined a sub-set of these as likely *in vivo* targets of CTBP-1-mediated repression.

Given the reported role of CTBP-1 in the regulation of lifespan and stress resistance[2], and other investigations implicating CTBP-1 in aspects of neuronal development (D. Yucel, unpublished), our identification of CTBP-1 target genes will make an important contribution to understanding the function of this transcriptional regulator in a range of contexts.

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947C

Characterization of the aminophospholipid translocase TAT-1 and phosphatidylserine asymmetry in plasma membrane. **Y. Niu[1]**, Q. Yuan[2], N. Xia[2], D. Xue[1,3]. 1) School of Life Science, Tsinghua University, Beijing, China; 2) National Institute of Diagnostics and Vaccine Development in Infectious Diseases, Xiamen University, Xiamen, China; 3) MCD Biology, University of Colorado, Boulder, CO 80309, USA.

Phosphatidylserine (PS) is asymmetrically distributed in several biological membranes and is normally restricted to the cytoplasmic leaflet of plasma membrane. Loss of PS asymmetry occurs in both normal and pathological conditions. For example, PS is externalized at the early stage of apoptosis and serves as an “eat-me” signal to trigger phagocytosis of the apoptotic cell. Disruption of PS asymmetry may contribute to various human diseases, including stroke and cardiovascular disorders. How PS asymmetry in biological membranes is established and maintained is poorly understood. We have examined the potential roles of C. elegans aminophospholipid translocases (named TAT proteins) in maintaining phospholipid asymmetry. In C. elegans animals deficient in tat-1, PS is ectopically exposed on the surface of all cells, indicating that tat-1 plays a critical role in preventing appearance of PS in the outer leaflet of plasma membrane. Moreover, ectopic PS exposure on the surface of normal cells results in random removal of living cells through a mechanism dependent on PSR-1, a PS-recognizing phagocyte receptor, and CED-1, which recognizes and engulfs apoptotic cells partly through a PS-binding bridging molecule TTR-52 (Darland-Ransom et al., Science 2008; Wang et al., Nature Cell Biology 2010). How the TAT-1 activity is regulated is unclear. Recently, a C. elegans Cdc50 homologue, CHAT-1, was identified and shown to act as a TAT-1 chaperone to regulate membrane PS asymmetry (Chen et al., PLoS Genetics 2010). To understand how TAT-1 is regulated and its roles in animal development, we raised several monoclonal antibodies against the TAT-1 protein. These antibodies detect two protein bands of approximately 130 kDa in N2 worm lysate, which are absent in lysates from tat-1(tm1034) and tat-1(tm3117) mutant animals. We also carried out co-immunoprecipitation (co-IP) experiments in C. elegans and identified several potential TAT-1-interacting proteins, one of which turns out to be CHAT-1. Immunostaining experiments are underway to examine the expression pattern and cellular localization of the endogenous TAT-1 protein. These experiments will help reveal how the activity of TAT-1 is regulated and how TAT-1 is involved in maintaining PS asymmetry on plasma membrane.

948A

Single-blastomere transcriptome profiling reveals asymmetric segregation of maternal transcripts in the first embryonic division. **Erin A Osborne[1,3]**, Jason D Lieb[2,3]. 1) Lineberger Comprehensive Cancer Center; 2) Department of Biology; 3) Carolina Center for Genomics, University of North Carolina, Chapel Hill, NC.

By coupling blastomere dissection with RNA-seq, we have characterized the identity and abundance of each RNA transcript in the AB and P1 cells at the 2-cell stage of development. Our results confirm the asymmetric segregation of many previously identified transcripts such as *mex-3*, which is enriched in the AB cell, and *cey-2*, enriched in the P1 cell. In addition, our study significantly expands the list of asymmetrically patterned transcripts. Our method of linear RNA amplification followed by RNA-seq has generated transcriptome profiles that are highly correlated with traditional RNA-seq protocols, and the technical reproducibility is high. We plan to expand this study to discover factors important for cell fate specification and to gain a mechanistic understanding of how a single pool of maternal RNA in the oocyte gives rise to distinct populations of RNA and proteins in individual cells during early development.

949B

Global and phenotype-specific suppressors of LIN-35/pRb synthetic phenotypes. **Stanley R. Polley**, david Fay. Molec Biol, Univ Wyoming, Laramie, WY.

A previously described genetic screen performed in our lab identified multiple mutations that exhibit strong synthetic interactions with loss of lin-35, the C. elegans Retinoblastoma protein ortholog. For example, LIN-35 and SLR-2, a C2H2 type Zn-Finger protein, co-regulate the expression of a large group of intestinal genes essential for nutrient utilization and intestinal function. However, single mutants of lin-35 or slr-2 exhibit no strong growth defects, lin-35; slr-2 double mutants uniformly arrest as starved L1 larva. A genome-wide RNAi screen for suppressors of lin-35; slr-2 larval arrest has identified 24 suppressors, most of which fall in to three functional classes: (1) ribosome biogenesis genes, (2) mitochondrial prohibitins, and (3) transcriptional regulators, most notably a class of chromatin remodeling genes shown to suppress the synthetic-multivulval (SynMuv) phenotype. In the case of prohibitins, recent work suggests a role in repressing fat store utilization. Consistent with this, Oil-Red-O fat staining of lin-35; slr-2 larva on phb-1/2(RNAi) reveal a decrease in available fat stores compared to controls, consistent with a mechanism for suppression that mobilizes fat stores in order to bypass L1 starvation induced arrest. Interestingly, a number of the identified lin-35; slr-2 suppressors are capable of suppressing other unrelated lin-35 synthetic phenotypes. Strikingly, RNAi of hcf-1, mes-4, sin-3, mrg-1, ZK1127.3, and raga-1 suppress the majority of lin-35 synthetic phenotypes tested. In fact, hcf-1 strongly suppresses six of the seven lin-35 synthetic phenotypes tested. Given that the majority of these promiscuous suppressors were characterized as SynMuv suppressors, we tested every member of this class on all available lin-35 synthetic strains. We identified 9 additional genes that while incapable of suppressing lin-35; slr-2 arrest, suppress other lin-35 synthetic phenotypes. Suppressors of multiple lin-35 phenotypes likely have roles specific to LIN-35 functions, and could therefore be potential targets for anti-cancer therapies. For example, both hcf-1 and mes-4 suppress the hyper-proliferation defect in distal tip cells and intestinal nuclei observed of lin-35; fzr-1 mutants. Current work includes an additional genome-wide RNAi for suppressors of lin-35; ubc-18 pharyngeal defects. As expected, there is significant overlap with suppressors identified in the lin-35; slr-2 screen, however a large number of novel genes suggest mechanistic roles that are specific to pharyngeal development. To our knowlege, this work represents the first comprehensive screen in metazoan for suppressors of diverse synthetic phenotypes whose commonality lies in a common altered gene product.

950C

Functional characterization of *swsn-2.1*, a SWI/SNF component and genetic interactor of *lin-35* Retinoblastoma. Iris Ertl[1], **Montserrat Porta-de-la-Riva[1]**, Laura Fontrodona[1], Eva Gómez-Orte[2], Lucía Suárez-López[3], Verónica Dávalos[4], Simó Schwartz Jr.[3], Juan Cabello[2], Julián Cerón[1]. 1) Genetics and Functional Genomics in *C. elegans*, Chemoresistance and Predictive Factors for Tumor Response and Stromal Microenvironment Group, IDIBELL, L'Hospitalet de Llobregat, Spain; 2) Center for Biomedical Research of La Rioja, Logroño, Spain; 3) CIBBIM-Vall d'Hebron Hospital, Barcelona, Spain; 4) Cancer Epigenetics and Biology Program (PEBC), IDIBELL, L'Hospitalet de Llobregat, Spain.

A genome wide RNAi-screen in *C. elegans* enabled the identification of genetic interaction partners of *lin-35*, the homologue of the human tumor suppressor Rb (Ceron et al. 2007). Since the Rb pathway is altered or abrogated in the majority of human tumors, the examination of these interactions could be an important step in the development of anti-cancer drugs. One of the identified genes was *swsn-2.1*. This gene is a homologue of the human genes SMARCD1, SMARCD2 and SMARCD3, which encode subunits of the SWI/SNF chromatin remodeling complex. We observed that *swsn-2.1* inactivation causes enhanced proliferation in certain cell lineages including the intestine. Furthermore, we have found that its human homologues show altered expression levels in colon carcinomas. Due to these preliminary observations, we chose to study *swsn-2.1* in more detail.

Two different *swsn-2.1* mutant alleles are at our disposal: one of them, *he159*, was isolated in the course of a screen in a deletion library. This mutation gives rise to various abnormalities, such as protruding vulva and sterility, whereby the penetrance is temperature-dependent. The second allele, *tm3309*, which we are backcrossing in our lab, was generated by the NBRP knock-out consortium. Animals that are homozygous for this mutation are arrested at early stages. We are employing these mutant alleles as well as RNAi for the functional characterization of *swsn-2.1*. We are generating transgenic strains for the *swsn-2.1* gene and have recently obtained a specific antibody for SWSN-2.1 that will help as to examine its expression pattern in addition to identifying interaction partners. Moreover, we are studying the functional link between *swsn-2.1* and its paralog *swsn-2.2*. Beyond that, we intend to investigate the conservation of *swsn-2.1* functions and study the implication of its human homologues in cancer development.

951A

Oxygen-sensitive gene expression in *C. elegans*. Dingxia Feng, **Jo A. Powell-Coffman**. Genetics, Development & Cell Biology Department, Iowa State University, Ames, Iowa.

Oxygen is essential to metazoan life, and animals have evolutionarily conserved strategies for adapting to changing levels of environmental oxygen during development, homeostasis and disease. The hypoxia-inducible factor (HIF) transcription complexes are the central regulators of oxygen-sensitive gene expression in animals as diverse as humans and *C. elegans*. In recent years, studies from several groups have discovered roles for *C. elegans hif-1* in adaptation to hypoxia, ageing, and resistance to heat and certain pathogens. HIF-1 also regulates neuronal development and certain behaviors. To better describe HIF-1 functions, we are combining genome-wide microarray studies with sequencing of DNA targets identified in chromatin immunoprecipitation studies. Additional studies aim to identify new components of the HIF-1 regulatory networks and to understand how they interact to influence stress response, pathogen resistance, and ageing.

952B

C. elegans TBX-2 is a SUMOylation dependent transcriptional repressor. Lynn Clary[1], **Tom Ronan[1,2]**, Peter Okkema[1]. 1) Biological Sciences, University of Illinois at Chicago, Chicago, IL; 2) Bioinformatics Program, Department of Bioengineering, University of Illinois at Chicago, Chicago, IL.

T-box transcription factors are crucial developmental regulators in all multicellular animals, and they have been implicated in a variety of human diseases and cancers. Despite their importance, few direct targets of T-box factors have been identified. *C. elegans* TBX-2 is a member of the Tbx2 sub-family of T-box factors, which in other species includes both transcriptional activators and repressors, and it is required for development of anterior pharyngeal muscles. We are interested in determining if TBX-2 is a transcriptional activator or repressor, and in identifying TBX-2 targets to characterize its molecular mechanism. To identify targets of TBX-2, we compared mRNA expression levels in wild-type and hypomorphic *tbx-2(bx59)* mutant embryos using Affymetrix microarrays. Of 19,885 genes examined, we found 980 mRNAs that were significantly up-regulated in *tbx-2(bx59)* and 175 mRNAs that were significantly down-regulated. We analyzed a subset of these differentially expressed genes and found the gene D2096.6 is directly repressed by TBX-2 at a variant T-box binding site in its promoter. Our previous genetic evidence suggests TBX-2 function is SUMOylation dependent, as reduction of the SUMO-conjugating enzyme UBC-9 produces pharyngeal phenotypes similar to loss of TBX-2. Consistent with these results we see that *ubc-9(RNAi)* causes over expression of D2096.6 similar to what we have observed in *tbx-2* mutants. By comparing mRNA expression levels in wild-type and *tbx-2(bx59)* mutant embryos we have identified the first direct target of TBX-2 in C. elegans. Our results suggest TBX-2 functions as a transcriptional repressor whose function is dependent on SUMOylation. We are identifying additional TBX-2 targets among the differentially expressed genes using bioinformatic techniques.

953C

Slimming Nematodes with Herbal Medicine: From pseudo-science to reality. **SOUDABEH SAHMEDDINI**, PETER HYLANDS, STEPHEN STURZENBAUM. King's College London, Biochemistry Department, London, SE1 9NH.

Obesity, defined as the abnormal accumulation of fat, is a key factor in a wide range of illnesses such as coronary heart disease and type II diabetes(1). Since western medication has struggled to identify effective but non-toxic anti-obesity drugs, East Asian medicine may offer promising new leads. Unfortunately, stringent scientific monitoring and post-treatment studies are rarely applied to herbal remedies. This shortcoming may possibly be addressed by exploiting the benefits of an invertebrate model organism such as *Caenorhabditis elegans*. The *C. elegans* genome is fully sequenced and has been shown to share many genes and pathways with mammals. In this study, *Hoodia gordonii*, an appetite suppressant extract from the South African cactus (2) was used as a target to study obesity. Age synchronized *C. elegans* (stage L1) were exposed to different doses of *H. gordonii* via the OP50 *E. coli* food source and maintained on nematode growth media (NGM). Size measurements (N=30 per condition) were determined using Image-Pro Express® (Media Cybernetics) software. Data was analyzed statistically using Prism GraphPad5® (GraphPad Software, Inc.) software to assess statistical significance at $p \leq 0.05$. Nematodes exposed to *H. gordonii* were significantly affected resulting in a dose dependant reduction in body fat and size but also progeny number. In addition, pharyngeal pumping (food consumption) was modulated in an age dependent manner. Moreover, a genome-wide microarray analysis performed to identify novel genes involved in fat metabolic pathways in response to the anorectic *H. gordonii*. This may lead to characterization of abundant gene families which may have been neglected in obesity-related studies. *H. gordonii* exerts noticeable dose dependent effects on the nematode. At this stage it is not yet known if these are a direct consequence or side effect of exposure, a question that is currently being investigated at the molecular level. (1). Obesity and overweight, 2008, World Health Organization, [online], (updated February 2011), Available at: http://www.who.int/mediacentre/factsheets/fs311/en/ [Accessed on 3rd March 2011]. (2). Lao L, Zhang RX, Zhang G, Wang X, Berman BM, Ren K, 2004, Brain Res 1020 (1-2): pp1-11.

954A

Regulation of gene expression by DBL-1/BMP. Jianghua Yin, Sushma Teegala, Edlira Yzeiraj, **Cathy Savage-Dunn**. Department of Biology, Queens College, and the Graduate Center, CUNY, Flushing, NY 11367.

The regulation of body size is a fundamental feature of animals critical to their survival

and fitness, yet its underlying mechanisms remain poorly understood. In *C. elegans*, the DBL-1 BMP-related signaling pathway is known to play a major role in growth control. To determine how DBL-1 signaling regulates body size, we used DNA microarray analysis to identify transcriptional targets of the pathway that potentially function as downstream effectors. Two categories of genes identified, cuticle collagens and fat metabolism regulators, were prioritized as plausible effectors of DBL-1 growth regulation. We determined the body size phenotypes of two DBL-1-activated collagen genes (*rol-6* and *col-41*) and one DBL-1-repressed collagen gene (*col-141*) by RNAi. Strikingly, knockdown of either *rol-6* or of *col-41* causes a reduction in body length, consistent with the activation of these genes by DBL-1, while knockdown of *col-141* causes an increase in body length, consistent with its expression being antagonized by DBL-1 to allow normal growth. We have generated reporter constructs for *col-41* that recapitulate regulation by the DBL-1 pathway. Deletion analyses have begun to identify sequences necessary for the regulation of *col-41* expression. We also used RNAi to determine whether identified target genes involved in regulation of fat storage have an effect on body size. We tested two genes that encode fatty acid desaturases, *fat-6* and *fat-7*, as well as the insulin-related ligand *ins-7*, which may encode an agonist of DAF-2, the insulin receptor. Two of these genes showed body size phenotypes: *fat-6(RNAi)* animals had reduced body length, while *ins-7(RNAi)* animals had increased body length. We hypothesize that the regulation of *fat-6* and *fat-7* expression may occur via DBL-1 repression of *ins-7*, which could result in decreased activation of DAF-2. Consistent with this hypothesis is the increased body size associated with *daf-2* mutations. Future studies of genetic and molecular interactions will test this hypothesis. We next made use of an existing *fat-7*p::GFP reporter to assay expression levels in DBL-1 pathway mutants or RNAi knockdowns. Expression of the reporter is reduced in *sma-9/Schnurri* mutant backgrounds but not in *dbl-1* mutants. We are currently creating additional reporters that may recapitulate the regulation of *fat-7* by *dbl-1* that is seen by other experimental approaches, such as microarray and qRT-PCR analyses. In summary, we have identified two classes of DBL-1 target genes whose loss-of-function phenotypes demonstrate a role in body size regulation. Using reporter constructs, we are dissecting the regulatory circuits by which these genes' expression levels are controlled.

955B

Comparison of Dosage Compensation Between Caenorhabditis briggsae and Caenorhabditis elegans. **Caitlin M Schartner**, Te-Wen Lo, Barbara J Meyer. University of California, Berkeley, CA.

Dosage compensation is a mechanism that equalizes gene expression from the X chromosomes between heterogametic sexes. In Caenorhabditis elegans, the dosage compensation complex (DCC) binds both hermaphrodite X chromosomes to repress transcription by approximately half, to equal the level expression from the single male X. Although C. elegans and C. briggsae diverged 15-30 million years ago, our analysis has shown that dosage compensation complex (DCC) subunits are conserved between species. Each C. elegans DCC component has a homolog in C. briggsae, and the DCC components DPY-27, MIX-1, and SDC-2 have been shown to have similar functions in C. briggsae dosage compensation. However, while DCC components appear conserved, DCC binding sites appear diverged. The C. elegans consensus motif (MEX, motif enriched on X) pivotal for C. elegans DCC recruitment to X is only enriched 0.6-2-fold on C. briggsae X compared to autosomes, in contrast to the 3.8-24-fold enrichment on the C. elegans X chromosome. Furthermore, we characterized the recruitment potential of several C. elegans recruitment sites and their C. briggsae homologous regions in both species. No C. elegans or C. briggsae sequences tested were able to recruit the DCC in C. briggsae to the same degree as in C. elegans. This suggests that the cis-acting DNA recruitment sites in C. briggsae have diverged. Ongoing ChIP-seq experiments to define the C. briggsae DCC binding sites will reveal the degree of divergence. The identification of DNA binding sequences in C. briggsae will set the stage to allow us to investigate the molecular co-evolution of the DNA sequence motif and the DNA-binding domain of the DCC.

956C

Carnosinase 1 (PES-9) and Carnosinase 2 (Y71H2AM.11) - two conserved dipeptidases are involved in regulation of embryonic development in C. elegans. **Katerina Sebkova**, Petr Yilma, David Kostrouch, Zdenek Kostrouch, Marta Kostrouchova. Laboratory of Model Systems, Inst Inherited Metab Disorders, First Fac Med, Charles University in Prague , Czech Republic.

Carnosine ((2S)-2-[(3-Amino-1-oxopropyl)amino]-3-(3H-imidazol-4-yl)propanoic acid, β-Alanyl-L-histidine) is a dipeptide found in high concentrations in vertebrate muscles and neuronal tissues. Carnosine is synthesized by carnosine synthases from histidine and β-alanine and is obtained also with food. Carnosinases were identified as two types of L-carnosine-hydrolyzing enzymes named CN1 and CN2, that share overall 49% of identity in their amino acid sequences. By sequence homology, carnosinases are found in genomes of distant species from bacteria to mammals. CN 1 is selective for carnosine, homocarnosine and anserine, while CN2 has a broader spectrum of substrate dipeptides. CN1 is a homodimeric dipeptidase secreted by liver into human blood plasma. Patients with carnosinemia, elevated levels of carnosine in blood plasma, nervous tissue and cerebrospinal fluid, bear an autosomal recessive mutation in CN1 and suffer with mental retardation, spastic paraplegy, retinitis pigmentosa and epilepsia. In this work, we characterized C. elegans carnosinase homologues that we identified by sequence similarity: CN1 (pes-9 which is on the chromosome V) and CN2 (Y71H2AM.11 localized on the chromosome III). Transgenic lines expressing GFP fusion proteins localized the expression of CN1 from two-fold embryos in the intestine and larvae and adults in the intestine, in pharyngeal and neuronal cells. CN2 was detected from 1.5 fold embryos to adulthood in intestinal cells. Inhibition of CN1 by RNAi induced lethality in approximately 10% of embryos. CN2 RNAi did not lead to the developmental phenotypes in separated experiments, but lead to markedly enhanced developmental defect in experiments with double inhibition of CN1 and CN2. We show that the bacterially expressed CN2 has carnosinase activity in vitro.Exposure to carnosine alone led to similar defects as the inhibition of CN1 and doubled the penetrance of phenotypical changes and embryonic lethality induced by CN1 RNAi. Our results suggest that modulations of carnosine concentration by carnosinases are constituents of a regulatory network that is critical for normal development of C. elegans. Acknowledgements: We thank Dr. A. Fire for vectors. The work was supported by the grant 0021620806 from the Ministry of Education, Youth and Sports of the Czech Republic.

957A

Characterizing the paternal RNA contribution of *C. elegans* sperm. **M. Shorrock**, T. Wu, D.S. Chu. Biology Dept., San Francisco State University, 1600 Holloway Ave, San Francisco, CA 94132.

Distinct transcriptional programs during gamete formation specify the differentiation of sperm and oocytes. In particular, while RNA is transcribed during meiosis and most of spermatogenesis, mature sperm become largely transcriptionally silent in later spermatogenesis as DNA is tightly compacted and cytoplasmic components are removed. However, a portion of RNA is retained in mature sperm. These paternal transcripts are delivered alongside DNA to the developing embryo, and have recently been implicated in successful fertilization and embryo development. Despite their potential contribution to early development, the RNA content of mature sperm is poorly characterized and their functional significance remains largely unexplored.

Using *C. elegans*, we are characterizing the RNA content of sperm. To do this, we first compared the profiles of RNA isolated from sperm, embryos, and whole worms. We purified RNA from mature sperm, embryo, and whole worm. As expected, we found embryo and whole worm expression profiles, determined by quantitative electrophoretic mobility, were dominated by two peaks characteristic of ribosomal RNAs. Conversely, mature sperm lacked discrete ribosomal peaks and had a comparatively diverse transcript population. These results verify that mature sperm retain RNA but are able to remove abundant ribosomal RNAs. This supports that sperm possess a set of RNAs distinct from somatic cells. We are now conducting microarray and next-generation sequencing analysis of RNA isolated from mature sperm to identify the specific paternal transcripts that are delivered to the embryo.

958B

Characterization of HRG-7, a novel protease involved in *C. elegans* heme homeostasis. **Jason Sinclair**, Iqbal Hamza. Animal & Avian Sci, Univ Maryland, College Park, MD.

Heme is an iron-containing prosthetic group that plays an essential role in a number of biological processes, including oxidative metabolism, signal transduction, and microRNA processing. Although the heme synthesis pathway has been well characterized, the mechanisms by which organisms maintain heme homeostasis downstream of synthesis remain poorly defined. The nematode *Caenorhabditis elegans* is unique among free living organisms because it is a heme auxotroph. It relies solely on environmental heme to survive and reproduce. Therefore, the worm is an excellent animal model to explore heme homeostasis without interference from endogenous heme production. Using *C. elegans* as a model organism, we previously discovered the first eukaryotic heme importers, HRG-1 and HRG-4, membrane-bound permeases. Because *C. elegans* are bacteriovorous, dietary heme acquisition is likely through degradation of heme-containing proteins in the worm intestine. To uncover the heme acquisition pathway in *C. elegans*, we analyzed results from Affymetrix microarrays and genome-wide RNAi screens. The combination of these experiments resulted in the identification of a conserved cathepsin-like protease, which we termed HRG-7. Here we show that under low heme conditions, *hrg-7* is highly upregulated in the worm intestine and depletion of *hrg-7* by RNAi results in heme-deficiency even though sufficient heme is available to the worm. Knockdown of *hrg-7* in the *hrg-1* / *hrg-4* double deletion strain resulted in growth retardation which was rescued by heme supplementation. These results suggest that HRG-7 is essential for heme homeostasis in *C. elegans*. Since HRG-7 is conserved in parasitic helminths that exacerbate iron deficiency, compounds that target the active site of HRG-7 may prove to be effective anthelminthic drugs.

959C

Transcriptional regulation of ciliary genes in C. elegans. J Burghoorn, BP Piasecki, KE Jeppsson, F Crona, S Sahlin, P Phirke, **P Swoboda**. Dept Biosciences & Nutrition, Center for Biosciences at NOVUM, Karolinska Institute, Huddinge, Sweden.

The successful development of every organism depends on the correct regulation and interaction of its gene products. Many regulatory networks hardwire these gene expression patterns through the use of specialized DNA sequences surrounding genes. These DNA sequences are bound by one or more specific transcription factors (TF). As such, TF are evolutionarily conserved crucial regulators of development. One example is the sole C. elegans member of the RFX (Regulatory Factor X) TF family, DAF-19. Recognition and binding of RFX TF/DAF-19 to a conserved 13-15 bp promoter sequence motif, the X-box, has proven to be the key regulatory step of cilia development in C. elegans and other organisms. Two thirds of the more than fifty direct target genes of DAF-19 identified to date in C. elegans are expressed in most or all ciliated sensory neurons (CSN), while one third are only expressed in specific subsets of CSN. In gene expression studies using transgenic C. elegans lines we investigated if the X-box itself or its position within promoter

regions had any influence on this phenomenon in three different ways: (i) by changing the positions of X-boxes within promoters; (ii) by swapping X-boxes, whereby X-boxes of ciliary genes expressed in all CSN were replaced with X-boxes from ciliary genes expressed in a only a few CSN, and vice versa; (iii) by testing a 14 bp X-box by itself, without any surrounding promoter sequences. Our experiments clearly demonstrated that the X-box itself is not the only factor that determines ciliary gene expression. Instead, we have identified additional cis-regulatory motifs that work in concert with the X-box to establish the correct expression pattern of a given ciliary gene. Through bioinformatics and in vivo dissection of the promoter regions of several X-box/ciliary genes, we have identified an enhancer motif, the C-box. This motif is typically located in close proximity to an X-box (within less than 80 bp). The C-box enhancer is conserved in related nematode species and in coordination with the X-box determines the expression patterns of ciliary genes. Ciliary genes that are expressed in most or all CSN are typically equipped with C-boxes, while genes that are expressed in only a few CSN typically have none. Our experiments clearly demonstrate that the proximity of the X-box relative to the translational start site does not play a significant role, as long as it is less than 300 bp upstream of the ATG. Instead, the X-box - together with additional cis-regulatory motifs - works in concert to establish the expression patterns of ciliary genes. We have identified one of these cis-regulatory motifs, the evolutionarily conserved C-box enhancer.

960A

Asymmetric regulation of the homeobox gene *ceh-5* in early embryogenesis of *C. elegans*. **Lois Tang**[1], Konstantin Cesnulevicius[1], Jürgen Hench[2], Daria Shlyueva[3], Akram Abou-Zied[4], Thomas Bürglin[1]. 1) Dept Biosci & Nutrition, Karolinska Institutet, Stockholm, Sweden; 2) Dept of Pathology, University Hospital Basel, Switzerland; 3) IMP, Vienna, Austria; 4) Faculty of Applied Medical Sciences, University of Tabuk, Saudi Arabia.

Homeobox genes are highly conserved transcription factors that play key roles in the development of humans and animals. From a 4D microscopy expression screen performed in the Bürglin lab, a homeobox gene - *ceh-5* - displaying a unique asymmetrical expression was revealed. This ortholog of the human VAX genes is expressed in three different groups of cells during gastrulation. An interesting aspect of *ceh-5* expression in the two bilaterally symmetric cell groups is a left-right asymmetry in their expression levels. Little is understood about cell fate specification after the beginning of gastrulation, and also how left-right asymmetries are generated; *ceh-5*, therefore, provides an excellent entry point to study these phenomena.

We are dissecting 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. The GFP expression patterns in the transgenic worms were monitored using 4D-imaging. 790bp upstream of the ATG is sufficient to initiate and maintain *ceh-5*'s expression during embryogenesis. Within this region, one of the motifs crucial to drive *ceh-5* complex expression is a SKN-1 binding motif.

Another key motif revealed by the investigation is a 21bp in the promoter region containing an E-box motif essential for the regulation of *ceh-5*. This motif is highly conserved among different *Caenorhabditis* species and is known to be a binding site for basic helix-loop-helix (bHLH) transcription factors (1). 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 not yet saturated, we have identified - *hlh-2*, *ngn-1* and *cnd-1* - when knocked-down, abolishes a part of the *ceh-5* expression pattern.

1) Yamada, K. and Miyamoto, K. 2005. Front Biosci.

961B

An integrated view of the organization of transcription factor binding sites in the *C. elegans* genome. **Florian Wagner**, Itai Yanai. Faculty of Biology, Technion, Haifa, Israel.

The realization that all nucleated cells in a multicellular organism contain the same genomic information, despite carrying out very specialized functions, is one of the hallmarks of cell biology. In the context of development, it is well-known that *cis*-regulatory elements (CREs) are crucial in recruiting transcription factors that ensure the precise spatio-temporal regulation of target genes. The DNA sequence of any higher eukaryote can therefore be viewed as a solution to the daunting challenge of arranging thousands of CREs with widely divergent roles in a linear and functionally coherent fashion. Studying the genome from this perspective can serve as a foundation for a broader understanding of the principles of gene regulation in development. To this end, we have integrated the location and magnitude of tens of thousands of genomic binding events from more than 40 *C. elegans* transcription factors (TF), acting at various stages of development, based on publicly available datasets from the modENCODE consortium. Our approach is unique in that it is fully quantitative and avoids the high false discovery rate associated with many previous studies. For the first time, this allows us to map out the *cis*-regulatory information content at any given location in the genome. We use this powerful analysis to estimate the extent to which CREs are generally organized into modules that integrate multiple regulatory inputs to produce cell-specific expression. Surprisingly, many promoters exhibit proximal regions with degenerate binding events. We provide evidence that these bindings do not mediate specific regulation and show that clustering genes based only on their specific CREs gives a much better correlation with their corresponding expression profiles, based on transcriptomic data from our lab and others. Our integrative approach provides a framework for understanding how individual sequence elements contribute to developmental expression programs. The analytical power of our approach will further increase with the integration of additional datasets.

962C

scla-1 Encodes a Nonreceptor Tyrosine Kinase Required for Antipsychotic Drug-induced Developmental Delay and Lethality in C. elegans. **X. Wang**, L. M. Hao, B. M. Cohen, E. A. Buttner. McLean Hospital & Harvard Medical School, Belmont, MA.

Pharmacogenomic studies in model systems have the potential to define previously unknown mechanisms of action of medications used in human illness and to discover new genes and signaling pathways in the model system, itself. We initiated pharmacogenomic studies in C. elegans to identify novel signal transduction pathways through which antipsychotic drugs (APDs), and in particular, the most effective APD, clozapine, exert their biological effects. These drugs have both growth stimulating and toxic effects in humans. Many of these drugs cause larval arrest and lethality in C. elegans. We took advantage of these phenotypes by performing a genome-wide feeding RNAi screen for suppressors of clozapine-induced larval arrest (Scla). The screen yielded 42 suppressors, one of which is F22B3.8, a gene we have named scla-1. scla-1 shares 31% identity with the nonreceptor tyrosine kinase IL-2-inducible T cell kinase (ITK), which is critical for the development, function and differentiation of T cells. We validated our RNAi result by backcrossing two deletions of scla-1 six times and then confirming the Scla phenotype of each strain. In addition to suppression of clozapine-induced larval arrest, we found that these mutants suppress both developmental delay and lethality induced by other APDs, such as chlorpromazine and fluphenazine. We also found that clozapine induces expression of scla-1 as assayed by q-RT-PCR. We are currently generating transgenic worms carrying transcriptional and translational GFP reporters for scla-1. We will test our translational construct for rescue of the Scla phenotype of our two knockouts. Previous work by our laboratory[1] and by Weeks et al.[2] showed that APDs activate the insulin/IGF-1 signaling (IIS) in C. elegans. In mammals, activation of ITK depends on phosphatidylinositol-3-kinase[3], which is a known target of the IIS effectors. Therefore, we are constructing an scla-1;daf-16::gfp animal to test the potential interaction of these pathways in C. elegans. The effects of APDs are likely determined by actions at multiple sites, and by defining the downstream interactions of pathways modulated by APDs, we may better understand their therapeutic and toxic effects. This knowledge may lead to improved treatments.

1. Karmacharya et al (2009) Neuropsychopharmacology 34, 1968-1978.
2. Weeks et al (2010) ACS Chem Neurosci 1, 463-473.
3. Fernandes et al (2009) MBC 20, 3690-3699.

963A

Linking dosage compensation complex binding to chromosome-wide gene regulation in *C. elegans*. **Bayly Wheeler**[1], Barbara Meyer[1,2]. 1) University of California, Berkeley , Berkeley, CA; 2) Howard Hughes Medical Institute.

Dosage compensation (DC) is an essential process required to balance levels of gene expression between the two X chromosomes of females and the single X of males. In *C. elegans*, like flies and mammals, DC modulates gene expression across an entire chromosome and thereby serves as an exemplary system to understand long-range mechanisms of gene regulation. To enact DC, the dosage compensation complex (DCC) is recruited to the X chromosome by recruitment elements, called *rex* sites, that act at a distance to control gene expression. How these recruitment sites function to recruit the DCC, facilitate spreading, and control gene expression are unknown. Progress here requires the ability to identify compensated genes with high confidence and resolution along X and to manipulate *rex* sites in their endogenous context. Whole transcriptome sequencing of wild-type and DC-defective animals is being used to identify compensated genes at multiple developmental stages with enhanced sensitivity over previous approaches. In addition, two novel technologies are being employed to insert and delete *rex* sites in the context of the X to link *rex* sites with their role in DCC binding, chromosome architecture, and DC. Lastly, parameters that influence the interaction between *rex* sites and compensated genes are being dissected by assessing expression of a reporter moved throughout the X and autosomes in different epigenetic contexts and at different distances from endogenous and engineered *rex* sites. Identification of the genes that are compensated coupled with the ability to manipulate *rex* elements and compensated genes will uncover mechanisms by which regulatory elements on the X enact appropriate patterns of gene expression chromosome-wide.

In *C. elegans* and mammals, DC is achieved by decreasing expression from the female X chromosomes to equal the level of expression from the single male X. While this process balances expression between the sexes, it could result in reduced expression from the sex chromosomes relative to the autosomes. It has been proposed that a secondary mechanism of chromosome-wide gene regulation exists to resolve this imbalance by increasing gene expression from the X in both sexes. Support for this hypothesis remains controversial. Therefore, the expression of both endogenous and engineered genes on the X and autosomes is being compared to test the existence of a secondary mechanism of chromosome-wide gene regulation; one that balances X gene expression in both sexes with the autosomes.

964B

The REF-1 Protein, HLH-29, Functions in the Somatic Gonad of Wild-Type C. elegans. **Ana M White**, Casonya Johnson. Biology, Georgia State University, Atlanta, GA.

HLH-29, like other members of the REF-1 family of transcription factor,s is a basic helix-loop-helix (bHLH) protein that contains two fully intact bHLH domains. Previously we demonstrated that HLH-29 affects development of the somatic gonad and the vulva in *C. elegans* and is expressed in the spermatheca of adult hermaphrodites. More recently, our results from yeast-two-hybrid screens suggested that HLH-29 interacts with the sexual identity factor, FKH-6. Our objectives are to demonstrate a genetic and molecular interaction between HLH-29 and FKH-6 *in vitro* and *in vivo*. First, we show by Far-western

blotting and by pull-down assays that HLH-29 and FKH-6 interact via the N-terminal most bHLH domain, Domain A. Second, we show that *hlh-29* animals have normal brood sizes, but also lay significantly more unfertilized oocytes than wild-type animals. We show that this phenotype is due at least in part to defective ovulation cycles that worsen as the animals age and that vary according to gonad arm. Third, we show that the interactions between FKH-6 and HLH-29 are likely to affect development and morphology of the gonad arm, in addition to ovulation, as HLH-29;FKH-6 (+/-) animals have more severe phenotypes than single mutant animals. Finally, our preliminary RT-qPCR data show that expression of the IP3 signaling pathway genes *ipp-5* and *ppk-1* is regulated by HLH-29 alone, while the expression of the genes C37H5.6, *dis-3*, and *emb-9* is regulated by HLH-29 together with FKH-6.

965C

Promoter Analysis of the GATA type transcription factor *elt-2*. **Tobias Wiesenfahrt**, Janette Berg, James McGhee. Department of Biochemistry and Molecular Biology, University of Calgary, Calgary, Alberta, Canada.

We have suggested that the GATA type transcription factor ELT-2 is the major regulator of transcription in the *C. elegans* intestine following endoderm specification, both embryonically and post embryonically. To understand the molecular details of how *elt-2* transcription is initiated during embryonic development and is maintained thereafter, we are analyzing the promoter region of *elt-2* in *C. elegans*. Comparison of 5 kb upstream sequences of the *elt-2* gene from 4 different Caenorhabditis species revealed three conserved regions (CRI- CRIII). Deletion series (both 3' and 5') as well as analysis of reporter constructs containing different combinations of the CRs suggested that CRI contains the basal promoter and CRIII contains the main enhancer of *elt-2*. The function of CRII is not yet clear. Rothman and Maduro have shown that the redundant GATA factors END-1 and END-3 are necessary for endoderm specification. Ectopic expression of END-1 can initiate ectopic expression of ELT-2, suggesting that END-1 can activate elt-2 expression in the earliest endoderm lineage. Previous experiments also showed that ELT-2 can bind to its own promoter *in vivo*. To find potential binding sites for END-1, END-3 and ELT-2, we searched for GATA sites within the CRs of elt-2. We identified 3, 3 and 4 conserved GATA sites within CRI, CRII and CRIII respectively. Band shift assays showed that END-1 and ELT-2 can bind to at least one and all four GATA sites within CRIII *in vitro* respectively. This suggests that END-1 (END-3 has not yet been tested) can activate *elt-2* expression directly. Mutating the GATA sites within CRIII individually and in different combinations, suggested that every CRIII GATA site contributes positively to *elt-2* expression. Reporter expression was absent in larvae and adult worms after mutating all GATA sites within CRIII. To test if *elt-2* expression relies solely on GATA factors we have engineered a reporter construct consisting of CRIII and CRI in which all GATA sites have been mutated. Results will be presented. To test if ELT-2 can drive intestinal specification and differentiation in the absence of END-1/END-3, we expressed *elt-2* under control of the *end-1* and *end-3* promoters in the *end-1*/*end-3* double mutant (kindly provided by Morris Maduro). Indeed, the *end-1p::elt-2* construct is able to rescue the *end-1*/*end-3* double mutant with reasonable penetrance, showing that ELT-2 can drive endoderm specification and further supporting the hypothesis that ELT-2 is involved in the regulation of every gene expressed in the intestine.

966A

Identification and characterization of protein phosphatases that regulate TGFβ signaling in *C. elegan*. **S. Xiong**, C. Savage-Dunn. Biology Dept, Queens College, CUNY, Flushing, NY.

TGFβ signaling is involved in various normal and abnormal cellular responses. Previous studies suggested the involvement of phosphatases in regulation of TGFβ signaling, but these studies were performed in cell culture rather than intact organisms. *C. elegans* is a tractable organism in which to study signaling in vivo. In *C. elegans*, growth is controlled by a conserved TGFβ pathway, Sma/Mab pathway. We used a *C. elegans* RNA interference library of phosphatases to identify genes that cause a body size phenotype. Library-wide screening was carried out in an RNAi-hypersensitive mutant background, *rrf-3*. 124 RNAi clones contributing to a body size phenotype were identified. To further narrow the candidate pool, we analyzed the body size phenotypes of these candidates using different genetic backgrounds: a strong loss-of-function type I receptor mutant *(sma-6(wk7))*, a weak loss-of-function type I receptor mutant *(sma-6(e1482))*, a loss-of-function mutant in a negative regulator of this Sma/Mab pathway *(lon-2)*, and in a strain that over-expresses the TGFβ ligand, DBL-1 (*dbl-1*++). These analyses allow us to narrow the 124- candidate pool down to an 80-candidate pool. A novel Sma/Mab pathway reporter, RAD-SMAD reporter (a kind gift from Dr. Jun Liu, Cornell University), was used to assess whether the candidates regulate body size phenotype in a Sma/Mab pathway-dependent manner or not. The reporter assay revealed 20 likely candidates regulate Sma/Mab signaling directly or indirectly. We're addressing the regulatory mechanism and physiological role of selected candidates via further genetic and molecular biological techniques.

967B

Repression of transgene expression by the Q/P-rich nuclear protein PQE-1. **Koji Yamada**, Jun-ichi Tsuchiya, Yuichi Iino. Department of Biophysics and Biochemistry, The University of Tokyo, Tokyo, Japan.

The LIM homeobox gene *lin-11* is expressed in several neurons including RIC and AIZ by its regulatory element located in the third intron (1). Compared to the constant expression in RIC, *lin-11* promoter drives unstable expression in AIZ. AIZ interneurons have many synaptic inputs from sensory neurons and are therefore considered as one of the sites for the integration of sensory information. We have taken a forward genetic approach to examine the basis for the variable expression of *lin-11* in AIZ.

To exclude the effect of genetic mosaicism, we generated strains in which *lin-11p::venus* reporter gene is integrated into a chromosome. Among the generated strains, JN304, which had an integrated transgene *peIs304* on Chromosome I, showed stable reporter expression in RIC and stochastic expression in AIZ. We mutagenized JN304 by EMS and screened 18,000 F2 genomes for mutants that showed stable reporter expression in AIZ. Through the screen, we isolated 12 independent mutant strains. The mutations of nine strains showed strong linkage to *peIs304*, suggesting that the mutations were variations in the transgene. Other three mutations, *pe334*, *pe335*, and *pe336* were mapped to the center of Chromosome III and identified as nonsense mutations of the *pqe-1* gene. The mutant phenotype was rescued by the introduction of a wild-type genome fragment spanning the *pqe-1* gene, confirming that *pqe-1* is the responsible gene.

pqe-1 encodes a Q/P rich nuclear protein and mutations in the gene were previously reported to enhance the toxic effect of ectopically expressed Huntingtin polyQ fragment, hence polyQ enhancer-1 (2). Because the expression of *lin-11p::venus* in AIZ turned out to be critically affected by the copy number of the transgene, we surmised that *pqe-1* suppresses the expression of the transgene. To test this hypothesis, we quantified the total fluorescence intensity of Venus expressed in RIC by *peIs304*, and found that the expression level of venus reporter was increased twice in each *pqe-1* mutant background. Furthermore, two lines with single copy insertion of *lin-11p::venus* transgene also showed stronger expression in the *pqe-1* mutant background. Because PQE-1 localizes in the nucleus (2), these results suggest that PQE-1 regulates the expression of the *lin-11p::venus* transgenes by an unknown transcriptional or post-transcriptional mechanism.

(1) Hobert O. et al., 1998. J Neurosci. 18:2084-96. (2) Faber P. et al., 2002. Proc Natl Acad Sci U S A. 99:17131-6.

968C

Identification of critical neurons for APL-1 expression in C. elegans. **Pei Zhao**[1,2], Collin Ewald[1,2], Chris Li[1]. 1) Biology department , City College of the City University of New York , New York , NY; 2) Graduate Center, the City University of New York New York, NY.

Alzheimer's Disease is a progressive neurodegenerative disorder whose pathology manifests as the accumulation of dense plaques and neurofibrillary tangles in the brains of patients. The major component of the plaques is the β-amyloid peptide, which is a cleavage product of the Amyloid Precursor Protein (APP). APP and the related proteins APLP1 and APLP2 are essential for neurodevelopment, but the specific cellular functions of the APP family remains unclear. Caenorhabditis elegans contains a single APP-related gene, apl-1. Loss of apl-1 results in larval lethality, indicating an essential function for apl-1. This lethality can be rescued by germline transformation with a genomic fragment encoding APL-1 or only the extracellular domain of APL-1 (APL-1EXT). Overexpression of APL-1 caused defects in brood size, movement, and an incompletely penetrant lethality. Hence, C. elegans presents an exceptionally attractive system to elucidate the function of APP family members. Pan-neuronal expression of APL-1 or APL-1EXT is sufficient to rescue the apl-1 loss-of-function lethality, suggesting that neurons are a critical source of APL-1. To determine from which neurons APL-1 must be released, we are using different promoters to drive APL-1 expression in different subsets of neurons. Furthermore, we using GFP and mcherry to tag APL-1 at the N- and C-terminus, respectively, to movement of different APL-1 fragments after cleavage. These experiments will allow us to determine whether sAPL-1, the secreted APL-1 fragment, can act as a long-range signaling molecule and/or through short-range cell-cell interactions.

969A

Identifying Regulatory Elements Controlling Spatial And Temporal Expression of *hlh-29* in *C. elegans*. **Huihui Zhu**, Casonya Johnson. Biology, Georgia State University, Atlanta, GA.

HLH-29 is one of six REF-1 family members that, like other HES proteins, act in a Notch dependent fashion to regulate the specification and development of the endoderm and mesoderm. Members of the REF-1 family also act in Notch-independent pathways to regulate animal behavior and development, but the molecular details of how and where these proteins function are not yet known. We do know that *hlh-29* mRNA is found throughout all developmental stages at varying levels, and the *hlh-29* promoter is active in both neuronal and non-neuronal tissues. We also know that loss of HLH-29 can result in underdeveloped gonad arms, yolk protein accumulation, and defective ovulation, and altered chemosensory behaviors. Our research objective is to better understand the regulation of *hlh-29* expression at the molecular level, by defining its minimal promoter and identifying important cis-regulatory elements needed for temporal and spatial regulation. To achieve this goal, we used bioinformatics tools to predict potential transcriptional factor binding sites. We have identified LAG-1, MED-1, SKIN-1 binding sites in the promoter region, which have been previously suggested as upstream regulators of hlh-29 expression. We also identified predicted binding sites for the homeotic selector protein Deformed, GATA-1 and homeobox binding proteins. We then generated five transcriptional fusions to GFP that consist of serial deletions of the hlh-29 promoter. These truncations have allowed us to sequentially remove each predicted cis-regulatory element. We are in the process of generating transgenic animals which we will use to monitor changes in green fluorescent signal as a result of deletion of each cis-regulatory element. These studies will allow us to determine which cis-regulatory elements are needed for tissue specific expression of *hlh*-29.

970B

Multi-platform sequencing identifies novel mirtron with an embryonic lethal phenotype in *Caenorhabditis elegans*. **Rina Ahmed**[1,2], Funda Sar[3,4], Zisong Chang[1], Kristin Gunsalus[5], Erik Miska[3,4], Christoph Dieterich[1]. 1) Berlin Institute for Medical Systems Biologie, Max Delbrueck Centre for Molecular Medicine, Berlin, Germany; 2) BIMSB-NYU PhD Exchange Program; 3) Wellcome Trust Cancer Research UK Gurdon Institute, University of Cambridge, UK; 4) Department of Biochemistry, University of Cambridge, UK; 5) Center for Genomics and Systems Biology, Department of Biology, New York University, USA.

MicroRNA genes (miRNAs) are post-transcriptional regulators of mRNA stability and/or translation and are regulated themselves on the level of gene transcription, processing and decay. MiRNAs regulate many biological processes and aberrant miRNA expression has been implicated in several disease states. Mirtrons are a special class of miRNAs since they originate from properly sized introns (~ 70 nt) of protein-coding genes. Precursor miRNAs (pre-miRNAs) of mirtrons are excised by the splicing machinery from the host gene, debranched, and directly processed by Dicer. The expression pattern of a mirtron is consequently similar if not identical to its host gene's expression pattern. Only a few mirtrons have been identified in vertebrates and invertebrates since their discovery in 2007. None of them has been linked to any phenotype so far.

We revisited the repertoire of miRNAs in *Caenorhabditis elegans* with a multi-platform sequencing approach (ABI SOLiD and Illumina GA II) to screen for novel miRNA gene candidates. Both platforms differ in sequencing bias, which is usually expressed in divergent normalized cross-platform read counts for any given miRNA. Consequently, both platforms complement one another in the gene discovery process. With this approach, we were able to extend the known set from four to six mirtrons. The modENCODE consortium (Chung et al., 2011) has independently confirmed our discovery.

However, one novel mirtron caught our attention and we started a functional characterization of this candidate gene. At the time of writing, we are certain that a knockout of the host gene has an embryonic lethal phenotype and shows greatly reduced levels of hatching worms. The knockout phenotype can be, at least partially, rescued by a mirtron-expressing transgene. Most surprisingly, this mirtron has been acquired recently and is not present in any of the other available *Caenorhabditis* genomes. We will give an update of our experimental findings at the International Worm Meeting.

References: Chung, W.J. *et al.* Computational and experimental identification of mirtrons in *Drosophila melanogaster* and *Caenorhabditis elegans*. Genome Res. 21, 286-300 (2011).

971C

RNA-binding Protein GLD-1 Genetically Interacts with Multiple miRNA Pathways in *C. elegans*. **Alper Akay**[1], Ashley Craig[1], Nicolas Lehrbach[2], Gyorgy Hutvagner[1], Eric Miska[2], Anton Gartner[1]. 1) Wellcome Trust Center for Gene Regulation and Expression, University of Dundee, Dundee, UK; 2) Wellcome Trust Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, UK.

GLD-1 is a member of a highly conserved RNA-binding protein family, characterised by the KH domain. In *C. elegans* GLD-1 functions throughout the germ-line in developmental processes such as mitosis/meiosis transition and spermatogenesis/oogenesis decisions, through translational regulation of various targets. In addition, GLD-1 has a key role in DNA damage-induced germ-line apoptosis through control of cep-1/p53 mRNA levels. In order to study GLD-1 target specificity and the molecular mechanisms that control GLD-1 function, we performed two genetic screens using the temperature sensitive hypomorphic *gld-1(op236)* allele. In our enhancer screen we found that RNAi-mediated knock-down of *nhl-2* and *vig-1* specifically caused sterility in *gld-1(op236)* worms, at the permissive temperature. As both *vig-1* and *nhl-2* have been shown to interact with the miRNA-induced silencing complex (miRISC) and are required for miRNA function, our results indicated a possible genetic interaction between *gld-1* and miRNA-mediated translational regulation. We indeed find that *vig-1* and *nhl-2* mutants have embryonic lethality and sterility in the *gld-1(op236)* mutant background. Furthermore, *gld-1(op236)* enhances the phenotypes associated with the loss of *let-7* and *mir-35* family miRNAs. In weak *let-7(mg279)* mutant worms *gld-1(op236)* enhances the phenotypes associated with larval to adult transition, suggesting a role for *gld-1* in the heterochronic pathway. In *mir-35* family mutants *gld-1(op236)* enhances the embryonic and larval lethality. Taken together, our data supports the hypothesis of a genetic interaction between GLD-1 and several miRNA pathways. In further support of our hypothesis, we identified ALG-1, CGH-1 and PAB-1 as direct interactors of GLD-1. We are currently investigating the roles of the direct interactors identified, in GLD-1 mediated translational regulation and the possibility of common targets between GLD-1 and miRNAs.

972A

Rab-7 can modulate microRNA activity in *C. elegans*. Zhiji Ren, **Victor R. Ambros**. Prog Molec Med, Univ Massachusetts Med Sch, Worcester, MA.

MicroRNAs (miRNAs) are endogenous non-coding small RNAs that post-transcriptionally regulate gene expression. They are known to play important roles in various developmental and physiological processes. Recent studies in *Drosophila* and mammalian cells have suggested that endosomal trafficking is directly associated with and functionally regulates miRNA activity. To investigate whether *C. elegans* miRNA pathways may be modulated by endosomal trafficking, we employed *C. elegans* miRNA mutants as sensitized backgrounds for an RNAi screen to identify ESCRT genes and RAB genes that modulate miRNA activity. These miRNA mutants from the screen have compromised miRNA pathway, which allows us to identify regulators of miRNA mediated gene silencing with distinct and quantifiable phenotypes. ESCRT genes function in endosomal trafficking and RAB genes are small GTPases that regulates membrane trafficking in general. We observed that RNAi knock down of *rab-7*, which regulates the transitions from early endosome to late endosome and late endosome to lysosome, decreases miRNA activity for several miRNA families in different tissues. First, knocking down *rab-7* enhances the developmental timing defect of a *let-7* family mutant *(nDf51)* and delays the downregulation of the corresponding target, HBL-1, in the hypodermis. In addition, *rab-7* RNAi aggravates the embryonic lethal phenotype associated with a *mir-35* family mutant *(gk262)*. Finally, knocking down *rab-7* enhances the phenotype of a *lsy-6* hypomorphic allele *(ot150)* in a set of bilaterally symmetric gustatory neurons (ASEL/R). Taqman qRT-PCR analysis of microRNA levels after *rab-7* RNAi did not reveal any dramatic impact on mature miRNA levels in wild type or the *let-7* family mutant *(nDf51)*. Therefore, our results suggest that *rab-7* can positively modulate miRNA activity downstream of biogenesis.

973B

RNAi-promoting Zinc Finger Protein, ZFP-1, is an essential factor required for chromatin integrity in *C. elegans*. **Daphne Anastasiades**[1], Germano Cecere[1], Morten Jensen[2], Jason Lieb[2], Alla Grishok[1]. 1) Department of Biochemistry & Molecular Biophysics, Columbia University, New York NY 10032 USA; 2) Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill NC 27599 USA.

Zinc Finger Protein 1 (ZFP-1), the *C. elegans* PHD finger-containing protein homologous to the human chromatin-associated protein AF10 (Acute Lymphoblastic Leukemia 1-Fused gene from chromosome 10), is one of the factors promoting RNAi-induced transcriptional gene silencing (TGS). Although the interaction between the C-terminal domain of AF10 and the methyltransferase Dot1 has been established, the function of the N-terminal portion of the protein, which contains the PHD fingers, is unknown. There are two distinct isoforms of ZFP-1 in *C. elegans*: a long isoform containing the N terminal PHD fingers and a C terminal leucine zipper, and a short isoform missing the PHD fingers but retaining the C terminus. We find that the long isoform is predominantly expressed in the germline and embryos while the short isoform is expressed in the soma, but not in the germline. Interestingly, the PHD fingers only present in the long isoform are retained in the *zfp-1* (*ok554*) mutant and can be detected in the germline by immunostaining. The two N-terminal PHD fingers of ZFP-1 (PHD1-PHD2) are essential for the viability of the worm since *zfp-1*(*ok554*)/nDf17 worms do not survive due to embryonic and larval lethality. When this portion is depleted in *zfp-1* (*ok554*) by RNAi, we also observe defects in the chromatin of oocytes suggesting that PHD1-PHD2 plays an essential role in chromatin integrity. Using *in vitro* binding assays we found that PHD1 specifically binds histone H3 dimethylated at lysine 4 (H3K4me2), while PHD1-PHD2 together bind histone tails less specifically with a preference for methylated H3K4. We confirmed the interaction of PHD1-PHD2 with nucleosomes using native gel-shift assays. In addition, we determined by ChIP that the localization of ZFP-1 to its target genes is diminished in COMPASS complex mutants, which are lacking proper methylation of H3K4. Also, we observe that PHD2 facilitates the dimerization of the protein both *in vitro* and *in vivo*. Downregulation of ZFP-1 has previously been reported to affect RNAi efficiency; however, we find that the *zfp-1* (*ok554*) mutant is not deficient in RNAi. In fact, depletion of PHD1-PHD2 in *zfp-1* (*ok554*) by RNAi leads to de-silencing of a transgenic array in an RNAi-TGS model, suggesting that this portion is required for RNAi. We propose that ZFP-1's essential role may be through stabilizing chromatin in conjunction with nuclear RNAi factors.

974C

In situ hybridization analysis of *let-7* microRNA. **Yoshiki Andachi**[1,2], Yuji Kohara[1,2]. 1) Genome Biol Lab, National Inst Genetics, Mishima, Japan; 2) Dept Genetics, SOKENDAI, Mishima, Japan.

MicroRNAs (miRNAs) are short RNAs that down-regulate target genes by forming base pairs with their mRNAs. miRNA genes are transcribed into long transcripts, primary miRNAs (pri-miRNAs), which are subsequently cleaved into mature miRNAs. Though miRNA expression is under transcriptional control, several miRNAs are also post-transcriptionally regulated. One of the miRNAs is *let-7* in *C. elegans*, which is temporally regulated. It remains unknown whether *let-7* is also post-transcriptionally regulated in a tissue-specific manner because of the unavailability of a method for *in situ* detection of miRNA in *C. elegans*.

We previously developed a strategy for isolating cDNA clones of target mRNAs that form base pairs with a miRNA of interest, in which the cDNAs are synthesized from the mRNAs using the miRNA as a reverse-transcription primer. Application of the method to *let-7* family miRNAs, *mir-48* and *mir-84*, provided clones derived from parts of the *let-7* pri-miRNA. In a region 3' adjacent to the clones, three sequences complementary to the seed of the *let-7* family miRNAs are included, and the seed complementary sequences are conserved in corresponding regions of the *let-7* genes in *C. briggsae*, *C. remanei* and *C. brenneri*. One possibility deduced from the findings is that the *let-7* family miRNAs are involved in *let-7* biogenesis by binding to the seed complementary sequences.

To examine tissues where *let-7* miRNA is produced, we developed an *in situ* hybridization (ISH) method, based on the ISH method to know mRNA expression in our lab. Using the ISH method, *let-7* miRNA was detected in vulva, rectum and pharynx of N2 worms at the L4 stage. ISH analysis of *let-7(mn112)* deletion mutants rescued by extra-chromosomal arrays of a *let-7* gene clone exhibited intense signals in seam, ventral neurons and distal tip cells in addition to the above tissues, presumably due to increased *let-7* gene copy number. As for *mir-48*, miRNA expression was observed in vulva, rectum, seam and a few neurons around pharynx of N2 worms, indicating that both *let-7* and *mir-48* miRNAs were co-expressed in several tissues. To test the requirement of the seed complementary sequences of the *let-7* pri-miRNA for *let-7* biogenesis, a *let-7* gene clone with all the complementary

sequences substituted was prepared and introduced into *let-7(mn112)* mutants. Extra-chromosomal arrays of the clone turned out to rescue the lethal phenotype of *let-7* mutants, and ISH analysis of the transgenic strains provided essentially the same *let-7* expression pattern as the transgenic strains of the wild-type clone. Further substitution studies are in progress.

975A

rde-4 is epistatic to *rde-4*. **Stephen A. Banse**, Craig P. Hunter. Dept Molecular & Cellular Biol, Harvard Univ, Cambridge, MA.

In C. elegans the introduction of dsRNA (double-strand RNA) down regulates genes in a process termed RNA interference (RNAi). RNAi acts via small interfering RNAs (siRNAs), which are generated by RDE-4/DCR-1 cleavage of long dsRNA. Interestingly, RDE-4 and a few other genes required for RNAi (e.g. *rde-1*) are also necessary for the inconvenient silencing of multi-copy transgenic arrays. In contrast, enhanced RNAi (*eri*) mutants show enhanced transgene silencing. These observations are consistent with a simple model whereby transgene derived dsRNA is processed into siRNAs by RDE-4/DCR-1 leading to RNAi-dependent silencing of the transgenic arrays.

Phenotypically, the ERI proteins act directly or indirectly to inhibit transgene silencing. The ERI proteins physically interact and are required for the production or stability of small RNAs that appear to silence endogenously expressed genes. It has been proposed that this eri-dependent small RNA silencing pathway competes with the RNAi pathway for limiting silencing resources. Our analysis shows that the *eri*-induced enhancement of transgene silencing requires the nuclear argonaute NRDE-3, indicating that not only is transgene silencing a nuclear RNAi process, but that NRDE-3 may be a limiting silencing resource. Interestingly, in addition to RNAi, RDE-4 is necessary for the generation of NRDE-3 associated endogenous siRNAs. This leads to the prediction that *rde-4* mutants should relieve competition for NRDE-3, and therefore be "Eri", if RDE-4 weren't also required for RNAi. Consistent with this seemingly contradictory prediction, our genetic analysis provides supports for RNAi promoting and RNAi inhibiting roles for RDE-4 in somatic transgene silencing. This conflict is resolved by spatial segregation of these functions, with RDE-4 repression of the NRDE-3-dependent somatic transgene silencing activity being limited to the germline of the previous generation in contrast to the known somatic role of RDE-4 in promoting transgene silencing.

Our work shows that transgene expression in the soma is synergistically repressed by the canonical RDE-1/RDE-4 RNAi pathway acting in the soma and by a second, a NRDE-3 dependent pathway most active in the parental germline. This germline process is inhibited by ERI-1 and RDE-4 and functions to generate a silencing signal which is inherited by the soma. Although loss of germline RDE-4 produces this silencing signal, it is typically masked by a requirement for somatic RDE-4 for efficient transgene silencing.

976B

A function for *mir-34* in gonad morphogenesis. **Samantha Burke**[1], Molly Hammell[2], Victor Ambros[1]. 1) University of Massachusetts Medical School, Worcester, MA; 2) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Mir-34 is a highly conserved microRNA whose function is not yet fully understood. Human cell culture and mouse model work has shown that *mir-34* plays a role in cell cycle progression, senescence, and apoptosis [1,2], while work in *C. elegans* has implicated *mir-34* in radiation-induced DNA damage response [3]. In transgenic *C. elegans* expressing GFP under the control of the *mir-34* promoter, expression is seen broadly throughout neural and muscle cells and in the hypodermis. Levels of both mature *mir-34* and the GFP reporter increase during adulthood. In starved L1 and dauer larvae mature *mir-34* levels are elevated compared to the developing L1 and L2 stage larvae, respectively [4]. We examined *mir-34* mutants for developmental phenotypes and determined that the mutants display incompletely penetrant gonad migration defect, including overextension of the gonad arms, improper contact between the arms and hypodermis, and extra turns. The incomplete penetrance of this phenotype suggested the presence of co-regulators. Therefore, we tested for genetic enhancement of the *mir-34* phenotype by loss of other microRNAS that share predicted targets with *mir-34*. Mutation of one such microRNA, *mir-83*, also causes a slight gonad migration defect. In *mir-34;mir-83* double mutant animals, the migration defect is significantly more pronounced, suggesting that *mir-34* and *mir-83* function redundantly in the regulation of targets involved in guiding the migration of the gonad during larval development. We are in the process of identifying transcripts co-targeted by *mir-34* and *mir-83* in order to better understand how the loss of both microRNAs can lead to such defects. References: [1] C. He et al., Biochemical and Biophysical Research Communications 388 (2009) 35-40. [2] M. Yamakuchi and C.J. Lowenstein, Cell Cycle 8 (2009) 712-715. [3] M. Kato et al., Oncogene 28 (2009) 2419-2424. [4] X. Karp et al., RNA 17 (2011) 639-651.

977C

A Conserved Motif Affecting Nucleosome Occupancy is Required for 21U-RNA Production in *C. elegans*. **Germano Cecere**[1], Grace Zheng[2], Katherine Klymko[1], Andres Mansisidor[1], Alla Grishok[1]. 1) Department of Biochemistry and Molecular Biophysics, Columbia University Medical Center, New York, NY 1003; 2) Koch Institute for Integrative Cancer Research, MIT, Cambridge, Massachusetts 02139, USA.

21U-RNAs in C. elegans are the newest addition to the growing number of endogenous short RNAs. Recent findings have revealed similarities between 21U-RNAs in C. elegans and piRNAs in other metazoans. 21U-RNAs, like piRNAs, exist in a complex with the Piwi subfamily Argonaute proteins and have a role in germline maintenance and transposon silencing. The biogenesis and molecular function of 21U-RNA and piRNA remain elusive. Here we show that previously identified conserved DNA motif located 20 nucleotide upstream of each 21U-RNA sequence is required for the production of the individual 21U-RNA. We demonstrate that this DNA motif is associated with low nucleosome occupancy characteristic of many promoters of protein-coding genes and that the low nucleosome occupancy, at least in part, is determined by the intrinsic property of poly(dA:dT) sequence present in the 34bp motif. Our findings suggests that transcription of each 21U-RNA is regulated separately through the upstream motif and illustrates evolutionary conservation of thousands identical regulatory DNA motifs underlying nucleosome positioning without the corresponding conservation in the transcribed short RNAs.

978A

RACK-1 acts in *let-7*-mediated heterochronic development of *Caenorhabditis elegans*. **Shih-Peng Chan**[1], Frank Slack[2]. 1) Graduate Institute of Microbiology, College of Medicine, National Taiwan University, Taipei 10051, Taiwan; 2) Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT 06520, USA.

In *Caenorhabditis elegans*, the *let-7* miRNA regulates the exit of cell cycle and terminal differentiation at the L4-to-adult switch. Dysfunction of *let-7* results in irregular hypodermal and vulval development of *C. elegans* and is also a feature of human lung cancer. Here we show that Receptor of Activated C Kinase (RACK-1) modulates *let-7*-mediated regulation in *C. elegans*. The RACK-1 protein co-immunoprecipitated with the nematode Argonaute protein, ALG-1. Reduction of *rack-1* gene expression by RNAi suppressed the aberrant vulva and hypodermis development phenotypes of *let-7(n2853)* mutant animals and promoted adult-specific gene expression. Our results indicate a relationship between RACK-1 and *let-7* in regulation of terminal differentiation that may help understand the mechanism of translational control by miRNAs.

979B

A Genome-wide analysis of genetic interactions in early embryogenesis in *C. elegans*. **P.G. Cipriani**, E. Munarriz, A. White, HL. Kao, J. Young, K. Erickson, S. Guzman, J. Lucas, K. C. Gunsalus, F. Piano. Dept Biol, New York University, New York, NY.

Genetic interaction studies have been useful in showing buffering between molecular pathways and predict function of non-lethal genes in many organisms. We undertook a large RNA interference (RNAi) based genome-wide screen to find genetic interactions (enhancers and suppressors) for genes involved in embryogenesis in *C. elegans*. To this end we have used 26 available temperature sensitive *(ts)* alleles of 24 essential genes *(dhc-1, div-1, dnc-1, emb-8, emb-27, emb-30, glp-1, hcp-6, lin-5, mat-1, mbk-2, mel-26, par-1, par-2, par-4, pod-2, rme-8, spd-5, spn-4, tba-1, zen-4, zyg-1, zyg-8, zyg-9)*, whose strong loss-of-function phenotype affects the early embryo. We have developed an image acquisition system [(1)] and have archived over 3 million images from all RNAi experiments.

To provide a rapid quantitative output, we have developed an image analysis system (DevStaR)[(2)]. DevStaR produces an automatic classification of the developmental stages of *C. elegans* animals from a population of mixed stages, and achieves near real-time scoring of image data in a fully automated manner. The output is equivalent to the quantification of progeny survival in every experiment (See poster by White *et al*).

Current analysis of the data shows that the distribution of the quantitative phenotypes allows us to compare the results of the ts mutant alleles and the controls, and to select potential positive genetic interactions for each *ts* allele with high accuracy. We will present data showing the trends of how genetic interaction networks are built around this set of essential genes. We are using these results to construct a comprehensive network of genetic interactions in early embryogenesis in *C. elegans*.

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980C

Yeast One-Hybrid Screen to Identify Factors Required for piRNA Biogenesis in C. elegans. **Amanda Day**, John Kim. Life Sciences Institute and the Department of Human Genetics, University of Michigan, Ann Arbor MI.

Piwi proteins and their associated small RNAs (piRNAs) have been implicated in germline maintenance and genomic surveillance in metazoans. C. elegans encodes two piwi related genes, prg-1 and prg-2, whose gene products bind a class of 21nt RNAs (21U RNAs) and function in the germline piRNA pathway(1,2). PRG-1 and piRNAs are required for fertility at elevated temperatures and repression of the Mariner transposon Tc3, but the mechanisms of these outcomes have not yet been explicitly determined(1,2). Little is known about the biosynthesis, transcription, and possible processing of piRNAs in the C. elegans germline. Unlike in fly, where piRNAs are processed from long, single-stranded precursors transcribed from relatively few discrete genomic loci (3), the 21U RNAs are encoded on both strands in two broad regions of chromosome IV (1,2,4). Beyond the defining 5' uridine, there is no discernable sequence motif present in the 15,000+ unique 21U RNAs identified thus far(4), but most 21U RNA-encoding loci exhibit a signature upstream motif, suggesting that each 21U RNA comprises a modular transcriptional unit(4). We hypothesize that the conserved upstream motif represents a binding site for factors that drives expression of each 21U RNA in the developing germline. To test this hypothesis, we are conducting a yeast one-hybrid screen to identify proteins that bind the conserved upstream motif of 21U RNAs. Multiple bait sequences were designed using this motif and surrounding genomic sequences. These bait sequences were integrated into the yeast genome and used to screen fusion libraries of C. elegans proteins expressed at the L4 and young adult stages through

selection for an antibiotic resistance factor encoded downstream of the bait sequence integration site. This resulted in the identification of 26 proteins thus far that allow yeast growth on antibiotic-containing media. To determine which of these interact specifically with the 21U RNA upstream motif, we are testing whether mutation of the bait abrogates the interaction. True positives are being investigated in vivo for a role in piRNA biogenesis through RNAi and/or mutant analysis by Taqman assay of 21U RNA expression levels. The effect on fertility, transposon activity, and germline development will also be characterized. This approach will provide insight into piRNA biogenesis and forge new paths for exploring the mechanisms of Piwi-piRNA function in the worm germline. 1. Das, P.P. et al., Cell 31: 79-90 (2008) 2. Batista, P.J. et al., Cell: 67-78 (2008) 3. Brennecke, j. et al., Cell 128: 1089-103 (2007) 4. Ruby, G. et al., Cell 127:1193-207(2008).

981A

Cel-bantam miRNA family regulates TGF-β Sma/Mab pathway. **María Pilar de Lucas**, Encarnación Lozano. Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.

We are interested in body size regulation of C. elegans as a model system to study the TGF-β Sma/Mab pathway. It is known that TGF-β controls gene expression and endoreduplication of hypodermis in worms, which in turn regulate their adult body size. Thus, null mutants of the TGF-β ligand, for instance, are small adult worms with lower hypodermal ploidy. We have asked whether microRNAs (miRNAs) may be involved in such regulation. It has been recently described that a mutant knockout worm of four of the five members of the bantam miRNA family (i.e., cel-mir-58) displays a small body size that resembles the size of TGF-β Sma/Mab pathway mutants. We have found that several members of this miRNA family are individually required for worms to acquire their wild-type size, although they act redundantly with each other, because the single null mutants are always larger than the four-mir-58 combined mutant. We also observed correlatively lower endoreduplication levels in the hypodermis of mir-58 mutants, which suggest that the small body size of those mutants is due to misregulation of the TGF-β Sma/Mab pathway (other growth pathways in worms are unrelated to ploidy levels). According to further sequence comparisons, we suggest that two genes of this pathway, dbl-1 (TGF-β ligand) and sma-6 (TGF-β receptor), may be targets of mir-58. In support of this hypothesis, mir-58 null mutants show a significant increase of sma-6 and dbl-1 mRNA levels.

982B

Germline expression, inheritance, and genomic characteristics of *Caenorhabditis elegans* 21U-RNAs. **Mallory Freeberg**[1,2], John Kim[2]. 1) Bioinformatics Graduate Program, University of Michigan, Ann Arbor, MI; 2) Life Sciences Institute and Department of Human Genetics, University of Michigan, Ann Arbor, MI.

21U-RNAs (21Us) are a class of *C. elegans* small RNAs characterized by 21 nt length, 5' uridine, and no sequence similarity or conservation.[1] 21Us represent the piRNAs of C. elegans, as they require Piwi protein PRG-1 for accumulation and are involved in germline maintenance and fertility.[2,3] In fly and mouse, piRNAs map antisense to repetitive elements and transposons, suggesting a silencing role;[4] however, 21Us map primarily to two broad regions of chromosome IV that are not enriched for selfish genetic elements. The targets of 21Us, and their mechanism of biogenesis, remain largely unknown. A recently characterized class of germline endogenous siRNAs, 26G-RNAs, show distinct expression in either spermatogenic cells or oocytes and embryos.[5,6] We wondered whether 21Us also show patterns of male or female germline specificity. Although some 21U species have been detected in both male and female germlines,[2,3] what might distinguish germline-specific patterns of enrichment among all 21Us is poorly understood. Here, we computationally analyze published sequencing datasets to assess germ cell specificity of 21Us. Unlike 26G-RNAs, 21Us do not exhibit discrete germ cell specificity, but more than 85% of the 21Us are two-fold or greater enriched in male or female germline. Initial 21U studies identified a conserved 8 nt motif (CTGTTTCA) located upstream of 21U loci.[1,2] Strikingly, 80% of male germline-enriched 21U upstream regions contain the 5 nt core motif GTTTC, compared to less than half of the female germline-enriched 21U upstream regions. Additionally, position 1 of the 8 nt motif upstream of male germline-enriched 21Us is strongly biased for C, whereas no preference is seen for female germline-enriched 21Us. We also observe that a C at position 1 of the 8 nt motif corresponds to higher expression levels of male, but not female, germline-enriched 21Us. Thus, we hypothesize that the CNGTTTCN upstream motif directs biogenesis of 21Us more efficiently in the male germline than the female. Together, these findings suggest that specific upstream motifs may direct male and female germline expression of 21Us in ways that are not yet understood. We are currently conducting experiments to elucidate the exact mechanism by which the upstream motif directs 21U expression (see poster by A Day and J Kim). 1. *Cell* **127**:1193 (2006). 2. *Mol Cell* **31**:67 (2008). 3. *Mol Cell* **31**:79 (2008). 4. *Nat Rev Genet* **10**:94 (2009). 5. *PNAS* **107**:3588 (2010). 6. *PNAS* **106**:18674 (2009).

983C

Single-worm RNA-seq as a tool for following epigenetic silencing. **Weifeng Gu**, Ahmed Ahmed Elewa, Craig Mello. Program Molec Med, Univ Massachusetts Med Sch, Worcester, MA 01605.

Epigenetic silencing is often unstable and thus can vary dramatically from one individual to the next. We are interested in learning more about the mechanisms that underlie this variation. For example are there small-RNAs that are differentially inherited or amplified from one individual to another? In order to explore this type of variation in C. elegans, we first needed RNA-seq cloning protocols that work on single worms. Thus far we have developed a very reliable cloning strategy to recover small RNA cDNA sequences from single animals. We have also developed a fully enzymatic approach for recovering mRNA sequences using 1 ug of total RNA and are optimizing the conditions to clone mRNA from single worms. Using these new tools, we are exploring the animal-to-animal variation in endogenous and experimentally-induced small RNA species. We will report on our findings at the meeting.

984A

Nuclear RNAi mediates off-target gene silencing. **Shouhong Guang**[1], Kirk Burkhart[2], Virginia Lamb[2], Scott Kennedy[2]. 1) School of Life Sciences, University of Science & Technology of China, Hefei, Anhui, China, WI., Anhui, China; 2) University of Wisconsin, Madison.

Off-target effect is one of the major concerns for the application of RNAi-based technology. While near perfect complementarity between siRNAs and their target RNA sequences is required for efficient gene silencing, exposure of eukaryotes to siRNAs frequently results in unintended silencing of genes exhibiting < 100% sequence identity to the trigger siRNAs. We developed a method to study this off-target effect in C. elegans and found that the components of nuclear RNAi pathway are required for this off-target gene silencing.

dpy-13 is a collagen gene, which belongs to a large gene family that contains more than 150 members with high sequence similarity. *dpy-13(e458)* mutant lacks most of the coding region, likely being a null mutation. *dpy-13(e458)* animals exhibit a dumpy phenotype, with a length roughly half of wild type N2 animals. Exposure of wild type N2 animals to dsRNA targeting dpy-13 induces a dumpy phenotype less severe than the *dpy-13(e458)* mutant. *eri(-)* animals exhibit enhanced sensitivity to RNAi. Feeding *eri(-);dpy-13(e458)* animals with dpy-13 dsRNA targeting the deleted region elicits a phenotype which is extremely more severe (super-dumpy) than *dpy-13(e458)*. This finding indicates that dsRNA targeting the dpy-13 gene is able to trigger an off-target silencing by targeting nucleic acid sequences outside of the dpy-13 locus, possibly other collagen genes with high sequence similarity.

We examined the genetic requirements for this off-target effect and found that RRF-1 and NRDEs are necessary for this RNAi off-target gene silencing. RRF-1 is a RNA-dependent RNA polymerase and generates secondary siRNA upon feeding RNAi. Our data suggest that RRF-1 is able to use off-target mRNAs as template for siRNA synthesis. NRDEs are necessary to silence nuclear localized RNAs. In nrde(-) animals, silencing of the nuclear localized RNAs is defective, while the cytoplasmic RNAi pathway remains functional. Interestingly, while several secondary Argonaute proteins are not critical for this RNAi off-target gene silencing, all NRDE proteins are essential for this effect.

Thus, our data suggest that at least some of the RNAi off-target gene silencing effects depend on the nuclear RNAi pathway.

985B

Casein kinase 2 (KIN-3/KIN-10) regulates microRNA activity in *C. elegans*. **Ting Han**[1], Vishal Khivansara[1], James Moresco[2], John Yates[2], John Kim[1]. 1) Life Sciences Institute, Department of Human Genetics, University of Michigan, Ann Arbor, MI; 2) Department of Chemical Physiology, The Scripps Research Institute, La Jolla, CA.

MicroRNAs (miRNAs) regulate diverse biological processes via repression of select target mRNAs bearing partial sequence complementarity. In addition to the core factors required for miRNA biogenesis and function, recent studies have revealed other mechanisms that modulate miRNA activity in response to developmental and environmental cues (1,2).

In a previous genome-wide RNAi screen to identify factors that could play a role in small RNA-mediated gene regulation, we found that depletion of *kin-3* and *kin-10* resulted in a supernumerary seam cell phenotype indicative of a developmental timing defect (3). In addition, *kin-3* and *kin-10* enhance the lethality associated with loss of *let-7* family miRNAs (*let-7*, *mir-48*, *-84*, and *-241*), suggesting that they are required for the full activity of *let-7* family miRNAs. KIN-3 and KIN-10, respectively, encode the catalytic and regulatory subunits of *C. elegans* casein kinase 2 (CK2), a serine/threonine kinase involved in an array of biological processes, including gene expression, cell proliferation and survival, and circadian rhythm. However, to our knowledge, a role of CK2 in the miRNA pathway has yet to be reported.

Employing multiple sensitized genetic and transgenic reporters, we found that inactivation of CK2 impairs the silencing of several miRNA targets, including *let-60*/RAS (*mir-84* target), *cog-1* (*lsy-6* target) and *mef-2* (*mir-1* target). In addition, CK2 enhances the temperature-sensitive embryonic lethality associated with loss of the *mir-35-41* cluster. These observations suggest that CK2 broadly regulates miRNA activity in multiple tissues. CK2 is not required for the accumulation of mature miRNAs, suggesting a function downstream of miRNA biogenesis. We hypothesize that wild-type CK2 phosphorylates components of the miRNA-induced silencing complex (miRISC) to promote miRNA activity. To understand how phosphorylation by CK2 could impact miRISC function, we are currently identifying the substrates of CK2 and mapping their phosphorylation sites.

1. Hammell et al. Cell 136, 926-938 (2009).

2. Bethke et al. Science 324, 95-98 (2009).
3. Kim et al. Science 308, 1164-1167 (2005).

986C

Regulation of *mir-34* expression by insulin signaling pathway in *C. elegans*. **M. Isik**, E. Berezikov. Hubrecht Institute, Uppsalalaan 8, Utrecht, Netherlands.

mir-34 is the founding member of an evolutionarily conserved miRNA family found in diverse species, including humans. Several studies demonstrated that *mir-34* is required for a normal cellular response to DNA damage in vivo resulting in altered cellular survival post-irradiation. To further explore the role of *mir-34* in *C. elegans*, we have created transgenic lines expressing P*mir-34::gfp* to examine the expression pattern at different stages of the animal. In larval stages, P*mir-34::gfp* was expressed throughout development in ventral nerve cord, dorsal nerve cord, subsets of head and tail neurons, excretory cell and excretory canal. In adults, P*mir-34::gfp* was also detected in vulva and seam cells. Expression was strongly upregulated in dauer larvae, especially in hypodermis, seam cells and amphid neurons. Examination of the reporter expression in *daf-2*(insulin receptor), *daf-1*(TGF-β receptor) and *daf-7*(TGF-β ligand) mutant backgrounds suggests that high *mir-34* expression in dauers is related to the differential gene expression at dauer stage but not to starvation conditions and that *mir-34* expression might be regulated by these signaling pathways. In correlation with its high expression in dauers, 8% of *mir-34(gk437)* formed partial dauers at 27 °C as compared to 1% in wild type worms, and *mir-34(gk437)* animals showed dauer maintenance defects. DAF-16, the single forkhead box O (FOXO) homologue, functions as the major target of the insulin signaling pathway, which is a determinant of dauer diapause. *daf-16(mu86); daf-7(e1372)*; P*mir-34::gfp* strain, which is dauer constitutive at 25 °C, lacks the high expression in dauers. Furthermore, GFP expression was seen in the intestine in response to stress conditions (starvation, heat, hypoxia, etc.) in Pmir-34::gfp transgenic lines. This increase, which was also validated by RT-PCR, was more prominent in *daf-16(mu86)* mutants as compared to wild type animals and it was missing in *daf-2(e1370)* mutants. These findings strongly imply the regulation of *mir-34* by insulin signaling pathway.

987A

miRNA regulation of stress responses. **Konstantinos Kagias**, Roger Pocock. BRIC, Copenhagen, Denmark.

miRNAs are a class of small non-coding RNAs involved in a variety of biological processes, including stress responses. In biological systems, stress can be any change in the internal and/or external environment that deviates from the normal steady state. Such stresses include temperature, radiation and oxygen. We have identified specific *C. elegans* miRNAs that show differential expression in hypoxic conditions (*hypoxia*) when compared to normal oxygen levels (*normoxia*). We are now performing phenotypic analysis of deletion mutants for two such miRNAs, namely *mir-87* and *mir-233*, in hypoxia and other stresses. We have found defects in the DNA damage response, in body morphology under hypoxia and in brood size. Further analysis suggests that *mir-87* and *mir-233* regulate *rnr-1* (RiboNucleotide Reductase-1) and high levels of this enzyme in the *mir-87; mir-233* mutant is responsible for the observed phenotypes. RNR-1 is the large subunit of ribonucleotide reductase and by homology is predicted to function in deoxyribonucleotide (dNTPs) biosynthesis. dNTPs levels have been associated with the DNA damage response and rates of mutagenesis and both excess and lack of dNTPs lead to similar effects. In addition to the work described above, we are using miRNA-sequencing to identify miRNAs that are responsible for the response of *C. elegans* to high temperature, radiation and hypoxia. Thus, we expect to identify new miRNAs with critical roles in stress responses. In conclusion, miRNAs, are small molecules whose mode of action is both rapid and reversible. This makes miRNAs excellent candidates as regulators of stress responses. In certain diseases like cancer, cells are exposed to different stresses (e.g hypoxia) and understanding more about the role of miRNAs in such conditions will allow us to develop better therapeutic strategies in the future.

988B

Variable Replication of a Flock House Virus Transgene in *Caenorhabditis* Natural Isolates. **EE Large**[1], G Broitman-Maduro[1], SW Ding[2], M Maduro[1]. 1) Department of Biology, University of California, Riverside, CA 92521; 2) Institute for Integrative Genome Biology and Department of Plant Pathology, University of California, Riverside, CA 92521.

Viruses of the nodavirus family are found primarily in insects and, more recently, in natural isolates of *Caenorhabditis* nematodes. Flock House Virus (FHV) is a positive-strand RNA virus of the nodavirus family that can infect a broad range of eukaryotic hosts such as insects, plants, mammalian cell culture, and yeast. FHV contains two genomic RNAs: RNA1 and RNA2. RNA1 encodes an RNA-dependent RNA polymerase as well as an RNAi suppressor, B2. Heat-shock induced expression of a hsp16-41::RNA1 fusion is sufficient to result in replication of RNA1 in *C. elegans* N2. We have previously shown that this replication requires expression of B2, and that B2 likely suppresses host RNAi. If the coding region for B2 is replaced with that of GFP (R1GFP), wild-type animals no longer replicate RNA1 efficiently but RNAi-defective strains have robust RNA1 replication. We wished to test the hypothesis that wild isolates of *C. elegans* might differ in their ability to replicate the R1GFP transgene. We used the R1GFP transgene assay to determine the effectiveness of FHV replication in *Caenorhabditis* natural isolates. Variable expressivity and unique patterns of GFP localization were observed. Our results suggest that natural isolates of *C. elegans* carry multiple differences in their ability to support FHV RNA1 replication. Subsequent assays for nodavirus infectivity and mapping of loci responsible for enhancement and repression of transgene expression will be reported.

989C

Genetic identification of post-transcriptional modulators of microRNAs. **Katherine McJunkin**, Victor Ambros. Department of Molecular Medicine, University of Massachusetts Medical School, Worcester, MA.

MicroRNAs are large class of small noncoding RNAs that regulate the expression of protein-coding genes in plants and animals. By repressing target mRNAs, microRNAs play important roles throughout normal development and in diverse processes in differentiated cell types. Accordingly, individual microRNAs display highly specific expression patterns, often restricted to a particular developmental stage or tissue. MicroRNA expression patterns are partially derived from transcriptional regulation; however, many examples of post-transcriptional regulation of microRNAs have also recently been described. All steps of the microRNA biogenesis pathway appear to be regulated in certain contexts in animals, including Drosha cleavage of the primary transcript and Dicer cleavage of the pre-microRNA. In conjunction with biogenesis, the regulated turnover of mature microRNAs (or pri- or pre-microRNA intermediates) could contribute to their complex spatio-temporal expression patterns. In comparison to biogenesis, very little is understood about the effectors or regulators of microRNA degradation. Finally, the activity of microRNA silencing complexes can be enhanced or dampened by the binding of accessory factors that do not affect microRNA processing or stability. The aim of this research is to elucidate novel modes of post-transcriptional control of microRNAs in animals, focusing on mechanisms of microRNA degradation. The accumulation of mature let-7 and ¬mir-35 and mir-51 family members are developmentally controlled at a post-transcriptional level. Thus, transgenic worms have been generated that bear fluorescent reporters to directly monitor changes in the activity of these microRNA families by simultaneously tracking microRNA transcription and target repression. Genetic screens are ongoing in these backgrounds to identify mutants in which the normal developmental patterns of target repression are disrupted, to identify genes that impact microRNA processing or turnover.

990A

Analysis of miR-35 stablity at the embryo-larval transition in *C. elegans*. **Kenneth J Murfitt**, Javier Armisen-Garrido, Nicholas J Lehrbach, Eric A Miska. Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, United Kingdom.

Perturbations in miRNA levels are known to have profound consequences in many biological processes; although the mechanisms underlying miRNA synthesis are well established little is known about how organisms regulate the steady-state levels of these molecules once they have been generated. Defining the factors and systems underpinning this regulation is essential to understanding the dynamics of miRNA activity. We have utilized the miR-35 family as a model for studying miRNA stability; these miRNAs show extensive intra-family redundancy and deletion of all eight members causes embryonic lethality. miR-35 is expressed strongly in *C. elegans* embryos but is essentially absent at all other developmental stages and it is hypothesized that this rapid transition in expression is achieved predominantly post-transcriptionally.

Following detailed analysis of the expression of miR-35 miRNAs, we carried out a candidate-based RNAi screen for factors that are required for the down-regulation of miR-35 at the embryo-larval transition. A single factor, the Pumilio-FBF RNA binding protein PUF-9 (but not the other members of this protein family), was identified in this way and validated in the relevant knockout strain. Double mutants of *puf-9* and *mir-35* have strong synthetic defects. In parallel, we have used a temperature-sensitive allele of the essential miRNA biogenesis factor PASH-1, and LNA miRNA microarrays, to study the half-life of miR-35 miRNAs. We find that these molecules have half-lives well below the average for all of the *C. elegans* miRNAs tested.

In conclusion, we have shown that depletion of an RNA binding protein, PUF-9, impairs the ability of *C. elegans* to fully down-regulate miR-35 at the embryo-larval transition. Although this impairment is subtle and transient (miR-35 levels return to baseline by the L2 stage in PUF-9 depleted animals) this finding suggests that rapid miRNA turnover may be actively regulated. Surprisingly, double knockout of PUF-9 and members of the miR-35 family causes a strong synthetic phenotype indicating that the protein and the miRNAs may share common targets or function in a common pathway; as such the impairment of miR-35 down-regulation following PUF-9 removal could be an indirect, or compensatory, response. We also show that the miR-35 family miRNAs have shorter than average half-lives, in vivo, following conditional blockade of miRNA biogenesis. This intrinsic instability may allow these miRNAs to act as finely balanced switches, controlling key events at the onset of larval life.

991B

Characterization of Transposon Silencing Pathways. **Carolyn M. Phillips**[1,2], Gary B. Ruvkun[1,2]. 1) Department of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Department of Genetics, Harvard Medical School, Boston, MA.

Small RNA mediated silencing of transposons is an important mechanism to prevent DNA damage and subsequent mutations in the germline. A subset of the exogenous and endogenous small RNA pathways genes have been demonstrated to be involved in transposon silencing. When these genes are mutated, transposons can be excised, leaving a double-strand break, which must be repaired by the cellular machinery, either through homologous recombination or non-homologous end joining.

I have developed a strategy to visualize double-strand breaks in the germline generated by unregulated transposons in the transposon silencing mutants. This assay will allow me to look at transposon hopping in mutants that are sterile and possibly 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. 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 small RNA pathways and genome integrity, and further elucidate the mechanisms of transposon silencing in the germline.

CMP is the Marion Abbe Fellow of the Damon Runyon Cancer Research Foundation.

992C

TDP-1, the C. elegans ortholog of ALS-associated protein TDP-43, modulates RNA editing and RNAi. **Tassa K. Saldi**[1], Christine M. Roberts[2], Peter E. A. Ash[3], Leonard Petrucelli[3], Christopher D. Link[2]. 1) Molec, Cellular, Dev Bio, University of Colorado, Boulder, CO., USA; 2) Institute for Behavioral Genetics, University of Colorado, Boulder, CO, USA; 3) Department of Neuroscience Research, Mayo Clinic, Jacksonville, FL, USA.

RNA binding protein TDP-43 is found in ubiquitinated inclusions in a number of neurodegenerative diseases, most prominently Amyotrophic Lateral Sclerosis (ALS) and some forms of Frontotemporal Lobar Dementia (FTLD). Importantly, mutations in TDP-43, have been shown to be causal in the development of ALS. TDP-43 is a mostly nuclear protein with numerous characterized roles in RNA metabolism including the modulation of transcription, splicing and translation. However, the mechanism by which TDP-43 meditates neurotoxicity is unclear. C. elegans expresses a single TDP-43 homolog, TDP-1, which can substitute for TDP-43 in mammalian alternative splicing assays. Recent immunoprecipitation experiments with anti-TDP-43 antibodies followed by Mass-Spectrometry have identified the enzyme adenosine deaminase acting on RNA (ADAR) as a binding partner of TDP-43. ADAR enzymes are known to have pivotal roles in the nervous system, and it has been shown that motor neurons degenerating in ALS fail to appropriately edit the mRNA coding for GluR2, a glutamate receptor subunit. We are therefore investigating a role for TDP-1 in adenosine-to-inosine RNA editing. We find that deletion of tdp-1 results in the mis-regulation of A to I RNA editing in published ADAR targets. Further, we find that deletion of tdp-1 results in somatic sensitivity and germline resistance to RNAi. The possible roles of the C. elegans ADARs, adr-1 and adr-2, in these RNAi effects are currently being investigated. Preliminary data indicates that deletion of tdp-1 and adr-1 results in synthetic neuronal deficits, suggesting that TDP-1 modulation of RNA editing does have biological consequences.

993A

RNAi screen to identify new genes and pathways regulating PKD-2 ciliary localization and function. **Bahareh Shanehsaz**, Cory Patrick, Juan Wang, Maureen Barr. Genetics, Rutgers University, Piscataway, NJ.

Cilia are of profound medical importance in human health, yet how sensory cilia develop and signal remains poorly understood. We focus on a subset of male-specific ciliated sensory neurons, which express the C. elegans polycystins (PCs) LOV-1/PC-1 and PKD-2/PC-2 and are required for male mating behavior. In humans, mutations in PKD1 and PKD2, encoding PC-1 and PC-2 respectively, cause autosomal dominant polycystic kidney disease (ADPKD). The evolutionarily conserved polycystin genetic pathway, ciliary localization, and sensory function make the nematode an attractive model to study ciliary formation, morphogenesis, specialization, and signaling in the context of human genetic diseases of cilia.

To identify molecules required for polycystin localization and/or function, we are performing a neuron-specific RNAi screen using a strain expressing the SID-1 dsRNA transporter under the control of the pkd-2 promoter, which is amenable to high-throughput and enables knockdown of otherwise essential genes (1). We performed a pilot RNAi screen of 70 candidate genes, identified from the literature based on interaction with PC-1 or PC-2. We observed a variety of PKD-2::GFP ciliary localization (Cil) defects, including abnormal accumulation of PKD-2 along the cilium proper, at the ciliary base, along the dendrite, and in the cell body. We are currently examining RNAi effects on male mating behavior. Our long-term goal is to complete a genome-wide RNAi screen once we work out the logistics of screening and validating candidates. This approach will provide a comprehensive picture of the molecules that influence polycystin channel assembly and localization, and will provide important insight to ciliary receptor trafficking in general.

(1) Calixto A, Chelur D, Topalidou I, Chen X, Chalfie M. Enhanced neuronal RNAi in C. elegans using SID-1. Nat Methods. 2010 Jul;7(7):554-9.

994B

The mevalonate pathway has a role in microRNA repression of target genes. **Zhen Shi**[1,2], Gary Ruvkun[1,2]. 1) Dept. of Molecular Biology, Massachusetts General Hospital, Boston, MA; 2) Dept. of Genetics, Harvard Medical School, Boston, MA.

The mevalonate pathway is present in all higher eukaryotes and many bacteria and mediates the production of isoprenoids. The isoprenoids feed into a wide range of biosynthetic pathway: sterols, dolichol, ubiquinone, heme A, and the lipid moiety for protein prenylation. C. elegans possesses a functional mevalonate pathway but lacks enzymes for the cholesterol synthesis, suggesting that mevalonate generates precursors for the other biosynthetic pathways. Inactivation of genes function in the synthetic steps in the mevalonate pathway as well as drugs that inhibit this pathway (the statins) strongly enhance the retarded phenotypes of the hypomorphic mutation in the let-7 miRNA. These phenotypes are suppressed by the inactivation of let-7 targets, suggesting that mevalonate is needed for normal let-7 repression of target genes. We found that the non-cholesterol biosynthetic outputs of the mevalonate pathway are required for the repression of these let-7 targets. In addition, inactivation of the mevalonate pathway also results in the de-silencing of lin-14, the target of lin-4 miRNA, compared to stage-matched control worms. To address which downstream branch(s) of the mevalonate pathway regulate(s) the developmental timing, we are performing a candidate-based RNAi screen for gene inactivations that cause retarded developmental timing. The cherry-picked RNAi library we are screening includes genes that function in protein prenylation, dolichol, ubiquinone and heme A biosynthesis.

995C

Sensitized Backgrounds Reveal Critical Roles for microRNA Families. **Elizabeth J Thatcher**, Victor Ambros. Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA.

MicroRNAs (miRNAs) are a class of small regulatory RNAs that have been implicated in the control of many cellular functions including cell specification, differentiation, proliferation, and metabolism. A smaller number of miRNAs are expressed in stem cells and at early stages of development, including some that are thought to maintain pluripotency. Interestingly, even though many C. elegans miRNAs are highly conserved, for many single deletion knockouts or even family knockouts, phenotypes are subtle or not evident. C. elegans have 23 miRNA families, 9 of which are conserved through humans. The Horvitz group showed that most miRNAs are not essential for development or viability on their own. This indicates a high degree of functional redundancy among miRNA family members, and between unrelated miRNA families in the worm. Recent findings from the Abbott group illustrated that sensitizing the worm may reveal several mutant phenotypes associated with loss of individual miRNAs or families. I examined the phenotypes associated with several critical miRNA effector complexes to determine whether combining miRNA family knockouts were able to alleviate or exacerbate the observed phenotypes. For instance, RNAi of Alg-1 or Alg-2 produces characteristic developmental delays and disruptions that include problems with molting, slow growth, absent or missing alae, and protruding vulva. These abnormalities are occasionally severe enough to produce embryonic or larval lethality. Examining the effects of Alg-1 or Alg-2 RNAi on miRNA family mutants produces an interesting picture. Most notably, the loss of some miRNAs appears to alleviate the impact of RNAi on Alg-1 or Alg-2 to produce a less severe phenotype as compared to RNAi on wild-type N2 animals. Moreover, some family mutants are uniquely sensitive to either Alg-1 or Alg-2 RNAi. These experiments should illuminate how microRNA pathways are integrated with broader gene regulatory networks, and are adapted to control the robust expression of diverse developmental events.

996A

Piwi proteins and piRNAs: targets, targeting requirements and pathway analysis. **E.-M. Weick**, L. D. Goldstein, M. P. Bagijn, A. Sapetschnig, E. A. Miska. Wellcome Trust/CRUK Gurdon Institute, University of Cambridge, United Kingdom.

Over the last few years, a variety of different classes of small RNAs have been found to regulate gene expression in metazoans. Piwi-interacting RNAs (piRNAs), which are bound to the germ line expressed Piwi subfamily of Argonaute proteins, are one of the latest additions to the family of short non-coding RNAs. The Piwi-piRNA effector complexes have been found to play a role in protection of the germ line from mobile genetic elements, a function which is conserved across several species. How this important role is executed mechanistically and whether there are any other targets of piRNAs remains to be determined.

Employing Mos1-mediated Single Copy transgene Insertion (MosSCI) in *C. elegans*, we have generated a piRNA sensor strain carrying a GFP transgene with a sequence complementary to an endogenous piRNA. In the germ line of wild-type worms, GFP expression is silenced, whereas silencing is abolished in a mutant lacking the nematode Piwi protein PRG-1. Furthermore, GFP is also expressed in a strain containing a transgene with a non-complementary control sequence.

Small RNA high-throughput sequencing data of the sensor strain revealed a *prg-1* dependent localised endo-siRNA (22G RNA) response surrounding the piRNA recognition site within the transgenic transcript. Using this presence of an endo-siRNA signature as an indicator of targets, we identified a number of endogenous piRNA targets, including protein-coding genes, repetitive elements and pseudogenes. These targets are up-regulated upon loss of *prg-1* as confirmed by global mRNA sequencing analysis and by qPCR of selected candidates.

To study the requirements for target recognition we generated several strains analogous to our sensor strain in which mismatches were introduced in sets of nucleotides along the piRNA recognition site. Analysis of these strains using large particle flow cytometry revealed potential importance of the 5' end of the piRNA in target recognition. In addition to our experimental data, we also identified a similar trend for reduced mismatch tolerance in the 5' end when looking at endogenous targets.

Overall, generation of an endogenous piRNA sensor has provided us with a tool to efficiently study the *C. elegans* piRNA pathway. In addition to identification of endogenous targets and elucidation of some of the requirements for target recognition, we are now using a forward genetics approach to identify further pathway components.

997B

Repression of germline RNAi pathways in somatic cells by multiple synMuv B chromatin

complexes. **Xiaoyun Wu**[1], Zhen Shi[1], Mingxue Cui[2], Min Han[2], Gary Ruvkun[1]. 1) Dept. of Molecular Biology, Massachusetts Gen Hosp, and Dept. of genetics, Harvard Medical School, Boston, MA; 2) Department of Molecular, Cellular, and Developmental Biology, and Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309.

The Retinoblastoma (Rb) protein is a chromatin regulator that represses transcription in tumor suppression. *C. elegans* genes encoding homologues of Rb and many of its chromatin cofactors are class B synMuv genes that repress *lin-3/EGF* transcription during vulva development. Mutations in several synMuv B genes, including *lin-35/Rb*, cause somatic misexpression of the germline RNA processing granules (P granules) and enhanced RNAi. Expression analysis reveals that multiple small RNA components are also misexpressed in the soma of these mutant animals, uncovering the mechanism for enhanced RNAi. Various synMuv B mutants, however, differ in the subcellular architecture of their misexpressed PGL-1 granules, their profile of misexpressed small RNA and P granule genes, as well as their enhancement of RNAi and the related silencing of transgenes. These differences define three classes of synMuv B genes, representing three chromatin complexes, a LIN-35/Rb-containing DRM core complex, a SUMO-recruited Mec complex and a synMuv B heterochromatin complex, suggesting that intersecting chromatin pathways regulate these soma *vs.* germline features. Consistent with this, the DRM complex and the synMuv B heterochromatin complex were genetically additive and displayed distinct antagonistic interactions with the MES-4 histone methyltransferase and the MRG-1 chromodomain protein, both are germline chromatin regulators known to be required for the synMuv phenotype and the somatic misexpression of PGL-1. Thus intersecting synMuv B chromatin pathways conspire with synMuv B suppressor chromatin factors to regulate the expression of germline specific RNAi genes, which enables heightened RNAi response that in part distinguishes germline from soma. Regulation of small RNA pathway genes by human retinoblastoma may also underlie its role as a tumor suppressor gene.

998C

The RDE-10/RDE-11 complex is required for RNAi-induced mRNA turnover in *C. elegans*. **Huan Yang**[1,2], Ho Yi Mak[1,2]. 1) Stowers Inst Med Res, Kansas City, MO; 2) Dept of Molecular & Integrative Physiology, The University of Kansas Med Center, Kansas City, KS.

We have established a transgene in *C. elegans* that undergoes periodic silencing during larval development. From a forward genetic screen, we isolated multiple mutant alleles that conferred a transgene de-silencing phenotype and identified two previously uncharacterized proteins involved in RNAi and transgene silencing. RDE-10 does not have any identifiable conserved domains while RDE-11 has a RING-type Zinc finger. Loss of *rde-10* or *rde-11* function specifically impairs cytoplasmic RNAi but not nuclear RNAi. Genetic and proteomic analyses indicate that RDE-10 and RDE-11 are components of a complex that acts synergistically with the NRDE-2/3 nuclear RNAi pathway. We further show that RDE-10 is recruited to mRNAs that have been targeted by RNAi in a RDE-11 independent manner. Differential enrichment of target mRNA fragments 5' to the RNAi trigger in wild-type but not *rde-11* mutant animals suggests that target mRNAs co-purified with RDE-10 are subject to RDE-11 dependent nucleolytic degradation. We will further investigate this catalytic event by cloning the 3' end of target mRNA molecules. We propose that the RDE-10/11 complex cooperates with secondary Argonaute proteins to mediate RNAi-induced mRNA turnover in the cytoplasm.

999A

A Role for Systemic RNAi in antiviral defense in C. elegans. **Jing Zhong**[1,2], Gao Zhihuan[2], Morris Maduro[3], Ding Shou-wei[2]. 1) CMDB Program, University of California, Riverside, Riverside, CA; 2) Department of Plant Pathology and Microbiology, University of California, Riverside, CA; 3) Department of Biology, Institute for Integrative Genome Biology, University of California, Riverside, CA.

RNAi-mediated antiviral immunity is induced in C. elegans by either replication of the Flock House Virus (FHV) RNA genome from an integrated transgene or infection of naturally occurring RNA viruses. As a result, robust virus replication in C. elegans requires expression of a virus-encoded suppressor of RNAi (VSR) from the cognate viral RNA genome. These findings indicate that C. elegans can be used as a small animal model to investigate the genetic requirements of RNAi-mediated antiviral immunity in animals. In this study, we performed an EMS mutagenesis screen in a transgenic C. elegans strain carrying an inducible GFP-expressing FHV replicon, which is highly susceptible to the RNAi-mediated antiviral immunity because of the removal of the VSR protein B2 of FHV. We found that worm mutants defective in the antiviral immunity included those either defective or susceptible to RNAi induced by exogenous dsRNA, consistent with earlier observations that exogenous RNAi and antiviral RNAi pathways have overlapping and distinct genetic requirements. Using SNP mapping, sequencing of candidate genes and genetic complementation, we determined that one worm mutant harbors a mutation in RNAi spreading defective 2 (rsd-2), known previously to be required for the transport of the silencing signal from somatic tissues to the germline. Both green fluorescence and Northern blot analyses showed that the FHV replicon accumulated to higher levels in the rsd-2 mutant than in worm mutants defective in the core RNAi pathway such as rde-1. These results suggest that systemic RNAi plays an important role in antiviral defense in C. elegans. We will report progress with our studies on other genes known to play a role in systemic RNAi.

1000B

An integrated approach for in vivo miRNA regulation studies in *C. elegans*. **Ilyass Zniber**, Marie Bothorel, Léo Guignard, Denis Dupuy. Genome Regulation & evolution, INSERM U869, Institut Européen de Chimie et biologie, Université de Bordeaux, Bordeaux, France.

MicroRNAs (miRNAs) are 20-22 nucleotides RNA molecules that function as negative regulators of gene expression in both eukaryotic and procaryotic organisms.

Single stranded mature miRNA bind to target mRNAs through partly complementary sequences that are localized in their 3' untranslated region. The first discovered miRNA genes were *lin-4* and *let-7*, which have been identified initially in *Caenorhabditis elegans*.

PicTar, a powerfull target prediction algorithm for miRNA regulation, predicts that 10% of *C. elegans* genes are regulated by this mechanism.

To date, very few of these predictions have been experimentaly validated.

The aim of this project is to develop an approach for large scale experimental validation of over a hundred of predicted targets.

I built a reporter cassette where two fluorescent proteins are expressed in a bicistronic manner under the target gene's promoter to mimic the spatio-temporal expression pattern of the studied gene.

The mCherry (transcriptional output reporter) is associated to the permissive UTR of the *unc-54* gene while the GFP (post-transcriptional output reporter) is regulated by the target gene's UTR. Taking advantage of *C. elegans* fosmid library, we can replace the ORF of the target gene by the two-color reporter cassette using recombineering (recombinaison ingeneering) for 200 miRNA regulated predicted genes by PicTar. Reporter fosmids will be injected to *C. elegans* and expression profile will be analysed by microscopy for fine expression and by flow cytometry using COPAS® (Union Biometrica) for quantitative analysis.

As a proof of principle, I present several studies performed on transgenic worms carrying the bicistronic cassette reporter specific of several *let-7* targets.

1001C

Towards a unique gateway for predicting genetic interactions in *C. elegans*. **Benjamin Boucher**[1,2,3], Anna Y. Lee[4,5], Michael Hallett[4,5,6], Sarah Jenna[1,2,3]. 1) Department of chemistry, Université du Québec à Montréal, Montreal, Quebec, Canada; 2) Pharmaqam, Université du Québec à Montréal, Montreal, Quebec, Canada; 3) Biomed, Université du Québec à Montréal, Montreal, Quebec, Canada; 4) McGill Centre for Bioinformatics, Mcgill University, Montreal, Quebec, Canada; 5) School of Computer Science, Mcgill University, Montreal, Quebec, Canada; 6) Rosalind and Morris Goodman Cancer Centre, Mcgill University, Montreal, Quebec, Canada.

Recent developments in computational methods have brought new insights on how genes influence each other in biological networks. So far, several in sillico approaches have been successfully used to predict genetic interactions (GIs) in different species using weighted data integrations. While experimental identification of genetic interactions in multicellular organisms is highly challenging, development of powerful predictive methodologies will allow the biologist to prioritize experimental validations of GIs and therefore uncover genetic interactomes of these organisms. We report here, a global survey of the performance and complementarity of three predictors generated to identify genetic interactions in *Caenorhabditis elegans*. We showed that general poor performance observed for each predictor was compensated by a high level of complementarity. We also demonstrated that GIs form a heterogeneous family of entities that are predicted with a higher accuracy through generation of specific predictors for subgroups of GIs with similar biological functions. We generated such predictors and identified more than 300,000 novel GIs genome-wide. Predictions from all available tools accounted for about 70% of the total estimated number of GIs genome-wide compared to 40% with previous predictors only. We are currently generating a web interface that will enable the investigator to identify GIs for a given gene of interest using all currently available tools.

1002A

Essentially C. elegans: A step towards defining the Essentialome of the Worm. **Jeffrey S C Chu**[1,2], Bob Johnsen[1,2], Domena Tu[1,2], Steven Jones[1,2,3], Marco Marra[1,3], David Baillie[2], Ann Rose[1]. 1) Medical Genetics, University of British Columbia, Vancouver, BC, Canada; 2) Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; 3) BC Cancer Agency Genome Sciences Centre, Vancouver, Canada.

We are investigating the role of essential genes in *C. elegans*. Several essential genes have been characterized by the *C. elegans* community and the published literature shows that the essential genes are of great interest, not only because of their biological importance to the development of *C. elegans*, but also because of their relevance to human health. Initial approaches to identifying essential genes involved screening for lethal mutations (lethals) using genetic balancers. This approach has yielded thousands of mutations defining more than 500 essential genes. We have mapped mutations to chromosomes I, III, IV and V. We aligned genetic mutations with physical coding regions in order to identify the molecular basis of the lethals. This involved positioning the lethals by using three-factor mapping and complementation to deletions and duplications. Subsequently, cosmid and fosmid transgenic rescues were used to identify candidate coding regions. Finally, PCR analysis and DNA sequencing confirmed the coding region containing a lethal mutation. This is a labour intensive and long term project.

Currently we are using whole genome sequencing to identify the coding regions corresponding to essential genes. The facts that the mutations are precisely mapped and that there are, in most cases, more than one allele per complementation group, makes the identification of the mutation for a given lethal strain relatively easy. The cost of Illumina sequencing and subsequent bioinformatics analysis makes this approach very competitive. Here we present the results of our initial sequencing of chromosome I lethals balanced by sDp2 and propose the feasibility of sequencing the entire essential gene collection. The sDp2 region covers the left half of chromosome I and contains approximately 1350 predicted coding regions. We have identified 237 of these as essential genes by lethal analysis. Statistical analysis predicts about 400 (Johnsen R.*et al*., Mol Gen Genet, 2000) and RNAi has identified 409 coding regions resulting in lethal phenotypes (Wormbase WS223). This may be an upper limit on the number of essential genes in the sDp2 region because RNAi can knockout whole families of closely related genes. Knocking out one member of the family may not be lethal. Correlating the lethal mutations with their corresponding coding regions would greatly increase the genetic information and tools available for analysis of the essential biology of *C. elegans*.

1003B

WormBase Gene Sets & Sequence Curation -. **Paul Davis**[1], Tamberlyn Bieri[2], Kevin Howe[1], William Nash[2], Philip Ozersky[2], Gary Williams[1], John Spieth[2], The WormBase Consortium. 1) WTSI/EBI, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK; 2) The Genome Center at Washington University School of Medicine, St Louis, MO, USA.

WormBase (**www.wormbase.org**) is an online resource of biological data that has been collated and curated from the literature and other scientific sources. The primary goal of the resource is to facilitate the use of *C. elegans* as a model biological system and to achieve this WormBase continues to add new types of data. Over the past decade the curation of *C. elegans* genes and its associated sequence data has served as a primary focus for the WormBase consortium but this is changing. During the current funding cycle, the scope of curational effort has broadened to include that of other *Caenorhabditids* and selected other worm species. The basis of our biological knowledge about any of these species is underpinned by the genomic sequences and associated gene sets. WormBase remains the reference point for the most up to date genome and gene set for *C. elegans* and we are constantly working to achieve this status for the growing number of closely related and significant roundworm species provided by the resource. In this poster we outline what is being done for *C. elegans* and the other worm species in WormBase. We give examples of curational activities being undertaken with an emphasis on gene curation.

WormBase encourages the research community to submit data, feedback and corrections so that we can make this resource as accurate and complete as possible. WormBase curators can be contacted via the **help@wormbase.org** email address or by filling in the web form (**http://www.wormbase.org/db/misc/feedback**).

1004C

Building better gene and orthology predictions across ten Caenorhabditis genomes. **Olivia Gardner**, Ana Rodrigues, Yufeng Zhai, Gerard Manning. Salk Inst, La Jolla, CA.

Sequence and function analysis of worm genes can be greatly helped by comparative genomics. Ten Caenorhabditis genomes have now been sequenced, but accurate prediction of gene sequences and determination of orthology are still major challenges. For instance, 74% of C. elegans genes have defined orthologs in C. briggsae, but this number is much lower in draft genomes. We are using evolutionary principles to synergistically address both problems. We established a computational pipeline capable of fine-tuning gene structure, using conservation of both protein sequence and intron-exon structure, local synteny and EST data, across all available genomes. Initial tests with the C. japonica assembly enabled improvements in gene prediction for 34% of all singleton orthologs, and a substantial improvement in orthology prediction. This approach will enable the reconstruction of the evolutionary history of each C. elegans gene, and the detailed analysis of sequence constraints acting on each protein.

1005A

Investigating the roles of a worm HP1 homolog during embryogenesis. **Jacob Garrigues**, Thea Egelhofer, Susan Strome. Dept. of Molecular, Cell, and Developmental Biology, University of California, Santa Cruz.

Proper formation of the germ line during animal development is essential for the propagation of species. In *C. elegans*, a gene that encodes a homolog of Heterochromatin Protein 1 (HP1), *hpl-2*, is required for a functional germ line at restrictive temperature (1). While previously reported that a null allele of *hpl-2* has a maternal-effect sterile (Mes) phenotype (2), we found that this sterility can be rescued by zygotic expression of *hpl-2(+)*, suggesting that HPL-2 serves a critical role during embryogenesis. The observed Mes phenotype and the possibility, suggested by findings in *Arabidopsis* (3), that HPL-2 may bind histone H3 trimethylated at lysine 27 (H3K27me3) generated by the Polycomb-like repressive complex MES-2/3/6 prompted us to assess the distribution of HPL-2 genome-wide in embryos, by chromatin immunoprecipitation followed by microarray analysis (ChIP-chip). The distribution of HPL-2 does not correlate with H3K27me3, but instead correlates well with H3K9me2, is highly enriched on autosome “arms”, and is depleted from the X chromosome. At the gene level, HPL-2 binds genes with developmental roles, suggesting a direct regulatory role in their expression; many of these genes are associated with reproduction. Interestingly, a subset of HPL-2 peaks overlap with peaks of SET-2 (also obtained by ChIP-chip), a histone methyltransferase that generates much of the H3K4me signal associated with transcription initiation in worms. Furthermore, loss of HPL-2 results in increased H3K4me3 levels at these overlapping regions, suggesting that one role of HPL-2 is to antagonize the activity of SET-2 at transcription start sites. This may explain the genetic interaction between *hpl-2* and *set-2* previously observed (4), and may demonstrate a new role for HP1 proteins during development. 1. Couteau *et al*. (2002) *EMBO Rep* 3, 235-241. 2. Coustham *et al*. (2006) *Dev Biol* 297, 308-322. 3. Zhang *et al*. (2007) *Nat Struct Mol Biol* 14, 869-871. 4. Simonet *et al*. (2007) *Dev Biol* 312, 367-383.

1006B

Identification of new DNA targets of nuclear envelope proteins in C. elegans using the DamID technique. **Cristina Gonzalez-Aguilera**, Peter Askjaer. Andalusian Center for Developmental Biology (CABD), Universidad Pablo de Olavide-CSIC, Seville, Spain.

The nuclear envelope (NE) has emerged as an important structure that serves numerous pivotal roles in the cell including compartmentalization, control of nuclear position and morphology, contribution to cell stability, chromatin organization and regulation of gene expression. The mechanism by which the NE controls gene expression is not well understood yet. Traditionally, the anchoring of chromatin to the nuclear periphery has been associated with silencing and heterochromatin formation. However, recent studies have shown that there is also actively transcribed chromatin at the NE, specially associated with the nuclear pore complexes. To unravel how the NE can regulate gene expression, we have developed tools to perform genome wide analysis using the DamID method. This method is based on the expression in vivo of chimeric proteins containing an adenine methyltransferase (Dam) from E. coli that methylates the DNA in the vicinity of native binding sites of a chromatin-interacting protein. Using the MosSCI technique, we have created Caenorhabditis elegans strains containing single copy insertions of Dam fused to NE and nuclear pore proteins such as emerin/EMR-1, lamin/LMN-1 and Nup98/NPP-10N. Employing a genetically amenable model system enables us to analyze nuclear architecture across several mutant backgrounds, and we are currently exploring methods to control expression of the Dam fusion proteins in a temporal manner and in specific tissues. We have confirmed the correct expression and localization of our fusion proteins and we are currently analyzing DNA binding sites using whole-genome tiling arrays and qPCR.

1007C

Links between histone modifications and splicing. **Moritz S Herrmann**, Paulina

Kolasinska-Zwierz, Julie Ahringer. The Gurdon Institute, Department of Genetics, University of Cambridge, United Kingdom.

Chromatin modifications comprise an essential layer of gene regulation. We previously showed that one particular chromatin modification, tri-methylation of lysine 36 on histone H3 (H3K36me3), is enriched on exonic regions of transcribed genes relative to intronic regions (Kolasinska-Zwierz et al., 2009). This observation suggests a link between chromatin modification and splicing. We are using a number of approaches to test the function of exon marking and to elucidate how it works.

First we are searching for enzymes involved in H3K36me3 exon marking. We are studying both histone methyltransferases and histone demethylases, as both could contribute to exon marking. Histone lysine methyltransferases are usually characterized by possession of a SET domain whereas histone lysine demethylases are characterized by JmjC domains. Previous work showed that mutants of the SET domain gene met-1 have reduced overall levels of H3K36me3 whereas mutants of the JmjC domain gene *jmjd-2* have increased H3K36me3 levels (Andersen and Horvitz, 2007; Whetstine et al, 2006). By ChIP-chip analysis, we find that exon marking is reduced but not eliminated in *met-1* mutants. We are currently screening for additional enzymes that contribute to tri-methylation of H3K36 and testing roles for C. elegans JmjC domain proteins in H3K36me3 exon marking.

Using ChIP-seq, we are also mapping the genome-wide locations of SET and JmjC domain proteins implicated in trimethylation of H3K36. Concurrently, we are using RNA-seq to test whether changes in exon marking result in altered splicing patterns.

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1008A

Quantitative epistasis map of genes regulating sex ratio. **Marta K Labocha**, Wenshan Luo, Boanerges Aleman-Meza, Weiwei Zhong. Biochemistry and Cell Biology, Rice University, Houston, TX.

Caenorhabditis elegans has two sexes, hermaphrodites (XX) and males (XO). Males are very rare among the self-progeny of wild-type hermaphrodites (1:500 to 1:2000). Several genetic pathways are known to affect sex ratios, including meiotic chromosome segregation, dosage compensation and sex determination pathways. When inactivated by RNAi, over 100 genes were found to give the high incidence of males (*him*) phenotype (WormBase WS212). Yet, the functional mechanisms and pathway information for the majority of these genes remain unknown. Therefore, we decided to conduct a quantitative epistasis study to uncover the genetic pathways and networks of these *him* genes.

To enable a high-throughput quantitative screen, we first developed a computer vision system to automatically measure *C. elegans* sex ratios. Using a motorized stage and a camera attached to the microscope, the system scans Petri plates and records images of animals. The system analyzes morphological features of the animals and outputs the numbers of adult males and hermaphrodites on each plate.

To identify pairwise interactions among genes, we inactivate each gene by RNAi or mutation, and measure the sex ratio of these single mutants using the automatic phenotyping system. We use these single mutant data to compute an expected sex ratio for the double mutant assuming that the two genes function independently. We then inactivate two genes by applying RNAi on mutants and examine whether the observed double mutant sex ratio is significantly different than the expected value. An enhancing interaction is identified if the observed phenotype is more severe than the expected value, and a suppressing interaction is denoted if the observed phenotype is milder.

In a pilot study, we selected mutants of five meiosis genes (*brc-1*, *brd-1*, *him-3*, *him-5*, and *zhp-3*) and screened them for possible interactions with 124 genes that gave the *him* phenotype. From over 600 gene pairs screened, we discovered over 100 genetic interactions. Among them, 55% interactions were suppressing, and 45% were enhancing. The majority (over 97%) of these interactions are novel ones that have not been reported before. These data suggest that our quantitative epistasis approach is a powerful tool to identify genetic interactions. 44 of the 124 *him* genes have homozygous viable mutants. We are now in the process of composing the 44x124 gene interaction map. We will report our results at the meeting.

1009B

A transcription factor specific RNAi screen to identify regulators of intestinal gene expression. **Lesley T. MacNeil**, H. Efsun Arda, A.J. Marian Walhout. Program in Gene Function and Expression, University of Massachusetts Medical School, Worcester, MA.

The assembly of gene regulatory networks has been accomplished through a number of different, complementary approaches. Techniques that identify protein-DNA binding events, including chromatin immunoprecipitations and yeast one-hybrid assays, have identified a number of physical interactions between genes and transcription factors (TFs). However, the regulatory consequences of these interactions remain largely unknown. We sought to develop a complementary *in vivo* approach to identify regulatory rather than physical interactions between genes and their regulators. We generated a TF-specific RNAi feeding library. Using both ORFeome clones, clones from the Ahringer library and additional clones made in house, we were able to generate constructs for a total of 913 TFs, which is 97% of all 940 predicted *C. elegans* TFs. In order to identify gene-specific regulators of gene expression, we used this library to screen transgenic animals carrying intestine specific transcriptional GFP fusion constructs. RNAi-treated animals were visually examined for changes in GFP expression; either lower expression, higher expression or a spatial change in expression. Screening was accomplished in a 96-well format with each transgenic line screened in three independent replicates. TFs scoring positive in at least two experiments were considered true positives. As expected, we found that *elt-2* regulates the expression of all intestinal transgenes assayed. This is consistent with the previous identification of *elt-2* as a global regulator of intestinal expression (McGhee et al., 2009. Dev.Biol. 327) and demonstrates that we are able to identify functionally relevant interactions using this approach. In addition, we have identified specific regulators for a number of intestinal transgenes. Together, our data indicate that our RNAi screening platform will be a valuable addition to the toolkit of regulatory network studies.

1010C

Characterization of the Transcriptome in L1 Arrest and Recovery with High Throughput Sequencing. **Colin Maxwell**[1], Igor Antoshechkin[2], Nicole Kurhanewicz[1], Jason Belsky[1], Ryan Baugh[1]. 1) Department of Biology, IGSP Center for Systems Biology, Duke University, Durham, NC; 2) Division of Biology, California Institute of Technology, Pasadena, CA.

For *C. elegans*, life in the wild is characterized by feast or famine. When the worm hatches in the absence of food it arrests in the first larval stage and increases resistance to numerous environmental stresses (L1 arrest, or L1 diapause). However, the transcriptional and post-transcriptional mechanisms that coordinate physiological responses to nutrient availability are not well understood. In particular, nutrient availability has a greater impact on transcription than known mechanisms can account for, and the extent of post-transcriptional regulation has not been explored. To characterize the influence of nutrient availability on the transcriptome and to discover mechanisms that differentiate L1 arrest from L1 development, we sequenced the transcriptomes (RNA-seq) of a time series of animals during L1 arrest and recovery. We find that RNA-seq is more sensitive than microarray analysis, has substantially more power to detect differential expression, and identifies differences in the behavior of transcript isoforms that would not be apparent with microarrays. Of the 20,985 Wormbase gene models, we detect expression for 17,508 (83%) of them. Of these, 6,679 (38%) are differentially expressed (FDR .1%). Of the 4,122 gene models with multiple transcript isoforms, we detect at least two of them in 2,643 (64%) cases. We rank these by the change in isoform composition across the time series and find hundreds of examples of isoforms in the same gene model with differing trajectories. We find that isoforms that change abundance relative to other isoforms in the same gene model tend to differ from those isoforms by coding regions, start sites, and non-coding regions simultaneously. In particular, we find no evidence that genes with varying isoform proportions are regulated exclusively transcriptionally or post-transcriptionally. Validation of these results is underway. By determining how the nematode transcriptome varies in response to this major environmental change, we lay the groundwork for mechanistic analysis of how nutrient availability governs post-embryonic development, influencing phenotypic plasticity and developmental robustness.

1011A

Analysis of mutation patterns in *C. elegans* strains with DNA repair deficiencies. **Bettina Meier**[1], Peter Campbell[2], Anton Gartner[1]. 1) WT Centre for Gene Regulation and Expression, University of Dundee, Dundee, UK; 2) Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK.

A number of human inherited cancer syndromes result from defects in DNA damage repair pathways such as mismatch (MMR), base excision (BER), nucleotide excision (NER) and double-strand break repair (DSB). Inefficient DNA repair, facilitated by mutations or changes in chromatin modification, can drive further genomic alterations required for cancer development. Moreover, certain genomic regions, such as repetitive sequences or sequences able to form secondary structures such as G4 quadruplexes, provide an obstacle for the replication and repair machineries in unchallenged cells. We know relatively little about spontaneous mutations rates and how this is prevented by the various DNA repair pathways when cells are not challenged by genotoxic agents. In addition, while telomerase is required for maintaining telomere length and integrity, the mutagenic burden induced by short, dysfunctional telomeres is not fully ascertained. To assess spontaneous mutation rates in various DNA damage response and telomere replication mutants compared to wild-type, we backcrossed the mutant strains extensively to wild-type to obtain nearly isogenic lines, which we are currently propagating for 20 generation before subjecting them to next generation sequencing. In the long term, we plan to initiate systematic studies to assay mutation patterns of a large number of known or suspected carcinogens in wild-type and DNA damage response defective mutants. Besides measuring mutagenesis rates, we aim to asses, if any of the mutation patters observed upon treatment of worms with distinct carcinogens reflect mutation patterns observed in human cancer whole genome sequencing projects. We will present the progress of our work. We note that access to thousands of mutations detected by systematic sequencing of a large number of “mutagenised’ worms would provide an invaluable tool for the *C. elegans* community.

1012B

Estimation of gene regulatory networks of C.elegans early embryo. **A. Okano**[1], K. Monobe[2], S. Nakayama[1], M. Ito[2], T. Maeshiro[1]. 1) University of Tsukuba, Tsukuba, Japan;

2) Ritsumeikan University, Kusatsu, Japan.

We have constructed a series of gene regulatory networks from microarray data of C.elegans early embryo, measured every ten minutes for initial 50 minutes starting from the single cell stage. Generated gene networks correspond to each measurement, i.e. 0, 10, 20 minutes and so on. Genome wide microarrays from Affymetrix were used. The generation method of gene regulatory network is based on the gene regulatory network of Sea urchin early embryo, which is experimentally verified network and thus of high confidence. Then genes belonging to the Sea urchin network is used as query sequence to search in C.elegans gene set. Highly homologous worm genes with significant expression value obtained from microarray data are included to the predicted gene regulatory network. Gene regulatory relationship is created if the Sea urhchin query genes are in regulatory relationship and if the expression values of C.elegans genes are not ambiguous from the gene regulatory types (activation or repression). Five gene regulatory networks were created. Network analysis is used to extract hub genes, the genes that regulate large number of other genes. The molecular function of hub genes identified based on wormbase database are: cell division, transcription factor, DNA binding, nucleotide binding, zinc ion binding, double helix binding, and enhancer binding. Temporal expression data was used for clustering process, resulting in 119 expression groups, which correspond to gene regulation groups.

1013C

Uncovering cryptic genetic variation for C. elegans embryogenesis. **Annalise Paaby**, Amelia White, Kris Gunsalus, Fabio Piano, Matt Rockman. New York University, New York, NY.

Cryptic genetic variation (CGV) is allelic variation that affects phenotype, but only under certain conditions: when the system is “perturbed” by changes in the environment or genomic background. Such conditional effects are probably common in biological systems, but they pose barriers to the identification of causal alleles that underlie complex traits.

In an to effort understand the nature of CGV, we are exploring the genetic architecture of early embryogenesis in C. elegans. Genome-wide screens have identified genes that affect embryogenesis in a single wild-type background (N2), providing a high degree of resolution in our understanding of the genetics underlying this process. We are utilizing this information to knock down embryonic genes in wild isolates, in order to identify natural allelic variants that affect early embryogenesis in perturbed animals. Embryogenesis is normally invariant, but using RNAi to silence critical embryonic genes reveals differences in embryonic lethality across strains.

We have used high-throughput phenotyping methods to evaluate differences in hatching across 64 wild C. elegans strains, silenced at 43 different genes. The patterns of lethality indicate significant levels of CGV for embryogenesis. Some genes reveal high variance in lethality, suggesting that these loci are particularly good perturbation targets for revealing CGV elsewhere across the genome. We also observe significant variation in sensitivity to germline RNAi in these worms.

1014A

Unique mono-nucleosome occupancy on the Caenorhabditis sex chromosome. **Christine G. Preston**[1], Yvonne Fondufe-Mittendorf[2,3], Jonathan Widom[2], Barbara J Meyer[1]. 1) Molec Cell Biol, UC Berkeley, Berkeley, CA; 2) Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston Il; 3) Molecular and Cellular Biochemistry, University of Kentucky, Lexington KY.

The mechanisms by which chromatin structure affect gene expression are under active study. Research in mammalian and Drosophila dosage compensation systems has revealed the importance of chromatin structure and histone modifications in the chromosome-wide regulation of gene expression. Caenorhabditis elegans dosage compensation provides another well-characterized model for studying long-range gene regulation. The DCC resembles mitotic condensin, a complex known to restructure and compact chromosomes in preparation for cell division. It remains unknown whether the condensin-like DCC restructures chromatin or chromosomes as part of the nematode dosage compensation mechanism. Recruitment of the DCC to specific locations on X is associated with a sequence motif. Once recruited to X, the DCC binds to non-recruiting sites, often in the promoters of actively expressed genes. The non-recruiting sites are not correlated with known sequence motifs, suggesting that other factors catalyze the targeting and dispersal of the DCC. To study the underlying chromatin architecture of C. elegans DCC binding sites and the X chromosome we sequenced mono-nucleosomes from several developmental stages, DCC mutant backgrounds, and nucleosomes reconstitued in vitro on genomic DNA. Supporting previous reports, we show a chromosome-wide bias favoring increased nucleosome occupancy directly upstream of gene start sites along the X chromosome. This additional X chromosome nucleosome occupancy is reflected in a sequence-based prediction model for both C. elegans and C. briggsae and recapitulated in in vitro reconstituted nucleosomes. Sites of DCC binding correspond with an increase in average measured and predicted nucleosome occupancy, suggesting that DNA-encoded nucleosome occupancy is not strongly inhibitory for DCC binding. We observe differences in nucleosome occupancy upstream of X and autosomal gene start sites that correlate with changes in developmental age and gene expression. Additionally we identified specific genetic loci with measurable changes in nucleosome occupancy or positioning between in vitro and in vivo nucleosomes. The unique chromatin architecture of the X suggests the sex chromosome has experienced distinct evolutionary pressures compared to the autosomes, and furthers our understanding of how chromatin affects specific biological processes.

1015B

Whole-genome analysis of alternative splicing in C. elegans. **Arun Ramani**[1,2], John Calarco[1,2], Qun Pan[2], Sepand Mavandadi[2], Ying Wang[3], Andrew Nelson[4], Leo Lee[2], Quaid Morris[2], Benjamin Blencowe[1,2], Mei Zhen[1,3], Andrew Fraser[1,2]. 1) Molecular Genetics, Univ Toronto, Toronto, ON, Canada; 2) Donnelly Centre, University of Toronto, ON, Canada; 3) Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 4) Department of Physiology, Development and Neuroscience, Univerisity of Cambridge, Cambridge, UK.

Alternative splicing (AS) is a crucial mechanism playing a prominent role in gene expression. This is a complex process aided by many proteins and protein complexes that is not well understood. Identification of these factors, genes they modify, their modes of action and the spatio-temporal regulation are of critical importance. We have developed a new resource to facilitate such analyses in Caenorhabditis elegans. To identify known events, discover novel splice events, we combined quantitative AS microarray profiling and next generation transcriptome sequencing data. Using data from various stages of development, we were able to identify thousands of novel splicing events, including hundreds of temporally regulated AS events. In addition, we have developed the C. elegans Splice Browser (http://splicebrowse.ccbr.utoronto.ca) to enable easy access to these data. This resource should aid researchers to extend large-scale exploration of splice variants to focused, high resolution in vivo functional studies.

1016C

The *C. elegans* transcriptome. **Valerie Reinke**[1], Brent Ewing[2], Mark Gerstein[3], Phil Green[2], S Henz[4], Amber High[2], LaDeana Hillier[2], Masaomi Kato[5], J Leng[3], Michael MacCoss[2], Gennifer Merrihew[2], David M Miller, III[6], Gunnar Ratsch[7], Rebecca Robilotto[3], Frank J Slack[5], W Clay Spencer[6], Pnina Strasbourger, Owen Thompson[2], Guilin Wang[1], Georg Zeller[8], Robert H Waterston[2]. 1) Dept Genetics, Yale Univ, New Haven, CT 06520; 2) Department of Genome Sciences, University of Washington, Seattle, WA, 98195; 3) Program in Computational Biology and Bioinformatics, Yale University, Bass 432, 266 Whitney Avenue, New Haven, CT 06520, USA; 4) Department of Molecular Biology, Max Planck Institute for Developmental Biology, 72076 Tubingen, Germany; 5) Dept MCDB, Yale University, PO Box 208103, New Haven, CT 06520; 6) Department of Cell and Developmental Biology, Vanderbilt University, Nashville, Tennessee 37232; 7) Friedrich Miescher Laboratory of the Max Planck Society, 72076 Tubingen, Germany; 8) European Molecular Biology Laboratory, 69117, Heidelberg, Germany.

As part of the modENCODE consortium, we are characterizing the C. elegans transcriptome using tiling arrays, RNA-seq, RT-PCR and mass spectrometry. Our earlier studies on whole animals of various stages and conditions and on specific cells and tissues led to a much improved set of protein coding genes covering greater than 95% of all genes including more than 12,413 trans-spliced leaders, 20,515 different trans-spliced transcript start sites, 28,199 polyA sites, 111,786 confirmed splice junctions, >7,000 inferred non-coding (nc) RNAs, and over 50 new miRNAs (1-5). More recently, we have (1) analyzed biological replicates with RNA-seq for different stages and conditions, validating the observed expression levels; (2) closed gaps in RNA-seq coverage of weakly expressed genes with RT-PCR; (3) characterized the RNA content of more finely staged embryos with RNA-seq; (4) tested methods that deplete rRNA to allow direct analysis by RNA-seq of ncRNAs and smaller samples, such as specific embryonic cells and tissues; (5) analyzed polyA+ RNA from selected stages of C. briggsae, C. remanei, C. brenneri and C. japonica; (6) analyzed miRNAs under additional stresses and conditions; and (7) characterized the proteins present in 12 size fractions from 16 different stages and conditions. All of the data are available through the modENCODE Data Coordinating Center and increasingly through WormBase. Our goal is to provide the community with a comprehensive description of the transcripts of the C. elegans genome, providing information about their specific utilization where possible. References 1. Hillier et al. Genome Research PMID: 19181841 2. Gerstein et al Science PMID: 21177976 3. Lu et al. Genome Reseaarch PMID: 21177971 4. Allen et al. Genome Research PMID: 21177958 5. Spencer et al. Genome Research PMID: 21177967.

1017A

Evolution of a Transcriptional Program: Tracking the Heat Shock Response Across Phyla. **Ana P. Rodrigues**, Gerard Manning. Salk Inst, La Jolla, CA.

In eukaryotes, heat shock (HS) induces an emergency stress response that involves a complex transcriptional program mediated by HS Factor-1 (HSF-1). HSF-1 also mediates several other external and internal stresses, and has recently been found to influence carcinogenesis, lifespan and promote disaggregation of amyloids in Alzheimer’s disease models. A large body of research on HSF-1’s canonical target, the hsp-70 promoter, has unveiled much detailed information on its binding site and binding mechanism. However, attempts to understand the full genomic scale and logic of its action have had limited success. We are leveraging available expression profiling data with large scale comparative promoter analysis to describe the HS response in four diverse eukaryotic phyla, characterize the variety of cis-response elements and track the evolution of both response elements and expression program within and between these phyla. We analyzed all publicly available whole-genome expression profiles involving HS and/or HSF-1 manipulations in S. cerevisae (177 chips), C. elegans (41 chips), D. melanogaster (77 chips), and H. sapiens (110 chips) to establish a set of core consistent HS responding genes, beyond its canonical targets. Comparison of such large numbers of relatively noisy experiments, combined with enhanced orthology detection, enabled us to identify both high confidence genes across the four model systems, as well as phylum specific genes. We found that the proteins encoded by these genes are enriched for expected stress-response functions, but also include many unexpected metabolic functions, frequently in key pathway positions. We integrated this information with the limited publicly available HSF-1 ChIP-chip data to investigate how

HSF-1 interacts with this larger set of putative targets. Our methodology for binding site identification exploits the cooperative nature of HSF-1 DNA binding to suggest degenerate binding sites, and uses the comparative information distilled from genome sequences to support them. This enabled us to expand the canonical HSF-1 binding site definition, and identify groups of genes enriched for novel site architectures, including various gapped multimeric sites. In addition, we found that despite the limited overlap between experimentally determined DNA-binding and transcriptional response, this overlap is substantially enriched in promoters with conserved binding sites, indicating that evolution can help distinguish productive from non- productive binding. Through experimental collaborations, we are also studying the targets and timing of HSF-1 activity in proteostasis, protection against amyloid formation and aging.

1018B

WORMFOOD GENOMICS: E. COLI DETERMINANTS OF C. ELEGANS' FITNESS. **Buck S. Samuel**[1], Tim Durfee[2], Holli Rowedder[1], Chris Carr[1], Justine Melo[1], Jeremy Glasner[2], Sean Sykes[3], Sarah Young[3], Carsten Russ[3], Guy Plunkett[2], Chad Nusbaum[3], Gary Ruvkun[1]. 1) Dept. of Molecular Biology, Mass. General Hospital and Dept. of Genetics, Harvard Medical School, Boston, MA; 2) Department of Genetics and Genome Center of Wisconsin, University of Wisconsin, Madison, WI; 3) Genome Sequencing and Analysis Program, Broad Institute of MIT and Harvard, Cambridge, MA.

Like other metazoans, C. elegans fitness (success) within its microbe-rich habitats depends on a tight balance of energy acquisition and expenditure. Thus, it is also highly tuned to microbial cues that allow it to separate potential food or friend from foe. Accordingly, some microbial signals have been postulated to influence fat storage in parallel to endogenous endocrine cues. Several studies also show that the E. coli-adapted N2-Bristol strain is especially sensitive to 'minor' differences in E. coli strains: faster growth rates, increased progeny delivery rates, and less fat retention is seen when worms consume HB101 compared to OP50. Perhaps due to this fitness benefit, worms also exhibit increased satiety and a behavioral preference for HB101. Thus, we have sought to identify the E. coli gene products that modulate C. elegans fitness. To this end, we have sequenced E. coli genomes routinely used in C. elegans cultivation: HB101 (2 isolates), OP50 (2 isolates) and HT115. Despite little variation among strain isolates, 350 and 412 genes are 'unique' to OP50 and HB101, respectively. Many are organized into clusters, and represent a range of gene functions: e.g., carbohydrate utilization (96), cell wall/LPS modification (42), amino acid metabolism (21), regulation (41), the Cascade system (6) and fatty acid metabolism (5). Phenotype microarrays were also used to confirm the metabolic defects. In order to systematically test the impact of these microbial gene products on C. elegans' fitness, we assembled nearly 200 single gene mutants with defined function in a 'neutral' and consistent genetic background (E. coli K12). We then used a number of assays to test a mutant's impact on N2 growth, broods, body size and fat storage. Our analyses indicate that both genes in core metabolism and transport/biosynthesis of conserved mediators of host interaction—autoinducers, biogenic amines, short-chain fatty acids and LPS—influence N2 fitness. Studies of these small molecules as sensory or nutritive cues to C. elegans directly or via regulation of E. coli metabolism are ongoing. However, results so far indicate that the microbial milieu of signals may be just as important of a determinant of C. elegans' fitness as the nutritional potential for supporting growth of a population within a given habitat.

1019C

An alternative analysis pipeline for mutation identification by next-generation sequencing. **Harold E. Smith**, Michael W. Krause. Genomics Laboratory, NIDDK, NIH, Bethesda, MD.

Next-generation sequencing provides a rapid and powerful means for identifying mutations on a genome-wide scale. Advances in sequencing technology have sparked similar improvements in software for mutation analysis. A combination of BFAST (for alignment), SAMTools (for mutation calling), and ANNOVAR (for annotation) was used to analyze Illumina short-read sequence data. BFAST utilizes a local alignment algorithm, similar to BLAST, that is more sensitive to small (1-4 base pairs) insertions and deletions than earlier alignment programs. SAMTools offers a number of user-defined parameters that allow for more selective screening of candidate mutations. ANNOVAR provides a simple, text-based annotation tool that parses the data for non-synonymous mutations in protein coding sequences. A number of mutations have been successfully identified and validated using this analysis pipeline. A novel approach for identifying larger deletions and insertions, including transposons, will also be presented.

1020A

High Occupancy Target (HOT) regions in C. elegans. **Eric Van Nostrand**[1], Stuart Kim[1,2], C. elegans modENCODE consortium. 1) Genetics, Stanford University, Stanford, CA; 2) Developmental Biology, Stanford University, Stanford, CA.

As part of the C. elegans modEncode project, regulatory targets for all C. elegans transcription factors (TFs) are being identified by ChIP-seq. In initial analyses of ChIP-seq for 23 transcription factors, we unexpectedly discovered that transcription factors have a continuum of downstream targets, ranging from targets that are bound specifically by that factor to a new type of DNA domain that is bound by most or nearly all TFs. 304 regions of clustered TF binding (bound by 15 or more TFs) were observed, which we term HOT (High Occupancy Target) regions. Genes located near HOT regions are characterized by ubiquitous and high expression, and tend to be essential for viability. Thus, large-scale analysis of worm ChIP-seq data has led to the discovery of an unexpected gene regulatory mechanism for essential, ubiquitous housekeeping genes. In contrast to HOT targets, the factor-specific targets were significantly enriched for genes with an expression pattern similar to the bound transcription factor, and encoded proteins with functions similar to the biological function of the bound transcription factor. For example, HLH-1 is a key regulator of muscle development, and the factor specific targets in the HLH-1 ChIP-seq experiment were enriched for genes expressed specifically in muscle and were induced in previous HLH-1 over-expression experiments. By contrast, the HOT regions bound by HLH-1 showed no association with muscle functions. Thus, for interpretation of target genes with specific biological functions from ChIP-seq experiments of tissue-specific transcription factors, it may be necessary to remove HOT regions from the analysis and focus on factor - specific targets.

1021B

Loss-of-Function genomic variations in wild *Caenorhabditis elegans*. **Ismael A. Vergara**, Maja Tarailo-Graovac, Jun Wang, Nansheng Chen. Molec Biol & Biochem, Simon Fraser Univ, Burnaby, BC, Canada.

As revealed by an explosive number of genome sequencing projects, humans carry an enormous amount of genomic variations (GVs). Strikingly, many of these GVs found in apparently healthy individuals are loss-of-function mutations previously associated with genetic diseases, including many types of cancer and mental retardations. How these GVs function in apparently normal and disease humans is not well understood, due to two major challenges. First, the large size of human genes, the large intergenic regions, and the large quantity of GVs residing within each gene together make it challenging to correlate GVs and affected genes. More importantly, it is difficult if not impossible to interrogate the impact of most functionally important GVs on the fitness of their carrier, hence impeding further understanding of their contribution to the pathogenesis of disease conditions in humans. Using Roche/454 (4X coverage) and Illumina sequence reads (72X coverage) in a complementary manner, we have identified various types of GVs with base pair resolution in the wild *C. elegans* strain CB4856, which was first isolated in an island in Hawaii. Using N2 strain as reference, we have identified 313,219 putative SNPs, 21,332 small indels, 533 large deletions and 208 large insertions, some of which are larger than 10,000 bp. Also, we have detected hundreds of insertions and deletions co-occurring at the same breakpoint. Inspection of the breakpoints has revealed patterns of local duplications as well as events of non-allelic homologous recombination. Among these GVs, we have identified candidate loss-of-function mutations in 141 different essential genes in *C. elegans*, nine of which are orthologs of human disease genes (OMIM). The biological impact of these loss-of-function GVs on the fitness of CB4856 is being analyzed.

1022C

A systematic examination of the effects of natural variation on perturbed phenotypes in Caenorhabditis Elegans. **Victoria Vu**[1,2], Arun Ramani[2], Tungalag Chuluunbaatar[2], Hong Na[2], Andrew Fraser[1,2]. 1) Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada; 2) Terrence Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada.

Common human disorders are complex. Disease risk depends on multiple inherited genetic variations. Differences in genetic background between individuals thus have a major effect on the outcome of inheriting any single disease-related allele, and the main goal of this project is to begin to assess directly the effect of genetic background on loss of function phenotypes. We will use RNA interference in Caenorhabditis elegans to generate loss-of-function phenotypes in multiple different natural isolates. We can thus systematically examine how a given monogenetic perturbation leads to a diversity of phenotypic consequences in different individuals of the same species. Having identified genes with differing RNAi phenotypes between any two isolates, we then use standard mapping strategies to identify the quantitative trait loci accounting for the diversity. In this way we can examine the genetic basis for the variation in loss-of-function phenotype of each gene examined. This will give us a comprehensive view of how genetic background impacts phenotypic variation. We have almost completed this first for two isolates, N2 and Hawaii, and will present the results of our screening and analysis here. To facilitate this large-scale study, we are using a manual screen to score lethality, brood size defects, and growth defects. Using a novel quantitative assay, we can measure precisely the quantitative effect on fitness of RNAi against any gene and this is a powerful tool for accurate comparisons between isolates. This assay uses a standard worm sorter to count progeny over an extended period of 4 days and we will presenting our experimental design and preliminary findings.

1023A

Tissue-specific identification of polarity protein complexes. Selma Waaijers, Javier Muñoz, Albert Heck, **Mike Boxem**. Utrecht University, Utrecht, The Netherlands.

The polarization of epithelial cells depends on the activity of three main polarity complexes: the Crumbs, PAR-3, and Scribble complexes. The domain architecture of these proteins suggests that they function as scaffolding proteins, recruiting and interacting with multiple protein partners. We are attempting to systematically identify novel interaction partners for these proteins through an affinity purification/mass spectrometry (AP/MS) approach.

AP/MS approaches to date have mainly been used in unicellular systems, such as yeast or tissue culture. In multicellular organisms such as *C. elegans*, the composition of protein complexes likely varies between tissues. For example, depending on the tissue type examined, PAR-3 is found in a complex with PAR-6 and aPKC, or PAR-3 and PAR-6/aPKC seem to have independent functions (e.g., in *C. elegans* epidermal epithelial cells or the *Drosophila* blastoderm epithelium). To accurately determine the composition of protein complexes, it is necessary therefore to develop technologies to purify proteins from specific tissues. In addition, this would allow us to compare protein complexes between different tissues, potentially revealing functional insights.

To purify proteins from specific tissues we are using an *in vivo* biotinylation based approach. Proteins of interest are tagged with GFP and a small (14aa) peptide (the Avi tag), that can be recognized and biotinylated by the bacterial biotin ligase BirA. To tag the proteins, we are using a fosmid-based recombineering approach, which allows us to maintain all the regulatory sequences controlling the expression of the protein. Transgenic lines expressing the GFP-Avi tagged protein are crossed with lines expressing BirA from tissue-specific promoters, resulting in biotinylation of the tagged protein only in the tissue of interest. Protein complexes are then purified from worm lysates using streptavidin beads. To identify the components of these complexes, we are using a quantitative mass spectrometry approach.

1024B

Sperm Mitochondria are Associated with Ubiquitinated Vesicles After Fertilization. Connie Hajjar[1], Andy Golden[2], **Lynn Boyd[1]**. 1) Dept Biological Sci, Univ of Alabama in Huntsville, Huntsville, AL; 2) Laboratory of Biochemistry and Genetics, NIDDK/NIH, Bethesda, MD.

Studies in *C. elegans* and other organisms have indicated that mitochondria are maternally inherited.[1] This has presented an interesting cell biological conundrum as to what becomes of sperm mitochondria that enter the egg upon fertilization. Studies in mammalian systems have suggested that sperm mitochondria are eliminated from the fertilized egg via a mechanism involving ubiquitination.[2] We have investigated this possibility in the worm. Using strains with germline expression of GFP::ubiquitin, we have observed ubiquitinated vesicles that cluster around the sperm DNA after fertilization. We propose that these vesicles are sperm-derived based on three observations: 1) they are not seen in oocytes, 2) they are closely associated with the sperm DNA, and 3) they stain positive with a sperm-specific antibody (see below for more details). Using Mitotracker tagging of sperm, we show that the sperm mitochondria are closely associated with the ubiquitinated vesicles but are not ubiquitinated themselves. The sperm vesicle-mitochondria conglomerate remains compact and close to the sperm DNA throughout the maternal meiotic divisions. Starting around prophase of the first mitosis, the sperm vesicles and mitochondria begin to disperse and their numbers diminish. By the 8-cell stage, we no longer detect either.

In order to learn more about the nature of the ubiquitinated vesicles, we have done additional labelling experiments. They stain with wheat germ agglutinin, indicating that they are indeed membrane bound vesicles. They do not stain with mitotracker or lysotracker. They stain positively with the 1CB4 antibody that recognizes the sperm-specific organelle, MO. 1CB4 stains the ubiquitinated vesicles, but not the sperm mitochondria in the embryo. Thus, the vesicles seen in the fertilized embryo may be derived from MO structures in the sperm. Linkage specific antibodies to polyubiquitin show that the vesicles harbor K63, but not K48, linked polyubiquitin chains. K63 chains can sometimes trigger autophagy suggesting that autophagic destruction may be involved in the elimination of the sperm-derived vesicles and possibly also the nearby sperm mitochondria. This interesting association between sperm mitochondria and these unusual ubiquitinated vesicles may reveal a novel mechanism for the elimination of paternal mitochondria from the fertilized egg.

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1025C

Isolation of novel molecules that regulate specialized ciliary morphologies. **Andrea Brear[1]**, Alexander van der Linden[2], Piali Sengupta[1]. 1) Dept Biology, Brandeis Univ, Waltham, MA; 2) Dept Biology, University of Nevada, Reno, NV.

Cilia are sensory organelles that project from the surface of most cell types in eukaryotic organisms. Primary cilia play critical roles in sensing external stimuli and generating an appropriate response. They are comprised of a microtubule-based axoneme and a surrounding membrane, and are generated by the highly conserved process of intraflagellar transport (IFT). IFT utilizes molecular motors to transport cargo such as axoneme precursors and membrane receptors required for the generation and maintenance of cilia. Most primary cilia exhibit simple rod shaped structures. However, some cell types can exhibit elaborate morphologies that are hypothesized to enable them to carry out specific functions. For example, the elaborate outer segment and connecting cilium in vertebrate rod and cone photoreceptors in important for sight, and the kinocilium in the ear is critical for hearing. Although much is now known regarding the formation of primary cilia, the mechanisms that give rise to specialized cilia are still unclear.

C. elegans contains about sixty neurons that exhibit ciliated endings; a subset of these neurons display elaborate ciliary morphologies. These specialized cilia are crucial for the unique sensory functions of the neuron types. Evidence from our lab and others suggests that cell specific cilia may arise from differential deployment of IFT proteins and motors. To identify molecules required for the generation of cell type-specific cilia, we carried out a forward genetic screen for mutants exhibiting aberrant morphology of the specialized cilia of the AWB olfactory neurons. Using SNP-mapping, we have mapped one promising mutant to a 0.9MB region on chromosome I, and will clone the affected gene via further mapping and whole genome sequencing. We are currently investigating whether this mutation specifically affects AWB cilia or whether additional cilia structures are also altered. Additional ongoing experiments are aimed at determining whether the localization of ciliary proteins is affected, and whether IFT is altered. We are also using an in silico approach to identify candidate molecules that may play roles in ciliary specialization in C. elegans. By characterizing novel molecules that regulate ciliary diversity, these studies can further our understanding of ciliogenesis in other organisms and may provide insights into cilia related diseases.

1026A

Identification of novel proteins involved in the regulation of IFT. **Joost Broekhuis**, Weng Yee Leong, Gert Jansen. Dept Cell Biol, ErasmusMC, Rotterdam, 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, and in the distal segments OSM-3 moves alone. The architecture of *C. elegans'* cilia suggests that cilia length and function can be dynamically regulated. We recently identified and characterized the *C. elegans* gene *dyf-5*. The *dyf-5* gene encodes a conserved MAP kinase. Loss-of-function and overexpression studies showed an affect of DYF-5 on the length of the cilia, as well as morphology. In addition we found several effects on IFT, including uncoupling of the two kinesins. Both *dyf-5* overexpression and loss-of-function result in a dye filling defect. To identify new proteins involved in the regulation of IFT we performed a screen for mutants that suppress the *dyf-5(lf)* or *dyf-5XS* Dyf phenotype. Thus far, we identified one mutant that suppresses *dyf-5XS*, but this inactivated the endogenous *dyf-5* gene. The *dyf-5(lf)* suppressor screen is ongoing. To identify binding partners of DYF-5 we are performing pull-down/mass spec experiments with N- and C- terminally Bio-tagged DYF-5. We are currently awaiting the mass spec results. In addition, we are investigating the three mammalian homologues of *dyf-5*, MAK, MRK, and MOK. In our model system, cultured kidney epithelial cells (IMCD3), MRK and MOK are both expressed. In knockdown and overexpression experiments with MRK we see similar effects on cilia length as we did in *C. elegans*. However, we see no effects on the velocities of IFT particles in the IMCD3 cells after knockdown of MRK. Thus far we did not observe any effects of MOK on cilia length. We are currently performing pull-down/mass spec on MOK::Bio and MRK::Bio to identify binding partners.

1027B

The GMAP210 homologue SQL-1 modulates Intraflagellar Transport in *C. elegans*. Suzanne Rademakers, **Joost Broekhuis**, Martijn Dekkers, Jan Burghoorn, Gert Jansen. Dept Cell Biol, ErasmusMC, Rotterdam, 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. We have previously shown that the expression of a dominant active G protein α subunit (GPA-3QL) in amphid channel neurons affects the coordination of OSM-3 and kinesin-2 and cilia morphology, resulting in a dye filling defect. We performed a genetic screen to identify mutants that suppress the *gpa-3QL* Dyf phenotype, and identified *sql-1*, which encodes the homologue of the mammalian Golgi protein GMAP-210. Using immunofluorescence we showed that SQL-1 also localizes to the Golgi in *C. elegans*. In *sql-1(lf)* animals we see no effects on cilia morphology, and also the Golgi appears normal. Overexpression of SQL-1 results in longer cilia. 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. 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. In the middle segment of *gpa-3QL; sql-1* double mutants we observed similar velocities as in *sql-1* single mutants, suggesting that in the middle segment the *sql-1* mutation is epistatic to *gpa-3QL*, which is in line with the suppression of *gpa-3QL*. Surprisingly, in the distal segment of *gpa-3QL; sql-1* double mutants OSM-3::GFP speed is significantly reduced. We are currently investigating if triple mutants can help us explain this result.

1028C

EXC Proteins Regulate Early Endosome Trafficking to Maintain Apical Surface in Narrow Tubules of *C. elegans*. Brendan Mattingly, Kelly Grussendorf, Elinor Brown, **Matthew Buechner**. Dept. of Molecular Biosciences, University of Kansas, Lawrence, KS.

Nematode excretory canals are thin hollow tubules that run the length of the animal, and are built from a single cell. Extension and maintenance of these tubules requires

concomitant growth of the basal outer surface of the canals and of the apical luminal surface. In *C. elegans*, loss-of-function mutations in any of a series of *exc* genes cause the canal lumen to swell into large fluid-filled cysts. These cysts form as a result of mechanical failure of the actin-based cytoskeleton lining the canal apical surface.

Several exc mutations (*exc-1*, *exc-9*, and *exc-2*) show genetic interactions with *exc-5*, which encodes a guanine exchange factor homologous to the mammalian FGD proteins that activate CDC42. We have examined constructs expressing subcellular organelle markers (including EEA-1, CHC-1, RAB-5, RAB-7, RAB-11, GRIP, and GLO-1, CDC-42, and the CDC-42-binding domain of WSP-1) within the excretory canals, and examined their placement in wild type, *exc-5* mutant, and *exc-5*-overexpressing strains. In *exc-5* mutants, early endosomes (marked by EEA-1) accumulate greatly in areas of the canals adjacent to and surrounding areas where large cysts formed. Most notably, we saw these accumulations of labeled EEA-1 in areas of the canal prior to the formation of cysts. Endocytic vesicles (labeled with RAB-5) also accumulated to some extent in these mutants. Recycling endosomes (marked by RME-1), conversely, were strikingly depleted in areas of cyst formation. In animals overexpressing *exc-5*, the RME-1 marker accumulated, while EEA-1 expression was lowered. We conclude that EXC-5 regulates trafficking of early endosomes necessary to recycle proteins that maintain the apical cytoskeleton. Mammalian FGD4 was reported by others to be necessary to maintain Schwann cells to maintain the myelin sheath to prevent the neuropathy Charcot-Marie-Tooth Syndrome Type 4H. Our results imply that FGD proteins may be needed to maintain efficient recycling of proteins to maintain the structure of these single-celled tubes as well.

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1029A

Identification of RhoGEFs that activate CDC-42 during radial polarization of the early C. elegans embryo. **Emily Chan**, Jeremy Nance. Developmental Genetics, New York University Skirball Institute, New York, NY.

Radial polarization defines the inside-outside axis of the embryo and is important for key morphogenetic and patterning events during early development. In C. elegans, cell contacts polarize early embryonic cells radially by excluding PAR-6, PKC-3, and PAR-3 from sites of cell contact, restricting these proteins to the outer, contact-free surface. We showed previously that radial polarity requires the RhoGAP protein, PAC-1, which localizes exclusively to cortical regions adjacent cell contacts and locally inactivates the Rho G protein CDC-42. PAR-6 interacts with active CDC-42 and is recruited to the contact-free surface where it is thought that CDC-42 remains active. The RhoGEF that is required to activate CDC-42 is unknown, but could play an important role in radial polarization if its localization or activity were dynamically regulated. In an initial RNAi knockdown screen of the 23 putative C. elegans RhoGEFs, no single GEF was required for PAR-6 localization, suggesting that GEFs for CDC-42 function redundantly. To identify the redundant GEFs, we performed an over-expression screen to select GEFs that could recruit PAR-6 to cell contacts where it is normally excluded by PAC-1 for further study. From a screen of 15 GEFs, we have identified two, ECT-2 and CGEF-1, which localize to the cortex when over-expressed in early embryos and can recruit PAR-6 to these sites. We are currently investigating the hypothesis that ECT-2 and CGEF-1 function redundantly to activate CDC-42 during radial polarization.

1030B

Functional Analysis of the AP-3 Clathrin-associated Adaptor Complex in *Caenorhabditis elegans*. **Carlos Chih-Hsiung Chen**[1], Anbing Shi[2], Donglei Zhang[1], Zhiyong Bai[2], Peter J. Schweinsberg[2], Lucy Mingchih Lee[1], Barth D. Grant[2], Christopher Rongo[1]. 1) Department of Genetics and Waksman Institute, Rutgers, The State University of New Jersey, Piscataway, NJ; 2) Department of Molecular Biology and Biochemistry, Rutgers, The State University of New Jersey, Piscataway, NJ.

Adaptor protein (AP) complexes mediate the association of cargo proteins with clathrin-coated vesicles (CCVs) to facilitate membrane trafficking along the secretory and endocytic pathways. Mammals possess four AP complexes (AP-1, -2, -3, -4), whereas three AP complexes (AP-1, -2, -3) are found in *Drosophila*, *C. elegans*, and yeast. All AP complexes form similar heterotetramers consisting of two large subunits (γ/β1, α/β2, δ/β3, ε/β4, respectively), which facilitate membrane association and clathrin binding; one medium subunit (μ1 ~ μ4), which mediates cargo recognition; and one small subunit (σ1~ σ4), which provides complex stability. While the AP-1 and AP-2 complexes have well characterized roles in secretion from the Golgi and endocytosis at the plasma membrane, respectively, the functions of AP-3, and AP-4 complexes are less well understood (1, 2). Previous genetic findings in *Drosophila*, mouse, human, and yeast demonstrate a role for AP-3 complexes in the trafficking of proteins from the Golgi directly to lysosomes and lysosome-related organelles (e.g., melanosomes and platelet dense granules). In humans, loss of AP-3 function results in Hermansky-Pudlak syndrome (HPS), with the clinical symptoms of hypopigmentation, prolonged bleeding, and pulmonary fibrosis due to defects in the maturation of melanosomes and platelet dense granules (3). In *C. elegans*, AP-3 function was shown to be essential for embryogenesis and larval development (4). In addition, defects in the formation of lysosome-related gut granules were reported in mutants for AP-3 complex genes (5). Although the AP-3 complex is clearly involved in trafficking to lysosomes and their related organelles, the precise trafficking steps conducted by AP-3 complex still remain uncertain. In this study, we examined the trafficking and subcellular localization of multiple fluorescently tagged cargo proteins and endosomal/lysosomal markers in both *C. elegans* epithelial cells and neurons. We show that in addition to regulating gut granule formation, AP-3 complex also regulates several key membrane trafficking steps within multiple cell types. **References** *1. Boehm, M et al., Mol Biol. Cell 12, 2907-2920 (2001). 2. Nakatsu, et al., Cell Structure and Function 28, 419-429 (2003) 3. Dell'Angelica, E. C. et al., Mol. Cell 3, 11-21 (1999). 4. Shim, J. et al., Mol. Cell 19(3), 452-457 (2005). 5. Hermann, G. J. et al., Mol. Biol. Cell 16, 3273-3288 (2005)..*

1031C

JIP3/UNC-16 has an UNC-101 dependent role in synaptic vesicle biogenesis. **Bikash C Choudhary**[1], Jitendra Kumar[1], Takashi Fukuzono[2], Li Chun[2], Kunihiro Matsumoto[2], Naoki Hisamoto[2], Sandhya P Koushika[1]. 1) Neurobiology, NCBS-TIFR, Bangalore-560065, Karnataka, India; 2) Department of Molecular Biology, Graduate School of Science, Nagoya University, Nagoya 464-8602, Japan.

UNC-16/JIP3 is a known cargo adapter for the Kinesin-I (UNC-116) motor and a Jun Kinase (JNK) scaffolding protein. unc-16/JIP3 mutants are able to bypass the requirement for the UNC-104/KIF1A kinesin motor for transport of pre-synaptic vesicle protein. In addtion, in unc-16 animals we observe (i) Synaptobrevin and RAB-3 moving in aberrant large tubular compartments that emerge from the cell body, (ii) Synaptobrevin is present at the dendritic tips of chemosensory neurons and (iii) resident golgi enzymes are mis-localized and present along the entire neuronal process. These data suggest a role for UNC-16 in trafficking likely at the golgi. The mis-localization of Synaptobrevin to dendrites of chemosensory neurons and large moving tubular compartments containing RAB-3 are also present in *lrk-1* mutants. However in *lrk-1* animals localization of resident golgi enzymes is not greatly altered and *lrk-1* is unable to bypass the requirement of UNC-104 in the localization of synaptic vesicle proteins. The dendritic localization of Synaptobrevin in *lrk-1* has been shown to be *unc-101* dependent. However, the dendritic localization of Synaptobrevin in *unc-16* animals is *unc-101* dependent only in an *unc-16* allele with a late stop in its ORF. Our data suggest a novel role for UNC-16 in the cell body where it acts either upstream or in parallel with LRK-1 in an UNC-101 dependent pathway in formation of the synaptic vesicle protein transport carriers.

1032A

The Role of Intracellular Trafficking in Modulating Ciliary Structure and Function in *C. elegans*. **David B. Doroquez**[1], Anique Olivier-Mason[1], Ali Sarkeshik[2], John R. Yates III[2], Piali Sengupta[1]. 1) Dept Biol, Brandeis University Waltham, MA; 2) Dept Chem Physiol, Scripps Research Institute, La Jolla, CA.

Primary cilia are organelles that serve as environmental sensors, and are present on most cell types in vertebrates. The structure and biogenesis of these organelles are conserved from algae to humans, allowing for parallel studies of cilia in many model systems. Defects in cilia are implicated in multiple sensory pathologies. *C. elegans* is an ideal system to study cilia with its tractability and the ability to analyze its 60 ciliated sensory neurons at high resolution. Individual chemosensory neurons exhibit highly specialized cilia structures that are essential for their sensory properties. 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 cilia, and this modulation is dependent on vesicular trafficking. Defects in trafficking are likely to affect cilia structure/function due to altered transport and localization of ciliary signaling molecules resulting in defective cellular homeostasis. The goal of this project is to study the role of intracellular trafficking in the generation and maintenance of cilia morphology. In order to identify components involved in cilia biology, we identified proteins associated with different IFT components via mass spectrometry and proteomics. Many predicted IFT-associated components were identified in this analysis, suggesting that this approach may allow us to identify new cilia-related components. We have identified trafficking proteins associated with IFT complex proteins. In particular, we identified the GTPase dynamin DYN-1 and localized it to the base of AWB cilia. Conditional *dyn-1* mutants have Y-shaped cilia branches with increased membranous areas, similar in phenotype to sensory signaling mutants. Knockdown of DYN-1 produces a bulbous AWB ciliary phenotype similar to that seen in clathrin AP2 adaptor *dpy-23* mutants. We also identified exocyst complex proteins in our IP-MS studies. Interestingly, we find that SEC-8 localizes to the base of AWB cilia. Our data suggest a role for endocytosis and exocytosis in contributing to ciliary function. Ongoing experiments are examining whether sensory signaling regulates rates of dendritic trafficking, exocytosis and endocytosis to maintain AWB ciliary structure. These experiments will elucidate the role of vesicular transport in regulating cilia structure and how the sculpting of specialized sensory ciliary architecture in an activity-dependent manner allows animals to respond to environmental cues.

1033B

Isolation and Characterization of *tat-1* suppressors. Xin Li, **Hongwei Du**, Baohui Chen, Xiaochen Wang. National Institute of Biological Sciences, #7 Science Park Road, Zhongguancun Life Science Park, Beijing 102206, China.

In eukaryotic cells, endocytic cargoes are transported to early endosomes where they are sorted to be recycled back to plasma membranes, degraded in lysosomes or delivered to trans-Golgi network. *tat-1*, which encodes a *C. elegans* P4-ATPase, is required for endocytic sorting and recycling by maintaining membrane phosphatidylserine (PS) asymmetry[1]. *tat-1(lf)* mutants accumulate big intestinal vacuoles and display various defects in endocytic transport [1,2]. To identify more regulators involved in this process, we performed a genetic screen to search for mutants which suppress the vacuolation phenotype in the intestine of *tat-1(qx30)* animals. From a screen of 12,000 haploid genomes, we have isolated two recessive mutations *qx49* and *qx71*, which completely suppress the appearance

of abnormal vacuoles in the intestine of *tat-1(qx30)*. *qx49* and *qx71* also suppress the vacuolation phenotype in animals lacking CHAT-1, the chaperon of TAT-1. By examining different steps of endocytic transport, we found that both *qx49* and *qx71* mutations efficiently reversed the cargo recycling defects in *tat-1(qx30)* mutants. In addition, the abnormally aggregated RAB-5-, RAB-7-, RAB-10- and RME-1-positive vesicles in the *tat-1(qx30)* intestine appeared to be normal in *qx49qx30* double mutants. We found that membrane PS asymmetry is still defective in *qx49qx30* and *qx30;qx71* worms, which indicates that the suppression of defective endocytic transport in the double mutants is not due to a correction of PS distribution. We have cloned the gene affected in *qx49* and *qx71* and are in the process of characterizing their functions in endocytic transport. We will report our progress in the meeting. References: 1. Chen B, Jiang Y, Zeng S, Yan J, Li X, et al. (2010) Endocytic Sorting and Recycling Require Membrane Phosphatidylserine Asymmetry Maintained by TAT-1/CHAT-1. *PLoS Genet* 6(12): e1001235. doi:10.1371/journal.pgen.1001235. 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.

1034C

qx42 affects lysosome function and embryonic development in *C. elegans*. Bin Liu, **Hongwei Du**, Xiaochen Wang. National Institute of Biological Sciences, #7 Science Park Road, Zhongguancun Life Science Park, Beijing, 102206, China.

Lysosomes are membrane-bound organelles that function to degrade cargoes received from various sources including endocytosis, phagocytosis and autophagy. Defects in lysosome function lead to rapidly growing numbers of human disorders, indicative of its important roles in animal development. From a forward genetic screen for regulators of programmed cell death, we isolated a recessive mutation *qx42*, which accumulated many refractile corpse-like objects at various embryonic stages. The appearance of corpse-like objects in *qx42* embryos is only partially suppressed by a loss-of-function mutation in the *ced-3* or *ced-4* gene which blocks almost all apoptosis in C. elegans. On the other hand, significantly higher numbers of Lysotracker Red-positive structures were observed in *qx42* mutants than in wild type, suggesting that the refractile corpse-like objects likely represent both cell corpses and abnormal lysosomes. To further examine this, we labeled lysosomes with NUC-1::mCHERRY, the C. elegans DNase II homolog that localizes to lysosomes. In wild type, NUC-1::mCHERRY stained many punctate and few tubule-like structures. In *qx42* embryos, however, the NUC-1-positive puncta were greatly enlarged, whereas tubular structures were mostly disrupted. Moreover, we partially released cells from C. elegans embryos by chitinase treatment followed by Lysotracker staining. We found that Lysotracker Red stained small punctuate structures in the cytoplasm of wild-type cells but labeled significantly enlarged compartments in *qx42* mutant cells. These data suggest that *qx42* mutants accumulate abnormally enlarged lysosomes. Consistent with this, we found that *qx42* mutants are defective in lysosomal degradation of various cargoes including apoptotic cells, yolk proteins and transmembrane cargoes like RME-2 and CAV-1, indicating that lysosome function is severely affected. *qx42* animals are viable but display a retarded embryonic development. We have cloned the gene affected in *qx42* and are in the process of characterizing its function in maintaining normal lysosome function and embryonic development.

1035A

An in vivo screen of localization and trafficking regulators of LET-23 EGFR. **Juan M. Escobar Restrepo**, Christina J. Herrmann, Langouet M, Hajnal A. Institute of Molecular Life Sciences, University of Zürich, Switzerland.

Proper EGFR localization in polarized cells is required for cell fate determination. Aberrant localization and changed receptor dynamics can cause human disease including various types of cancer. *In C. elegans*, the conserved LIN-2, LIN-7 and LIN-10 complex retains LET-23 EGFR at the baso-lateral compartment of the Vulval Precursor Cells (VPCs), allowing the receptor to receive the inductive LIN-3 EGF signal secreted from the gonadal Anchor Cell. Loss-of-function mutations in components of the LIN-2, LIN-7 and LIN-10 complex cause apical mis-localization of LET-23 (Kaech et al 1996). As a result, the LIN-3 signal is not efficiently received, leading to a reduction in the number of induced VPCs and consequently a Vulvaless (Vul) phenotype. Several screens have been performed based on the Vul phenotype to identify new components required for proper activation and regulation of LET-23 EGFR. However, it has so far not been possible to observe LET-23 EGFR localization *in vivo*. Additionally, most of the knowledge of kinetics and trafficking of the EGFR comes from studies of mammalian cell cultures and not from whole organisms. We have therefore created a functional LET-23::GFP translational reporter to visualize the receptor in the VPCs of live animals. Using this reporter we are performing a forward RNAi screen for genes controlling LET-23 localization and trafficking during vulval development. We are currently screening 740 RNAi clones that have been annotated to give a Protruding vulva (Pvl) phenotype and 110 RNAi clones of candidate genes that are implicated in receptor regulation or trafficking. This approach allows us to identify new components of the LET-23 localization machinery and to investigate in live animals changes in receptor dynamics during vulval development.

1036B

PAR proteins regulate the localization of LET-99 during asymmetric division. Jui-Ching Wu, **Eugenel B. Espiritu**, Lesilee S. Rose. Molecular and Cellular Biology, University of California, Davis, Davis, CA.

Positioning of the mitotic spindle is essential for a number of developmental processes, including asymmetric divisions. During such divisions, the spindle must be aligned with the axis of cell polarity and the spindle is often displaced so that division gives daughters of unequal size. In many systems, PAR polarity proteins establish polarization of the cell and regulate spindle movements via a complex including Gα, GPR and LIN-5. In *C. elegans* one-cell embryos, GPR and LIN-5 are asymmetrically localized and activate cortical forces that pull on astral microtubules to position the spindle. We previously showed that LET-99 is a key regulator of GPR asymmetry. LET-99 is asymmetrically localized at the cortex in a lateral-posterior band pattern, where it inhibits GPR localization. Quantitative analysis of LET-99 localization in mutant backgrounds shows that PAR-3 inhibits cortical LET-99 localization at the anterior cortex, while a gradient of PAR-1 inhibits LET-99 at the posterior-most cortex. Additionally, cytoplasmic polarity mediators are not required. PAR-1 is a Ser/Thr kinase, which associates with LET-99 in immunoprecipitations from embryo extracts and in pull-down assays. In other systems, phosphorylation of targets by PAR-1 is known to generate binding sites for 14-3-3 proteins, which causes dissociation of the targets from the cortex. We therefore tested LET-99 for association with the *C. elegans* 14-3-3 protein, PAR-5, using bacterially expressed His-tagged LET-99 incubated with embryo extracts. PAR-5 bound to His-LET-99 in wild-type extracts, but PAR-5 binding was greatly diminished in extracts from *par-1(RNAi)* embryos. We used computer predictions for 14-3-3 binding sites followed by yeast-two hybrid (Y2H) assays to identify two LET-99 serine residues essential for PAR-5 binding. To determine the in vivo relevance of these sites, we introduced S to A mutations into an otherwise full-length rescuing LET-99 transgene (LET-99-AA). When transferred into a *let-99* deletion mutant background, the transgene encoded LET-99-AA protein was mislocalized. Instead of being present in a band pattern, LET-99-AA localized to the entire posterior cortex of the one-cell embryo, similar to what was observed for LET-99 in *par-1* mutant embryos. These and other results strongly support the model that PAR-5 binds to LET-99 to dissociate it from the cortex, and that this interaction is regulated by phosphorylation of LET-99 by PAR-1 at the posterior. In the future, we will test the hypothesis that PAR-1 directly phosphorylates LET-99 using in vitro kinase assays. We will also test LET-99 interacting proteins identified in Y2H screens for a role in anchoring LET-99 at the cortex.

1037C

Intracellular trafficking of the type I and type II TGFβ receptors, SMA-6 and DAF-4, in *C. elegans*. **Ryan Gleason**[1,2], Adenrele Akintobi[2], Barth Grant[2], Richard Padgett[1,2]. 1) Waksman Inst., Rutgers Univ, Piscataway, NJ; 2) Dept. of Molecular Biology and Biochemistry, Rutgers Univ, Piscataway, NJ.

Receptor-mediated endocytosis regulates the internalization, degradation, and recycling of a diverse group of membrane bound, cell-signaling receptors. This regulation of cell signaling receptors modulates receptor availability, duration of signaling, and signaling strength. We have been developing *C. elegans* as a model to study the trafficking of TGFβ receptors SMA-6 and DAF-4. Interestingly, using endocytic mutations that block recycling and transport to the trans-Golgi network (TGN), we find that trafficking of the type I and type II receptors are handled differently in the animal. Mutations in *vps-35*, a component of the retromer, block C. elegans TGFβ signaling, indicating that retromer recycling of one or both receptors is necessary for full signaling strength. This work was prompted by the identification of *sma-10*, which was discovered through a genetic screen in the Padgett Lab, and which we show also affects the trafficking of the type I and type II receptors, *sma-6* and *daf-4*. Experiments were done to distinguish between a role in receptor secretion or in internalization of receptors. Using double mutants for the AP-2 complex (required for entry into the cell) and *sma-10*, we show that *sma-10* does not act in secretion of the receptors but acts after receptor internalization. In *sma-10* mutants, both receptors accumulate in the cytoplasm, indicating that recycling and/or degradation are altered. Further data will be presented to show how TGFβ traffics in *C. elegans* and what role *sma-10* plays in this process.

1038A

The EXC-1 GTPase Is Required for Maintenance of Tubule Shape in the Excretory Canals. **Kelly Grussendorf**, Amanda Riss, Matthew Buechner. Dept. of Molecular Biosciences, University of Kansas, Lawrence, KS.

Tubulogenesis involves the formation of tubule shape and diameter along both apical (lumenal) and basal sides. Once the tubule is formed, this structure needs to be regulated and maintained. The excretory canals provide a simple model to study these processes. The excretory canal cell, located near the terminal bulb of the pharynx, 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. The set of EXC proteins maintain 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.

The Exc-1 phenotype exhibits canal cysts of variable size and number that are frequently located at the distal ends of the canals. Cysts can be very small, or as large as the entire diameter of the worm. We have cloned the *exc-1* gene via fosmid and gene rescue, RNAi phenocopy, expression pattern, and allele sequencing, and found that this gene encodes a homologue of the family of mammalian Interferon-Inducible GTPases (IIGP), proteins that include a Ras GTPase domain. Overexpression of *exc-1* causes the canal cell to form normal-diameter tubules, but with a defective basal surface. This phenotype also occurs through overexpression of other *exc* genes. *exc-1* shows genetic interactions with other members of the exc family; the results imply that EXC-1 acts downstream of the LIM-domain protein EXC-9, but upstream of the guanine exchange factor EXC-5 (See poster by B. Mattingly).

We have crossed *exc-1* (*rh26*) mutants to marker strains that show the position of subcellular organelles within the canals. *exc-1* mutants appear to have the same effect on

subcellular organelles as does loss of EXC-5, a buildup of apical early endosomes with concomitant loss of recycling endosomes, leading to blockage of recycling of material to the apical membrane and presumed weakening of the apical surface. Current work is investigating possible direct protein-protein interactions between EXC-1 and EXC-5, and EXC-1's role in the tubular amphid sheath cells.

We are grateful for marker constructs and advice supplied by Barth Grant, John White, Brian Ackley, Erik Lundquist, and Monica Driscoll, as well as support from NINDS R03-NS067323 and the Inez Jay Fund of the University of Kansas.

1039B

Molecular characterization of TGF-β regulation by multiple intracellular vesicle trafficking processes in *C. elegans*. Katherine Beifuss, **Tina Gumienny**. Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX.

Cell-to-cell signaling by TGF-β pathways is critical for the development and homeostasis of eukaryotes. What receiving cells do with the TGF-β signal is still not entirely clear. The objective of our study was to identify molecular players and pathways that regulate TGF-β within receiving cells. To accomplish this goal, we first generated a *C. elegans* strain expressing functional, fluorescently tagged TGF-β superfamily member DBL-1. We then used this strain to screen an RNAi library for genes required for proper DBL-1 localization *in vivo*. Results from this screen indicate that DBL-1 is endocytosed by both clathrin-mediated and lipid raft-mediated processes. Ubiquitin, SUMO, and cytoskeletal components are required for the normal restricted subcellular localization of DBL-1-positive vesicles. We have identified a Patched-like Hedgehog receptor that is normally required to prevent basal-to-apical transcytosis of DBL-1. We conclude that a balance of intracellular trafficking events that include signaling through early endosomes and recycling/planar transcytosis, degradation in lysosomes, and basal-to-apical transcytosis regulates TGF-β signaling in *C. elegans*.

1040C

Regulation of the RAB-2 GTPase involved in Dense-Core Vesicle Maturation. **Mandy Hannemann**, Stefan Eimer. Molecular Neurogenetics, European Neuroscience Institute, Göttingen, Germany.

Fast synaptic transmission is mediated by the release of neurotransmitters from synaptic vesicles. However, for efficient neurotransmission neurons also rely on dense-core vesicles (DCVs) that are co-released with SVs from axons. Despite their importance for neuronal activity little is known about the function of DCVs as well as how their release and biogenesis are regulated. DCVs are thought to be generated at the late Golgi apparatus as immature DCVs which subsequently undergo a maturation process through rounds of homotypic fusions and clathrin mediated membrane remodelling events. This maturation process is required for efficient processing of neuropeptides within DCVs and removal of factors that would interfere with DCV release. Previously, we have shown that the GTPase RAB-2 and its effector, RIC-19, are involved in DCV maturation in *C. elegans* neurons. In *rab-2* mutants specific cargo is lost from maturing DCVs and mis-sorted into the endosomal/lysosomal degradation route. This cargo loss could be prevented by blocking endosomal delivery. This suggests that RAB-2 is involved in retention of DCV components during the sorting process at the Golgi-endosomal interface. To understand how RAB-2 activity is regulated at the Golgi we screened for GTPase activating proteins (GAPs) that would inactivate RAB-2. We identified a potential RAB-2 GAP that is exclusively neuronally expressed showing similar DCV maturation defects as *rab-2* mutants. We could demonstrate that RAB-2 binds specifically to the TBC-domain of its cognate GAP. Interestingly, the RAB-2 GAP also binds to the RAB-2 effector, RIC-19, as well as to its human ortholog, ICA69. Therefore, it is proposed that a dynamic ON/OFF cycling of RAB-2 at the Golgi induced by the GAP/effector complex is required for proper DCV maturation.

1041A

Investigating the function of the GLO-1 Rab GTPase in lysosome-related organelle biogenesis. Hannah Somhegyi[1], Thomas Curtin[2], Brian King[2], Travis Walton[2], **Greg J. Hermann[1,2]**. 1) Dept of Biology, Lewis & Clark College, Portland, OR; 2) Program in Biochemistry & Molecular Biology, Lewis & Clark College, Portland, OR.

Lysosome-related organelles (LROs) comprise a class of cell-type restricted compartments with specialized functions. In mammals, they include melanosomes, platelet dense granules, and lamellar bodies; 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. Our studies have shown that GLO-1, a homologue of Rab32/38, is required for the formation of *C. elegans* gut granules, lysosome-related organelles containing autofluorescent and birefringent material found only within intestinal cells. Presently, the function of Rab32/38 proteins in any system remains unknown. In an effort to better understand the role of GLO-1, we have analyzed the activity of 16 different alleles of *glo-1*. All of the alleles result in the complete loss of birefringent material from embryonic intestinal cells and its mislocalization into the embryonic intestinal lumen. Interestingly, a missense allele *glo-1(kx8)*, which changes an invariant residue in the G3/switch II domain of small GTPases, retains weak activity. We carried out a genetic screen for mutations the suppress the gut granule biogenesis defect associated with *glo-1(kx8)*. We identified two alleles, *kx105* and *kx106*, which map to similar locations, that are semi-dominant, gain-of-function, and allele-specific suppressors of *glo-1(kx8)*. Notably, the gut granules that are restored in *glo-1(kx8); kx105* lack some gut granule associated proteins, which are mistargeted to conventional lysosomes. However, the gut granules that are restored utilize previously identified gut granule trafficking pathways. Together, these results indicate that the suppressor restores some but not all GLO-1 activities and that the identification of the suppressor will provide insights into the function of the protein. Rabs act via interactions with effector molecules when they are in the GTP bound state. We have found that a GTP-locked and functional form of GLO-1 is present on organelles that are distinct from mature gut granules. They do not appear to be early or late endosomes and are thus divergent from organelles participating in trafficking to conventional lysosomes. These compartments are the likely site of GLO-1 activity and identify intermediates in gut granule trafficking.

1042B

GLO-2 is a nematode specific factor functioning in gut granule biogenesis. Daniel Saxton[1], Emily Scavarda[1], Allison M. Weis[1], Becca Salesky[1], Laura Thomas[2], Kaila Warren[1], Alec Barret[2], **Greg J. Hermann[1,2]**. 1) Dept of Biology, Lewis & Clark College, Portland, OR; 2) Program in Biochemistry & Molecular Biology, Lewis & Clark College, Portland, 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 evolutionarily conserved AP-3 subunits, HOPS complex, and GLO-1/Rab38, genes whose homologues function in trafficking to lysosomes and lysosome-related organelles. In addition, we have identified nematode specific factors, such as the gut granule associated GLO-3 protein, that function in gut granule biogenesis. 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 another nematode specific gene required for gut granule biogenesis, *glo-2*. *glo-2* is defined by two alleles that exhibit maternal effect gut granule biogenesis defects. *glo-2* mutant adults and embryos lack multiple markers associated with gut granules, while other endolysosomal organelles do not appear to be similarly affected. Our genetic and cellular studies implicate *glo-2* function in gut granule biogenesis pathways distinct from those currently implicated in gut granule formation. *glo-2* encodes a small cytoplasmically localized protein that is not obviously conserved outside of nematodes. *glo-2(-)* is partially suppressed by overexpression of RAB-7, which is not obviously required for gut granule formation, suggesting overlap in trafficking pathways to lysosome-related and conventional lysosomal organelles, which co-exist in *C. elegans* intestinal cells. To better understand the function of GLO-2 we are currently carrying out studies of intracellular trafficking pathways in *glo-2(-)* animals and searching for GLO-2 interacting proteins.

1043C

Zygotic expression of *par-1* suggests multiple functions. **Daryl D. Hurd**. Dept Biol, St John Fisher Col, Rochester, NY.

PAR-1 (cytoplasmic partitioning defective) is a member of the MARK (microtubule affinity regulating kinase) subfamily of serine/threonine kinases that is implicated in a wide range of processes including the establishment of embryonic/epithelial/neuronal polarity, microtubule dynamics, metabolism and signaling. PAR-1 was originally identified as a maternally provided protein that functions to polarize the one-cell embryo prior to the first asymmetric cell division. It has subsequently been shown that missense alleles that cause embryonic lethality are not null. The *par-1* gene encompasses an atypical, large locus of about 40kB, and it encodes ten potential transcripts through multiple translational starts and alternative splicing. To understand when and where PAR-1 might be used beyond its well-characterized role in early embryogenesis, I fused yellow fluorescent protein to three of the potential start codons in the locus using PCR-based sequence overlap extension. In addition, a fourth transcriptional fusion created in large-scale promoterome projects was obtained and analyzed. *par-1*a is predominantly expressed in body wall muscle cells with faint expression in multiple neurons. *par-1*b is expressed in vulval epithelial cells, the excretory cells, alimentary epithelial cells and various cells of the somatic gonad. *par-1*c is expressed in multiple neurons including cholinergic motor neurons and certain amphid sensory neurons. *par-1*d is also expressed in multiple neurons including sensory neurons in the head and B-type male tail ray neurons. These expression patterns and the observation that a deletion allele of *par-1* causes early larval lethality support the idea that the maternal role of *par-1* is not its only function. Further study of the zygotic roles of *par-1* will shed light on the various functions of this multi-purpose kinase.

1044A

Intracellular PLA_1 and acyltransferase, which are involved in *C. elegans* stem cell divisions, determine the *sn*-1 fatty acyl chain of phosphatidylinositol. **Rieko Imae[1,2,3]**, Takao Inoue[1,3], Masako Kimura[1], Eriko Kage-Nakadai[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, Tokyo, Japan.

Phosphatidylinositol (PI), an important constituent of membranes, contains stearic acid as the major fatty acid at the *sn*-1 position. This fatty acid is thought to be incorporated into PI through fatty acid remodeling by sequential deacylation and reacylation. However, the genes responsible for the reaction are unknown, and consequently, the physiological significance of the *sn*-1 fatty acid remains to be elucidated. Here, we identified *acl-8, acl-9, acl-10*, which are closely related to each other, and *ipla-1* as strong candidates for genes involved in fatty acid remodeling at the *sn*-1 position of PI. In both *ipla-1* mutants and *acl-8 acl-9 acl-10* triple mutants of *C. elegans*, the stearic acid content of PI is reduced and asymmetric division of stem cell-like epithelial cells is defective. The defects in asymmetric division of these mutants are suppressed by a mutation of the same genes involved in intracellular retrograde transport, suggesting that *ipla-1* and *acl* genes act in the same pathway. IPLA-1 and ACL-10 have phospholipase A_1 and acyltransferase activity,

respectively, both of which recognize the *sn*-1 position of PI as their substrate. We propose that the *sn*-1 fatty acid of PI is determined by *ipla-1* and *acl-8, -9, -10* and crucial for asymmetric divisions.

1045B

Possible role of *pak-1*/Pak and *ced-10*/Rac in Wnt/Ror signaling. Kah Yee Goh, Natalie Ng, Thilo Hagen, **Takao Inoue**. Biochem, National Univ Singapore, Singapore, Singapore.

Wnt signaling pathways regulate diverse cellular processes in multiple species. Ror, a class of receptor tyrosine kinases, binds to Wnts and transduces the signal by a mechanism that is not well understood. To identify mediators of Ror signaling, we examined the role of P21-activated kinases (PAKs) and Rac GTPases in *C. elegans* vulval development and dauer formation, two processes that require *cam-1*/Ror function. We found that *pak-1*/Pak and *ced-10*/Rac promote posterior orientation of P5.p and P7.p during vulval development, consistent with a positive role in the *cam-1* pathway. Additionally, *pak-1* and *ced-10* mutants displayed the 27°C constitutive dauer phenotype, similar to *cam-1* mutants, suggesting that *pak-1* and *ced-10* function in the *cam-1*-mediated dauer regulation. In the HEK293T cell line, Wnt5a/Ror2 signaling inhibits canonical Wnt signaling and downregulates TCF-mediated transcription. We found that transfection of dominant negative Pak1 or Rac1 into HEK293T cells caused upregulation of TCF-mediated transcription. Together, these results suggest a conserved function of Pak and Rac in the Wnt/Ror signaling pathway.

1046C

Characterization of the exocyst in C. elegans. **Yaming Jiu**, Jussi Jäntti. Institute of Biotechnology, University of Helsinki, Helsinki, Finland.

The exocyst is an evolutionarily conserved protein complex required for cell polarity regulation and the targeting and tethering of transport vesicles to the plasma membrane. The complex is composed of eight-protein subunits sec-3, sec-5, sec-6, sec-8, sec-10, sec-15, exoc-7 and exoc-8. The functions of individual exocyst components are poorly understood. Furthermore, the regulation of the exocyst function and the mechanisms by which this tethering complex interact with other cell polarity components are poorly understood. We characterized exoc-7 and exoc-8, two apparently nonessential subunits of exocyst complex in C. elegans that display mild phenotypes. The exoc-7, exoc-8 and exoc-7;exoc-8 double mutant worms show differential behavior defects. These phenotypes resemble those observed in cilia mutants (uncoordinated movement, chemosensation and thermosensation). However, no obvious defects in cilia structure were observed. A targeted RNAi screen for enhanced phenotypes was performed for small GTP-binding proteins in exoc-7 and exoc-8 and in exoc-7;exoc-8 double mutant worms. This screen identified in total eleven GTPases with enhanced phenotypes when combined with exoc-7 and exoc-8 mutations. The hits verified previously in other systems identified functional links between GTPases and the exocyst. Both exoc-7, exoc-8 and exoc-7;exoc-8 double mutants enhanced the size of vacuoles in the epithelial intestine cells caused by the rab-10 knock down. These findings suggest that exoc-7 and exoc-8 are functionally linked to rab-10 in vesicular transport regulation in C. elegans. In addition, the screen identified novel GTPases functionally linked to exocyst. The work is underway to further characterize the functional links between these GTPases and the exocyst.

1047A

Physiological roles of ESCRT complexes in *Caenorhabditis elegans*. **Dong-Wan Kim**[1], Hyun Sung[1], Donghyuk Shin[2], Haihong Shen[3], Joohong Ahnn[1,4], Sun-Kyung Lee[1,4], Sangho Lee[2]. 1) Department of Life Science, Hanyang University, Seoul 133-791, Korea; 2) Department of Biological Sciences, Sungkyunkwan University, Suwon 440-746, Korea; 3) Department of Life Science, Gwangju Institute of Science and Technology, Gwangju 500-712, Korea; 4) The Research Institute for Natural Sciences, Hanyang University, Seoul 133-791, Korea.

ESCRT (Endosomal Sorting Complex Required for Transport) complexes are required for protein degradation, cytokinesis and viral budding. Extensive genetic, biochemical and structural studies on the ESCRT system have been carried out using yeast and mammalian models. However, the functions of ESCRT system at whole organism-level are less well studied. In *C. elegans*, we performed RNAi experiments to knock-down the gene expression of ESCRT components and profiled their effects on protein degradation and endocytosis of YP170, a yolk protein. Knockdown of ESCRT-I (*vps-23* and *vps-28*) and ESCRT-III (*vps-24* and *vps-32.1*) components interfered with protein degradation. knockdown of ESCRT-II (*vps-25* and *vps-36*) and ESCRT-III (*vps-20* and *vps-24*) components hampered endocytosis. In contrast, the knock-down of *vps-37*, another ESCRT-I component, showed no defect both in YP170 uptake and degradation. Depletion of at least one component from each complex - ESCRT-0 (*vps-27*), ESCRT-I (*vps-23, vps-28*, and *vps-37*), ESCRT-II (*vps-36*), ESCRT-III (*vps-24*) and Vps4 (*vps-4*) - resulted in abnormal distribution of embryos in uterus of worms, possibly due to abnormal ovulation, fertilization and egg-laying. These results suggest differential physiological roles of ESCRT-0, I, II and III complexes in organism context of *C. elegans*.

1048B

Centrosome centration is driven by dynein-dependent movement of intracellular organelles along astral microtubules in *C. elegans* early embryos. **Kenji Kimura**, Akatsuki Kimura. Cell Arch Lab, National Inst Gen, Mishima, Japan.

The centrosome is a major microtubule organizing center in animal cells, and its position is generally maintained at the cell center. This centrosome centration is driven through the microtubule pulling force, which is dependent on cytoplasmic dynein. The pulling force exerted upon each microtubule is estimated to be proportional to its length. It has been unclear, however, how dynein motors pull microtubules for centrosome centration in a length dependent manner. We provided evidence that centrosome-directed movement of intracellular organelles along microtubules by dynein generates force for centrosome centration in *C. elegans* embryo. We identified *dyrb-1*, a dynein light chain subunit, as a potential subunit involved selectively in centrosome centration. DYRB-1 is also required for organelle transport toward the centrosome along astral microtubules. We found that the strong temporal correlation between the net movement of centrosome centration and organelle transports. Centrosome centration was impaired when the proteins such as *rilp-1*, *rab-7*, and *rab-5* that mediate association between organelles and dynein were knocked down. These results suggest that centrosome-directed movements of intracellular organelles produce a reaction force against the movements that pulls the centrosome toward the cell center. Because the organelles are distributed equally throughout the embryo, the resultant pulling force per microtubule will be proportional to the microtubule length. This is the first model providing a mechanical basis for the microtubule length-dependent pulling force in the cytoplasm for centrosome centration.

1049C

Tubulin polyglutamylation in the sensory cilia is flexibly regulated in response to the environmental stresses. **Y. Kimura**[1], O. I. Kaplan[2], S. Hameed[1], H. Kunitomo[3], Y. Iino[3], O. E. Blacque[2], M. Setou[1]. 1) Dept Mol Anat, Hamamatsu Univ Sch Med, Hamamatsu, Shizuoka, Japan; 2) Sch Biomol Biomed Sci, UCD Conway Inst, Univ Colg Dublin, Ireland; 3) Dept Biophy and Biochem, Grad Sch Sci, the Univ Tokyo, Tokyo, Japan.

The primary cilium is a microtubule (MT)-based organelle that is significant for sensory signal transduction. Sensory channels and receptors are transported and condensed on the sensory cilia through intraflagellar transport (IFT) along MT. Polyglutamylation is a reversible post-translational modification (PTM) seen in a specific glutamate residue near the carboxyl terminus of α- and β-tubulins. We employed a genetic approach using *C. elegans* to analyze the regulatory mechanisms and physiological roles of this unique PTM. We recently identified the enzyme genes for tubulin polyglutamylation genetically and biochemically. Adding glutamates is accomplished by a member of tubulin tyrosine ligase-like (TTLL) family *ttll-4*, whereas shortening polyglutamate chains is performed by *ccpp-6*, a gene encoding a cytosolic carboxypeptidase.

Since in *C. elegans*, tubulin polyglutamylation is exclusively detected in the sensory cilia, we studied the phenotypes observed in ciliated sensory neurons using *ttll-4* and *ccpp-6* mutants. The modification is completely lost in *ttll-4* mutant worms. In contrast, *ccpp-6* mutant showed a mild increase of the modification. Both mutants did not have severe morphological defects in sensory cilia, however, whose anterogade movement of IFT particles was affected. In *ttll-4* mutants, the speed of particle movement was decreased. Conversely, *ccpp-6* mutants worms showed an increase of the movement velocity. Thus, the efficiency of IFT could be controlled by the extent of polyglutamylation on MT. Additionally, *ttll-4* mutants showed the sensitivity reduction measured by behavioral analyses such as chemotaxis for NaCl and osmotic avoidance, indicating tubulin polyglutamylation is required for chemosensory behavior.

Based on the results above, we hypothesized that this bidirectional PTM could be used to modify the sensitivity of sensory neurons. Intriguingly, the extent of tubulin polyglutamylation is increased by the treatment of various environmental stresses such as physical vibration, temperature, and high osmotic pressure. We also identified the signaling pathways to control this stress-responsive enhancement. Taken together, tubulin polyglutamylation in sensory cilia is not a rigid system but a flexible modification in response to environmental stimulations and could be used for the modulation of sensory plasticity.

1050A

Centrosome positioning and the establishment of the anterior-posterior axis in the one-cell embryo. **Zachary Klock**, Jessica Meeker, Margaret Williams, Liza Agayeva, George Pellegrino, Eva Jaeger, Rebecca Lyczak. Biology Dept., Ursinus College, Collegeville, PA.

In the one-cell *Caenorhabditis elegans* embryo, the anterior-posterior (A-P) axis is established when the sperm donated centrosome contacts the posterior cortex. While this contact appears to be essential for axis polarization, little is known about the mechanisms governing centrosome positioning during this process. Recently we showed that the puromycin sensitive aminopeptidase, PAM-1, is required to position the centrosome during the time of axis establishment. In *pam-1* mutants, the centrosomes move prematurely from the cortex, and the A-P axis is not established. However, we can bypass the requirement for PAM-1 in polarity by preventing centrosome movement through inactivation of dynein. In order to further study the requirement of the centrosome, we are using time-lapse confocal microscopy of one-cell stage embryos to examine the time during the cell cycle and the duration of the centrosome contact in wild-type and *pam-1* mutant embryos. Additionally, we are examining these events in *zyg-12* mutant embryos in which centrosomes lose association with the pronuclei but the embryos polarize normally. To find additional proteins required for centrosome positioning, we are using a genetic suppressor screen and a candidate gene approach to identify PAM-1 target proteins. Through these approaches we hope to identify proteins that regulate centrosome dynamics and hence anterior-posterior polarity in the early embryo.

1051B

Identifying mechanisms of contact-mediated cell polarization. **Diana Klompstra**, Dorian Anderson, Jeremy Nance. Skirball Institute, NYU School of Medicine, New York, NY.

During gastrulation, cells move to a position in the embryo that is appropriate for the type of tissue or organ that they will form. The directional movements of gastrulation require that cells acquire a polarity that allows them to asymmetrically localize cytoskeletal components. Most cells polarize when PAR proteins develop a restricted cortical localization, which allows them to interact asymmetrically with the downstream effectors that mediate polarity. In *C. elegans* and mammalian embryos, the polarity of early embryonic cells is determined by cell-cell contacts, which restrict PAR polarity proteins to contact-free surfaces. The goal of my project is to determine how cell contacts induce the PAR protein asymmetries that polarize early embryonic cells, preparing them for gastrulation. We previously identified the RhoGAP protein PAC-1 as an upstream regulator that is required to exclude PAR proteins from contacted surfaces of early embryonic cells. PAC-1 itself is recruited by cell contacts, where it locally inhibits the Rho GTPase CDC-42 to direct PAR protein asymmetries. How PAC-1 is able to sense where contacts are located and localize specifically to these sites is unknown. We have identified an N-terminal fragment of PAC-1 that is sufficient for localization to cell contacts, and showed that localization of this fragment depends on HMR-1/E-cadherin, a homophilic adhesion molecule that is restricted to sites of cell contact. These findings suggest that E-cadherin interactions between cells recruit PAC-1 directly to cell contacts. However, in contrast to the PAC-1 N-terminus, full-length PAC-1 can localize to cell contacts when HMR-1/E-cadherin is removed, indicating that a redundant signal functions with HMR-1/E-cadherin to recruit PAC-1 to contacts. I will report on our progress in characterizing the linkage between PAC-1 and HMR-1/E-cadherin and in identifying factors that function redundantly with HMR-1 to localize PAC-1 and polarize cells.

1052C

Dissection of the LATS kinase pathway in *C. elegans*. **Hanee Lee**, Junsu Kang, Junho Lee. IMBG, School of Biological Sciences, Seoul National University, Seoul, Korea.

The Hippo(Hpo) pathway is a conserved signaling pathway regulating organ size homeostasis via controlling cell proliferation and apoptosis. We previously reported that *wts-1*, the *C. elegans* LATS homolog which is a core gene in the Hpo pathway, showed an early larval arrest phenotype due to the defective functions of the intestine(1). In this study, we tried to find the downstream genes of *wts-1* by using EMS random mutagenesis. As a result, we obtained 18 suppressor lines that suppress the larval arrest phenotype of *wts-1*. We identified *yap-1* and *egl-44*, the *C.elegans* homolog of YAP/Yki and TEAD respectively, as suppressors of *wts-1* by SNP mapping. Mapping of the remaining mutations is in progress. Because it is known that LATS kinase acts through YAP/yki, a transcription co-activator, which interacts with the TEAD transcription factor, our results suggest that the core components of the Hpo pathway and their genetic interaction are conserved in *C.elegans*. Therefore, *C. elegans* is a good model organism to study the Hpo pathway. To delineate the Hpo pathway in *C. elegans*, we are looking for upstream and downstream genes using RNA*i* screening and microarray, respectively. Our work will contribute to further understanding of the Hpo pathway. (1)Kang J, et al. (2009), Lats kinase is involved in the intestinal apical membrane integrity in the nematode *Caenorhabditis elegans*. Development 136(16), 2705-15.

1053A

SUMOylation of ARL-13 regulates ciliogenesis and cilia sensory function. **yujie Li**, jinghua hu. Nephrology & Hypertension, Mayo Clinic, Rochester, MN.

Cilia are microtubule-based sensory organelles protruding from most cell surfaces. An evolutionarily conserved bi-directional intraflagellar transport (IFT) process builds and maintains all cilia. In previous study, we reported that small GTPase ARL-13, whose human homologue is mutated in ciliopathy Joubert syndrome, acts in concert with another small GTPase ARL-3 in regulating IFT integrity and ciliogenesis. Here, we identified one ciliary interactor of ARL-13 as UBC-9, the only known conjugating enzyme for the SUMO (small ubiquitin-related modifier)-ylation pathway. We found that UBC-9 physically associates with ARL-13 in Y2H and in vitro biochemical studies. Using in vitro sumoylation assay, we mapped the lysines responsible for the sumoylation of ARL-13 protein. We confirmed that UBC-9 strongly expresses in ciliated cells and colocalizes with ARL-13 in cilia. By analyzing the transgenic animals expressing various mutant ARL-13, we found that SUMOylation-deficient ARL-13 proteins still localize to cilia, but they can only partially rescue the ciliogenesis defect when introduced into arl-13 null background. Furthermore, absence of ARL-13 sumoylation results in the mislocalization of all examined cilary sensory receptors (including polycystins and ODR-10), as well as compromised cilia sensory function. Finally, we demonstrated that the cilia-related roles for SUMOylation of ARL-13 are probably highly conserved in mammals. The information got from our studies in worm and mammalian models provides insights into how cilia form and function, and the pathophysiologies of human ciliopathies.

1054B

Coordination of Arls in cilia. **yujie Li**, qing wei, yuxia zhang, jinghua hu. Nephrology & Hypertension, Mayo Clinic, Rochester, MN.

Joubert syndrome (JS) is the most common inherited cerebellar malformation disorder that belonging to a rapidly expanding group of human diseases called ciliopathies. All identified JS loci encode cilia-related proteins, however, with the precise roles enigmatic. Small GTPase Arl13B was identified as one JS locus. Here, we report the function of ARL-13, the sole C. elegans ortholog of human Arl13B, in regulating cilia biogenesis. In C. elegans, ARL-13 specifically localizes to ciliary doublet segment in its proline-rich C-terminus dependent manner. arl-13 animals exhibit shortened cilia with various cilia ultrastructural defects as well as disrupted association between intraflagellar transport (IFT) subcomplex A and B. Intriguingly, depletion of ARL-3, another evolutionarily conserved ciliary small GTPase, can suppress ciliogenesis defects in arl-13(lf) via a microtubule associated deacetylase HDAC-6 dependent pathway by restoring the binding efficiency between IFT subcomplex A and B. BBS proteins are a group of proteins mutated in another human ciliopathy Bardet-Biedl syndrome. It was reported that IFT-A and IFT-B dissociate in several worm bbs mutants. By checking BBS-7 and BBS-8 in arl-13 null worms, we found BBS proteins move with integrate IFT particles, but not with dissociated IFT-A or IFT-B subcomplex. These observations suggest the gene products encoded by different ciliopathy loci can have a functional crosstalk in same cellular process involved in ciliogenesis.

1055C

Tracing Endocytosis in the Nematode Gut. **Willisa Liou**[1], Barth Grant[2], Ayesha Hossain[3], David Hall[3]. 1) Anatomy, Chang Gung University, Kwei-Shan, Tao-Yuan, Taiwan; 2) Molecular Biology and Biochemistry, Rutgers University, Piscataway, New Jersey; 3) Center for C. elegans Anatomy, Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York.

Although marking the endocytic route with gold tracer has been successfully demonstrated in many mammalian cell types, its application to the intestinal cells of *C. elegans* has yet to be realized. In this study we fed the worm with 6 nm cationic gold particles in conjunction with OP50 for 30 minutes to two hours. Ultrathin sections of resin embedded as well as cryoprotected samples were prepared and examined with electron microscopy. The majority of gold tracer remained in the lumen of the gut in association with degrading bacteria. Rarely were gold particles found among the microvilli and much less in membrane-bound vesicles inside the cell. The apparent inaccessibility of gold tracer to endosomes from the apical pole of the intestinal cells may be inherent to this particular cell type, or our tracer may be too large. We are redoing the experiments with a smaller tracer. However, to rule out the possibility of an initial aldehyde fixation artifact, high pressure freeze fixation is also being tested. Here we report two advances in the handling of *C. elegans* for high pressure freezing: 1. Single worm confinement in cellulose capillary tubes for oriented frozen hydrated sectioning. 2. Rehydration of high pressure frozen/freeze substituted sample amenable for subsequent immunolocalization studies. In addition, we are testing standard post-embedding immunolocalizations on plastic-embedded tissues to identify the precise positions of actors in endocytosis.

1056A

Identification of novel factors antagonizing endocytic organelle fusion in C. elegans. **Kai Liu**, Zhiyang Gao, Xiaojuan Sun. Institute of Genetics and Developmental Biology, CAS, beijing.

Appropriate fusion and fission of endocytic organelles are critical to endocytosis, phagocytosis and other cellular processes. In C. elegans, the HOPS complex was found to be required for fusion of endosomes, lysosomes, and formation of phagolysosomes, mutants of which exhibit fragmented endosomes and lysosomes, decreased lysosomal degrading activity, and accumulation of apoptotic cells. To better understand the mechanisms underlying endocytic organelle fusion and phagolysosome formation, we carried out a genetic screen for mutants that can suppress either the small organelle phenotype or the accumulation of germ cell corpses in vps-18(tm1125) mutants. From a pilot screen of 3000 haploid genomes, we isolated two alleles, yq4 and yq5, both of which partially rescued the fragmented organelle phenotype in vps-18(tm1125) coelomocytes while yq5 reduced the number of germ cell corpses. We further found that yq4 suppressed the defects in endosome/lysosome fusion in arl-8(tm2388) mutants. Moreover, double mutants of yq4 with rab-7(ok511) display a giant granule which has the early endosome property in coelomocytes. Because loss of rab-7 function abrogates the early-to-late endosome conversion and arl-8 is mainly involved in late endosome/ysosome fusion, these observations suggest that yq4 may act negatively at multiple steps to regulate organelle fusion. We are currently cloning the genes affected by yq4 and yq5, and investigating their roles in regulating endocytic organelle fusion as well as apoptotic cell degradation.

1057B

Identification of a New Gene Required for the Proper Localization of PKD-2 to Cilia. **Julie Maguire**, Maureen Barr. Dept Gen, Rutgers, The State Univ NJ, Piscataway, NJ.

PKD-2 and LOV-1 constitute a putative mechanosensory transient receptor potential (TRP) channel-receptor polycystin complex that is required for male mating behaviors. PKD-2 and LOV-1 localize to cilia found at the ends of dendrites of male-specific sensory neurons, including the head CEM and the tail RnB and HOB neurons. *pkd-2* and *lov-1* mutants are defective in hermaphrodite contact response and exhibit location of vulva (Lov) defects. Mutations in the human homologs of *lov-1* and *pkd-2*, PKD1 and PKD2, lead to autosomal dominant polycystic kidney disease (ADPKD). The mammalian polycystins localize to cilia on renal epithelial cells. We use *C. elegans* as a tool to identify molecules required for polycystin ciliary localization.

We previously screened for mutants exhibiting PKD-2::GFP mislocalization (Bae et al. 2008). In the ciliary localization (Cil) defective mutant *my16*, we observe an abnormal accumulation of PKD-2::GFP in CEM cilia and along the distal dendrites of the RnB and HOB neurons. We are interested in mapping *my16* and in determining the effects of the mutation on male sensory behaviors. We are also examining genetic interactions between *my16* and other components of the PKD pathway.

With SNP and deficiency mapping, *my16* was determined to be on LGI between -2.26 cM and +0.08 cM. In conjunction with Whole Genome Sequencing, this limited *my16* to one of two ORFs. Both a fosmid and a single gene construct containing ORF1 but not ORF2

rescued the *my16* Cil phenotype. We conclude that *my16* is a missense mutation in ORF1, which we hereafter refer to as *cil-7*. *cil-7* appears to encode a nematode-specific protein with orthologs in *Caenorhabditis* species and *Pristionchus pacificus*. CIL-7 has four coiled-coils, but no other distinguishing domains. Reinke et al. 2004 found *cil-7* expression to be male sex-specific. We are currently determining gene expression and protein subcellular localization of CIL-7. We hypothesize that *cil-7* affects microtubule-based ciliary transport and will determine the mechanism by which *cil-7* regulates PKD-2 ciliary localization. These studies will provide important insight into how sensory receptors, including PKD-2, localize to cilia.

1058C

Putative De-ubiquitylating Enzymes MATH-33 and USP-47 are Required for Polarity Establishment in *C. elegans*. **Richard J. McCloskey**, Kenneth Kemphues. Molec Biol & Gen, Cornell Univ, Ithaca, NY.

The *C. elegans* one-cell embryo polarizes in response to a cue from the sperm centrosome, and this signal leads to a cascade of events that localizes cell fate determinants to execute the developmental program of the worm. In the course of investigating novel genes that may be involved in polarity we found that simultaneous mutation/RNAi of the genes *math-33* and *usp-47* leads to the inability of embryos to correctly establish and maintain asymmetry as defined by posterior and anterior markers PAR-2 and PAR-3. Protein homology indicates that MATH-33 and USP-47 are putative de-ubiquitylating enzymes whose similar structure and common expression in the germline and early embryos strongly suggests they have functional overlap. We determined that polarity phenotypes result from an impaired establishment phase in which we observe reduced PAR-2 recruitment, and a decrease or failure of posterior to anterior myosin flow. Our observations on the position of the centrosomes lead us to suggest that the centrosomal interaction with the posterior cortex is defective. The polarity defects can be suppressed by compromising the proteasome, suggesting that MATH-33 and USP-47 affect protein stability rather than protein activity. Lastly, we found that USP-46, the homolog of a DUB with a role in polarity in *Schizosaccharomyces pombe*, is potentially redundant with MATH-33 and USP-47, suggesting that de-ubiquitylation is a required and evolutionarily conserved mechanism to control polarity. .

1059A

FGF signaling cooperates with WNT signaling in C. elegans vulval cell lineage polarity. **Paul Minor**[1], Anand Asthagiri[2], Paul Sternberg[1]. 1) HHMI/Biology Division, Caltech, Pasadena, CA; 2) Department of Chemical Engineering, Northeastern University, Boston, MA.

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 P7.p display mirror symmetry about the vulval center. Previous data shows the orientation of these cells is determined by the interaction of multiple Wnt signals. Two separate signal cascades beginning with Wnts from the anchor cell promote the wild-type, anterior-facing P7.p, vulval lineage, whereas the Wnt/EGL-20 signal from the tail promotes the daughter cells of P7.p to face the posterior. Here we show that EGL-20 acts through a member of the LDL receptor superfamily, LRP-2, in addtion to Ror/CAM-1, Van Gogh/VANG-1. All three proteins act together to control vulval polarity through the localization of the beta-catenin-like transcriptional coactivator SYS-1. We also show that the FGF/EGL-17 pathway acting through Raf/LIN-45 is also required for the P7.p wild-type lineage orientation. The role of FGF in the P7.p orientation decision presents the first evidence that the 1° daughter cells of P6.p influence the polarity of the neighboring 2° cells. Finally by observing SYS-1 localization we show that the FGF pathway converges with Wnt in determining P7.p orientation.

1060B

C.elegans rootletin homolog, *che-10*, is required for intraflagellar transport and cilia maintenance. **Swetha Mohan**, Michel Leroux. Department of Molecular Biology and Biochemistry, Simon Fraser University.

Non-motile or primary cilia are specialized microtubule-based organelles that protrude from many cell types in metazoans. They are adapted to serve many sensory functions, including transducing mechanical, chemical and visual stimuli. The formation and maintenance of all cilia depends on a process termed intraflagellar transport (IFT), which mobilizes ciliary precursor proteins from the base of the cilia (basal body) to the growing end of the ciliary compartment. IFT involves two anterograde molecular motors, heterotrimeric Kinesin-II (KIF3) and homodimeric Kinesin-II (KIF17). The retrograde motor, cytoplasmic dynein 1b/2, is loaded on to the axoneme during anterograde transport and is activated upon arrival at the tip of the cilium, where it is required for retrograde transport of all IFT machinery. Primary cilia are mainly composed of the ciliary axoneme, the basal body from which the axoneme nucleates, the ciliary membrane and a cytoskeleton-like structure called ciliary (or striated) rootlets. Rootlets are associated with the basal body and extend proximally towards the cell nucleus. Striated rootlets are evolutionarily-conserved organelles whose components and function remain unclear. Rootletin is a core structural component of the striated rootlets; in the absence of the protein, the rootlets do not form. Although rootlets are known to be required for the stability of some cilia (e.g. photoreceptor cilia), their mechanism of function remains unknown. Here, we demonstrate that the *C. elegans* Rootletin homolog, *che-10*, is required for the maintenance of cilia. In the absence of CHE-10, only some cilia are present post-embryogenesis but these degenerate over time. *che-10* mutants have varying lengths of cilia, wherein IFT is absent from shorter (<5 microns) cilia. Furthermore, velocity analyses of IFT proteins suggests that IFT is deregulated in *che-10* mutants. The strict regulation of IFT velocities may be compromised due to defects in motor coordination. We hypothesize that CHE-10 (Rootletin) regulates IFT by assembling the IFT components at the base of the cilium, such that disruption of the cytoskeletal protein leads to improper assembly and eventual degeneration of cilia.

1061C

Immunoglobulin domain containing isoforms of UNC-89 (obscurin) are required for myofilament organization and calcium signaling in *Caenorhabditis elegans*. Patrick Spooner[1], M. Berenice Duran[2], Jennifer Bonner[3], Guy Benian[2], **Kenneth Norman**[1]. 1) Center for Cell Biology & Cancer Research, Albany Medical College, Albany, NY; 2) Department of Pathology, Emory University, Atlanta, GA; 3) Department of Biology, Skidmore College, Saratoga Springs, NY.

The force generating machinery in muscle is regulated by the influx of calcium ions into the muscle cytoplasm. Initially, depolarization of the sarcolemma leads to calcium entry via voltage gated calcium channels, which rapidly triggers the release of calcium from the sarcoplasmic reticulum via ryanodine receptors by calcium induced calcium release. Thus, calcium needs to be rapidly, accurately and reliably regulated and the calcium channels and contractile machinery must be maintained in a highly ordered arrangement for efficient and effective muscle contraction to occur. The mechanism underlying this highly organized configuration is not fully understood. Using a combination of genetic and cell biological techniques, we have found that the *C. elegans* ortholog of the giant muscle protein obscurin, UNC-89, is required for muscle cell organization. The *unc-89* locus encodes at least 6 isoforms as large as 900 kDa, 4 large Immunoglobin (Ig) domain rich isoforms and two smaller tandem kinase containing isoforms (Small et al. 2004; Ferrara et al. 2005). From our analyses, we have found that large Ig domain rich isoforms of UNC-89 are critical for sarcomere and SR organization. Furthermore, we have found evidence that *unc-89* mutants lacking the large Ig rich isoforms have defects in excitation-contraction coupling in the pharyngeal and body wall muscle, through the coordination of calcium influx. Thus, our data implicates the large Ig domain rich isoforms of UNC-89 in maintaining muscle cell architecture that is critical for efficient and effective muscle contraction.

1062A

Role of the cullin CUL-5 in the regulation of polarity and asymmetric division. **Anne Pacquelet**, Grégoire Michaux. Institut de Génétique et de Développement de Rennes, France.

PAR proteins are essential regulators of polarity in a variety of cell types, including neurons, epithelial cells as well as asymmetrically dividing cells. We use the first division of the *C. elegans* embryo to study the mechanisms regulating PAR proteins and polarity in the context of asymmetric cell division. In the one-cell embryo, PAR-3, PAR-6 and PKC-3 define an anterior cortical domain while PAR-1 and PAR-2 define a posterior domain. We previously showed that an ubiquitin ligase formed by the cullin CUL-2, the substrate specific adaptor FEM-1 and the cofactors FEM-2 and FEM-3 contributes to polarity in the one-cell embryo by regulating PAR-6. Interestingly, we found that another cullin, CUL-5, also regulates polarity in the early embryo. In particular, loss of *cul-5* enhances the lethality and polarity defects of *par-2(ts)* mutant embryos grown at semi-restrictive temperature. Preliminary results indicate that these defects are further enhanced when *fem-3* is inactivated, suggesting a possible redundancy between the CUL-2/FEM-1,2,3 and CUL-5 based ubiquitin ligases. We are currently further characterizing the role of CUL-5 during polarity establishment and asymmetric cell division.

1063B

Molecular and genetic characterization of suppressor of *bec-1* lethality *(sub)* mutants. **Nicholas J Palmisano**, Alicia Meléndez. Biology, Queens college, The Graduate Center, CUNY New York, NY.

Autophagy is the major intracellular pathway for the degradation and recycling of long-lived proteins and organelles and is thus crucial for cell homeostasis. It is induced by stress, over-crowding, or starvation conditions. C. elegans *bec-1* is the ortholog of *beclin1/ATG6/VPS*30 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. In mammals, *beclin 1* has been shown to be a haploinsufficient tumor suppressor gene. 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, insights on the function of *bec-1* in C. elegans will likely shed light on its role in tumorigenesis in humans. We and others have found that *bec-1* functions in various biological processes, including survival, longevity, fat accumulation, dauer and reproductive development. In addition, we have recently shown that *bec-1* is important for the proper localization of MIG-14 to the Golgi network and is required for germ cell corpse clearance, implicating a role for *bec-1* in phagosome maturation. Thus, *bec-1* serves a role in both autophagy and endocytosis. Complete loss of *bec-1* results in lethality, therefore highlighting the importance of *bec-1* in development. Whereas many functions and interactions of Atg6/Beclin 1/BEC-1 have been described, little is known about the genes that regulate or act downstream of Atg6/Beclin 1/BEC-1 during development. A complete loss of function mutation of C. elegans *bec-1(ok691)* is essential for viability. We have isolated two dominant suppressor mutations that suppress the *bec-1* lethal phenotype, and named these mutants *sub* for *sup*pressor of *bec-1* lethality. Importantly, both *sub* mutations suppress all *bec-1* loss of function phenotypes tested, including the decrease in fat accumulation in *bec-1* homozygous mutant animals, as well as the shortening of lifespan associated with heterozygous *bec-1* mutants. Using whole

genome sequencing combined with SNP mapping, we have found various candidate mutations for *sub-1* and *sub-2*. Since both mutations act dominantly in their suppression for *bec-1*, they may represent either gain of function or haploinsufficient mutations. We will report on the molecular and genetic characterization of the *sub* mutations. We hypothesize that these suppressor mutations represent novel loci that may regulate *bec-1* mediated activity. Such novel loci would further our knowledge of BEC-1 and its role in development as well as provide new therapeutic targets for cancer treatment and age-related diseases.

1064C

Arp2/3 dependent branched actin regulates intracellular trafficking to maintain apical junctions. **Falshruti B. Patel**, Yelena Bernadskaya, Martha Soto. Dept Pathology, UMDNJ/RWJMS, Piscataway, NJ.

Trafficking of proteins during development is highly regulated for proper polarity. The apical adherens junctions define the apical-basal boundary in a polarized epithelial cell. Regulated trafficking is required for formation and maintenance of apical junctions throughout the life of an animal. We have previously shown that the CIP4/TOCA proteins that induce membrane curvature during trafficking regulate junctional protein abundance at the membrane. In vitro data shows that TOCA proteins directly interact with the WAVE/SCAR complex of the conserved Rac1-WAVE/SCAR-Arp2/3 (GEX) pathway that regulates branched actin nucleation. Mutation in the C.elegans homologs of the TOCA and GEX pathway proteins lead to similar morphogenesis defects. Loss of the GEX pathway causes a drop of the apical F-actin levels correlating with intestinal lumen expansion. We also observed cell shape defects in the gex and arp-2 mutants prompting us to study the effects of loss of GEX pathway on adherens junctions. Live imaging and EM studies show that loss of the GEX pathway affects junctional protein maintenance. In live embryos, loss of the GEX pathway causes a drop in the levels of some apical junction proteins. EM studies on animals depleted of the GEX pathway components post-embryonically show thin elongated junctions compare to the short, electron dense junctions of wild type animals. This suggests a role of the GEX pathway in maintenance of the apical junctions. We performed apical uptake assays in the intestine to test if shift in the junctions and lumen expansion can be explained by apical endocytosis defects. Preliminary data suggests that the GEX pathway is not required for apical uptake suggesting no defects in endocytosis. To test if defects at the apical junction are due to abnormal recycling, we analyzed a recycling endosome protein, RME-1. Live imaging shows that depletion of the GEX pathway dramatically affects RME-1 localization. Fractionation of the GEX mutants also shows changes in the RME-1 subcellular distribution. Furthermore, loss of RME-1 affects junctional protein levels in the epidermis similarly to the GEX pathway components. These data indicate that WAVE and Arp2/3 dependent branched actin regulation of trafficking contributes to the maintenance of apical adherens junctions.

1065A

Identification and characterization of novel Wnt signaling pathway components. **Woon Cheng Poh**, Yanqing Shen, Takao Inoue. Biochemistry, NUS, Singapore.

C. elegans vulval development is a well-established model for the study of signal transduction networks and morphogenesis. In animals lacking Wnt receptors *lin-18*/Ryk or *lin-17*/Fz, P7.p descendants show reversed polarity and form a pseudovulva posterior to the normal vulva. To identify novel components of the *lin-18*/Ryk-mediated signalling pathway, we are using EMS mutagenesis to generate mutations that cause P7.p polarity reversal (P-Rvl) and sterility and mutations that suppress the *lin-18;lin-17* double mutant. In a F1 clonal screen of 2000 mutagenized haploid genomes, two mutants with a Pn.p reversal-like phenotype were isolated, in addition to a *lin-17* allele. Both mutants were viable as homozygotes and showed 20% anterior or posterior pseudovulva. We also screened 7260 mutagenized haploid genomes for suppressors of the *lin-18;lin-17* double mutant which shows 100% P7.p reversal and lethality. Candidate mutants that suppress the P-Rvl and lethal phenotypes were obtained, and are being analyzed. In mammalian cells, Ryk is cleaved at the transmembrane domain and the intracellular domain (ICD) is transported into the nucleus. We generated *Plin-18::lin-18 ICD::gfp* and *Plin-18::lin-18KD::gfp* (KD=kinase domain) transgenic animals and found that LIN-18 ICD and KD were nuclear localized. We are using RNAi to identify components that regulate LIN-18 cleavage and nuclear localization.

1066B

Endocytic regulation of LET-23 EGFR signaling during vulva induction. Olga Skorobogata, **Christian E. Rocheleau**. Dept Med, McGill Univ, Montreal, PQ, Canada.

The Rab7 GTPase regulates late endosome trafficking of the Epidermal Growth Factor Receptor (EGFR) to the lysosome for degradation. However, little is known about how Rab7 activity, functioning late in the endocytic pathway, affects EGFR signaling. We are using *C. elegans* vulva cell fate induction, a paradigm for genetic analysis of EGFR/Receptor Tyrosine Kinase (RTK) signaling, to assess the genetic requirements for *rab-7*. Using a *rab-7* deletion mutant, *ok511*, we found that RAB-7 antagonizes LET-23 EGFR signaling to a similar extent as previously described negative regulators. Epistasis analysis places *rab-7* upstream or in parallel to *lin-3 EGF-like* and *let-23 EGFR*. However, *rab-7* expression in the Vulva Presursor Cells (VPCs) is sufficient to rescue the *rab-7(ok511)* VPC induction phenotypes indicating that RAB-7 functions in the signal receiving cell and suggests that RAB-7 regulates ligand-dependent signaling. Components of the Endosomal Sorting Complex Required for Transport (ESCRT)-0, and -I, complexes, *hgrs-1 Hrs*, and *vps-28*, also antagonize signaling, suggesting that LET-23 EGFR likely transits through Multivesicular Bodies (MVBs) en route to the lysosome. Consistent with RAB-7 regulating LET-23 EGFR trafficking, LET-23::GFP is present in larger puncta in the VPCs of *rab-7* mutant animals. Our data imply that Rab7, by mediating EGFR trafficking and degradation, plays an important role in downregulation of EGFR signaling. Failure to downregulate EGFR signaling contributes to oncogenesis, and thus Rab7 could possess tumor suppressor activity in humans.

rab-7(ok511) strongly suppresses the *lin-2(-)* Vulvaless phenotype, but would not have been identified in previous *lin-2(-)* suppressor screens due to its maternal effect lethal phenotype. We performed a clonal *lin-2(-)* suppressor screen to identify additional maternal effect lethal suppressors. We identified two suppressors, *vh4* and *vh22*, which map to distinct regions of chromosome I. *vh4* fails to complement *agef-1(-)* and *agef-1(RNAi)* suppresses *lin-2(-)*, however no lesion was identified in the *agef-1* coding sequence. AGEF-1 is a putative Guanine nucleotide Exchange Factor for ARF-1/-3 GTPases that could function with UNC-101 and the AP-1 adaptor complex to modulate LET-23 EGFR signaling. We are using whole genome sequencing to identify the causative DNA lesions for *vh4* and *vh22*.

1067C

Novel players of cell polarity and asymmetric cell division in *C. elegans* identified through analysis of a polarity genetic network. **Josana Rodriguez**, Bruno Fievet, Julie Ahringer. Gurdon Inst, Cambridge, United Kingdom.

Cell polarity and asymmetric cell divisions are essential for the generation of cellular diversity. The importance of these processes during development has been emphasized by recent findings, which show that defects lead to the formation of tumors. Towards identifying most of the factors involved in cell polarity and asymmetric cell division, we carried out 17 large scale RNAi suppressor screens of temperature sensitive mutants of genes involved in actomyosin contraction, par polarity and microtubule pulling forces governing spindle positioning. This allowed us to generate a polarity genetic interaction network of 186 genes with 229 genetic interactions (see Fievet et al., abstract). Of these genes, 23 have been previously shown to have a role in the asymmetric first cell division, validating our approach. For the rest of the candidates (87% of our network), no early polarity phenotype has been reported for knock-down in a wt background.

To identify functions of novel candidates, we have been studying effects of their knockdown in sensitized backgrounds. This strategy has successfully identified specific functions for genes in the network. For example, out of the 24 identified suppressors of *nmy-2(ts)* (non-muscle myosin II), we found that eight enhance embryonic lethality when knocked-down in a gain of function mutant of *act-2* (actin) and seven out of these eight enhancers show defects in the actomyosin cytoskeleton during the first cell division. In collaboration with Stephan Grill we are using biophysical methods to characterize actomyosin dynamics upon loss of function of these candidates. We are using a similar approach to characterize the suppressors of *par-2(ts)* and *pkc-3(ts)*. Functional antagonism between PAR-2 and PKC-3 is critical for *C. elegans* asymmetric first cell division and robustly found in our screens. Therefore, we are studying the enhancement phenotype of *par-2* suppressors in *pkc-3(ts)* and vice versa. In another strategy, we have been using patterns of genetic interactions to predict functions for novel genes in the network. This analysis identified several potential new components and/or regulators of the anaphase-promoting complex (APC). In addition, the network functionally implicates different signalling pathways in polarity. For example, we have identified a role for PKA signalling in the regulation of the asymmetric first cell division, which we are currently studying.

Due to the conservation of cell polarity mechanisms, we expect that the *C. elegans* polarity network we have built will be relevant for cell polarity events in other model organisms.

1068A

Characterizing the adrenomedullin homologue in C. elegans. **Beatriz Sáenz-Narciso**, Eva Gómez-Orte, Alfredo Martínez, Juan Cabello. Oncology, CIBIR, Fundacion Rioja Salud, Logroño, La Rioja, Spain.

Adrenomedullin (AM) is a peptide hormone that shows multiple physiological functions in vertebrates, such as bronchodilation, neurotransmission, hormone regulation, antimicrobial activity or growth regulation. Deregulation of AM has been shown in different human pathologies such as diabetes, cancer, hypertension, heart failure, and sepsis. Research on molecules capable of modulating AM and its effects may lead to the discovery of new pharmacological agents to treat these important human diseases. Highly interesting is the role of AM in cancer. It has been proven that AM acts as an autocrine/paracrine tumor cell survival factor and its elevated expression in cancer cells can promote angiogenesis leading to tumor progression. While AM has been well characterized in other research models, little is known about this molecule in C. elegans. We have found and characterized a mutant in the AM homologue of C.elegans, here called wam-1. 4D microscopy analysis of wam-1 mutants shows defects in the migration of the hermaphrodite gonad. There are also several defects in embryonic development. Some embryos have cells that are excluded out of the worm during development while fate specification, as well as proliferation is normal. This suggests a function of wam-1 in cell adhesion. To localize the expression of the worm AM homologue protein, we performed inmunostaining using mouse anti-AM antibodies. The AM protein was localized in the hypodermis. In contrast, we also generated transgenic lines expressing GFP under the control of the wam-1 promoter. GFP expression was detected in the mouth of the worm, although due to its homology with secreted hormones, the protein could play a role far away from the secretory cells. At the moment, we are performing a detailed molecular and genetic characterization, in order to understand the function of this molecule.

1069B

Dynein-dependent microtubule sliding is not sufficient for anaphase spindle-pulling force in the *C. elegans* embryo. Eva Gusnowksi, **Martin Srayko**. Biological Sci, Univ Alberta, Edmonton, AB, Canada.

Most multicellular organisms utilize asymmetric cell division to generate cellular diversity throughout development. In *C. elegans*, the first cell division results in two daughters that are developmentally distinct and unequal in size. The size asymmetry is due to precise positioning of the mitotic spindle towards the posterior side of the cell. In order for posterior spindle pole displacement to occur properly in the one-cell embryo, the motor protein dynein and members of a heterotrimeric G-protein pathway are required to generate asymmetric forces that originate at the inner embryonic cortex[1]. However it is not clear how the different components of this pathway contribute to force generation to accomplish spindle displacement.

Dynein is a minus-end directed microtubule motor that moves along the microtubule lattice in a processive "walking" manner. Dynein appears to localize throughout the anterior-posterior cortex, therefore, it is possible that the motors are asymmetrically activated, with a higher level of motor activity at the posterior cortex. In order to address how dynein could be asymmetrically activated to achieve posterior spindle displacement, we developed an assay to measure dynein's activity on individual microtubules at the embryo cortex. This assay utilizes the microtubule end-binding protein EBP-2 to track individual polymers as well as a method to release microtubules from the centrosomes so that they are free to move along the cortex in response to external motor forces. Our study reveals that cortical dynein motors maintain a basal level of activity that propels microtubules along the cortex, even under experimental conditions that drastically reduce anaphase spindle forces. This suggests that dynein-based MT sliding is not sufficient for anaphase spindle-pulling force. Instead, we find that this form of dynein activity is most prominent during spindle centering in early prophase. We propose a model whereby different dynein-mictrotubule interactions are utilized for specific spindle-positioning tasks in the one-cell embryo.

1. Gönczy et al., 1999 J Cell Biol.; Gotta and Ahringer, 2001 Nat. Cell Biol.; Gotta et al., (2003) Current Biology; Grill et al., (2001) Nature; Srinivasan et al., (2003) Genes & Dev.

1070C

Spindle asymmetry produced by Wnt signaling regulates asymmetric nuclear localization of β-catenin. **Kenji Sugioka[1,3]**, Kota Mizumoto[1], Hitoshi Sawa[1,2]. 1) Laboratory for Cell Fate Decision, CDB, RIKEN, Hyogo, Kobe, Japan; 2) Multicellular Organization Laboratory, National Institute of Genetics, Mishima, Japan; 3) Dept. Biol., Grad. Sch. Sci., Kobe Univ., Kobe, Japan.

Asymmetric division is a fundamental process to produce cellular diversity. In *C. elegans*, many asymmetric divisions are regulated by the Wnt signaling pathway. After the EMS cells are polarized by a Wnt signal from the posteriorly located P2 cell, 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 during the division and found that the numbers of astral microtubules were asymmetric during telophase; higher at the anterior spindle pole than the posterior. This spindle asymmetry was regulated by Wnt signaling. Because APR-1/APC localizes asymmetrically to the anterior cortex and colocalizes with microtubules, APR-1 is likely to stabilize astral microtubules to produce the asymmetry of spindle. 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.

1071A

Identification of SORF-1 (suppressor of organelle fusion) as a negative regulator of endocytic organelle fusion in *C.elegans*. **Xiaojuan Sun[1]**, Zhiyang Gao[1], Jing Xu[1], Yudong Jing[1], Xuezhao Liu[1], Hui Xiao[2], Chonglin Yang[1]. 1) Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No. 1 West Beichen Road, Chaoyang District, Beijing 100101, China; 2) The Department of Genetics The Scripps Research Institute 10550 North Torrey Pine Road La Jolla 92037, USA.

Endocytosis plays important roles in diverse physiological processes such as nutrient uptake, signal transduction and development. During endocytosis, appropriate homotypic fusion of early/late endosomes and conversion from early to late endosomes are critical for successful delivery of endocytic cargoes to lysosomes for degradation. Although many factors were found to promote the early-to-late endosome conversion or homotypic organelle fusion, it is less understood how these events are negatively regulated. Here we report a novel gene named *sorf-1*(suppressor of organell fusion) that antagonizes endocytic organelle fusion in *C. elegans*. In *sorf-1* loss-of-function mutants, endosomal/lysosomal compartments are slightly, but significantly, enlarged in the macrophage-like coelomocytes. In the absence of *rab-7*, however, *sorf-1* mutation results in the appearance of a giant granule reminiscent of early endosome in coelomocytes, indicating that loss of *sorf-1* function caused inappropriate fusion of early endosomes. Similar phenotype was observed in double mutants of *sorf-1* with *sand-1* or *ccz-1*, which are essential for *rab-7* to mediate the early-to-late endosome conversion. We also found that *sorf-1(lf)* rescued the small late endosome and lysosome phenotype in *arl-8(lf)* mutants in which lysosome biogenesis is defective, suggesting that *sorf-1* also antagonizes late endosome/lysosome fusion. Moreover, SORF-1 forms complex with SAND-1, which is disrupted by CCZ-1. Altogether, these findings suggest that SORF-1 may play a dual role in early-to-late endosome conversion and homotypic fusion for proper biogenesis of endocytic organelles.

1072B

Arf GTPases antagonize the Frizzled/Planar cell polarity pathway to regulate the asymmetric cell division of the Q.p neuroblast. **Jerome Teuliere**, Aakanksha Singhvi, Shaun Cordes, Karla Talavera, Gian Garriga. Department of Molecular & Cell Biology, Univ California, Berkeley, Berkeley, 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 divisions of neuroblasts 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 caused the Q.p division to produce two precursors that were more similar in size. Several of these genes encode components of the Arf GTPase cycle, which is involved in membrane trafficking. Loss of the Arf GAP CNT-2 in particular strongly altered the Q.p division, generating two daughters of equivalent size. Analysis of RME-2 in *cnt-2* mutant oocytes showed that CNT-2 regulates receptor-mediated endocytosis, and RNAi knockdown of endocytosis genes disrupts the Q.p division, suggesting that CNT-2 mediates endocytic events necessary for the Q.p ACD. What might CNT-2 regulate? We have also implicated the LIN-17 and MOM-5 Frizzled receptors in the Q.p ACD: *lin-17 mom-5* double mutants but not *lin-17* or *mom-5* single mutants displayed Q.p lineage defects, but Wnts were not needed for this regulation. From genetic interactions analysis, cell-autonomy studies and protein localization data, we propose that CNT-2 negatively regulates a Frizzled/Planar Cell Polarity pathway.

1073C

Sensory input modulates ciliary receptor localization and IFT activity. **Juan Wang[1]**, Natalia Morsci[2], Maureen Barr[1]. 1) Rutgers University, Piscataway, NJ; 2) University of Wisconsin-Madison.

Cilia are sensory organelles that house sensory receptors and signaling transduction complexes. Intraflagellar transport (IFT) is required for maintaining cilia structure and function. Here, we investigate how ciliary proteins localization and IFT activity are regulated by sensory input. *Caenorhabditis elegans* possesses sensory cilia at distal dendrites of sensory neurons, which detect a repertoire of environmental stimuli. Four male-specific CEM ciliated sensory neurons are required for mate detection. C. elegans LOV-1 and PKD-2 are TRP polycystin (TRPP) receptor/channel complex enriched in CEM cilia and required for CEM sensory function. By culturing adult males raised in isolation or in the presence of adult hermaphrodites, we defined the basal or stimulated condition of CEM cilia. We found that GFP tagged PKD-2 is dynamically regulated in response to sensory input. PKD-2 protein accumulated at ciliary base under stimulated conditions. Using IFT reporters, we visualized IFT activity in CEM cilia and observed changes in IFT velocities under different conditions. In contrast to wild type, *lov-1* mutant males fail to upregulate IFT activity when exposed to mates, indicating that *lov-1* is required for the regulation of the IFT dynamics in the CEM neurons. We conclude that IFT activity and ciliary membrane protein localization are dynamically regulated by sensory input, and that the polycystins regulate IFT activity.

1074A

Regulation of Rab GTPase-mediated endosomal trafficking by TBC-2. **Xiaolin A Wang**, Farhad Karbassi, Marc-André Sylvain, Krittika Bhende, Anna Chavlovski, Christian E Rocheleau. Medicine, McGill University, Montreal, PQ, Canada.

The RAB-5 and RAB-7 GTPases are key regulators of endosome to lysosome trafficking, whose activities are positively regulated by Rab Guanine nucleotide Exchange Factors and negatively regulated by GTPase Activating Proteins (GAP). We previously demonstrated that TBC-2 functions as a RAB-5 GAP. Loss of tbc-2 activity results in the formation of enlarged late endosomes in the intestine that require the activities of RAB-5, RAB-7 and components of the HOPS complex and enhanced degradation of Yolk during embryogenesis. TBC-2 colocalizes with RAB-7 on late endosomes, and requires RAB-7 for membrane localization. We hypothesize that RAB-7 recruits TBC-2 to late endosomes to inactivate RAB-5 and possibly RAB-7, to facilitate early to late endosome maturation.

We are currently testing if membrane localization is important for TBC-2 function and how TBC-2 is recruited to endosomal membranes. We have yet to detect a significant physical interaction between TBC-2 and RAB-7. Rac1 is an interesting potential candidate to bridge or stabilize this interaction, as it has been found to physically interact with both

Rab7 and Armus (a TBC-2 homolog) in mammals. We found that TBC-2 interacts with all three C. elegans Rac proteins, CED-10, RAC-2, and MIG-2. This is interesting in light of the fact that both TBC-2 and CED-10 are required for efficient phagocytic degradation of apoptotic corpses. We are currently testing the biological significance of these interactions.

To identify additional regulators of endosome to lysosome trafficking, we performed a *tbc-2(tm2241)* suppressor screen. We identified *vh8* as a mutant that not only suppressed the large late endosome phenotype, but also resulted in a loss of GFP::RAB-7 from endosome membranes, suggesting that *vh8* defines a regulator of RAB-7 activity and/or membrane localization. We mapped *vh8* to chromosome III, close to the *tra-1* gene. We are using RNAi to test candidate genes in this region for similar phenotypes as *vh8*. Preliminary data indicates that *obr-1*, a homolog of an oxysterol binding and Rab7 interacting protein, ORP1L, as a candidate.

1075B

The BBSome regulates IFT particles turn-around at cilia tip. **Qing Wei**, Yuxia Zhang, Yujie Li, Jinghua Hu. Dept Med, Mayo Clinic, Rochester, MN.

Cilia act as motile or sensory devices on the surface of most eukaryotic cells, and cilia dysfunction results in a variety of severe human pathologies, now collectively termed ciliopathies. Phylogenetically conserved intraflagellar transport (IFT) machinery, which is composed of IFT-A and IFT-B subcomplexes, mediates the bidirectional movement of IFT cargos that are required for the biogenesis, maintenance, and signaling of cilia. However, how IFT particles assemble at cilia base and turnaround at cilia tip are poorly understood. From a mutagenesis screen searching for C. elegans mutants with defective IFT turnaround at the ciliary tip, we identified a new dyf-2 allele, dyf-2(jhu616), which encodes a mutant DYF-2 with a G361R alteration in conserved WD40 domain. In contrast to the reported null dyf-2 allele in which cilia are severely truncated, dyf-2(jhu616) mutants possess superficially normal cilia, and IFT-A and IFT-B move together anterogradely, suggested that this mutation itself doesn't affect the IFT-A and IFT-B complex integrity. However, IFT-B components and its associated OSM-3 motor, but not IFT-A components and its associated kinesin-II motor, accumulate at the ciliary tip. Kymograph analysis indicates that only IFT-A moves retrogradely in dyf-2(jhu616). Surprisingly, the BBSome are not found associating with moving IFT particles in dyf-2(jhu616). Furthermore, bbs-1(jhu598), which encodes a mutant BBS-1 altered at conserved G207 site (G207D), was characterized as the mutant that fully recapitulates the defective IFT turnaround phenotypes of dyf-2(jhu616) in our screen. Further analyses support a model that the BBSome controls IFT complex assembly at the ciliary base, and then associate with anterograde IFT particles in a DYF-2 dependent way to the ciliary tip to regulate IFT reassembly and turnaround. The absence of the BBSome at the ciliary tip impairs the IFT retrograde integrity.

1076C

A novel basal body protein DYF-17 controls the ciliary entry of DYF-11 and IFT assembly. **Qing Wei**, Jinghua Hu. Dept Med, Mayo Clinic, Rochester, MN.

Cilia, motile or sensory devices protruding from most eukaryote cell surfaces, are microtubule-based hair like organelles that extend from the modified mother centriole (basal body). Cilia dysfunction has been linked to a wide spectrum of human disorders, now collectively termed ciliopathies. Intraflagellar transport (IFT), a bidirectional movement of protein particles along the cilia axonemal microtubule, is required for cilia assembly and maintenance. However, little is known about how IFT particles assemble at the ciliary base. Here, we isolated a new C.elegans ciliogenesis mutant jhu455, which encodes an uncharacterized protein DYF-17. GFP-tagged DYF-17 only label cilia base. Double staining with other cilia base markers indicated DYF-17 locates between the dendritic tip and the proximal end of the transition zone. In jhu455 animals, cilia are truncated. Interestingly, most IFT-B components could enter into the residual cilia, whereas IFT-A proteins only accumulate around the ciliary base and show no ciliary staining. IFT cargo proteins (ciliary membrane receptors) were also found to mislocalize in jhu455 background. These observations indicate a role for DYF-17 in regulating proper assembly of IFT particles at the ciliary base. Remarkably, we found that, in all IFT components examined, only DYF-11 (an IFT-B polypeptide) exhibits a unique mutant phenotype with abnormal and strong accumulation below the transition zone, the exact site where DYF-17 locates. DYF-11 was reported to be essential for IFT assembly and bridge the association of IFT machinery and membrane cargos. Our data showed that dyf-11 mutants completely photocopy the mutant phenotypes of jhu455 animals. Furthermore, we found that the mammalian homolog of DYF-17 locates at the distal appendage of mother centriole in immuno-EM assay and co-immunoprecipitated with mammalian DYF-11 homolog MIP-T3. Knockdown of mammalian DYF-17 abolishes the ciliary localization of MIP-T3 and compromises the cilia formation. Taken together, our data support a highly conserved ciliogenesis pathway in which recruiting of DYF-11 by distal appendage protein DYF-17 is a prerequisite for the ciliary entry of DYF-11 and the downstream IFT assembly.

1077A

Unraveling the VAP MSP secretion mechanism. **Jessica L Winek**, Michael Miller. University of Alabama at Birmingham, Birmingham, AL.

VAPs are evolutionarily conserved proteins with an N-terminal MSP (major sperm protein) domain and C-terminal transmembrane domain. A P56S substitution in the VAPB/ALS8 MSP domain is associated with amyotrophic lateral sclerosis (ALS) and late-onset spinal muscular atrophy (Nishimura et al., 2004). We have shown that VAP MSP domains are cleaved from the transmembrane domain and secreted into the extracellular environment (Tsuda et al., 2008). The P56S mutation inhibits secretion. Therefore, MSP secretion may play a critical role in ALS pathogenesis and understanding this secretion mechanism could lead to novel therapeutic strategies. MSP domains do not contain a signal peptide and are secreted by an unconventional mechanism (Kosinski et al., 2005). The goal of this project is to elucidate the mechanism by which neurons secrete VAP MSP domains. Recent work in yeast has shown that the Acb1 protein is secreted by a novel mechanism that depends on autophagy genes (Duran et al., 2010; Manjithaya et al., 2010). We are testing the hypothesis that this mechanism is essential for VAP MSP secretion. Our initial data suggests that MSP domains may have a similar secretion mechanism to the Acb-1 protein in Saccharomyces cerevisiae.

1078B

Cis- and *trans*-regulatory mechanisms of ciliary targeting in a subset of chemosensory neurons in C. elegans. **Martin Wojtyniak**, Piali Sengupta. Department of Biology, Brandeis University, Waltham, MA.

Most eukaryotic cells possess primary cilia which are sensory organelles specialized for environmental sensation. Cilia house signaling proteins such as transmembrane receptors and channels. Ciliary localization of these proteins is essential for cilia function and cellular homeostasis, and mislocalization of these proteins leads to ciliopathies. For example, mutations resulting in the mislocalization of rhodopsin have been linked to retinal and macular degenerative diseases in mammals. In *C. elegans*, the cilia of chemosensory neurons contain G protein-coupled receptors (GPCRs) and channels required for sensing environmental cues. Although some of the *cis*- and *trans*-acting factors required for ciliary localization of these proteins have been identified in nematodes and other systems, it is likely that additional mechanisms also play a role, and that different mechanisms operate in different cell types. The goal of this project is to identify the sequences and factors that are essential for targeting membrane proteins to the cilia of specific chemosensory neuron types in *C. elegans*. The cyclic nucleotide gated channel beta subunit TAX-2 is localized to the proximal segment of the cilia in the AWB olfactory neuron type. We find that this localization is dependent on a C-terminal *cis*-acting sequence and are determining whether this sequence is also sufficient for ciliary targeting. Via expression of GPCRs in different sensory neurons, we have described cell-type specific differences in their ciliary localization suggesting that different sets of *trans*-acting factors may be required to target GPCRs to cilia in different neurons. Current work is aimed at identifying the sequences in GPCRs required for cilia targeting, as well as analysis of candidate *trans*-acting factors.

1079C

LET-99, a novel G protein regulator for asymmetric division. Dae Hwi Park, **Anna Ye**, Lesilee S. Rose. Molecular and Cellular Biology, UC Davis, Davis, CA.

Asymmetric divisions that generate cell diversity are required for normal development and stem cell maintenance. Spindle positioning is a key aspect of asymmetric division. The mitotic spindle must be aligned with the axis of cell polarity in order for cell fate determinants to be differentially segregated to daughter cells to give them different fates. The conserved PAR polarity proteins establish cell polarity, and they also regulate spindle movements via a complex involving Gα subunits, GPR and LIN-5. The Gα/GPR/LIN-5 complex is necessary for the cortical forces the pull on astral microtubules, potentially by recruiting regulators of the microtubule motor dynein. In *C. elegans* one-cell embryos, GPR and LIN-5 are asymmetrically localized in a dynamic pattern that correlates with changes in the pulling forces. GPR and LIN-5 first show an overall anterior enrichment during prophase, when pulling forces center the nuclear-centrosome complex and rotate it on to the polarity axis. GPR/LIN-5 enrichment then switches to the posterior during the spindle displacement movements that produce an unequal division. We previously showed that LET-99 is a key component of this pathway and acts downstream of the PAR proteins during both nuclear centration/rotation and spindle displacement. LET-99 antagonizes G protein signaling by inhibiting the localization of GPR at the cortex. The highest levels of LET-99 are present in a posterior-lateral cortical band, which results in the lowest levels of cortical GPR in this region; nonetheless, LET-99 function is also needed for the overall anterior and posterior enrichment of GPR through the cell cycle. To determine the mechanism of LET-99 action, we tested LET-99 for interaction with other components of the pathway. In vitro pull down and yeast two-hybrid assays show that LET-99 can interact with GOA-1 and GPA-16, the two Gα subunits used in this system. Direct binding of LET-99 to Gα could directly interfere with formation of a Gα/GPR/LIN-5 complex; alternatively, LET-99 could regulate an upstream step involving other pathway components such as Gβ or RIC-8. To further elucidate the molecular mechanism of LET-99 action, we are carrying out additional genetic and interaction studies with Gβ and Ric-8.

1080A

RAB-6 and the Retromer Complex Regulate Glutamate Receptor Recycling Through A Retrograde Transport Pathway. **Donglei Zhang**[1,3], Nora R. Isack[1], Doreen R. Glodowski[1], Barth D. Grant[2], Christopher Rongo[1]. 1) The Waksman Institute, Department of Genetics, Rutgers The State University of New Jersey, Piscataway, New Jersey, USA; 2) Department of Molecular Biology and Biochemistry, Rutgers The State University of New Jersey, Piscataway, New Jersey, USA; 3) Graduate Program in Molecular Biosciences, Rutgers The State University of New Jersey, Piscataway, New Jersey, USA.

Synaptic plasticity is regulated by the collective endocytosis, membrane recycling, and degradation of AMPA-type glutamate receptors (AMPARs), yet the specific intracellular trafficking pathways available to AMPARs, and the mechanism by which such pathways are selected for AMPARs in neurons, is poorly understood. Here we show that the AMPAR subunit GLR-1 in *C. elegans* utilizes the retrograde transport pathway to regulate its synaptic abundance. GLR-1 is localized on post-synaptic membranes, where it regulates

reversals of locomotion in a simple behavioral circuit. GLR-1 synaptic abundance is regulated by a combination of endocytosis and membrane recycling, although the specific pathways have not been fully elucidated. One possible pathway is retrograde transport via Rab6 GTPases and the retromer complex, which shepherd certain membrane proteins from endosomes back to Golgi; in the absence of retrograde transport, cargo proteins are degraded. We find that in mutants for *rab-6.2*, or the retromer genes *vps-35*, *snx-1*, and *rme-8*, GLR-1::GFP receptors are degraded in neurons, and mutant animals show behavioral defects indicative of diminished GLR-1 function. By contrast, expression of a constitutively active RAB-6.2 drives the retrograde transport of GLR-1 back to cell body Golgi. Mutations that block endocytosis suppress the effects of *rab-6.2* mutations, suggesting that RAB-6.2 functions downstream of GLR-1 endocytosis. Mutations that block GLR-1 turnover in *rab-6.2* mutants cause the accumulation of GLR-1 in endosomes, suggesting that GLR-1 is directed to lysosomes when retrograde trafficking is blocked. MIG-14, which undergoes retrograde recycling, undergoes a similar degradation in *rab-6.2* mutants, consistent with a loss of retrograde transport. In its GTP-bound state, RAB-6.2 physically interacts with, colocalizes with, and directs the subcellular localization of the PDZ/PTB domain protein LIN-10, and the regulation of GLR-1 retrograde transport by RAB-6.2 requires LIN-10 activity. Specific retrograde cargo and a function for the retromer in neurons have not been previously demonstrated. Our results indicate that RAB-6.2 and LIN-10 recycle AMPARs along a retrograde transport pathway in neurons so as to maintain synaptic strength.

1081B

qx193 affects lysosome dynamics and motility in *C. elegans*. **Wei Zou**, Baohui Chen, Bin Liu, Xiaochen Wang. NIBS, Beijing, China.

Lysosomes are specialized compartments that degrade endocytosed and intracellular materials. Defects in lysosome function lead to a rapidly growing numbers of human disorders, indicating the important role of lysosomes in cellular homeostasis and animal development. However, how lysosome biogenesis and dynamics are regulated remains poorly understood. *qx193* mutation was generated by gamma irradiation, which affects lysosome morphology in several different cell types in Caenorhabditis elegans. In wild type, lysosomes which are labeled by either the lysosomal enzyme NUC-1 or a lysosomal membrane protein LAAT-1 or are stained by lysotracker blue appear as both small puncta and thin tubules. In *qx193* mutants, however, the tubular structures are completely disrupted and lysosomes appear to aggregate as big puncta. This phenotype is more evident in late embryonic and early larval stages. In addition, lysosomes which were quite dynamic in wild type became static in *qx193* mutants, suggesting that lysosomal motility may be affected. We examined degradative activity of lysosomes and found that *qx193* mutants affect degradation of several different kinds of cargoes including yolk protein, autophagic cargoes and apoptotic cells. To investigate how *qx193* affects lysosome morphology and motility, we examined lysosomes in *qx193* animals that are also defective in *rab-5*, *rab-7* or *hgrs-1* which acts at different steps of endolysosomal transport. Our results suggest that the gene affected by *qx193* likely functions downstream of these genes. We are now in the process of cloning of this gene and further characterizing its function in regulating lysosome dynamics.

1082C

In vivo analysis of epithelial cell polarization. **Stephen Armenti**, Jeremy Nance. NYU School of Medicine, New York, NY.

Establishing epithelial cell polarity and cell-cell junctions are critical steps in tissue morphogenesis. In order to form mature junctions, epithelial cells must (1) respond to polarity cues and localize junction proteins to the site of future junction formation and (2) coalesce these proteins into fully-formed junctions. This process is essential during organogenesis, where the establishment and maintenance of junctions enables cells to segregate functional membrane domains. Our lab uses C. elegans to study the highly conserved process of epithelial polarization and junction formation. We have shown that the scaffolding protein PAR-3 is required to polarize the embryonic intestine but is dispensable for epidermal polarization. However, additional loss of apical membrane determinant EAT-20/Crb in PAR-3 mutant embryos compromises epidermal polarization. We are currently investigating how EAT-20 localizes and functions with PAR-3 in junction formation. Our findings will provide new insights as to how distinct cell types can employ unique methods to polarize. In other systems, PKC-3/aPKC regulates junction stability downstream of PAR-3. However, the molecular mechanism for this process has yet to be fully described. We have performed a genetic suppressor screen to identify genes functioning with pkc-3 to regulate epithelial junctions. We are cloning two unknown pkc-3 suppressors identified in this screen and characterizing how they regulate epithelial junctions. We have shown that these suppressors specifically rescue junction defects seen in pkc-3ts mutants. Findings from this study will provide new insights into the basic mechanisms of junction formation.

1083A

Two parts of Caenorhabditis elegans glypican LON-2 negatively regulate a BMP-like pathway and require membrane proximity. **S. Bageshwar**, T. Gumienny. Molec & Cellular Medicin, Texas A&M Hlth Sci Ctr, College Station, TX.

Several members of the heparan sulfate proteoglycan (HSPG) family regulate growth factor signaling, but the molecular mechanisms for this regulation remain largely unknown, especially for TGF-β signaling. *Caenorhabditis elegans* glypican LON-2 negatively regulates the DBL-1/TGF-β signaling pathway by directly binding DBL-1. Loss of *lon-2* results in animals longer than wild type, caused by increased signaling by the TGF-β superfamily member DBL-1. Glypicans consist of a globular domain containing 14 conserved cysteines and a linker domain consisting of heparan sulfate glycosaminoglycan (GAG) chains. They are tethered to the outer cell surface by a glycosylphosphatidylinositol (GPI) anchor. Through rationally designed mutagenesis studies, we report that GAG chains are not critical for the function of full length LON-2; however, the LON-2 linker region with GAG-attachment sites is sufficient to antagonize BMP signaling. Therefore, either the globular protein core or the GAG chains on the linker are sufficient for LON-2 activity *in vivo*. We also showed that membrane localization of LON-2 is required for its function. LON-2, like some other glypicans and several other extracellular TGF-β regulators, contains an arginine-glycine-aspartic acid (RGD) tripeptide sequence towards the C-terminus of its globular domain. The RGD sequence binds integrins, transmembrane heterodimeric proteins that mediate interactions of cells with extracellular matrix and intracellular cytoskeleton. To be functional, membrane localization of LON-2 must be provided either indirectly through the RGD motif (and presumed integrin binding) or directly through GPI linkage.

1084B

CPNA-1, a novel copine domain containing protein, links the integrin associated protein PAT-6 (Actopaxin) to the giant protein UNC-89 (obscurin) in *C. elegans* muscle. Ge Xiong[1], Adam D. Warner[2], Hiroshi Qadota[1], Donald G. Moerman[2], **Guy M. Benian[1]**. 1) Dept Pathology, Emory Univ, Atlanta, GA; 2) Dept Zoology, Univ British Columbia, Vancouver, BC.

CPNA-1 was discovered independently by two strategies: (1) It was found as one of 4 new Pat mutants in an RNAi screen (Meissner et al. PLoS Genet. 2009). (2) It was identified in a two-hybrid screen as a binding partner for the N-terminal region (Immunoglobulin domains (Ig) 1-5) of the giant protein UNC-89 (obscurin). When CPNA-1 was used to test for interaction with two-hybrid clones covering all of UNC-89-B (8,081 residues), we detected binding only with the UNC-89 region containing Ig1-5. The minimal region of UNC-89 required for interaction is Ig1-3, and its interaction with CPNA-1 was confirmed by two biochemical methods. The N-terminus of CPNA-1 has a predicted transmembrane helix and near its C-terminus is a "copine domain", an ~180 residue long region with weak homology to the extracellular region of integrins, and unknown function. CPNA-1 is an "atypical" copine family protein as it has a copine domain but no C2 domains. We have found that *C. elegans* has 7 genes encoding proteins with copine domains: *cpna-1* through *cpna-5* are atypical, and *nra-1* and *gem-4* are typical. When tested by 2-hybrid against copine domains from each of these proteins, UNC-89 Ig1-5 interacted with only the copine domain from CPNA-1. CPNA-1 specific antibodies localize the protein to integrin adhesion complexes (M-lines and dense bodies) of nematode body wall muscle. We found that CPNA-1 binds to the M-line proteins UNC-89, LIM-9 (FHL), SCPL-1 (a CTD-type phosphatase), UNC-96, and a protein common to the M-line and dense body, PAT-6 (actopaxin). PAT-6 is a member of a 4-protein complex (including UNC-112 (Kindlin), PAT-4 (ILK) and UNC-97 (PINCH)) that bind the cytoplasmic tail of integrin. Previously it was shown that PAT-4 binds the C-terminal half of PAT-6 (Lin et al. Curr. Biol. 2003). We have shown that the N-terminal half of PAT-6 binds CPNA-1. A yeast 3-hybrid assay demonstrates a ternary complex can form between PAT-6, CPNA-1 and UNC-89. A null mutant for *cpna-1*, *gk266*, displays the typical Pat (Paralyzed arrested at two-fold) phenotype. The Pat embryonic lethal phenotype has been found in loss of function mutants of many components of muscle focal adhesion structures. By localizing previously characterized muscle adhesion complex proteins in *cpna-1* mutant embryos, and localizing CPNA-1 in other Pat mutants, we have placed CPNA-1 in the M-line/dense body assembly pathway of embryonic muscle: CPNA-1 lies between PAT-6 and MYO-3. We hypothesize that PAT-6 recruits CPNA-1, and in turn, CPNA-1 recruits additional proteins (UNC-89, LIM-9, SCPL-1, UNC-96) to muscle focal adhesions.

1085C

The giant protein UNC-89 (obscurin) interacts with CUL-1: implications for ubiquitin mediated protein degradation in muscle. M. Berenice Duran, Hiroshi Qadota, Kristy J. Wilson, **Guy M. Benian**. Dept Pathology, Emory Univ, Atlanta, GA.

unc-89 mutants display reduced locomotion, and disorganization of muscle thick filaments, and usually lack M-lines. *unc-89* encodes 6 major isoforms, ranging in size from 156,000 to 900,000 Da. The largest of these isoforms, UNC-89-B, consists of 53 immunoglobulin (Ig) domains, 2 fibronectin type III (Fn3) domains, a triplet of SH3, DH and PH domains near its N-terminus, and two protein kinase domains called PK1 and PK2 near its C-terminus. The human homolog is called "obscurin". UNC-89 is localized to sarcomeric M-lines. To clarify how UNC-89 is localized and how it performs its functions, we are using a yeast 2-hybrid approach to identify binding partners. UNC-89 region Ig8-13 was used to screen a yeast 2-hybrid library and this yielded the protein CUL-1. CUL-1 is one type of cullin, a component of SCF complexes that direct the action of E3 ubiquitin ligases. A crucial protein for vertebrate muscle protein turnover is Atrogin-1, which is another component of an SCF complex that contains cul1 (CUL-1 of vertebrates). When CUL-1 was used to screen 15 additional segments that cover all of UNC-89-B (8,081 amino acids), two other segments of UNC-89 were found to interact. The following regions of UNC-89 were found to be necessary and sufficient for interaction with CUL-1: Ig9-11, Ig18-23, and Ig50-51. A binding assay using purified recombinant proteins verified the interactions. With reference to the crystal structure of the human Cul1-Rbx1-Skp1 complex (Zheng et al. 2002), Ig8-13 requires structural Repeat 2, Ig18-23 requires Repeats 1, 2 and 3, and Ig48-52 requires Repeats 1 and 2 for binding. The interaction of UNC-89 with this N-terminal half of CUL-1 is compatible with the idea that UNC-89 influences interaction of CUL-1 with its adaptor protein SKR-1 (Skp1 in worms). RNAi knock down of *cul-1* results in disorganization of myosin thick filaments, a phenotype that is very similar to that of *unc-89(su75)*, which lacks all the large UNC-89 isoforms and therefore lacks all CUL-1 binding sites. In addition, *cul-1(RNAi)* results in nearly normal localization of UNC-112 to M-lines and dense bodies, but with some abnormal accumulations of UNC-112 protein. Interestingly, *unc-89* mutants display a thinner sarcomeric region, and we hypothesize that this might result from increased degradation of sarcomeric proteins. Furthermore, we hypothesize that normally UNC-89 sequesters CUL-1 and prevents it from promoting ubiquitin mediated degradation of sarcomeric proteins. Thus, potentially, an obscurin/cullin interaction may have an important role in preventing human muscle atrophy, a condition found in normal aging (sarcopenia), and in many chronic diseases. We are now conducting experiments to test our hypothesis.

1086A

Morphogenesis of the *C. elegans* intestine depends on the ability of Arp2/3 to promote membrane association of apical proteins. **Yelena B. Bernadskaya**, Falshruti Patel, Hiao-Ting Hsu, Martha Soto. Dept Pathology & Lab Medicine, UMDNJ/RWJMS, Piscataway, NJ.

The establishment of the apical domain of *C. elegans* intestinal epithelia requires the apical enrichment of actin and formation of junctions at the apicolateral domain. The contribution of different actin regulators to junction formation is poorly understood. We have found that Arp2/3, a regulator of branched actin formation, is required for normal intestinal morphogenesis. Loss of Arp2/3 or of its activating WAVE/SCAR complex leads to reduced levels of apical actin in the intestine during development along with a widened intestinal lumen. Interestingly, overall polarity of the tissue is initiated, suggesting a more subtle regulation of epithelial integrity. It has previously been proposed that Arp2/3 is only required for protrusion formation during the establishment of adherens junction but plays no role in junctional maintenance. However, we have observed similar apical defects when Arp2/3 or WAVE/SCAR are depleted from adult worms, suggesting that Arp2/3 is required continuously throughout the life of the animal to maintain the apical domain. Loss of Arp2/3 in embryos results in reduced apical accumulation of DLG-1, a component of the apical adherens junction. During embryonic development, the DLG-1/AJM-1 complex rather than the cadherin/catenin complex is required for enrichment of apical F-actin. The role of Arp2/3 in promoting apical enrichment of F-actin and DLG-1 is supported by ERM-1, a protein that connects F-actin to membranes. Loss of Arp2/3 shifts both ERM-1 and DLG-1 to a subcellular fraction enriched in recycling endosome proteins and loss of a regulator of recycling endosomes shifts ERM-1 and DLG-1 back toward pellet fractions enriched in plasma membrane. Taken together, these data support a model in which Arp2/3 is recruited to the apical region of the intestine by ERM-1 where it promotes and maintains junction formation by regulating the membrane association of proteins that establish and maintain the apical domain.

1087B

Control of Distal Tip Cell Migration in *C. elegans* through the Interaction of Dishevelled Proteins and CACN-1. **Lauren Byrnes**, Melissa LaBonty, Erin Cram. Biology, Northeastern University, Boston, MA.

Cell migration is a vitally important process in both embryonic development and adult tissue repair and regeneration. The migration of two specific cells, called distal tip cells

(DTCs), is of special importance in gonadal development in the nematode *C. elegans*. In a genome wide screen, the *cacn-1* gene was shown to be required for correct DTC migration and gonad formation. Recent studies in the lab have shown the MIG-5 protein to interact biochemically with CACN-1. MIG-5/Dishevelled is an adaptor protein in the highly conserved Wnt pathway. In addition to *mig-5*, *C. elegans* has two other disheveled homologs, *dsh-1* and *dsh-2*. Because of the functional and sequence similarity between these genes, we hypothesize that DSH-1 and DSH-2 interact genetically and biochemically with CACN-1 to control distal tip cell migration in a manner similar to that shown for MIG-5. To investigate this hypothesis, RNA interference (RNAi), was used to inhibit the synthesis of DSH-1 or DSH-2 and DTC phenotypes were monitored. While *dsh-1* RNAi worms showed no evidence of gonad defects, some *dsh-2* RNAi worms were observed to have no migration from one or both of the gonad arms, indicating that *dsh-2* is involved in DTC migration and/or specification. Genetic interactions with *mig-5* and *cacn-1* are currently being determined. In addition, a Yeast Two Hybrid assay is being conducted to determine if DSH-1 and DSH-2 can interact directly with CACN-1. Studying the genetic and biochemical interactions between CACN-1 and these Wnt pathway genes will deepen our understanding of cell specification and migration.

1088C

TOCA-1 and actin polymerization contribute to P-cell nuclear migration. **Yu-Tai Chang**, Daniel A. Starr. Molec & Cellular Biol, Univ California, Davis, Davis, CA.

Moving the nucleus to an intracellular location is essential for a wide variety of cell and developmental processes, including the formation of polarized cells, fertilization, differentiation, and cellular migration. Defects in nuclear migration block development and lead to disease. However, the mechanisms of how nuclei are moved are poorly understood. We employ larval P-cells in C. elegans as a model for nuclear migration. 12 P-cell nuclei migrate from lateral to ventral positions during the mid-L1 stage. They subsequently divide to form neurons and the vulva. Null mutations in *unc-83* or *unc-84* inhibit nuclear migration by disrupting interactions between nuclei and microtubule motors. Nuclear migration defects lead to the death of P-cells resulting in uncoordinated (Unc) and egg-laying defective (Egl) animals missing P-cell derived lineages. Interestingly, the *unc-83* and *unc-84* 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, but at 15°C, P-cell nuclear migration occurs normally. We therefore hypothesize that an additional pathway functions in part redundantly to the *unc-83/unc-84* pathway to migrate P-cell nuclei at 15°C. To test our hypothesis, we carried out forward genetic screens and isolated nine *emu* (enhancer of the nuclear migration defect of unc-83 or unc-84) alleles at 15°C in *unc-84* null animals in the background of an *unc-84* rescuing array. To quantify the severity of the P-cell nuclear migration defect of *emu* alleles, we scored the number of UNC-47::GFP-positive GABA neurons in adults. Compared to *unc-84* null animals, *emu; unc-84* double mutants had significantly fewer GABA neurons at all temperatures. The *emu* alleles are recessive and have been partially mapped. Using whole-genome sequencing, we have determined that the *yc20* allele is a lesion in *toca-1* and are currently sequencing other alleles. *toca-1(RNAi)* phenocopied *yc20*, suggesting that TOCA-1 functions in parallel to the UNC-84/UNC-83 pathway to move P-cell nuclei. TOCA-1 is conserved in flies and mammals. It consists of an F-bar domain that binds to curved membranes and two domains that recruit cdc42 and WAVE to induce F-actin polymerization. Thus, TOCA-1 functions to regulate actin dynamics at the membrane-cytoskeleton interface; previous studies show that TOCA-1 facilitates clathrin-mediated endocytosis (Giuliani et al., 2009. PLoS Genetics). That same study also had data suggesting that TOCA-1 localizes to the nuclear envelope of oocytes. We are currently investigating the subcellular localization of TOCA-1 in P-cells. Together, our results implicate a novel mechanism for nuclear migration based on the nucleation of actin filaments.

1089A

The Anal Depressor Undergoes Morphological and Functional Changes during Caenorhabditis elegans Male Development. **Xin Chen**. Department of Biology, Texas A&M University, College Station, TX.

The anal depressor is a sexually dimorphic muscle in C. elegans. It is an H-shaped cell in hermaphrodites and larvae males and alters its shape and function significantly during L4 male development. In adult males, the anal depressor functions as an auxiliary spicule protractor muscle while in hermaphrodites and larvae males it participates in defecation. We are interested in exploring the mechanism through which the anal depressor extends its attachment on the dorsal spicule protractor muscles and how do the myofilaments in the anal depressor reorient during L4 development. Using fluorescent markers driven by the lev-11 promoter and the unc-103E promoter, we observed the morphological change of the anal depressor during mid and late L4 stages. We found that the anal depressor becomes slimmer during the developing process and the ventral attachment moves forward from the rectum to the dorsal protractor muscles. The process might involve the disassembly of the dorsal-ventral myofilaments and new assembly of anterior-posterior F-actins structures. Therefore signals that activate the Cdc42/Rac and the Rho protein may contribute to the morphological change of the anal depressor. These signals might be cell autonomous which are resulted from the expression of genes that influence the cell development. These might also be external signals which serve as instructive cues for the cytoskeleton changes. To test the first hypothesis, we are going to observe the morphological changes of the anal depressors in males that contain mutations in developmentally-essential genes and identify the ones that exhibit abnormal anal depressor development. To address the second hypothesis, currently we are considering the signal as short-range and muscles, neurons or epithelial cells that are adjacent to the anal depressor might be the potential structures that send the signals. To explore this, we ablated cells that give rise to these muscle or neurons during larvae stages and observed the development of the anal depressor in the male. We found that the ablation of M cell during L1 stage resulted in an abnormal morphology of the anal depressor in the adult male. We observed slimmer anal depressor but only part of the attachment had moved to the dorsal protractor muscle. This suggests that the sex muscles which the M cell gives rise to might send signals that are partially responsible for the change of the anal depressor. To further address the problem, we are going to ablate single sex muscle to examine which specific muscle is responsible for this. We are currently ablating the B cell descendants during L2 or L3 stages and observing the anal depressor development during early, mid and late L4.

1090B

An E-Cadherin-mediated hitchhiking mechanism for *C. elegans* germ cell internalization during gastrulation. **Daisuke Chihara**, Jeremy Nance. Skirball Inst, NYU, New York, NY.

We are using the *C. elegans* primordial gonad to understand how stem cells assemble into a niche during development. The *C. elegans* primordial gonad contains two somatic gonad precursor cells (SGPs) and two primordial germ cells (PGCs). The primordial gonad assembles during embryogenesis when PGCs and SGPs come together adjacent the intestine.

As a first step in understanding niche assembly, we investigated how PGCs move to the site where the primordial gonad forms. PGCs and somatic cells move into the interior during gastrulation. Gastrulation begins when the endodermal precursor cells ingress into the interior of the embryo. Subsequently, mesodermal cells and PGCs ingress in a spatial and temporal sequence that is highly orchestrated. Because somatic cells require transcription to ingress whereas PGCs are transcriptionally quiescent, we hypothesized that somatic cells might push or pull the PGCs into the embryo. We used videomicroscopy to identify cells that contact the PGCs, and used laser killing to determine if the contacting cells are required for PGC ingression. The PGCs are surrounding by adjacent mesodermal cells and internal endodermal cells. We found that the only contacting cells necessary for PGC ingression were the endodermal cells, which ingress into the embryo an hour before the PGCs. Killing or altering the fate of the endodermal cells prevented PGC ingression but not ingression of other somatic cells. Using membrane markers, we showed that PGCs and endodermal cells maintain contact throughout gastrulation, and that the internal endodermal cells undergo a morphogenetic movement that appears to pull the adherent PGCs into the embryo. PGCs express high levels of HMR-1/E-cadherin, and knocking down HMR-1 caused PGCs to detach from endodermal cells and remain on the surface of the embryo. Finally, we show that the enrichment of HMR-1 protein in the PGCs is not due to transcriptional upregulation, but is instead due to an increase in protein expression mediated by the *hmr-1* 3' UTR. We propose that PGCs upregulate E-cadherin to maintain tight adhesion with endodermal cells, which pull the PGCs into the embryo and position them at the site of primordial gonad assembly. Our results highlight the importance of germ cell - gut interactions during development and of E-cadherin-mediated adhesion in niche formation.

1091C

The minus-end actin capping protein, TMD-1/tropomodulin, regulates the morphology of the intestine and excretory cell. Corey Hoffman, Clarence Ling, Gary Mantione, Nicole Vissichelli, Vincent Cannataro, Thomas Gallagher, **Elisabeth (Abbi) Cox**. Biology, SUNY College at Geneseo, Geneseo, NY.

Tropomodulins are actin regulatory proteins that interact with the slow-growing end of actin filaments and also have actin nucleating activity. *C. elegans* has a tropomodulin homolog, TMD-1/UNC-94 that encodes two isoforms with sequence similarity to vertebrate tropomodulins. TMD-1 is involved in body wall muscle development[1,2]; and we have found that it is also needed for proper morphology of the intestine and excretory cell. In the intestine, TMD-1 localizes to the terminal web, which is an actin and intermediate filament rich structure that underlies the apical membrane facing the lumen. In the intestine, loss of *tmd-1* function results in flattened morphology of the lumen and a reduction in intestinal lumen volume. Interestingly, the intestinal defects can be rescued by performing weak RNAi for the myosin phosphatase, *mel-11*, suggesting a potential role for TMD-1 in promoting proper actomyosin contractility levels in the terminal web. More globally, this points to an important and previously undocumented role for actomyosin contractility in regulating lumen shape of simple endothelial tubes. *tmd-1(tm724)* mutants, which carry a large deletion in the *tmd-1* gene, also have excretory cell defects; specifically the canals fail to extend properly. Improper structure of the intestine and excretory cell may account for the larval lethality, patchy appearance, and slow growth exhibited by *tmd-1(tm724)* mutants. This work indicates an important role for TMD-1/tropomodulin in morphogenesis of tubular epithelial tissues. [1] Yamashiro et al. (2008) J. Cell Sci. 121: 3867-77. [2] Stevensen et al. (2007) J. Mol. Bio. 374: 936-50.

1092A

Roles for anillin (*ani-1*) in regulating cell shape changes during *C. elegans* embryogenesis. **Nellie Fotopoulos[1]**, Neetha Makil[2], Yun Chen[1], Alisa Piekny[1]. 1) Biology, Concordia University, Montreal, Quebec, Canada; 2) Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA.

Changes in cell shape involve reorganization and contraction of the actin-myosin cytoskeleton. During *C. elegans* embryogenesis, both cytokinesis and elongation use Rho-mediated shape changes. Elongation is where lateral epidermal cells (seam cells) change shape from cube-like to cylindrical, which is transmitted to the ventral and dorsal epidermal cells, transforming the ovoid embryo into the long, thin worm. In the seam cells, actin

filaments become highly organized and are shortened by myosin contraction via the Rho kinase LET-502. Myosin phosphatase (MEL-11) inactivates myosin in the dorsal and ventral cells and is sequestered to cell boundaries in the seam cells, which limits its interaction with myosin. LET-502 is likely regulated by RHO-1, but the lack of *rho-1* alleles precluded studying its role in elongation. Using a deletion allele generated by the Knockout Consortium, we found that *rho-1* is required for elongation and other morphogenetic events, including ventral enclosure.

Cytokinesis is the final stage of cell division and requires the Rho-mediated formation and ingression of an actin-myosin ring. The same genes regulate both elongation and cytokinesis, suggesting they use analogous shape change mechanisms. In other eukaryotes, anillin is required for cytokinesis by coordinating actin and myosin filaments. It can also bind to RhoA, scaffolding the upstream regulator to actin and myosin. *ani-1* (anillin in *C. elegans*) is required for polar body extrusion during meiosis and asymmetric furrow ingression during cytokinesis. Since elongation and cytokinesis may use similar shape change mechanisms, we hypothesize *ani-1* functions in elongation. Using *ani-1* RNAi, we observed phenotypes consistent with elongation defects. We also performed zygotic-specific *ani-1* RNAi (by rescuing maternal RNAi) and observed elongation defects, suggesting this phenotype does not arise from earlier defects in meiosis or cytokinesis. We found genetic interactions between *ani-1* and genes that function in elongation using zygotic alleles for *rho-1* and *mlc-4*. Furthermore, using GFP:ANI-1 (gift from A. S. Maddox), we observed non-cytokinetic localization patterns for ANI-1 during embryogenesis. These findings may be the first evidence to support developmental-specific functions for anillin outside cytokinesis in any organism.

1093B

Isolation, characterization, and whole genome sequencing of a mutant defective for the pharyngeal g1a gland sub-type. **Vikas Ghai**[1], Stephane Flibotte[2], Jeb Gaudet[1]. 1) Department of Biochemistry & Molecular Biology, University of Calgary, Calgary, AB, Canada; 2) Canada's Michael Smith Genome Sciences Centre, Vancouver, BC, Canada.

Our goal is to use the *C. elegans* pharynx as a model to understand the complexities of organogenesis. We have previously identified a sub-set of pharyngeal gland expressed Mucin-related *phat* genes, which are secreted by the glands into the pharyngeal lumen. The loss of PHAT secretion results in early larval arrest due to a severe pharyngeal feeding blockage. This is the case in mutants for *hlh-6*, the bHLH transcription factor that directly activates the *phat* genes, and when we ablate the gland cells (Smit et al. 2008), alluding to a role for the glands in feeding and transport of bacteria. Each of the three gland sub-types secretes these proteins at a unique position along the pharyngeal lumen, suggesting that one of the functions of the gland sub types is to create a local concentration of PHAT proteins, completely coating the pharyngeal lumen.

From a genetic screen we have isolated a mutant, *glad-25*, where the g1a gland sub-types are missing, or displaced from the pharynx. The pharynx appears morphologically normal, and linearly related pharyngeal cells are correctly specified, suggesting this seems to be specific loss of the g1a gland cells.

Coincidental with the loss or displacement of the g1a gland cells and loss of PHAT-1 expression, is the presence of a feeding blockage initiating during the later larval stages and through adulthood. This blockage is more progressive and less severe when compared to the early larval arrest due to blockage in hlh-6 mutants. Feeding defective *glad-25* animals grow slower, and are smaller. Previously we've had success rescuing feeding defects in *hlh-6* mutants using the viscous *E. coli* strain HB101. Feeding *glad-25* mutants HB101 was able to rescue the feeding defects, the small body size, and slow growth. This would suggest that loss of a gland sub-type results in a spatial disruption of secreted PHAT proteins, and subsequently a unique feeding defect.

We used array CGH to map the mutation between 7.5-9.5 Mb on chromosome IV, and performed whole genome sequencing identifying ~20 variants in the region that cause amino acid changes, with several of theses variants possibly causing a loss-of-function. We are currently testing theses candidates by cosmid rescue.

1094C

Investigations into the collective movement of substrate neurons and epidermis during ventral enclosure. **Claudiu Giurumescu**, Andrew Chisholm. Division of Biological Sciences, University of California San Diego, La Jolla, CA, 92093.

In the embryonic development of the *C. elegans* nervous system, neuronal precursors undergo several collective movements, including closure of the ventral cleft following gastrulation. We are interested in how these collective movements are controlled and their relationship to other morphogenetic processes such as epidermal enclosure. To visualize such cell movements we have used computer-aided tracking of histone-GFP labeled nuclei. We have developed new software tools that allow semi-automated tracking of all 667 nuclei (554 live nuclei + cell deaths) up to the 1.5-fold stage (see abstract by Giurumescu et al, 2010 Development meeting). Using these tools, we find that during epidermal enclosure neurons undergo widespread collective movements (see abstract by Giurumescu and Chisholm, 2010 Development meeting). Neuronal collective migrations during epidermal enclosure could be a cause or effect of epidermal movements, or could be independently driven. To address the relationship of movements in epidermis and substrate we are ablating neuronal precursors and analyzing the effects on enclosure. Systematic studies by Sulston et al. (1983) showed that laser killing of specific neuronal precursors such as ABprpap prevented hatching. We have confirmed these results and find that the ablated embryos arrest during epidermal enclosure. We are investigating whether specific descendants of ABprpap are important for the enclosure process. Identification of substrate neurons critical for enclosure was enabled by the analysis of the NK2 homeobox gene *ceh-27*. RNAi of *ceh-27* has been reported to cause defects in epidermal enclosure (B. Harfe and A. Fire, 1998 East Coast meeting). We have extended these findings using a *ceh-27* deletion allele. Preliminary analysis of the *ceh-27* expression pattern indicates that *ceh-27* is expressed in substrate neurons that include those defined by ablation as being essential for enclosure. We are currently characterizing the behavior of the substrate neuron population in *ceh-27* mutants.

1095A

Searching for interactors and substrates of the PXN-2 extracellular matrix peroxidasin. **Jennifer R. Gotenstein**, Andrew D. Chisholm. Division of Biological Sciences, University of California San Diego, La Jolla, CA 92093.

Embryonic elongation of *C. elegans* involves cooperation of the epidermal cytoskeleton, muscle tissue, and the extracellular matrix. In screens for elongation-defective mutants we identified *pxn-2*, a member of the peroxidasin family of extracellular matrix peroxidases (Ref. 1). Null alleles of *pxn-2* cause 100% lethality at embryonic or early larval stages. PXN-2 is also required postembryonically for maintenance of basement membrane integrity. Peroxidasins are extracellular peroxidases thought to catalyze cross-linking of as-yet unknown substrates in the basement membrane. To identify genes that interact with *pxn-2* we have analyzed a spontaneous extragenic *pxn-2* suppressor, *ju958*. In parallel, we are examining basement membrane proteins as candidate substrates for PXN-2-dependent crosslinking. The *ju958* suppressor arose spontaneously during passage of a *pxn-2(ju358)* stock. *ju358* is a viable partial loss of function allele of *pxn-2*. *ju958* behaves as a recessive autosomal suppressor. We find that *ju958* can suppress *pxn-2(tm3464)* null mutants to partial (45%) viability, suggesting *ju958* partially bypasses the requirement for PXN-2. We used SNP mapping to localize *ju958* to a 1.3 Mb region on chromosome I. Whole genome sequencing of *ju958* strains (kindly performed by Alex Boyanov and Oliver Hobert) revealed a single missense change in this region, affecting the *vab-10* locus. *vab-10* encodes large cytoskeletal cross-linking proteins known as spectraplakins (Ref. 2). The *vab-10* mutation in suppressed *ju958* strains causes a D to N change in a linker region between the spectrin repeats, common to all *vab-10* isoforms. The affected residue is conserved in other nematodes and *Drosophila* but not in mammals. Preliminary analysis suggests that *vab-10(ju958)* does not cause elongation defects in a *pxn-2(+)* background. We are currently testing whether *vab-10(ju958)* can suppress other elongation-defective mutants. As *vab-10* is itself essential for epidermal elongation it is unclear why a mutation in *vab-10* should suppress the elongation defect in *pxn-2* mutants. Basement membrane function is important for transduction of muscle contractions essential for elongation (Ref. 3). *vab-10(ju958)* may cause an altered function of *vab-10* that allows epidermal cell shape changes to occur despite reduced muscle tension.

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1096B

Mechanisms Involved in Regulating the Activity and Localization of a Microtubule-depolymerizing Kinesin in the One-cell Stage *C. elegans* Embryo. **X. Han**, K. Cheung, M. Srayko. Biological Sci, Univ Alberta, Edmonton, AB, Canada.

Microtubules are required for multiple cellular processes including mitosis, cytokinesis, and vesicle transportation. Factors that regulate microtubule dynamics in the cell help determine the final form and precise cellular role of many cytoskeletal structures. Among the known modulators of microtubule dynamics, we are specifically interested in the microtubule-depolymerizing kinesins of the kinesin-13 family, such as KLP-7 (CeMCAK).

In *C. elegans*, KLP-7 locates to the kinetochore and the centrosome. It has been implicated in regulating microtubule outgrowth at the centrosome because loss of KLP-7 results in an increase in the number of centrosomal microtubules (Srayko *et al.*, 2005, Schlaitz *et al.*, 2007). Extensive work from other labs on the vertebrate homologues of KLP-7 indicates that they are negatively regulated through phosphorylation by the Aurora kinases (Andrews *et al.*, 2004, Lan *et al.*, 2004, Ohi *et al.*, 2004, Schlaitz *et al.*, 2007, Zhang *et al.*, 2007 and 2008). In order to understand how KLP-7 is regulated in the cell, we tested the possibility that Aurora kinases are directly involved. 2D gel-electrophoresis revealed an alteration in the ratio of potential KLP-7 phosphorylation variants in lysates from either *air-1(RNAi)* (Aurora A-depleted) or *air-2(RNAi)* (Aurora B-depleted) embryos, compared to wild type. Furthermore, we found that both AIR-1 and AIR-2 kinases phosphorylate KLP-7 *in vitro*. We used a combination of mass spectrometry, similarity to data on the vertebrate homologues, and an Aurora kinase phospho-site prediction algorithm to identify potential *in vivo* phosphorylation sites within KLP-7 (Zhou *et al.*, 2004). We are currently performing a structure-function analysis to determine which putative Aurora sites are required for KLP-7's intracellular location and/or its depolymerase activity at the centrosome. Results obtained thus far indicate that mutating one of the C-terminal Aurora sites from serine to glutamic acid (to mimic constitutive phosphorylation) or to alanine (to mimic non-phosphorylation) interferes with KLP-7 function but not its ability to target to centrosomes or kinetochores.

1097C

DPY-24 regulates the timing of DTC dorsal turn. **Tsai-Fang Huang**, Chun-Yi Cho, Jheng-Wei Huang, Yi-Chun Wu. Institute of Molecular and Cellular Biology, National Taiwan University, Taipei, Taiwan.

Cell migration plays a key role in animal development and requires integration of temporal and spatial information. In the *C. elegans* hermaphrodite, two somatic distal tip cells (DTC) undergo a stereotyped migration pattern during larval development. DTCs are

born in the ventral mid-body in L1 and migrate to the ends of the body in L2. During L3, DTCs migrate to the dorsal side and then re-orient to the mid-body. They continue moving to the mid-body during L4 and halt in adult. The heterochronic genes *daf-12, dre-1*, and *lin-29* act redundantly to specify the L3 temporal fate of the DTCs[1]. The guidance receptor UNC-5 is both necessary and sufficient for the dorsal migration of the DTCs away from the ventrally concentrated UNC-6/netrin[2, 3]. We isolated a *dpy-24* mutant with a precocious DTC dorsal migration defect. We found that *dpy-24* encodes a zinc-finger-containing protein and negatively regulates *unc-5* transcription prior to DTC dorsalward turning. Loss-of-function mutations in *dpy-24* cause a precocious *unc-5* transcription and premature DTC dorsal turn, whereas a constitutive *dpy-24* expression delays *unc-5* transcription and leads to retarded dorsal turn. Immunostaining of worms at different developmental stages with anti-DPY-24 antibodies showed that DPY-24 protein is present in the DTCs only before DTCs turn dorsalward. Using a transcriptional gfp reporter assay, we found that *daf-12* and *lin-29* act together to repress *dpy-24* transcription. This repression may be responsible for the timely disappearance of DPY-24 prior to DTC dorsalward turning. These results suggest that a transcriptional repression cascade may regulate the timing of DTC dorsal turn.

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1098A

A C.elegans pathology model of Leucine-rich repeat kinase 2 (LRRK2). **Xu Huang**, Enrico Schmidt, Ralf Baumeister. Bio 3, University of Freiburg, Freiburg im Breisgau, Germany.

Mutations in the Leucine-rich repeat kinase 2 (LRRK2) were shown to be associated with both juvenile and sporadic form of Parkinson's disease (PD). LRRK2 is a multidomain protein containing several protein-protein interaction surfaces, a GTPase domain followed by a MAP kinase-like domain. Although LRRK2 was suggested to play a role in several cellular processes like vesicle trafficking and autophagy, little is known about the pathological pathways associated with LRRK2. Mutations within the kinase domain were shown to be responsible for the induction of the pathology observed in patients. Among them the most prominent substitution from G to S at position 2019 in the kinase domain was shown to significantly increase the kinase activity of human LRRK2 in vitro. How hyperactivation of the kinase activity however contributes to the pathology of PD and which pathways are involved in the pathogenesis is not known. In order to investigate the Parkinson's disease pathogenesis caused by the mutation of LRRK2 in vivo and identify new pathways associated with the LRRK2-mediated pathogenesis, we generated transgenic C.elegans strains expressing the G2019S variant of human LRRK2. As a control kinase dead version of human LRRK2 was expressed.. Overexpression of human LRRK2 (G2019S) in C.elegans driven from the endogenous promoter of lrk-1 - the C. elegans homologue of human LRRK2 caused a complex germline defect finally resulting in sterility of adult worms. In detail, the gonad is generally misshaped trough DTC migration defects, the oocytes in the byIs170[LRRK2(G2019S)::gfp] transgenic lines are highly disorganized and have little or no discernable rechis. Furthermore, oocyte DNA was found in large clumps in the proximal gonad arms shown by DAPI staining in the byIs170[LRRK2(G2019S)::gfp] transgenic lines, suggesting developmental defects of the oocytes. These defects were not observed in the corresponding control strain expressing the kinase dead version of byIs178[LRRK2(Kinase dead)::gfp] transgenic lines. Our studies indicated that the pathological mutant of LRRK2 affects several aspects of the gonadal development is related to its kinase domain. Our LRRK2 (G2019S) model can be used to study cellular defects caused by the pathological variant G2019S of human LRRK2.

1099B

The *C.elegans* spectraplakin VAB-10 regulates nuclear migration by linking actin and microtubule cytoskeleton in the gonadal distal tip cells. **H-S. Kim[1]**, R. Murakami[2,3], S. Quintin[4], M. Mori[1], K. Tamai[2], K. Ohkura[2], M. Labouesse[4], H. Sakamoto[3], K. Nishiwaki[1]. 1) Bioscience, Kwansei-Gakuin Univ, Sanda, Hyogo, Japan; 2) RIKEN, Center for Developmental Biology, Japan; 3) Graduate School of Sci and Tech, Kobe University, Japan; 4) Development and Stem Cells program, IGBMC, CNRS/INSERM/ULP, France.

Cytoskeletal regulation is important in cell migration. In *C.elegans*, U-shaped hermaphrodite gonads are formed by directed migration of gonadal distal tip cells (DTCs), which offer a simple model to investigate the mechanism of cell migration in organogenesis. The *vab-10* gene encodes VAB-10 spectraplakins, cytoskeletal linker proteins, and *vab-10(tk27)* mutants exhibit abnormal gonad formation caused by defective DTC migration. Here we report that one of the spectraplakin isoforms, VAB-10B1, plays an essential role in DTCs and their nuclear migration by regulating the actin and microtubule cytoskeleton. When wild type DTCs turn dorsally, the rotation of migratory axis from anteroposterior to dorsoventral is preceded by nuclear migration within DTCs. Although DTCs are attracted dorsally by the UNC-6/netrin guidance in the *vab-10(tk27)* mutant lacking VAB-10B1, their nuclei stayed at their anterior or posterior ends rather than were relocated to the dorsal side of DTCs as in the wild type, suggesting that VAB-10B1 is required for nuclear migration of DTCs. We also found that the alignment of filamentous (F)-actin was weakly and of microtubules was severely disorganized in mutant DTCs. A microtubule plus tip marker EBP-2-GFP revealed polarized microtubule growth toward nuclei in the leading edge of DTCs, while those in *vab-10(tk27)* mutant DTCs showed randomized patterns, where the numbers of EBP-2-GFP comets were significantly reduced, suggesting that polarized alignment of microtubules is required for nuclear migration of DTCs. Moreover, a *vab-10* mini-gene encoding only the actin- and microtubule-binding domains significantly rescued the gonadal defects, suggesting the VAB-10B1 activity in linking actin and microtubule filaments. These results suggest that VAB-10B1/spectraplakin regulates the polarized alignment of microtubules by linking F-actin and microtubules, which facilitates normal cell migration and nuclear translocation of DTCs.

1100C

Investigating the role of CACN-1 in the Canonical Wnt Signaling Pathway. **Melissa LaBonty**, Mouna Ibourk, Erin Cram. Northeastern University, Boston, MA.

Development of the C. elegans gonad is directed by the migration of two leader cells, the distal tip cells (DTCs), and provides an excellent model to study cell migration *in vivo*. CACN-1, a novel and highly conserved C. elegans protein, is required for proper migration of the DTCs. We have conducted a genome-wide yeast two-hybrid screen, and identified the Dishevelled homolog, MIG-5, as a possible CACN-1 interacting protein. MIG-5 is a modular signaling scaffold protein that plays an integral role in the Wnt signaling pathways. In canonical Wnt signaling, MIG-5 works upstream of POP-1, which is the only C. elegans member of the TCF/LEF family of transcription factors. To investigate the role that CACN-1 plays in canonical Wnt signaling, we used an integrated line containing POPTOP, a TOPFLASH-like reporter that allows for measurement of POP-1 activity *in vivo*. Early results indicate that knockdown of *cacn-1* by RNAi in POPTOP worms causes an increase in POPTOP expression levels. Further experiments will explore the effect of CACN-1 on other components of the canonical Wnt signaling pathway and may help to elucidate its role in C. elegans development.

1101A

The F-Bar and Rho GTPase-activating protein RGA-7 controls CDC-42 activity during embryonic elongation in Caenorhabditis elegans. **Germain Lacoste-Caron**, Sarah Jenna, Emmanuel Martin. Chimie - Biochimie, Université du Québec à Montréal (UQAM), Montréal, Québec, Canada.

Embryonic elongation is a late stage of C. elegans embryonic development required to transform an ovoid embryo into a vermiform larva. This process implies some major morphological changes controlled by Rho GTPases within embryonic hypodermal cells. We recently identified the RhoGTPase CDC-42 as a novel component of a signalling pathway controlling elongation. We report here the identification and characterization of a novel CDC-42-regulator controlling embryonic elongation:RGA-7. rga-7 codes for a RhoGTPase-activating Protein (RhoGAP). This domain presents an in vitro GAP activity towards CDC-42 and to a lesser extent towards RHO-1 and MIG-2. Consistently with this activity, genetics studies revealed that cdc-42 and rga-7 have antagonistic functions during embryonic elongation. Characterization of the genetic structure of rga-7 locus revealed that this gene code for three transcripts, all expressed in embryos. Interestingly, the two larger proteins coded by these transcripts contain a consensus F-Bar domain, suggesting the involvement of RGA-7 in membrane trafficking.

1102B

A genetic screen to elucidate the role of a PP2A phosphatase required for mitotic spindle assembly. **Karen I. Lange**, Martin Srayko. Biological Sciences, University of Alberta, Edmonton, AB, Canada.

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* a specific protein phosphatase 2A (PP2A) complex, termed RSA (regulator of spindle assembly), is required for robust microtubule outgrowth from the centrosome. This heterotrimeric complex is composed of RSA-1, PAA-1, and LET-92 and is located at the centrosomes. 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[1]. However, the centrosomes in *rsa-1* mutants appear to have all major components and they are otherwise competent to support microtubule outgrowth[1]. In order to find potential targets of this complex, we performed a genetic screen for suppressors of a recessive temperature sensitive allele of *rsa-1(or598ts)*[2]. Progeny from *or598ts/or598ts* adults exhibit 100% lethality at 26°C but only 20% lethality at 15°C, making this allele ideal for our suppressor screens. We have screened an estimated 150,000 haploid genomes in three independent rounds of EMS mutagenesis. Twenty-two candidate suppressors were isolated and 19 have since been identified. Currently all identified suppressors are missense mutations that are intragenic or in *paa-1*, the structural subunit of the PP2A complex. These suppressors suggest that the *rsa-1(or598ts)* lesion disrupts the protein-protein interactions between RSA-1 and PAA-1. Yeast-two-hybrid analysis has been used to confirm this hypothesis. The remaining suppressors are currently being mapped and further characterized.

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1103C

An enhancer screen to identify genes that function with UNC-6/UNC-40 (netrin) to promote anchor cell invasion. **Lauren R. Lilley[1,2]**, Quiyi Chi[1], David R. Sherwood[1]. 1) Biology Department, Duke University, Durham, NC; 2) University Program in Genetics and Genomics, Duke University, Durham, NC.

Cell invasion through basement membrane is essential during key developmental and physiological processes including blastocyst implantation, neural crest and myoblast migration, leukocyte transmigration, and capillary formation. The signaling cues that target

invasion through basement membrane barriers remain poorly understood, largely due to the tissue and genetic complexity of vertebrate experimental models. The experimentally tractable model of *C. elegans* anchor cell (AC) invasion into the vulval epithelium during uterine-vulval attachment is a paradigm to study cell invasion through basement membrane *in vivo*. We have previously shown that UNC-6 (netrin) secreted from the ventral nerve cord polarizes its receptor (UNC-40), actin, and actin regulators (Rho GTPases, Ena/VASP, PI(4,5)P2) to the invasive cell membrane of the AC that contacts the basement membrane. This polarization appears necessary for the AC to generate cellular protrusions that breach the basement membrane. However, null mutations in both *unc-6* (netrin) and *unc-40* cause delays, but not complete blocks in AC invasion, suggesting that other genes act with UNC-6/UNC-40 signaling to promote AC invasion. To indentify these genes, we performed a genome-wide RNAi modifier screen for genes whose RNAi-mediated knockdown enhances the invasion defect of animals harboring a null mutation in *unc-40*. We have found that knockdown of 10 genes previously not known to be involved in AC invasion (including two uncharacterized putative G-Protein coupled receptors) show an invasion defect in the *unc-40* null background. We are beginning to characterize these genes in detail and are working to elucidate the aspects of cell invasive behavior they control.

1104A

Molecular characterization of the *C. elegans* anillin isoforms. **Michael Loloyan**, Alisa Piekny. Biology, Concordia University, Montreal, Quebec, Canada.

Processes that involve cell shape changes such as cytokinesis and morphogenesis are vital for the development of an organism and are driven by the cytoskeleton. Cytokinesis occurs due to the contraction of an actin-myosin ring to form two daughter cells, while morphogenesis describes coordinated actin-myosin cell shape changes within a tissue. Both of these events require controlled regulation of cytoskeletal dynamics by the RhoA GTPase. In *C. elegans* cytokinesis, the conserved GEF ECT-2 activates RHO-1 (RhoA) to drive actin polymerization through CYK-1 (formin) and activation of MLC-4 (myosin light chain) by LET-502 (Rho kinase) to form and ingress a contractile ring. ANI-1 (anillin) is required for asymmetric furrow ingression and contains conserved actin and myosin binding domains in its N-terminus through which it influences actin and myosin localization. In human cells, the AHD (Anillin Homology Domain), a conserved region in the C-terminus of anillin, binds to RhoA and Ect2. Through these multiple interactions, anillin may function as a scaffold to coordinate cytoskeletal components together with their regulators. We hypothesize that anillin's function may not be restricted to mitotic cells, and that the molecular interactions of the C-terminus of anillin are conserved in the *C. elegans* homologues, which include ANI-1, ANI-2 and ANI-3. ANI-2 is required for proper gonad formation and recently was shown to antagonize ANI-1 if it is stabilized in the early embryo, and ANI-3 has no known function. ANI-1, ANI-2 and ANI-3 contain the AHD region, but ANI-2 and ANI-3 lack the N-terminal actin and myosin binding domains. We found that the AHD region of ANI-1 and ANI-2 interact with RHO-1 and ECT-2. Residues that are required for the human anillin-RhoA and anillin-Ect2 interactions have been identified and we are performing mutational analyses to determine their conservation. Furthermore, ANI-1 and ANI-2 interact with human RhoA and Ect2, but cannot interact with the *Drosophila* ECT-2 homologue Pebble. *Drosophila* anillin interacts with the partner for Pbl, RacGAP50C (CYK-4), and it is possible that evolution has favored an anillin-Ect2 interaction in some species and an anillin-Cyk-4 interaction in others and the reason for this selection is not clear.

1105B

The roles of *rhgf-2*/Rho GEF and *fhod-1*/formin in regulating the actin cytoskeleton during embryonic elongation. Simon K. Rocheleau[1], Christopher A. Vanneste[1], Eko Raharjo[1], Robert Steven[2], David Pruyne[3], **Paul E. Mains[1]**. 1) Dept Biochem & Molec Biol, Univ Calgary, Calgary, AB, Canada; 2) Department of Biological Sciences, University of Toledo, Toledo, OH; 3) Department of Cell and Developmental Biology, SUNY Upstate Medical University, Syracuse, NY.

Embryonic elongation, which transforms the roughly spherical embryo into a long, thin vermiform larva, is mediated in part by a smooth muscle-like contraction of an actin/myosin network in the epidermal cells. Here we describe two new players that regulate this process. Previous work has shown that contractile force is generated by two redundant pathways that activate non-muscle myosin. Contraction is triggered by the phosphorylation of MLC-4, which activates the non-muscle myosins NMY-1/NMY-2. This is counteracted by MLC-4 dephosphorylation by MEL-11/myosin phosphatase. One of the two contractile pathways involves the small GTPase RHO-1, which activates LET-502/Rho-binding kinase, which in turn inhibits the MEL-11 brake to contraction. A second, parallel pathway involves FEM-2/protein phosphatase 2c.

In a suppressor screen of *mel-11*, we isolated an allele of a Rho GEF (guanine exchange factor), *rhgf-2*. As expected, *rhgf-2* genetically behaves as an upstream activator of the *let-502*/Rho-binding kinase branch of the elongation pathway and appears to function in parallel to *fem-2*. RHGF-2 binds to and acts as a GEF for RHO-1 (See abstract by Tran et al.).

Even though circumferential actin filaments are present in all epidermal cells, most of the contractile force is generated by the lateral (seam) cells. The lateral cell microfilaments are qualitatively different than those in their dorsal and ventral neighbours in that they are found further from the apical surface. We have identified a actin nucleator in the formin family that may be in part responsible for these differences. FHOD-1 (formin homology domain) is expressed only in the lateral epidermal cells and mutations cause actin defects only in those cells. Curiously, genetic results indicate that *fhod-1* acts downstream of *let-502* and in parallel to *fem-2*, implying that the two pathways may act on different sets of microfilaments.

1106C

Extracellular leucine-rich repeat proteins and maintenance of epithelial integrity. **Vincent P. Mancuso[1]**, Jean Parry[1], Luke Storer[1], Corey Poggioli[1], David Hall[2], Meera Sundaram[1]. 1) Dept Genetics, Univ Pennsylvania, Philadelphia, PA; 2) Dept Neuroscience, Center for C. elegans anatomy, Albert Einstein College of Medicine, Bronx NY.

Elron (extracellular Leucine-Rich Repeat only) proteins are known to have roles in synapse formation, axon guidance, and cell-cell adhesion. Here we describe distinct roles for the two transmembrane Elron proteins K07A12.2 and LET-4/SYM-5/C44H4.2 in C. elegans epithelial cells. Both K07A12.2 and let-4 single mutants have lethal defects in embryonic elongation and excretory system function. In the low-penetrance embryonic phenotype, epidermal cell morphology appears normal until embryonic elongation, at which point epidermal lesions form. This suggests a role for K07A12.2 and LET-4 in the maintenance of epidermal cell integrity during the stress of elongation. Another transmembrane Elron, SYM-1, acts redundantly with LET-4 during elongation (Davies et al., 1999). Both K07A12.2 and LET-4 GFP fusion reporters localize to the apical face of epidermal cells during elongation, suggesting an interaction with the embryonic sheath. Individuals that do not have the embryonic elongation defect develop a lethal excretory system defect. In the excretory system defect phenotype, both K07A12.2 and let-4 single mutants accumulate fluid in the excretory system and die as fluid-filled L1s. The excretory system includes three tandem unicellular epithelial tubes: the excretory canal cell, the duct cell, and the pore cell. Although both K07A12.2 and LET-4 are required for a functioning excretory system, each functions in a different aspect of the excretory system. K07A12.2 mutants lack a pore cell autojunction, though it is currently unknown whether it fails to form or is lost during development. This phenotype suggests a role for K07A12.2 in initial formation of the pore cell, or in maintenance of the tube shape. A failure of either process could explain the excretory system defect. In let-4 mutants, the pore autojunction is unaffected, but the duct cell lumen becomes distorted and discontinuous just before hatch, indicating a role for LET-4 in lumen integrity. LET-4::GFP is localized to the luminal (apical) side of the duct cell, and may interact with the extracellular matrix inside the lumen of the duct and pore cells. This work suggests roles for Elron proteins in mediating interactions between epithelia and the apical extracellular matrix to help maintain cell shape and integrity. Davies AG, et al. (1999) Genetics 153, 117-134.

1107A

The PCP pathway components vang-1 and prkl-1 are required for the Wnt dependent migration of the Q neuroblasts descendants. **Remco A Mentink**, Hendrik C Korswagen. Korswagen Group, Hubrecht Institute, Utrecht, Utrecht, Netherlands.

The Q neuroblast lineage of C. elegans provides a sensitive system to study the function of Wnt signaling in cell migration. During early larval development, the descendants of the left and right Q neuroblasts migrate in opposite directions along the anteroposterior axis. The posterior migration of the left Q cell descendants (QL.d) is controlled by canonical Wnt/beta-catenin signaling, whereas the anterior migration of the QR.d is mediated by a beta-catenin independent signaling mechanism. Our aim is to gain further insight into the non-canonical Wnt signaling pathway that controls QR.d migration and to discover novel pathway components. In agreement with previous studies, we found that the Wnt receptors mom-5/Frizzled and cam-1/Ror2 are required for QR.d migration. Although cam-1 has been described as a non-autonomous inhibitor of Wnt signaling, our expression analysis using single molecule mRNA FISH shows that cam-1 is specifically upregulated in the QR lineage, consistent with a cell-autonomous role in Wnt signaling. In addition, we found that components of the planar cell polarity (PCP) pathway are required for the correct migration of the QR.d. The non-canonical Wnt/PCP pathway has key functions in defining polarity along the plane of the tissue and also controls cell movement during convergence and extension in vertebrates. Our results show that the core PCP components vang-1 and prkl-1 are required for the correct positioning of the QR.d and that these genes function as inhibitors of the Wnt dependent migration, most likely at the level of the Frizzled receptors.

1108B

madd-2 regulates anchor cell invasion. **Matthias Morf[1]**, Ivo Rimann[1], Stefanie Nusser[1], Mariam Alexander[2], Peter Roy[2], Alex Hajnal[1]. 1) Institute of Molecular Life Sciences, University of Zurich, Winterthurerstrasse 190, 8057 Zürich, Switzerland; 2) Department of Molecular Genetics, The Terrence Connelly Centre for Cellular and Biomolecular Research, University of Toronto, 160 College Street, Toronto, ON M5S 3E1, Canada.

Invasion, the process during which cells cross tissue borders, needs to be tightly regulated since uncontrolled invasion can lead to metastatic cancer growth. During C. elegans larval development, a specialized cell in the somatic gonad called anchor cell (AC) breaks two basal laminae and then invades the adjacent vulval tissue in order to form a connection between the developing vulva and uterus. Multiple signals from the vulval cells and the ventral nerve cord regulate AC invasion, however, how these signals are integrated by the AC is largely unknown. We have identified madd-2, a conserved RING finger gene and E3 ubiquitin ligase, as a regulator of AC invasion. madd-2 has independently been identified by both the P. Roy lab as a gene acting downstream of the UNC-40 netrin receptor in controlling muscle arm extensions and the C. Bargmann & M. Tessier-Lavigne groups that showed that the same is true in axon branching. The human madd-2 homologue, MID1, is mutated in most cases of Opitz syndrome, a disease characterized by ventral midline defects. Analysis of basal laminae breaching in madd-2 mutants demonstrates that the timing of AC invasion is changed. madd-2 single mutants show delayed invasion, but AC

invasion in double mutants for madd-2 and either unc-40 or unc-6 occurs precociously. We thus postulate that AC invasion is not only activated at the late L3 stage, when invasion normally begins, but also repressed at earlier stages. Additionally, by examining markers for the invasive membrane, we find that AC morphology is altered in madd-2 mutants. The AC in madd-2 mutants forms ectopic protrusions at random locations, suggesting a loss of directed invasion. RNAi mediated knock-down of the proposed GEF K07D4.7, a homolgue of human Ephexin, suppresses the ectopic AC protrusions of madd-2 mutants but does not affect AC shape in wild-type animals. Thus, the GEF K07D4.7 might be negatively regulated by madd-2, directly or indirectly, to prevent the formation of ectopic AC protrusions at the time of invasion.

1109C

Infection-induced plasticity of hindgut development. **Frederick A. Partridge**, Jonathan Hodgkin. Dept Biochem, Univ Oxford, Oxford, United Kingdom.

The genome is often thought to be a blueprint for the development of an organism. In nature however, a single genotype can often give rise to different phenotypes depending on environmental conditions. This is called phenotypic plasticity. Understanding the genetic architecture underlying such choices is an important challenge for developmental biology.

When *C. elegans* and certain other rhabditid nematodes are infected by various pathogens, notably the coryneform bacterium *Microbacterium nematophilum,* they respond by altering the development of the hindgut, producing a swollen tail. Our goal is to understand the cellular and molecular mechanism of this response.

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 easier to isolate than mutants affecting the response to infection. To avoid this problem we have stably expressed activated MAP kinase pathway genes in the hindgut, using a promoter fragment derived from *egl-5.* This phenocopies tail swelling in the absence of infection. We then used RNA interference to search for suppressors of this constitutive tail swelling, screening the 16750 genes targeted by the Ahringer library in triplicate in an RNAi hypersensitive background. Known ERK MAPK components such as *mpk-1* suppress tail swelling in this assay, and we additionally identified many new genes involved in the swelling response. The predicted functions of these genes show that distinct cellular processes such as endocytosis, ion transport, and control of the extracellular matrix act together during development in the regulated modulation of cell shape that underlies tail swelling.

We will also present the progress we have made in understanding the cell biology of the process.

1110A

A possible role for TRY-2/plasminogen during C. elegans embryonic development. **Melissa Ponce**, Austin Howard, Saeideh Sarraf-Mamouri, Martin Hudson. Biology and Physics, Kennesaw State University, Kennesaw, GA.

How neurons modify the extracellular matrix during nervous system development is poorly understood. Plasminogen-like proteases are required for cell migration and axon outgrowth of the dorsal root ganglia (1, 2) and are implicated in mossy fiber axon branching in the hippocampus (3). The use of proteolytic activity to facilitate axon outgrowth must be carefully controlled and coordinated with instructions from multiple axon guidance molecules. How does a neuron integrate these extracellular cues with protease activity to accurately permit axon growth through time and space?

The kal-1/anosmin gene is implicated in multiple aspects of neuronal development including cell migration and axon branching in both vertebrates and invertebrates (4-7). In C. elegans, KAL-1 has high affinity for heparan sulfate proteoglycans (HSPGs); key components of the cell surface and extra cellular matrix. In addition, human anosmin exhibits high affinity binding to urokinin-related plasminogen activator (PLAU) in vitro. As such, KAL-1/anosmin is well positioned to integrate HSPG and extracellular matrix cues with the proteolytic activity of plasminogen activators, facilitating accurate axon outgrowth.

PLAU contains a plasminogen protease and kringle domain. Our preliminary data indicates that C. elegans TRY-2 may be orthologous to human PLAU as this is the only C. elegans protease that contains both of these domains. In addition, try-2(ok2531) embryos exhibit neuroblast migration defects suggesting a role for plasminogen activity in embryonic development. Genetic analysis is ongoing to establish whether try-2 functions with other genes required for neuroblast migration, axon outgrowth and KAL-1 dependent axon branching.

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1111B

The *C. elegans* Hailey-Hailey disease homolog *pmr-1* is essential for embryonic development. **Vida Praitis[1]**, Adityarup Chakravorty[1], Charlotte Feddersen[1], Dae Gon Ha[1], Leah Imlay[1], Sarah Kniss[1], Walter Liszewski[1], Michael Miller[1], Juliet Mushi[1], Angela Schacht[1], Tyson Stock[1], Alexander Sullivan-Wilson[1], Zelealem Yilma[1], Lensa Yohannes[1], Jeffrey Simske[2]. 1) Dept Biol, Grinnell Collegel, Grinnell, IA; 2) Rammelkamp Center for Education and Research Cleveland, OH.

ATP2C1/PMR1 is a Ca2+/Mn2+ ATPase that localizes to the golgi where it acts in protein processing, metal homeostasis, and Ca2+ signaling. In humans, lesions in the gene cause Hailey-Hailey disease or benign familial pemphigus (MIM# 169600), which shows a semi-dominant inheritance pattern and is marked by severe skin lesions that typically occur in the second decade of life. Defects in the gene have also been associated with squamous cell carcinomas. In two independent screens designed to identify temperature-sensitive alleles of genes essential for embryonic enclosure, we isolated *jc10* and *ru5*. Complementation analysis demonstrated these mutations were allelic and several independent lines of evidence indicate that it is lesions in the *C. elegans* ATP2C1 homolog *pmr-1* that are responsible for the phenotypes observed in these mutants. Whole-genome and directed sequencing revealed mutations in *pmr-1* in both *jc10* and *ru5*, the phenotypes are rescued by fosmids carrying the *pmr-1* gene, and the *ru5* allele fails to complement *pmr-1(tm1840)*, which contains a deletion in the first exon of the gene. Embryos homozygous for mutations in *pmr-1* have defects in epidermal cell migration and adhesion during enclosure, as well as later pharynx unattached (PUN) and body morphogenesis phenotypes at 25C. Temperature shift experiments indicate that the temperature sensitive period occurs earlier in development, just after ventral cleft formation and during a period of extensive cell birth. Using lineage analysis and GFP markers that are expressed in a subset of cells during and just after the temperature-sensitive period, we determined that the timing of divisions and cell fate are normal in *pmr-1(ru5)* embryos, but that the position of some cells is defective. This analysis demonstrates that the *C. elegans* PMR-1 Ca2+/Mn2+ ATPase plays an important role in cell positioning during embryonic development.

1112C

PAT-4 (ILK) regulates the binding of UNC-112 (Kindlin) to PAT-3 (β-integrin) in *C. elegans*. **Hiroshi Qadota[1,2]**, Donald G. Moerman[2], Guy M. Benian[1]. 1) Dept Pathology, Emory Univ, Atlanta, GA; 2) Dept Zoology, Univ British Columbia, Vancouver, BC.

Myofilaments within *C. elegans* 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 adhesion complexes. To clarify the molecular mechanisms regulating integrin mediated attachment structures, we are investigating protein-protein interactions between and among integrin-associated proteins using yeast two-hybrid methodology.

From genetic and cell biological studies, many proteins including PAT-3 (β-integrin), UNC-112 (Kindlin), and PAT-4 (ILK) have been located to adhesion structures. Previously, it was shown that PAT-4 binds to the N-terminal half of UNC-112. Here we demonstrate using a yeast two-hybrid assay that the cytoplasmic tail of PAT-3 binds to full-length UNC-112, and that GST-PAT-3 can pulldown an UNC-112/PAT-4 complex from a worm lysate. We also demonstrate that the UNC-112 N-terminal and C-terminal halves bind each other. This was demonstrated using a two-hybrid assay and an *in vitro* binding assay with purified UNC-112 N and C terminal halves. Since full-length UNC-112 could not bind to either the N-terminal half or C-terminal half, an intramolecular interaction within UNC-112, is most likely. These results led us to hypothesize that UNC-112 exists in two interconvertible states, a closed inactive state and an open active state, and that upon binding of PAT-4 to the UNC-112 N-terminal half, UNC-112 opens, and consequently the UNC-112/PAT-4 complex binds to the cytoplasmic tail of PAT-3.

To further support this hypothesis, we obtained both *in vitro* and *in vivo* evidence: (1) Using purified proteins, we observed competition between the UNC-112 C terminal half and PAT-4 for binding to the UNC-112 N-terminal half. (2) By using PCR-based random mutagenesis, we isolated a point mutation (D382V) in the N-terminal half of UNC-112 that showed that it does not bind to PAT-4 by two-hybrid. When HA-tagged UNC-112 was expressed in worms and immunoprecipitated, wild type, but not D382V mutant UNC-112, was able to co-pellet PAT-4. Most importantly, HA tagged wild type, but not D382V mutant UNC-112, localized to M-lines and dense bodies *in vivo*. These results suggest a molecular mechanism by which PAT-4 (ILK) can regulate the binding of UNC-112 (Kindlins) to PAT-3 (β-integrin).

1113A

Microtubules in embryonic morphogenesis. **Sophie Quintin**, Ambre Bender, Michel Labouesse. IGBMC, ILLKIRCH, France.

During C. elegans morphogenesis, the embryo elongates from a bean to a worm shape within 3 hours, without any cell division. Elongation, which ultimately reduces embryo circumference and lenghthens its A-P axis by 4-fold, depends on 3 processes: 1- actomyosin tension 2- a mechanotransduction pathway between muscles and the epidermis 3- microtubules (MT). In 1986, J. Priess showed that treatments with MT-depolymerising drugs affect elongation, however how MT precisely act is still unknown. We address this question by inducing the expression of spastin —a MT-severing protein— in various cell types. We use an operon-like expression system in order to co-express the spastin protein (SPAS-1) with an mCherry reporter, and express it in all epidermal cells (lin-26 promoter), in the lateral seam cells (P_ceh-16), in the dorso-ventral cells (P_elt-3 and P_dpy-7), or in all cells using a heat-shock promoter (hsp). MTs are indeed disrupted in mCherry-expressing cells, as visualised with a tubulin::GFP reporter. MT degradation is proportional to the dose of spastin expressed in the cells, the best results being obtained with the strong hsp and the dpy-7 promoters. Besides, early SPAS-1 induction causes cell division defects in the early blastomeres, suggesting that MT function is efficiently disrupted. We found that embryonic lethality is higher with the hsp promoter vs any epidermal promoter (35% vs below 10%). Importantly, a deleted version of SPAS-1 did not cause lethality. Using videomicroscopy, we could define the time window in which SPAS-1 induction causes embryonic phenotypes, namely just before the onset of elongation. Interestingly, if SPAS-1 is induced after the lima-bean stage, it no longer affects morphogenesis. Similar results were

observed when SPAS-1 was expressed under the dpy-7 promoter, which becomes active after the onset of elongation; the embryos elongate normally although they lack MTs. However, MTs might provide some stiffness, since a transient and local bulge is often observed where they are degraded. Finally we found that the Rho kinase let-502 mutants are hypersensitive to SPAS-1 expression, arguing that actomyosin tension could compensate for the lack of MTs. In parallel, to test if some genes required for elongation also affect MT dynamics, we have generated an integrated P_dpy-7::EB-1::GFP line. We observed that the MT growth rate is higher in seam cells than in dorso-ventral cells. Interestingly this difference is almost abolished in the let-502 mutants, which are blocked in elongation and lack actomyosin tension. In conclusion, there would be a close link between actin and MTs in morphogenesis: i) actomyosin tension could compensate the lack of MTs in the epidermis, ii) MT growth rate in the seam cells depends on a normal actomyosin tension. Further tests should confirm or infirm this hypothesis.

1114B
Somatic gonad precursor migration in *C. elegans*. **Monica R. Rohrschneider**, Jeremy Nance. Developmental Genetics Program, Skirball Institute, New York University-School of Medicine, New York, NY.

In many species, including mouse, *Drosophila*, and *C. elegans*, the somatic gonad precursor cells (SGPs) and the primordial germ cells (PGCs) are born at a distance from one another, and must migrate in order to coalesce and form the proper structure of the gonad. In *C. elegans*, the SGPs migrate nearly half the length of the embryo in order to reach the PGCs. This migration is critical, as the SGPs are required for survival and proliferation of the germ cells. However, little is known about what drives the SGPs to migrate, and what triggers them to stop.

To address these questions, we are constructing transgenic strains that will allow us to better characterize the migration of the SGPs and their interactions with neighboring cells. We have found that the SGPs extend projections as they migrate posteriorly along the edge of the endoderm cells, and when they reach the PGCs, their projections appear to wrap around the PGCs. We are using both genetic and physical ablations of the PGCs and of endoderm cells to test the hypothesis that these cells are required for normal SGP migration. Surprisingly, SGP migration is grossly normal in *mes-1* mutants which lack PGCs, suggesting that PGCs do not provide a long-range attractive cue to the SGPs. However, SGP migration is disrupted in *end-1 end-3* mutants, which lack endoderm, suggesting that endoderm development or morphogenesis is required for normal SGP migration.

1115C
The role of extracellular ZP-domain protein DYF-7 in body size development. **Robbie D. Schultz**, Tina L. Gumienny. Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX.

Body size development is strictly regulated in *C. elegans* through genetic and environmental components. The most well studied of these genetic regulators is the TGF-β DBL-1 pathway. Loss of DBL-1 signaling leads to decreased body size as well as abnormal development of male-specific structures. Mutants lacking DYF-7 display a small body size and do not properly sense their environment. DYF-7 is a transmembrane zona pellucida (ZP) domain-containing protein expressed in head and tail neurons as well as seam cells. Previous studies have shown that DYF-7 is involved in neural tip anchoring, which explains its sensing defect, but its role in body size development has remained unexplored. To determine if DYF-7 regulates body size though DBL-1 signaling, we performed epistasis and expression studies. Our epistasis analyses between *dyf-7* and *dbl-1* pathway members reveal that double mutants are significantly smaller than either single mutant, indicating DYF-7 regulates body length independent from the TGF-β DBL-1 pathway. We also found GFP-tagged DBL-1 is properly expressed and localized in animals lacking DYF-7 signaling. Further, male-specific structures appear normal in *dyf-7* mutants. An additional mode of body size regulation is through organization of the cuticle, which surrounds the exterior of the animal. The cuticle in *dyf-7* mutants has morphological defects that could contribute to the small body size phenotype. These results indicate that DYF-7 acts through cuticle organization, independent of the TGF-β pathway, to regulate body size.

1116A
UNC-83 and UNC-84 bridge the nuclear envelope to move nuclei. **Daniel A. Starr**[1], Erin Tapley[1], Heidi Fridolfosson[1,2], Marina Meyerzon[1,3], Nina Ly[1,4]. 1) Mol & Cellular Biol, Univ California, Davis, CA; 2) Anesthesiology, Univ of California, San Diego, CA; 3) Mol & Cellular Biol, Univ California, Berkeley, CA; 4) Biosciences Graduate Program, Stanford Univ, Palo Alto, CA.

Nuclear migration is important for a wide variety of cellular and developmental processes. We employ nuclear migration in the embryonic hyp7 precursors as a model to study nuclear migration. We have shown that UNC-84 localizes to the inner nuclear membrane with its conserved SUN domain in the perinuclear space. The SUN domain then interacts with the KASH domain of UNC-83 to recruit it to the outer nuclear membrane. Thus, UNC-83 and UNC-84 form a bridge across the nuclear envelope, connecting the nucleoskeleton to the cytoskeleton. Here we report a series of findings about the function and regulation of this bridge. First, we show that UNC-83 recruits kinesin-1 (UNC-116) and dynein (DHC-1) to the surface of the nucleus using KLC-2, BICD-1, NUD-2, LIS-1, and EGAL-1. Live filming of nuclear migrations in various genetic backgrounds showed that kinesin-1 provides the major force to move nuclei forward while dynein regulates backwards movements to bypass roadblocks. We also showed that the microtubule network in these cells is polarized using EBP-1::GFP. Second, we have identified multiple signals that function to recruit UNC-84 to the inner nuclear membrane. Importantly, mutations in an INM-SM or a novel SUN-NELS disrupt the efficient localization of UNC-84, suggesting that an active process exists to traffic UNC-84 from the peripheral ER to the nuclear envelope. Perhaps this is through a small isoform of IMA-3. Third, using deletion analysis of the lumenal domain of UNC-84, we have defined the minimal region of UNC-84 required for nuclear migration, which is surprisingly small. Together, these three findings have greatly enhanced our understanding of the molecular mechanisms of how UNC-83 and UNC-84 mediate nuclear migration.

1117B
The Rac-GEF UNC-73/TRIO Mediates Multiple and Genetically Distinct Pathways to regulate excretory cell migration. Nancy Marcus[1,2], **Eve Stringham**[1,2]. 1) Dept Biology, Trinity Western University, Langley, BC, Canada; 2) Dept. of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada.

The actin cytoskeleton regulator UNC-53/NAV2 is required for both the anterior and posterior outgrowth of several neurons as well as that of the excretory cell (Stringham, et al., 2002; Schmidt et al., 2009) while the kinesin like motor VAB-8 is essential for most posteriorly directed migrations in *C. elegans* (Wightman et al., 1996). Genetic analysis of putative interactors of UNC-53 or VAB-8, and cell specific rescue experiments, suggest that VAB-8, SAX-3/ROBO, SLT-1/Slit and EVA-1 are functioning together in the outgrowth of the excretory canals, while UNC-53 appears to function in a parallel pathway with UNC-71/ADAM. The known VAB-8 interactor UNC-73/TRIO operates in both pathways, as isoform specific alleles exhibit enhancement of the phenotype in double mutant combination with either UNC-53 or VAB-8. Collectively, our results suggest a bipartite model for UNC-73/TRIO function, where the UNC-73E isoform, together with UNC-53 and UNC-71 functions cell autonomously within the excretory cell to promote outgrowth, while the UNC-73B isoform through partnering with VAB-8 and the receptors SAX-3 and EVA-1 has a cell non-autonomous function in excretory cell outgrowth.

1118C
The Nm23-H1/H2 homologue NDK-1 activates Ras/MAPK signaling interacting with kinase scaffold of Ras during vulval development in *C. elegans*. **Krisztina Takacs-Vellai**[1], Neda Masoudi[1,2], Luca Fancsalszky[1], Ehsan Pourkarimi[1,2], Tibor Vellai[1], Anton Gartner[2], Anita Alexa[3], Attila Remenyi[3], Anil Mehta[4]. 1) Department of Genetics, Eotvos Lorand University, Budapest, Hungary; 2) Wellcome Trust Centre for Gene Regulation and Expression, University of Dundee, UK; 3) Department of Biochemistry, Eotvos Lorand University, Budapest, Hungary; 4) Division of Medical Sciences, Centre for CVS and Lung Biology, Ninewells Hospital Medical School, UK.

nm23-H1(currently *nme1*), the first identified metastasis inhibitor, encodes a nucleoside diphosphate kinase (NDPK). Despite the wide biological significance of NDPK deletion (heart failure, defective immunity, insulin release and postnatal survival) the molecular mechanisms by which Nm23-H1 exerts its pluripotent roles are still unclear. Here, we focus on the role of *ndk-1* (which corresponds to *F25H2.5*), the *Caenorhabditis elegans* orthologue of *nm23-H1/H2* in Ras-mediated signaling events during development. Deletion of *ndk-1* results in a protruding vulva phenotype. *ndk-1* null mutants display a severely reduced diphosphorylated MAPK level in somatic tissues, indicating defects in Ras/ERK signaling. Therefore we examined the interaction of *ndk-1* and the EGFR/Ras/MAPK pathway that is central to vulval development. Reduced expression of the Ras/MAPK target gene *egl-17(FGF)* in the *ndk-1* null mutant background further suggests that NDK-1 is needed for full Ras/MAPK activation. Our epistasis analysis demonstrates that *ndk-1* acts downstream of *let-60/Ras* and upstream of *mpk-1/ERK* during vulval development, at the level of kinase suppressor of Ras (*ksr*). KSR-1 and KSR-2 proteins act as scaffolds to approximate Ras/MAPK components permitting downstream signaling. Mechanistically we show that NDK-1 binds physically to KSR-2, and we suggest that NDK-1/NDPK activates Ras/MAPK signaling through modification of the KSR-2 scaffold during vulval development in *C. elegans*. This study provides the first *in vivo* evidence for the significance of the NDPK/KSR interaction and could provide a model system to understand the pleiotropic effects of NDPK in human diseases.

1119A
Mutations Affecting Cessation of Gonadal Leader Cell Migration in *C. elegans*. **Kikuchi Tetsuhiro**, Shibata Yukimasa, Nishiwaki Kiyoji. Dept of Bio, Graduate School of Scien, Kwansei Gukuin Univ, Sanda, Japan.

Organs are often formed by elongation and branching of epithelial tubes. Although regulation of initiation and direction of epithelial tube migration is an important aspect in organogenesis, there must be mechanisms that terminate epithelial migration to generate organs of appropriate size. These mechanisms remain mostly unexplored, however. The gonadal development in *C. elegans* offers a simple model system to study the morphogenesis by epithelial tubes. The migration of the anterior and posterior gonadal leader cells called distal tip cells (DTCs) promote directional elongation of gonad arms during larval development and form a pair of U-shaped gonad arms in rotational symmetry. We placed Venus under the control of the DTC specific *mig-24* promoter and fluorescently visualized DTCs. In the wild type adult worms, two DTCs stopped around the dorsal side of the vulva. We isolated two mutants, *tk102* and *tk107* by EMS mutageneis. About 80% of DTCs in these mutants overshot the vulval region, but fewer than 10% of DTCs did in the wild type. Both of these mutants are recessive and they partially complement each other. These mutants seem not to affect growth rate, animal size and behavior. To understand the difference in migration modes of DTCs between the wild type and the mutants, we analyzed the timing of DTC turns and migration speed of DTCs. We found that DTCs in these mutants make two turns at the same timing as in the wild type, but that the deceleration rates of mutant DTCs after the second turn were decreased compared to the wild type. Thus

the DTCs in the mutants fail to stop at the normal timing. Interestingly, we found that the expression of *mig-24::Venus*, which is normally weakened after stopping of DTCs at the young adult stage, was retained to be strong even in the 2-day-old adults in *tk107*. *mig-24* encodes a transcription factor of the bHLH family. Genetic mapping experiments assigned *tk102* and *tk107* to 2473kb and 1853kb overlapping regions of the chromosome III, respectively. Deep sequencing analysis revealed a nonsense mutation in the predicted gene *F42H10.5* in the *tk107* genome. RNAi knockdown of this gene resulted in an overshoot phenotype similar to that observed in *tk107*. We also successfully rescued *tk107* by a fosmid clone. *F42H10.5* appears to encode a Zink-finger DNA-binding protein having weak homology to ZBED4 in mammals. Therefore, it is possible that *F42H10.5* is a negative transcriptional regulator of *mig-24* which could act in promotion of DTC migration.

1120B
GSK-3 Regulates Left/Right Symmetry Breaking. **Michael G. Tiongson**, Christian Pohl, Zhirong Bao. Dept Dev Biol, Memorial Sloan-Kettering, New York, NY.

Breaking left-right (LR) symmetry is a fascinating process in bilaterian embryogenesis as the asymmetry arises without apparent outside spatial cue. In C. elegans, LR symmetry is broken at the 4 to 6 cell division. As ABa and ABp divide synchronously, their spindles are initially aligned to the LR axis, but skew in such a manner that by the end of the division, the left side daughters are situated anterior to the right side daughters. This positional bias between the left and right side AB cells is sufficient to specify the handedness of the animal. Using micromanipulation to force the right AB daughters more anterior to the left is enough to reverse all normal L/R asymmetries of the worm. However, the actual molecular mechanism underlying this initial AB spindle skew is largely unknown. We used high-resolution time lapse fluorescent microscopy to delineate the cellular level events of the spindle skew. We found that this skew appears to be fundamentally different from spindle repositioning events found at the one- and two-cell stages, where the spindle rotates within the cell and determines the cleavage plane before cytokinesis starts. Instead, the ABa/p spindle skew occurs as the cytokinesis furrow forms and the contractile ring begins to ingress. This observation indicates that the skew is a whole cell movement rather than the spindle rotating within the cell. In previous experiments, we showed that in the events directly after the ABa/p skew, GSK-3, the C. elegans glycogen synthase kinase ortholog, is required in the global rearrangement of cells to translate the initial LR asymmetry into a LR body plan. Here we show that in about 9% of gsk-3(nr2047) embryos, ABa/p skew in the reversed direction, suggesting that gsk-3 plays a role in the initial LR symmetry breaking cue. In an enhancer screen we found that the reversal rate can be increased to 30-40%. Currently, we are using quantitative analysis to uncover the molecular mechanism of these reversal cases.

1121C
Caenorhabditis elegans RHGF-2 is an essential RHO-1 specific RhoGEF that binds to the multi-PDZ domain scaffold protein MPZ-1. **Thuy Tran**, Li Lin, Shuang Hu, Todd Cramer, Vera Hapiak, Richard Komuniecki, Robert Steven. University of Toledo, 2801 W. Bancroft Street Toledo, OH 43606.

Members of the Rho family of small GTPases, including Cdc42, Rac and Rho, are activated by a large number of different RhoGEFs and play a key role in the regulation of fundamental cellular processes such as cell morphology and polarity, cell cycle progression and gene transcription. We identified a *Caenorhabditis elegans* RhoGEF protein, which we named RHGF-2, as a binding partner of the *C. elegans* multi-PDZ domain scaffold protein MPZ-1. MPZ-1 and its mammalian homolog MUPP1 function in multiple signaling pathways through their interactions with several receptors including the serotonin and glutamate neurotransmitter receptors. RHGF-2 exhibits significant identity to the mammalian RhoGEFs Tech/SYX-1/GEF720 and contains a class I C-terminal PDZ binding motif (SDL) that interacts specifically with MPZ-1 PDZ domain eight. RHGF-2 RhoGEF activity is specific for the *C. elegans* RhoA homolog RHO-1 as determined by direct binding, GDP/GTP exchange and luciferase assays. *rhgf-2* deletion mutants arrest development during embryogenesis and hatch as short immobile animals. Labeling of cell junctions in *rhgf-2* embryos with JAM-1::GFP revealed that epidermal cells do not elongate as they do in wild type. Surprisingly, a functional *rhgf-2::gfp* construct appears to be expressed exclusively in a subset of neurons with no expression observed outside the nervous system at any time from the start of development. *rhgf-2* overexpression results in loopy movement with a high amplitude waveform indicating RHGF-2 influences the control of locomotory behavior with effects similar to those observed with constitutive activation of the Rho GTPase pathway. Transient expression of RHGF-2 in N1E-115 neuroblastoma and NIH3T3 fibroblast cells prevents neurite outgrowth and causes cell rounding, respectively, which is consistent with the reported effects of the mammalian RHGF-2 homologs. Together, these observations indicate neuronally expressed RHGF-2 is an essential RHO-1 specific RhoGEF that binds specifically to MPZ-1 PDZ domain eight and is required for wild-type C. elegans morphology.

1122A
SUMO proteases in C. elegans. **A Tsur**, L Broday. Cellular and developmental biology, Tel-Aviv University, Sackler faculty of medicine, Tel-aviv, Israel.

The covalent attachment of SUMO to its target proteins is a highly dynamic and reversible post-translational modification due to the activity of SUMO proteases. Both de-conjugation of SUMO (deSUMOylation) and the processing of SUMO precursors prior to conjugation are carried out by the same family of proteases called Ubl specific proteases (ULP) in yeast, Drosophila and C. elegans and Sentrin-specific proteases (SENP) in vertebrates. ULP/SENPs are therefore key modulators of SUMOylation dynamics. In order to perform a comparative analysis of the five known C. elegans ULP proteins (ULP-1-5) we generated a phylogenic tree of over 70 different ULP/SENP sequences. The tree shows that ULP-3 which was considered to be a SUMO protease is an ortholog of the mammalian SENP8, a Nedd8-specific protease. ULP-1 appears to be the most closely related to SENP1 and SENP2. ULP-2 is highly related to the mammalian SENP6 and SENP7 suggesting a possible preference towards de-conjugation of SUMOylated species and not SUMO processing [1]. Expression of a translational reporter ULP-2::GFP is mainly nuclear and appears at late gastrulation. ulp-2(RNAi) resulted in ~30% embryonic lethality. Analysis of DLG-1 and AJM-1 fluorescence reporters showed severe defects in epidermal morphogenesis. Our findings suggest a role for ULP-2 in epithelial development. 1. Mukhopadhyay D, Dasso M. Modification in reverse: the SUMO protease. Trends Biochem Sci. 2007 Jun;32(6):286-95. Epub 2007 May 17.

1123B
VAB-3 and VAB-5 are involved in mediating embryonic anterior muscle development. **Ryan D. Viveiros**[1], Robert Barstead[2], Donald G. Moerman[1]. 1) Dept Zoology, Univ British Columbia, Vancouver, BC, Canada; 2) Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, U.S.A.

C. elegans body wall muscle is formed after a series of well-orchestrated steps. Initially, embryonic muscle cells accumulate under the hypodermal seam cells along the left and right lateral line. Shortly thereafter, the muscle cells in these two quadrants begin to migrate either dorsally, or ventrally, depending on cell fate to form the final four muscle quadrants present upon hatching. As the muscle cells are migrating they are still dividing, as are many other cells in their immediate environment. This means the cell-cell contact of the cells during migration is dynamic and can vary from animal to animal. This creates a dynamic environment where the extracellular matrix and cell surface contacts are in constant flux, which begs the question as to how thee cells navigate unerringly to their final destination.

As well as these dorsal and ventral migrations, we have previously identified a second migratory event, in which the anterior muscles cells in each of the developing four quadrants extend membrane processes to the anterior tip of the embryo. These membrane extensions are required for proper anterior migration of the muscle quadrant as loss of the ventral membrane processes in *ina-1, sax-3, vab-1/vab-2* or laminin mutant backgrounds leads to a posterior displacement of the ventral muscle cells, most likely due to steric interference from mislocalized neurons.

Continuing our investigation into the factors mediating these processes, we have identified the Pax-6 homologue VAB-3 and the uncloned gene *vab-5* as additional genes/proteins affecting anterior muscle cell extensions and migrations. We are currently attempting to determine how *vab-3*, which is known to play a role in hypodermal and glial patterning in the head, can affect anterior muscle migration, as well as, mapping the *vab-5* allele and using whole genome sequencing to determine the causative mutation in *vab-5(e108)*, the only extant allele of this gene.

1124C
Analysis of UNC-6/netrin's effectors during C. elegans anchor cell invasion. **Zheng Wang**, David Sherwood. Duke University, Durham, NC.

Cells invasion through basement membrane (BM) occurs in various contexts, including embryonic development, immune surveillance, and cancer metastasis. The mechanisms underlying cell invasion remain poorly understood. Anchor cell (AC) invasion into the vulval epithelium in C. elegans is an experimentally tractable model that combines powerful in vivo cell biological and genetic analysis to reveal the mechanisms underlying invasion. Utilizing this model, we previously reported that UNC-6, a guidance cue orthologous to vertebrate netrins, directs AC invasion by orienting the formation of a specialized invasive membrane domain toward its BM target. Secreted from the ventral nerve cord, UNC-6 concentrates its receptor UNC-40 and a number of actin regulators including the Rac GTPases MIG-2 and CED-10, the Ena/VASP ortholog UNC-34, and the phospholipid phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) at the AC's cell membrane in contact with BM. How netrin signals through these and other potential effectors to promote invasion remains unclear. Combining genetic and cell biological analyses, we have examined the role of these and other known effectors of UNC-6/UNC-40 in axon guidance during AC invasion. Surprisingly, we have found that the organization of effectors acting downstream of UNC-6/UNC-40 signaling in neurons is largely conserved during the seemingly distinct process of cell invasion. Similar to neurons, our analyses reveal two branches. In one, UNC-34 (Ena/VASP) acts downstream of UNC-40 and plays a role in promoting F-actin formation and maintaining the polarized localization of other invasive membrane components. The second branch is composed of the Rac GTPases MIG-2 and CED-10, the PAK (p21-activiated kinase) ortholog MAX-2 and UNC-115. The effectors in this branch act together to promote F-actin formation at the invasive membrane, but do not regulate polarity. Interestingly, our analysis indicates that MIG-10, an UNC-6 dedicated effector in axon guidance in the HSN neuron, has activity outside of UNC-6 signaling during AC invasion. Together, this work provides new insight into the molecular mechanism by which the UNC-6/UNC-40 pathway establishes invasive polarity and suggests that netrin signaling has dedicated effectors that mediate diverse cell biological processes.

1125A
Screening for essential muscle genes and synthetic lethality using high-throughput RNAi. **Adam D. Warner**, Teresa Rogalski, Aruna Somasiri, Iasha Chaudhry, Donald G. Moerman. Dept. Zoology, University of British Columbia, Vancouver, BC, Canada.

The sarcomere is an 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 a dense body, the nematode muscle homolog and analog of a Z-disc are required for the development and function of body wall muscle in *C. elegans*. Without these essential attachment complex proteins including among others, 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]. Studies of beta-integrin/PAT-3[2] and kindlin/UNC-112[3] in the nematode first implicated these proteins as being essential in muscle, and demonstrated that the assembly adhesion complexes at the muscle membrane are the first steps in sarcomere assembly.

Focusing on the paralyzed and arrested at two-fold stage (Pat) phenotype that occurs with loss of essential muscle genes in nematodes, we previously carried out a muscle transcriptome-wide RNAi screen on N2 animals to identify genes with an essential role in muscle development[4]. Using a similar high-throughput approach, we are redoing the screen in a sensitized background with strains harbouring hypomorphic alleles for known important muscle genes including *unc-97* and *unc-95*. By using RNAi to screen strains that have weakened or disrupted muscle, we hope to identify synthetic lethal interactions and possibly novel Pat genes. The identification of new genes affecting sarcomere assembly should help unravel the mystery of this complex example of cytoarchitecture.

[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]Meissner et al (2009). PLOS Genetics: Jun 5(6).

1126B

Essential Functions of IFA-2 Domains in *C. elegans* Fibrous Organelles. **Kyle C. Williams**, John Plenefisch. Department of Biological Sciences, The University of Toledo, Toledo, OH 43606.

The *C. elegans* fibrous organelle (FO) complex consists of apical and basal hemidesmosomes linked by cytoplasmic intermediate filaments (IFs). FOs are found most prominently in the epidermis where they overlie body wall muscle and serve to transmit force from the muscle to the cuticle. IFA-2, one of four epidermally expressed IFs whose loss results in epidermal fragility and failure of muscle-cuticle force transmission, localizes to FOs. Unlike IFB-1 and IFA-3 that are required embryonically, IFA-2, although expressed in the embryo, is not essential for FO function until postembryonic stages. This distinctive phenotype allows us to specifically explore the functions of IFA-2 domains, and map their interactions with other FO associated proteins. All IFs contain three domains: a central rod domain and globular head and tail domains. The rod is essential for assembly of IFs into mature filaments while less is known about the functions of the head and tail domains. Roles of the head and tail domains of IFA-2 were examined by expressing GFP-tagged IFA-2 variants in transgenic animals and examining their phenotype and localization in wild-type and null backgrounds. Mutant variants included IFA-2 deleted for the head domain (ΔH), deleted for the tail domain (ΔT), deleted for both (RO), and variants that contained only the head domain (HO) or tail domain (TO). The expression and function of ΔH is virtually indistinguishable from full-length IFA-2. ΔT results in a lowered incorporation of IFA-2 into FOs and is unable to rescue a null allele of ifa-2, suggesting the tail domain is essential to IFA-2 function. Though both HO and TO are expressed well in the hypodermis at all stages, incorporation into FOs is variable between individual animals. High levels of early-stage HO incorporation into FOs correlate with a dominant-negative muscle detachment phenotype, while low-level incorporation results in healthy animals. This suggests the head domain interacts with FO components essential to tissue integrity. To determine the protein components of the FOs that interact with the head and tail domains, yeast two-hybrid and co-localization experiments are in progress. This work should help to elucidate the role of IFA-2 in FO function and reveal the underlying defects leading to tissue fragility.

1127C

Novel regulation of the CUL-3/MEL-26 protein degradation pathway by the giant protein UNC-89. **Kristy J. Wilson[1]**, Hiroshi Qadota[1], Paul E. Mains[2], Guy M. Benian[1]. 1) Dept Pathology, Emory Univ, Atlanta, GA; 2) Dept Biochem & Mol Biol, Univ of Calgary, Calgary, Alberta.

The sarcomere contains a number of extraordinarily large polypeptides (700,000 Da—4 MDa) composed of multiple Ig and Fn3 domains, and one or two protein kinase domains. In *C. elegans*, one of these giants is UNC-89 (obscurin in humans). The largest UNC-89 isoform (~900,000 Da) consists of 53 Ig domains, 2 Fn3 domains, a triplet of SH3, DH and PH domains, and two protein kinase domains. Antibodies localize UNC-89 to the M-line. To understand how UNC-89 is localized and performs its functions, we are taking a systematic approach to identifying its binding partners. When UNC-89 segment Ig1-Ig5 was used to screen a yeast 2-hybrid library we pulled out MEL-26. When MEL-26 was used to screen a collection of 2-hybrid clones spanning the entire UNC-89-B coding sequence, one more region showed interaction, 1/3 interkinase-Ig53-Fn2. Minimally to interact with MEL-26, Ig2-Ig3, and Ig53-Fn2, are required. MEL-26 is one type of "substrate recognition protein" (and contains a BTB domain) that interacts with cullin 3. Cullin 3 is one type of cullin, highly conserved proteins that act as a scaffold for assembly of the ubiquitination machinery. Anti-MEL-26 antibodies (kindly provided by Lionel Pintard) localize to muscle M-lines and I-bands. RNAi of *mel-26* or *cul-3* beginning at the L1 larval stage (to avoid embryonic lethality), resulted in adult animals with body wall muscle having disorganization of GFP::MHC-A, in a pattern very similar to that observed in *unc-89(su75)* loss of function. We next examined sarcomeric organization in the temperature sensitive allele of *mel-26*, *ct61sb4*, marked with the neuronal Unc, *unc-29*. This strain, *mel-26(ct61sb4) unc-29(e1072)*, was grown at the permissive temperature, and resulting adults were stained with anti-MHC A. These animals, but not *unc-29(e1072)* showed disorganization of thick filaments similar to that observed in *mel-26(RNAi)*. MEI-1 (katanin) is the target of MEL-26 in *C. elegans* embryos (Pintard et al. Nature 2003). As adults, *mei-1(ct46)*, a dominant gain of function and ts allele, that encodes a protein that cannot bind MEL-26, also shows disorganization of thick filaments. All but one of the *unc-89* mutant alleles (~20 total) display a thinner sarcomeric region, which we speculate results from increased degradation of sarcomeric proteins. When UNC-89 is deficient, we would expect that more MEL-26 would be available to promote the degradation of the sarcomere. Therefore, we hypothesize that normally the interaction of UNC-89 with MEL-26 prevents the CUL-3/MEL-26 complex from promoting the ubiquitin mediated degradation of sarcomeric proteins.

1128A

Analysis of immunogenic proteins in *C. elegans* (IV). **A. Yamakawa[1]**, M. Ushiro[1], T. Tanaka[1], J. Fukumoto[1,2], A. Tamura[1,3]. 1) Dept Material Sci, Wakayama National Col of Technol, Gobo, Japan; 2) Tokushima Univ, Tokushima, Japan; 3) Tokyo Univ of Agric and Technol, Tokyo, Japan.

To construct monoclonal antibodies covering *C. elegans* immunogenic proteins, we immunized Balb/c mice with crude extracts from heterogenous population of *C. elegans*. The antibody producible spleen cells from these immunized mice were fused with P3U1 myeloma cells.

Up to this day, the 21 kinds monoclonal anti-*C. elegans* antibodies were selected based on the strong staining in the immunoblotting for total *C. elegans* protein and were classified into four types (I, II, III and IV) based on the immunostained-band sizes detected on the Western blots.

On the other hand, the 19 kinds monoclonal anti-*C. elegans* antibodies were additionally selected based on the weak staining in the immunoblotting for total *C. elegans* protein. These antibodies were analyzed by Western blotting and were classified into the following eleven types. Antibodies (1 kind) belonging to type A detected the 80 kDa-band. Antibody (2 kinds) belonging to type B detected several bands in addition to the 100 kDa-band. Antibody (3 kinds) belonging to type C detected the 180 kDa-band. Antibody (1 kind) belonging to type D detected the 150 kDa-band. Antibody (4 kinds) belonging to type E detected 40 kDa-band and 150 kDa-band. Antibody (3 kinds) belonging to type F detected several bands in addition to the 150 kDa-band. Antibody (1 kind) belonging to type G detected the 175 kDa-band. Antibody (1 kind) belonging to type H detected the 35 kDa-band. Antibody (2 kinds) belonging to type I detected the 20 kDa-band. Antibody (1 kind) belonging to type J detected the 110 kDa-band. Antibody (1 kind) belonging to type K detected several bands in addition to the 175 kDa-band.

1129B

Analysis of Protease-Dead Separase: Development of a New Method to Study Dominant-Negative Transgenes. **Joshua N. Bembenek**. MCDB, University of Michigan, Ann Arbor, MI.

The *C. elegans* embryo is an ideal system to study several important processes including egg activation and cell division. We are studying the role of separase, a protease required for chromosome segregation, in regulation of RAB-11 vesicle trafficking during anaphase. A key question is whether the protease activity of separase is required for its role in vesicle exocytosis. To address this question we generated a protease-dead separase mutant (GFP::SEP-1^{PD}) and found that it accumulated more strongly at sites of action within the cell during anaphase, suggesting that it traps substrates. GFP::SEP-1^{PD} expression causes high rates of embryonic lethality and severe adult phenotypes, suggesting that it is dominant-negative and interferes with endogenous separase function. Using the generally applicable method described below to propagate strains with dominant-negative transgenes, we plan to investigate the protease-dependent functions of separase in the *C. elegans* embryo.

The most common method for expression of fluorescent fusion proteins in embryos involves generation of integrated transgenes under the control of the *pie-1* promoter, using *unc-119* as a selection marker. However, this system is not inducible and prevents generation of lines expressing dominant-negative proteins because strains are inviable. Using standard methods, we obtained lines that express GFP::SEP-1^{PD} which could not be propagated more than a few generations. However, *gfp(RNAi)* feeding eliminated transgene expression and allowed propagation of GFP::SEP-1^{PD} lines for many generations without obvious deleterious defects. Animals removed from *gfp(RNAi)* for 5 generations recover transgene expression and associated phenotypes. To obtain more severely dominant-negative or over-expressing transgenes, animals can be rescued after bombardment directly on *gfp(RNAi)* feeding bacteria. We also took advantage of the fact that the *pie-1* promoter has little expression in the male germline. Backcrossing GFP::SEP-1^{PD} males to *unc-119* females allows propagation of the line without using *gfp(RNAi)* and circumventing the 5 generation delay to obtain expression. F1 Heterozygous GFP::SEP-1^{PD} hermaphrodites show high levels of embryonic lethality and 50% of the surviving F2 animals are sterile or lay 100% dead embryos. Transgenic males can also be crossed to mutant lines to examine genetic interactions. Bombardment of an *unc-119(ed3);him-5(ok1896)* mutant (made by Judy Yanowitz) may also facilitate isolation of dominant-negative transgenes. These methods are immediately applicable for studies of dominant-negative transgenes using widely established techniques and should open new lines of investigation in the *C. elegans* embryo.

1130C

Breaking It Down: Investigating Synaptonemal Complex Disassembly In C. elegans Using RNAi. **Heather M. Brockway**[1], Sarit Smolikove[1,2]. 1) Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA; 2) Department of Biology, University of Iowa, Iowa City, IA.

The events in meiotic prophase I are essential for proper chromosome segregation. In prophase I, homologous chromosomes pair, synapse, and form crossovers to recombine the genetic material. Once these events are completed, the homologous chromosomes segregate in preparation for meiosis II, at which time the sister chromatids separate. This process is highly regulated by a series of molecular checkpoints, ensuring that each step proceeds correctly. The synaptonemal complex (SC) is involved in most prophase I events and is a highly conserved protein structure formed between homologous chromosomes during meiosis. The absence of a functional SC leads to missegregation of chromosomes and generates aneuploid gametes. We utilize the model organism C. elegans to elucidate the underlying molecular mechanisms of the SC disassembly pathway. Our research employs RNAi methodology as a means of discovering the proteins associated with SC disassembly. Prior screens conducted in our lab have uncovered a novel mutant possessing meiotic defects specific to SC disassembly. Using this new mutant, we have initiated an RNAi screen to search for enhancers and suppressors of the embryonic lethal phenotype associated with this mutation. The goal of this screen is to identify SC disassembly proteins via their interaction with this new mutant. To date, 4% of the RNAi library has been screened. In this pilot, we identified three suppressors and seven enhancers that were mutant-specific. Eight of these clones have not shown an embryonic lethal phenotype in other screens, including a screen on the wild-type background, thus indicating we are capable of identifying novel clones with this method. Of these eight clones, two are known genes while the rest are uncharacterized ORFs. Cytological analyses are conducted to determine if the changes in embryonic lethality are associated with defects in SC disassembly. Identifying meiotic genes involved in SC disassembly will lead to a better understanding of the molecular mechanisms involved with the disassembly process and chromosome segregation in general.

1131A

Cytosolic Aminopeptidase P (APP-1): a possible role in meiotic progression in *Caenorhabditis elegans*. Hannah Craig[1], Enrique Martinez-Perez[2], **Darren Brooks**[3], R. Elwyn Isaac[1]. 1) Faculty of Biological Sciences, Miall Building, University of Leeds, Leeds LS2 9JT, UK; 2) MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, London, W12 0NN, UK; 3) Biomedical Sciences Research Institute, School of Environment and Life Sciences, University of Salford, Salford M5 4WT, UK.

Aminopeptidase P (APP) is a metallopeptidase that specifically removes amino acids from the N-terminus of peptides with a penultimate N-terminal proline residue. Of the 20 proteogenic amino acids, Pro is unique in that it is the only amino acid whose side chain links to the α-amino group, forming a pyrrolidine ring. The conformational restraints thus imposed mean that peptide bonds in the vicinity of Pro tend to be resistant to general peptidases. However, there is a need for peptidases to cleave peptide bonds involving Pro, not only for digestion of dietary peptides but also for processing of peptide hormones and in the catabolism of cytosolic peptides generated by, for example, proteasome activity and autophagy. 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. A *C. elegans app-1* null mutant (tm1715; Japanese National Bioresource Project), that has a 452 bp deletion disrupting exons 2 and 3, has a higher (18-fold) number of males generated through self-fertilisation of the adult hermaphrodite, and also a 50% reduction in brood size. These phenotypes are similar to those seen in a sub-set of *him* mutants defective in segregation of the autosomes and also the X chromosome during meiosis. The APP-1 antibody generates diffuse staining inside meiotic nuclei of the female gonad. Staining tm1715 with anti-RAD-51 antibodies suggests an altered pattern in the repair of DNA double-strand breaks generated during meiotic prophase and indicates an important biological role for APP-1 in meiotic progression in *C. elegans*. The strong expression of *app-1* within the intestinal cells of *C. elegans* suggests that worms lacking APP-1 might experience nutritional and caloric stress, especially during periods of high demand for Pro (eg. for collagen synthesis at moults). Since it is well established that dietary restriction extends life-span in *C. elegans*, we will report the consequence of the absence of APP-1 peptidase activity upon longevity of the adult hermaphrodite.

1132B

Distinct Roles for the Kinesins BMK-1 and KLP-18 in Bipolar *C. elegans* Oocyte Meiotic Spindle Assembly. Valerie R. Osterberg, Meredith H. Price, **Sara N. Christensen**, Bruce A. Bowerman. Institute of Molecular Biology, University of Oregon, Eugene, OR.

To investigate the mechanisms that control assembly of acentrosomal oocyte meiotic spindles, we have isolated temperature-sensitive, embryonic-lethal mutants, and then examined them using Differential Interference Contrast (DIC) optics for abnormal numbers of maternal pronuclei as indicative of meiotic spindle defects. We found alleles of two widely conserved kinesins, *bmk-1(or627ts)/kinesin 5* and *klp-18(or447ts)/kinesin 12*. Worms heterozygous for either of these mutations produce viable embryos at the restrictive temperature, suggesting they are recessive. Studies using both RNAi and two deletion alleles have suggested that *bmk-1* is not essential, but our allele suggests it is. Although vertebrate orthologs of BMK-1 and KLP-18 are partially redundant and involved in bipolar mitotic assembly, bipolar mitotic spindles assembled in *bmk-1(or627ts)* and *klp-18(or447ts)* single mutant and *klp-18(or447ts); bmk-1(or627ts)* double mutant early embryos. However, both single mutants exhibit a partially penetrant absence of the maternal pronucleus, based on DIC microscopy. We therefore used spinning disk confocal microscopy with GFP:tubulin and mCherry:histone fusions to examine spindle assembly dynamics during Meiosis I in live zygotes. In both mutants we observed monopolar spindles that often extrude all chromosomes into the first polar body. However, the dynamics of monopolar spindle assembly in each mutant are very different. After nuclear envelope breakdown (NEB) in *klp-18(or447ts)* oocytes, chromosomes are clustered around a single focus of microtubules (MTs) that condenses and is fully extruded. After NEB in *bmk-1(or627ts)* oocytes, a large multipolar spindle forms external to disorganized chromosomes and then collapses into a monopolar spindle, with MTs all projecting inward from the cytoplasmic face of a cortical chromosomal cluster. All chromosomes but not all MTs are then extruded. To examine meiotic spindle poles more directly, we used recombineering to make a GFP:ASPM-1 fusion that marks meiotic spindle poles. In *klp-18(RNAi)* zygotes expressing GFP:ASPM-1 and mCherry:histone, GFP:ASPM-1 localized to one focus surrounded by chromosomes, confirming that a monopolar spindle forms early in Meiosis I. The distinct spindle morphologies suggest that BMK-1 and KLP-18 act at different steps in meiotic spindle assembly. To understand how BMK-1 and KLP-18 function together to promote bipolar oocyte meiotic spindle assembly, we are using live cell imaging with GFP and mCherry fusions to further compare spindle dynamics and morphology in *bmk-1(-)* and *klp-18(-)* single and double mutants.

1133C

A member of the DOG-1 family is essential for normal cell division. **George Chung**, Ann Rose. Department of Medical Genetics, University of British Columbia, Vancouver, Canada.

The Rose laboratory has been studying genes encoding members of a helicase subfamily that includes dog-1 (1,2 and see poster by Jones & Rose, this meeting) and rtel-1 (3,4). A third member of this family has orthology to the yeast gene, CHL1. In the absence of the gene function, worms grow to become sterile adults (Ste) with a movement defect (Unc). Using the D-neuron-specific GFP marker (unc-47::gfp, constructed by the Jorgensen lab), we visualized these neurons with fluorescence microscopy. Both newly hatched wild-type and mutant animals showed 6 DD neurons derived from embryogenesis. Additional 14 D neurons (13 VD and DVB) developed in the wild-type animals prior to adulthood. In mutants, however, we observed an average of only 12 D neurons rather than the expected 20. In order to determine if other cell types were affected in a similar manner, we used a seam cell marker (scm::gfp, constructed by the Rothman lab) to visualize the presence of seam cells during L4. The homozygous mutant worms only had an average of 10 seams cells per row, fewer than the 16 in wild-type worms. Furthermore, many mutant animals did not have mature oocytes. In animals that did have mature oocytes, DAPI staining revealed diakinetic chromosome spots in excess of the expected six. Taken together, this gene is

requireed for cell division. (1)Youds, J.L., O'Neil, N.J. & Rose, A.M. Homologous recombination is required for genome stability in the absence of DOG-1 in Caenorhabditis elegans. Genetics 173, 697-708 (2006). (2)Youds, J.L. et al. DOG-1 is the Caenorhabditis elegans BRIP1/FANCJ homologue and functions in interstrand cross-link repair. Mol. Cell. Biol 28, 1470-1479 (2008). (3)Barber, L.J. et al. RTEL1 maintains genomic stability by suppressing homologous recombination. Cell 135, 261-271 (2008). (4)Youds, J.L. et al. RTEL-1 enforces meiotic crossover interference and homeostasis. Science 327, 1254-1258 (2010).

1134A

GSP-3 and GSP-4 are required for sperm meiotic chromosome segregation in *C. elegans*. **Thais Godoy Cintra**, Jui-Ching Wu, Aiza Go, Diana Chu. Department of Biology, 1600 Holloway Avenue, San Francisco State University, San Francisco, California 94132, USA.

Errors in meiotic chromosome segregation during spermatogenesis have serious consequences: male infertility, embryonic lethality, and developmental abnormalities. Meiosis is distinctly regulated in spermatocytes and oocyes to control the developmental timing, size, and number of gametes produced. However, the molecular factors that differentiate chromosome segregation events during sperm meiosis from oocyte meiosis or mitosis are largely unknown. We have found that two nearly identical PP1 phosphatases in *C. elegans*, GSP-3 and GSP-4 (GSP-3/4) are required for male infertility and sperm meiosis. Using live-imaging of histone-GFP tagged strains deleted for both *gsp-3/4*, we find multiple defects in chromosome segregation during sperm meiosis. First, in the absence of GSP-3/4 during meiosis I, the partitioning of the X chromosome is delayed, causing the X chromosome to become stretched before finally resolving to one pole. Second, in the second meiotic division, sister chromatids are unable to segregate properly, forming bridged nuclei. These bridged nuclei either end up in the same spermatids or the residual body, a repository for cellular components, resulting in either multinucleated sperm or anucleated sperm, respectively. AIR-2 (Aurora kinase) and REC-8 (meiotic cohesin) are normally localized and removed in *gsp-3/4* double mutant spermatids, confirming that cohesin removal does not contribute to chromosome segregation defects. Immunolocalization experiments show GSP-3/4 localization around sperm meiotic chromosomes in a pattern similar to that of kinetochore proteins, key connectors of chromosomes and microtubules. The chromosome segregation defects and localization pattern of GSP-3/4 suggest these PP1 phosphatases may regulate chromosome-microtubule dynamics. Thus, we are investigating microtubule dynamics during sperm meiosis in the absence of GSP-3/4. In addition, we are also characterizing the role of GSP-3/4 regulation of kinetochore components in sperm meiosis. In particular, we are investigating changes in HCP-2 and HCP-4 localization, as we have previously shown they are differentially enriched in sperm chromatin compared to oocyte chromatin.

1135B

FBF-mediated repression of the Cip/Kip cell cycle inhibitor CKI-2 promotes self-renewal of C. elegans germline stem cells. Irene Kalchhauser[1], Brian Farley[2], Sandra Pauli[1], Sean Ryder[2], **Rafal Ciosk[1]**. 1) Friedrich Miescher Inst, Basel, Switzerland; 2) University of Massachusetts Medical School, Worcester, USA.

The transition of stem cells from self-renewal to differentiation has been linked to modifications of the cell cycle. However, the relation between key stem cell regulators and cell cycle changes remains poorly understood. RNA-binding proteins of the PUF family regulate stem cells in diverse animals. For example, Caenorhabditis elegans FBF maintains self-renewal of stem cells in the germ line. Here, we report that FBF promotes self-renewal by repressing the mRNA encoding CKI-2$^{Cip/Kip}$, a Cyclin E/CDK2 inhibitor. We have previously shown that repression of *cye-1* (cyclin E) mRNA by the STAR-domain protein GLD-1 inhibits CYE-1/CDK-2 activity, promoting germ cell differentiation. Based on these and current findings, we propose that a post-transcriptional regulatory circuit mediated by FBF and GLD-1 controls the self-renewal versus differentiation decision in the germ line by promoting high CYE-1/CDK-2 activity in stem cells, and repressing CYE-1/CDK-2 activity in differentiating cells.

1136C

A screen for C. elegans oocyte meiotic spindle-defective mutants. **Amy Connolly**, John Yochem, Josh Lowry, Bruce Bowerman. Institute of Molecular Biology, University of Oregon, Eugene, OR., 97405.

We are interested in identifying and investigating essential genes involved in oocyte meiotic spindle assembly, as part of a large-scale screen for temperature-sensitive, embryonic lethal-mutants. We initially identify mutants based on temperature-sensitive embryonic lethality, and then examine their early embryonic cell divisions using time-lapse DIC imaging. In a small number of these mutants, we detect an abnormal number of maternal pronuclei in the one-cell zygote, suggestive of oocyte meiotic spindle assembly or function defects. This far we have found several mutants with either multiple or no maternal pronuclei. One new mutant, *or1129ts*, has multiple maternal pronuclei, while another *or974ts* sometimes lacks a maternal pronucleus. We have narrowed the locations of *or1129ts* and *or974ts* to linkage group IV and V, respectively, by using Restricted-site Associated DNA polymorphism (RAD) mapping. RAD mapping relies on crossing the mutant into the polymorphic Hawaiian strain (CB4856), and then isolating and pooling F2s that are again homozygous for the mutation. The genomic DNA is purified then cut with a restriction enzyme (EcoRI) and ligated to barcoded adaptors. Finally, Illumina DNA sequencing is used to sample SNPs throughout the genome and narrow the mutation to a 1-3 Mb region. The *or1129ts* and *or974ts* mutations complement the kinesin mutants *klp-18(or447ts)* IV and *bmk-1(or627ts)* V, respectively, the only other meiotic spindle mutants we know of that map to these linkage groups (see abstract by Osterberg et al), suggesting that these mutations are alleles of other genes. We are now using whole genome sequencing to determine the identity of the affected gene in each mutant. We will characterize their mutant phenotypes using GFP and mCherry fusions to microtubules, histones, and other proteins for live cell imaging, as part of an ongoing effort to define the genetic pathways and networks that control C. elegans meiotic spindle assembly.

1137A

Characterization of the role of TLK-1 in kinetochore assembly in *C. elegans*. **Jessica M. De Orbeta[1,2]**, Tokiko Furuta[1], Jill M. Schumacher[1,2]. 1) Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, TX; 2) Program in Genes and Development, University of Texas Graduate School for Biomedical Sciences, Houston, TX.

The highly conserved Aurora B kinase is a key player in the regulation of kinetochore-microtubule attachments. An important Aurora B function is to monitor the formation of incorrect attachments and promote their disassembly. This leads to the release of the microtubules, allowing for kinetochore reattachment in the correct orientation. Aurora B activity is influenced by several factors including direct phosphorylation of the C-terminal domain of INCENP, which leads to an allosteric switch to an active kinase conformation. In addition to INCENP our laboratory has discovered a novel Aurora B substrate, the Tousled-like kinase (TLK-1). The phosphorylation of TLK-1 at serine 634 by the *C. elegans* Aurora B homolog AIR-2, increases AIR-2 activity and changes the subcellular localization of TLK-1 from the chromatin to the outer kinetochore. We hypothesize that AIR-2 phosphorylation of TLK-1 targets TLK-1 to the kinetochore and that phosphorylated TLK-1 (pTLK-1) localization at the kinetochore affects kinetochore function. Immunolocalization experiments in *C. elegans* embryos showed that pTLK-1 kinetochore localization was abolished when a protein necessary for kinetochore establishment, KNL-2, was reduced, indicating that pTLK-1 is bona fide kinetochore component. Also, we determined that a protein at the microtubule interface, HCP-1, is also required for pTLK-1 localization to the kinetochore, showing that pTLK-1 localizes to the outer kinetochore, suggesting that pTLK-1 may function in microtubule attachment. Additionally, to determine whether TLK-1 affects the organization of the kinetochore, TLK-1 depleted embryos were stained for BUB-1, an outer kinetochore protein. These embryos had misshapen or “twisted” metaphase kinetochores, indicating that pTLK plays a role in maintaining proper kinetochore architecture. Currently, transgenic worms expressing phospho-mutant versions of TLK-1 are being generated to determine the functional role of TLK-1 S634 phosphorylation during the cell cycle.

1138B

Identifying the role of the Tousled-like kinase during the cell cycle. **Jason R. Ford[1,2]**, Tokiko Furuta[1], Henry Adams[1], Jill M. Schumacher[1,2]. 1) U.T. M.D. Anderson Cancer Center, Department of Genetics, Houston, TX, USA; 2) Program and Genes and Development, U.T. Houston GSBS, Houston, TX, USA.

The Tousled kinase (Tsl) was initially identified in *Arabidopsis thaliana* via a mutation responsible for aberrant floral organ.. There are two mammalian orthologues of Tsl and one orthologue in *C. elegans*, TLK-1; the human kinases are nuclear proteins with maximal activity tied to ongoing DNA replication during S-phase. Very few substrates of TLK have been described with the most reported being the chromatin assembly factor ASF. Aside from its S-phase functions, we have shown that TLK-1 also contributes to chromosome segregation as a substrate and activator of the AIR-2/Aurora B kinase. AIR-2-dependent phosphorylation of TLK-1 is detectable at the centrosomes, kinetochore (KT), and KT-microtubules (MTs) from early prophase to metaphase. TLK-1 depletion phenotypes include embryonic lethality, a spindle assembly checkpoint (SAC)-dependent mitotic delay, chromosome segregation defects, and distended nuclei in late multicellular embryos. To further determine the consequences of TLK-1 depletion in developing embryos, we used live-cell analysis of early embryos subjected to *tlk-1(RNAi)*. Our analysis revealed a spindle rotation defect in 50% of a TLK-1-depleted population during the first mitotic division. More specifically, the centrosome-pronuclear complex rotation in TLK-1-depleted embryos is significantly delayed with respect to nuclear envelope breakdown. This striking phenotype suggests a nascent role for a known chromatin regulator, TLK-1, in regulating microtubule behavior during mitosis, and the epistatic relationships between *tlk-1* and other genes required for spindle rotation are being investigated. It has been reported that P_0 embryos depleted of regulatory dynein subunits exhibit an inability to correctly rotate the centrosome-pronuclear complex in a manner strikingly similar to that of the TLK-1-depleted- P_0 embryos, suggesting that TLK-1 functionality may influence dynein-dependent processes in the early embryo. Interestingly, co-depletion of TLK-1 with certain dynein subunits results in an inability to completely separate centrosomes and correctly rotate the mitotic spindle, an effect that forces MTs emanating from centrosomes to attach KTs in a monopolar-like fashion. With these data taken together, we hypothesize that TLK-1 is affecting vital cytoskeletal processes relating to centrosome dynamics and other dynein-related processes during mitosis, and relating the established roles of TLK-1 to its functions in cytoskeletal/spindle dynamics and cell cycle regulation will shed light on the consequences of TLK-1 action during development.

1139C

Asymmetric chromosome behavior during oocyte meiosis. **Christina M Glazier[1]**, Abby F Dernburg[1,2]. 1) Department of Molecular and Cell Biology, UC-Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

Meiosis is the specialized cell division that gives rise to haploid gametes. In order to reduce the chromosome complement by half, meiosis involves one DNA replication event

followed by two chromosome segregation events. For proper segregation to occur, homologous chromosomes must pair, synapse, and undergo crossover recombination to form physically linked bivalents. A microtubule-based spindle then separates the bivalents so homologous pairs are pulled to opposite spindle poles. Sister chromatids then realign at the metaphase plate and segregate to opposite poles during meiosis II. In animals, oocyte meiosis gives rise to a single functional gamete, rather than four, and the other meiotic products are typically extruded into a polar body that does not contribute to the developing zygote.

In cases where the two homologous copies of a chromosome missegregate, or nondisjoin, at the first meiotic division, the expectation is that each gamete would have an equal probability of receiving either zero or two copies of that chromosome. However, in *C. elegans*, it has long been recognized that XO progeny (males) greatly exceed the number of triplo-X (Dumpy) hermaphrodites, both in wild-type broods and in mutants with nonrecombinant (achiasmate) X-chromosomes, such as *him-5* or *him-8*. I am exploring the basis for this unusual inheritance pattern to learn more about the mechanisms underlying meiotic chromosome segregation.

One hypothesis that I am testing is that the excess of males over triplo-X hermaphrodites could result from a preferential missegregation of achiasmate X chromosomes into the polar body. I am developing genetic and cytological assays to determine whether this asymmetric chromosome behavior is specific to the X chromosome, or instead reflects an intrinsic asymmetry of the spindle. Eventually I also plan to identify genetic factors that alter the segregation behavior, perhaps resulting in more random behavior.

1140A

A Polo-like kinase coordinates meiotic chromosome dynamics. **Nicola C. Harper**[1,2], Regina Rillo[1,2], Sara Jover-Gil[1], Needhi Bhalla[3], Abby F. Dernburg[1,2,4]. 1) Dept of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute; 3) Dept of Molecular, Cell and Developmental Biology, University of California, Santa Cruz, Santa Cruz, CA; 4) Dept of Genome Sciences, Lawrence Berkeley National Laboratory.

Accurate segregation of homologous chromosomes in meiosis relies on their pairing, synapsis and recombination. Previous work from our lab has characterized special regions of *C. elegans* chromosomes termed pairing centers (PCs) which are specifically bound by a family of zinc finger (ZnF) proteins and facilitate homolog pairing and synapis. Homolog interactions are first established in the “transition zone” region of the gonad, which is marked by nuclei with clustered chromosomes. In the transition zone, PCs colocalize with patches of the nuclear envelope (NE) proteins SUN-1 and ZYG-12 to form a bridge between chromosomes and the cytoplasmic microtubule network. This connection facilitates homolog pairing and restricts synapsis to occur specifically between properly paired homologs. PC-mediated chromosome connections to the cytoskeleton appear to be a variation of the widely conserved meiotic bouquet, which is typically mediated by telomeres. Major unanswered questions have been how PCs exert their functions and how components of the NE bridging patches are established at meiotic entry and maintained until homolog pairing and synapsis are complete.

We have defined essential roles for the Polo-like kinase PLK-2 in coordinating these meiotic processes. We find that PC function is imparted by the recruitment of PLK-2 to PCs through an interaction with the ZnF proteins. This recruitment promotes SUN-1 phosphorylation at a key serine residue (Ser12), NE patch aggregation, and homolog pairing and synapsis. Loss of PLK-2 results in partial defects in homolog pairing and synapsis because the closely related PLK-1 can partially substitute for its role at PCs. Deletion of all four PC ZnF proteins, or the absence of both PLK-1 and PLK-2, completely abolishes chromosome clustering, phosphorylation of SUN-1 at Ser12, NE patch formation, and homolog interactions. We also find that PLK-2 is required for two critical responses to unsynapsed chromosomes: a cell cycle delay that maintains chromosome connections with the cytoskeleton to facilitate continued homology search, and the preferential apoptosis of nuclei containing unsynapsed chromosomes. This work reveals novel roles for Polo-like kinases and expands our understanding of meiotic regulatory mechanisms that ensure accurate transmission of genetic information from parents to progeny.

1141B

$CRL2^{ZYG-11}$ and cyclin B degradation. **Cassandra S. Heighington**, Edward T. Kipreos. University of Georgia, Athens, GA.

The cullin 2-RING finger ubiquitin ligase, $CRL2^{ZYG-11}$, is a key regulator of meiosis. The ZYG-11 protein, which contains leucine-rich and ARM-like repeats, functions as the substrate-recognition subunit for the CRL2 complex to bind substrates for ubiquitylation. ZYG-11 is required for the metaphase II-to-anaphase II transition during meiosis. *zyg-11* mutant embryos have a significant delay in meiosis II and are defective in the degradation of cyclin B through both meiosis and mitosis in the early embryo[1]. RNAi of cyclin B1 partially rescues the meiotic delay, indicating that the failure to degrade cyclin B1 contributes to this phenotype[1]. Cyclin B2.1 and B2.2 act redundantly with cyclin B1[2]. In a large-scale screen for suppressors of *zyg-11(ts)* mutants, we identified a mutation in cyclin B2.1. Reducing the level of cyclin B2 with RNAi in *zyg-11(ts)* mutants rescues the embryonic lethality at the semi-permissive temperature (72% hatched vs. 20% hatched), suggesting that an increase in the overall level of cyclin B contributes to the *zyg-11* mutant phenotype. ZYG-11 also regulates the level of cyclin B1 during somatic cell divisions. The level of cyclin B1 is higher in vulval cells after their final mitotic divisions in *zyg-11(ts)* larvae relative to wild-type or *mat-3(ts)* larvae. The requirement of ZYG-11 for cyclin B1 degradation is surprising because it is widely accepted that a different ubiquitin ligase, the anaphase-promoting complex/cyclosome (APC/C), is the sole ubiquitin ligase that targets cyclin B1 for degradation. We are working to establish whether ZYG-11 works upstream of APC/C to allow it to target cyclin B for degradation, or if ZYG-11 and APC/C work in parallel pathways to degrade cyclin B1. In an effort to define the region of cyclin B1 that is required for its degradation, we made GFP fusion constructs of three regions of cyclin B1: the N-terminal region (NTER), cyclin box 1 (CBOX1), and cyclin box 2 (CBOX2). We found that the level of CBOX1::GFP decreases substantially in wild-type embryos during meiosis. *zyg-11* RNAi stabilizes the level of CBOX1::GFP, suggesting that ZYG-11 targets cyclin B through the CBOX1 region. RNAi of the APC/C subunit *mat-3* does not stabilize the CBOX1::GFP construct to a statistically significant level. These results suggest that ZYG-11 specifically targets the CBOX1 region of cyclin B1 for degradation and may act in a pathway that is parallel to that of APC/C. (1) Liu et al., 2004, Development. (2) van der Voet et al., 2009, Cell Cycle.

1142C

SEP-1 and CED-3 regulate apoptosis and separation of sister chromatids in *C. elegans*. **Pan-Young Jeong**, Kumar Ashish, Pradeep M Joshi, Joel H Rothman. NRI, UCSB, Santa Barbara, Santa Barbara, CA.

Separation of sister chromatids at the metaphase-to-anaphase transition is crucial for accurate chromosome transmission during cell proliferation, and defects in chromosome segregation are associated with many cancers. Separase, a cysteine protease, mediates this process. The separase enzyme cleaves Scc1, a member of the cohesion complex that holds sister chromatids together, thereby allowing chromosomes to separate during anaphase and be pulled towards the spindle poles. Programmed cell death (PCD) is an active and tightly controlled process that acts as the first line of defense against the formation of cancers. We have found that the *C. elegans* CED-3caspase, which had been known strictly for its role in activating PCD, may also perform a separase-like function during cell proliferation and germline meiosis. However, we found that this separase-like function of CED-3 is not regulated by CED-4. We have also found that the *C. elegans* separase SEP-1 may play a caspase-like role in activating PCD during normal development, as it is required for the occurrence of PCD in embryos. Both caspases and separases belong to the CD clan of the cysteine protease family and our findings should illuminate the interrelationship between apoptosis and mitosis.

1143A

Genetic and molecular investigation of the Fanconi Anemia pathway in *C. elegans*. **Martin R. Jones**, Ann M. Rose. Med Gen, Univ British Columbia, Vancouver, Canada.

Fanconi Anemia (FA) is a rare autosomal recessive cancer susceptibility syndrome associated with various congenital abnormalities and a predisposition to developing various types of cancers. The Fanconi pathway prevents chromosome instability by resolving DNA lesions that block replication and transcription, such as interstrand crosslinks (ICL). It is thought that the FA pathway acts to coordinate the repair of DNA damage through different DNA repair effectors such as the Homologous Recombination repair (HR), Nucleotide Excision Repair (NER), and Translesion Synthesis (TLS) pathways.

C. elegans has a simplified Fanconi Anemia pathway which has proven to be a valuable model for the discovery and investigation of conserved FA-associated genes. Our lab previously identified DOG-1 as the functional orthologue of the human FANCJ helicase. Animals mutant for a null allele, *dog-1(gk10)*, share many of the hallmarks of FA cells such as sensitivity to interstrand crosslinks (ICLs). Using a combination of genetic and molecular techniques we are exploiting *C. elegans* genetics to investigate the role of FA-associated genes in maintaining genome stability. We are combining this approach with a variety of screening strategies to identify additional gene functions that when disrupted exacerbate the genomic instability of animals defective for FA-associated genes.

We have identified a number of genetic interactions with *dog-1(gk10)* mutants using the increased frequency of small G-tract deletions that occur in the absence of DOG-1 function as an assay. These include homologous recombination repair components, translesion synthesis (TLS) polymerases, and the Fanconi anemia pathway gene *fcd-2/FANCD2*. In addition, we have examined the viability of *dog-1(gk10)* animals with other mutants, revealing interactions with the RecQ helicase HIM-6/BLM, which is implicated in the resolution of stalled replication forks, and the anti-recombinase RTEL-1/RTEL, which results in synthetic lethality.

The variety of genetic and molecular tools combined with a conserved, though simplified, FA pathway makes *C. elegans* an excellent animal model in which to identify new FA-associated genes and to elucidate the function of the FA pathway.

1144B

Local meiotic recombination rate variation in *Caenorhabditis elegans*. **Taniya Kaur**, Matthew Rockman. Center for Genomics & Systems Biology, New York University, New York, NY.

Meiotic recombination plays a pivotal role in shaping the genetic diversity in a population upon which natural selection may act. However, meiotic recombination rates are rarely constant across different regions of the genome in eukaryotes. A variety of factors (including DNA sequence, chromatin state, sex, environmental conditions) are postulated to explain the observed genome wide meiotic recombination rate variation. The meiotic recombination rate variation observed in *Caenorhabditis elegans* chromosomes is quite striking. Typically, the chromosome arms show high recombination rates whereas the chromosome centers are domains of low recombination. Pronounced boundary regions separate these distinct recombination rate domains. The causes underlying this consistent domain structure interspersed by distinctive boundary regions of transitioning recombination rates is unknown. It also remains to be determined whether the observed

'near constant' domains are in fact constant or on closer inspection of local recombination rate patterns would be irregular and punctuated with recombination hotspots. Consequently, another important unaddressed question is whether the distinctive recombination rate boundaries are discrete or diffuse. A closer inspection of the local recombination rate landscape surrounding the boundary regions is thus warranted to gain a clearer understanding of the chromosome wide recombination rate pattern. Previous work suggests that the boundary between the center and right arm of chromosome II resides at approximately position 12,020,000 base pairs (bp). To precisely map this boundary and obtain a detailed view of the local recombination landscape, recombinants between two visible markers flanking the site, *unc-4* and *rol*-1, were selected, and 183 SNPs within this 2 Mbp interval were identified. Recombination rates were calculated after scoring crossover breakpoints in recombinant worms using the Illumina GoldenGate genotyping assay. Recombination rates from both hermaphrodite- and male-specific meiotic events were compared to investigate the effect of sex on local recombination rate patterns and the position of the recombination rate boundary. Our data for hermaphrodite specific recombination rates demonstrates at a kilo-base pair (kbp) resolution a discrete recombination rate boundary in the region of interest. Also, the high marker density spanning the recombination rate boundary region will allow us to identify potential recombination hot spots in both hermaphrodites and males at an unprecedented resolution.

1145C

ZTF-8 is required for DNA double-strand break repair and DNA damage response in the germline in coordination with the 9-1-1 DNA damage checkpoint. **Hyun-Min Kim**, Monica Colaiacovo. Genetics, Harvard Med School, Boston, MA.

Several distinct processes occur during meiosis I to ensure that homologous chromosomes accurately segregate away from each other. These include homologous pairing, synapsis, recombination and checkpoint regulation of meiotic progression. However, the mechanisms underlying accurate chromosome segregation during meiosis I are poorly understood at the molecular level. We have investigated the roles of ZTF-8, a novel and highly conserved protein identified through an RNAi screen for meiotic candidates. We have found that ZTF-8 plays a role in DSB repair in the germline, in coordination with the DNA damage response 9-1-1 complex. ZTF-8 is observed localizing in nuclei at the pre-meiotic tip (mitotic nuclei) in the germline. ZTF-8 signal then decreases upon entrance into meiosis followed by an increase in pachytene, which persists throughout late prophase. Interestingly, we identified UBC-9 and SMO-1 as binding partners for ZTF-8 in a yeast two-hybrid screen. Moreover, ubc-9 mutants exhibit an altered localization of ZTF-8. Taken together, these data suggest a tight regulation of ZTF-8 at the protein level, potentially via ubiquitination, between exit from mitosis and entrance into pachytene. Several observations implicate ZTF-8 as playing an important role in DNA repair. First, ztf-8 mutants exhibit activation of a DNA damage checkpoint as supported by the presence of enlarged mitotic nuclei and elevated levels of ATL-1, a DNA damage checkpoint protein, in these germline nuclei. Second, levels of RAD-51 foci are elevated both in mitotic as well as in meiotic pachytene nuclei in ztf-8 mutant germlines compared to wild type, suggesting either increased DSB formation or an impaired ability to repair DSBs. In addition, ztf-8 mutants exhibited increased levels of germ cell apoptosis, further suggesting a defect in DNA repair and activation of a DNA damage checkpoint. Exquisite specificity in DNA damage sensitivity is observed in ztf-8 mutants. Specifically, embryonic lethality (decreased hatching %) is induced following exogenous DSB formation by γ-IR, but not following exposure to UV, nitrogen mustard and camptothecin. Moreover, replication arrest is observed following hydroxyurea (HU) treatment. In addition, both γ-IR and HU treatments altered the localization pattern of ZTF-8 in the germline. Finally, clk-2 mutants are synthetic lethal with ztf-8, suggesting a link to the DNA damage checkpoint. This is further supported by epistasis analysis which places ztf-8 acting downstream of hus-1, a member of the 9-1-1 complex. Taken together, our studies identify ZTF-8 as a novel protein required for the maintenance of genomic integrity in the germline and reveal a link with the DNA damage response pathway.

1146A

Investigating the role of Aurora kinases during meiotic prophase in C. elegans. **Y. Kim**[1,2], A. Dernburg[1,2]. 1) Department of Molecular and Cell Biology, University of California, Berkeley, Berkeley, CA 94720; 2) Howard Hughes Medical Institute.

The widely conserved family of Aurora kinases regulates key aspects of both mitosis and meiosis. Although the contribution of these Ser/Thr kinases during mitosis has been extensively studied, analysis of their meiotic functions has been hampered by strict requirement for Aurora kinases during development. C. elegans expresses two Aurora kinases, AIR-1 and AIR-2. While inhibition of AIR-2 in the germline results in a failure of meiotic chromosome segregation, its roles in earlier prophase events have not been reported. AIR-2 is observed along the synaptonemal complex (SC) on pachytene chromosomes, raising the possibility that it may have a role in modulating chromosome dynamics in early prophase. We found that SYP-2, an essential transverse component of the SC, contains a conserved motif for Aurora phosphorylation, which overlaps a conserved docking motif for its opposing phosphatase, Protein Phosphatase 1 (PP1). Purified SYP-2 is indeed phosphorylated by AIR-1 in vitro, and the phosphorylation is abolished when the predicted site (S22) is mutated to alanine. To determine the consequences of preventing SYP-2 phosphorylation in vivo, we have generated C. elegans strains expressing various syp-2 transgenes at a defined genomic locus by Mos1-mediated single-copy insertion (MosSCI). While a wild-type syp-2 transgene complements a syp-2 null mutation (ok307), strains expressing a phosphorylation-defective or a PP1 docking-defective SYP-2 in a syp-2 (ok307) background produce a many dead eggs and a high incidence of male progeny, indicative of defects in meiosis. We are currently investigating how this putative Aurora/PP1 phospho-switch regulates aspects of SC assembly and disassembly, and will present our current findings.

1147B

Determining Whether Autosomes and the X Chromosome have Distinct Genetic Requirements for Synapsis Checkpoint Activation in C. elegans. **Piero Lamelza**, Needhi Bhalla. University of California, Santa Cruz, Santa Cruz, CA.

Meiosis is a specialized cell division in which diploid cells give rise to haploid gametes by undertaking a single round of replication and two rounds of chromosome segregation. During meiosis, chromosomes pair, synapse and recombine to generate the chiasmata necessary for proper chromosome segregation. Pairing Centers (PCs), cis-acting sites toward the end of each chromosome, promote pairing and synapsis. Checkpoints monitor proper prophase I progression, curbing the production of aneuploid oocytes by inducing apoptosis of nuclei with meiotic defects. We study the synapsis checkpoint, which is activated by unsynapsed chromosomes bearing a PC and results in increased germline apoptosis. Checkpoint activation occurs when the X-chromosome is unsynapsed (*meDf2* heterozygotes) or when all chromosomes are unsynapsed (*syp-1* mutants). MES-4, a histone methyl-transferase (HMT) which lays down active H3K36me3 marks, localizes on autosomes and the PC of the X-chromosome. Mutation of *mes-4* abolishes the checkpoint when the X is unsynapsed. In contrast, *syp-1* mutants require the mutation of another H3K36me3 HMT, MET-1, in conjunction with *mes-4* for decreased levels of apoptosis. My project aims to determine whether autosomes and the X-chromosome have different genetic requirements for synapsis checkpoint activation. The model predicts MES-4 is needed for checkpoint activation if the X is unsynapsed while MET-1 is needed for checkpoint activation if the autosomes are unsynapsed. We predict that the active methyl marks laid down by MES-4 and MET-1 are required at PCs to maintain active chromatin on unsynapsed chromosomes which would normally be silenced by meiotic silencing of unpaired chromatin (MSUC).

1148C

Spindle assembly checkpoint plays a role in DNA-damage-induced cell cycle arrest in C. elegans male germline. **Kate Lawrence**. Molecular and Cellular Biology, University of California Davis, Davis, CA.

Persistent DNA damage in germline stem cells leads to embryonic lethality, progeny inviability or germline tumors. Consequently, cells closely monitor genomic integrity and can delay their progress through the cell cycle so that repair precedes division. In C. elegans, genotoxic perturbations to proliferative cells in the distal tip of the gonad activate checkpoints that initiate a cell cycle arrest. When this arrest is in response to stalled replication forks induced by hydroxyurea (HU), it is characterized by enlarged nuclei and can be visualized cytologically. HU damage is sensed by the C. elegans homolog of ATR, a PI3-related protein kinase, and launches a signaling cascade that results in a G1/S phase arrest. The signal transducers and downstream effectors of this DNA-damage-response (DDR) pathway have been studied extensively in hermaphrodites, but have not been investigated fully in males. We saw that while RNAi knockdown of several of these genes disrupts checkpoint output in hermaphrodites, the same treatment does not prevent HU-induced arrest in males. Our preliminary results strongly suggest that not all components of the DDR are essential for male cell-cycle arrest in response to stalled replication forks. We next investigated functional redundancy between the DDR and the spindle assembly checkpoint (SAC), which is most often associated with regulating kinetochore attachment to spindles during prometaphase/metaphase of mitosis and meiosis. We found that RNAi knockdown of several SAC components alone did not affect HU-induced cell-cycle arrest in males; however, knockdown of both ATR and SAC resulted in a failure to arrest in the presence of HU. This result suggests that, in males, the DDR and SAC work together to elicit arrest in the presence of stalled forks. To analyze this differentially regulated HU-induced arrest, we identified markers that characterize the stages of the cell cycle. Preliminary data suggests that the SAC, like the DDR, mediates an S phase arrest not predictive of its expected role as an inhibitor of cdc20 at metaphase. Future work aims to understand this novel role for SAC components and investigate the mechanisms used by the SAC to induce an S phase arrest.

1149A

SPD-1-centralspindlin interaction is critical for maintaining the mechanical integrity of the central spindle against cortical pulling forces. **K.-Y. Lee**, M. Mishima. Gurdon Institute, University of Cambridge, UK.

In anaphase, a number of factors important for cytokinesis localise to the central spindle, which consists of antiparallel microtubules between the segregating chromosomes. Two evolutionarily conserved microtubule bundling factors, SPD-1 microtubule-associated protein and the centralspindlin ZEN-4 kinesin/CYK-4 RhoGAP complex, are essential for central spindle formation. Although they independently show microtubule bundling activity *in vitro*, it is unclear how they cooperate to assemble a stable central spindle *in vivo*. In mammalian cells, a direct interaction between PRC1 (SPD-1 ortholog) and the RhoGAP subunit of centralspindlin has been reported. To dissect the significance of this interaction on central spindle formation, we first confirmed that the interaction is conserved in *C. elegans* by yeast two-hybrid and *in vitro* pull-down assays. After narrowing down the interacting regions, we determined that the R83W mutation of *spd-1(oj5)* allele, which leads to central spindle defects and embryonic lethality, falls into the CYK-4-binding domain of SPD-1. Strikingly, while retaining other known functions, SPD-1 R83W showed significantly reduced binding to CYK-4, indicating that the *spd-1(oj5)* phenotypes are due

to loss of interaction between SPD-1 and centralspindlin. In addition, we identified several residues in CYK-4 important for SPD-1 binding. We then generated transgenic worms expressing GFP-tagged CYK-4, either wild type or mutants defective in SPD-1 binding. The wild-type *cyk-4::gfp* transgene efficiently rescued the lethality of homozygous *cyk-4*-null embryos. However, the mutant transgenes could only partially rescue the *cyk-4*-null lethality, further indicating the biological significance of SPD-1-centralspindlin interaction. Live imaging of the *cyk-4*-null embryos expressing both CYK-4::GFP and mCherry::tubulin revealed that the CYK-4 mutants transiently localise to the central spindle, but later the central spindle breaks into two half spindles. Interestingly, a reduction in cortical pulling forces by RNAi depletion of several factors, such as LIN-5, GPR-2 or GPA-16, partially restored the stability of the central spindle and prolonged the accumulation of the CYK-4 mutants. Our observations suggest that SPD-1-centralspindlin interaction is critical for stabilising the central spindle under mechanical tension.

1150B

βpat-3 integrin is involved in regulation of CKI-1. Shingo Kihira, Eunjeong Yu, Jessica Cunningham, **Myeongwoo Lee**. Dept Biol, Baylor Univ, Waco, TX.

Integrins are αβ heterodimeric cell surface receptors that coordinate interactions between the extracellular matrix (ECM) and the cell via bidirectional signalings. Function of integrins include cell migration, adhesion, growth, and differentiation. *C. elegans* is an ideal model to study integrin functions. The β1C integrin, an alternatively spliced variant of the mammalian β1 subunit, has been previously found to upregulate a cyclin-cdk comlex inhibitor, $p27^{kip1}$ and inhibit cell proliferation. However, studies on the β1C integrin have been limited to mammalian tissue, while it suggested a potential role of growth suppression in cancer cells. In this study, we investigated $p27^{kip1}$ regulation using a β1C-like mutation in *pat-3* integrin, *pat-3 (β1C)*, by creating rescue lines co-injecting with CKI-1::GFP, a *C. elegans* homologue of $p27^{kip1}$. Molecular analyses revealed that the level of CKI-1::GFP was elevated in the *pat-3 (β1C)* rescue lines. In addition, the CKI-1::GFP showed disrupted localization patterns in the *pat-3* mutant and was accumulated in the nuclei of *pat-3 (β1C)* while it localized to nucleoli in *pat-3 (+)* rescues. Further RNAi analyses revealed that integrin signaling molecules such as *pat-4/ILK*, *unc-52*, and *unc-97/PINCH* as well as other proteins such as *cul-1*, *skpt-1/SKP2*, and *lin-23* were involved in the CKI-1::GFP localization. These findings set a framework for studying linkage between integrin signaling and CKI-1 regulation *in vivo*.

1151C

Condensin-mediated chromosome architecture & crossover regulation. **Teresa W. Lee**, Barbara J. Meyer. HHMI & Dept of Mol & Cell Biol, UC Berkeley, Berkeley, CA.

During meiosis, chromosomes undergo complex morphological changes that ensure their proper segregation. Crossover recombination (CO) supplies a physical connection between homologs critical for successful meiosis. Due to their importance, COs are subject to strict control that guarantees at least one per homolog and ensures wide spacing between multiple COs (interference), which requires communication along an entire chromosome's length. *C. elegans* exhibits an extreme form of interference: only one CO occurs on each homolog. Meiotic disruption of condensin I or condensin II - complexes that structure chromosomes in preparation for cell division - perturbs CO regulation: CO frequencies are increased, and their distribution along the chromosome is altered. This increase is strongly correlated with an overall extension of the chromosome axis, an increase in DNA double-strand breaks (DSBs), and a shift in DSB distribution to the same genetic intervals as the shifted COs. Condensins have non-redundant roles in CO regulation. Disruption of each causes a different distribution of DSBs and COs, and disruption of both perturbs axis length greater than disruption of either. Both complexes may be deployed differentially while sharing an underlying mechanism for structuring meiotic chromosomes.

To evaluate the independent roles of condensin I and II, and possible functional redundancy between subunit paralogs, we are analyzing changes in CO and DSB distributions, and effects on chromosome structure, in different backgrounds that disrupt both complexes. We have also found that depletion of the post-translational modification SUMO perturbs regulation of COs and DSBs, while lengthening chromosomal axes. However, the means by which loss of sumoylation increases COs remains unclear. Sumoylation and condensins can influence global chromosome architecture, permitting chromosome-wide communication that may inform CO regulation.

1152A

A Genetic Screen for Temperature-Sensitive, Embryonic Lethal Mutations in *C. elegans*. **Josh Lowry**, John Yochem, Amy Connolly, Bruce Bowerman. Inst. of Mol. Bio., University of Oregon, Eugene, OR.

We are conducting a genetic screen for temperature-sensitive, embryonic lethal mutants, with a goal of identifying 2000 new mutants. Initial characterization of these mutants involves using Differential Interference Contrast (DIC) microscopy to quickly examine early embryonic cell divisions. New mutants are then sorted into one of four broad categories: (i) wild-type early divisions, (ii) meiotic and mitotic cell division defective, (iii) eggshell defective, or (iv) delayed P1 division. We are most interested in the cell division defective and eggshell defective classes.

For cell division defective mutants we use DIC videomicroscopy to make time-lapse movies of the first two mitotic divisions. Thus far we have found several mutants with novel phenotypes. For example, 1/3 of or1180ts embryos were missing a paternal pronucleus. The or1190ts mutation results in both a loss of AP polarity in the one-cell zygote, and in P0 mitotic spindle assembly and chromosome segregation defects. Lastly, or1167ts mutants appear to have a monopolar spindle in the first division, and a bipolar spindle in the second division.

From the eggshell defective class we have defined a new sub-class based on gene requirements at multiple stages of development. When shifted to the restrictive temperature as L4 larvae, adults produce embryos that lyse in water, while upshifting L1 larvae results in adult sterility. This mutant class may provide a model for investigating the role of membrane trafficking and its regulation in gonad morphogenesis.

We are also exploring next generation DNA sequencing methods to rapidly identify affected loci. To this end we employ Restriction-site Associated DNA (RAD) mapping. RAD mapping takes advantage of single nucleotide polymorphisms (SNPs) that exist between our parental N2 strain and the Hawaiian strain CB4856. Mutant strains are crossed to Hawaiian males. F2 outcross progeny that are homozygous for the mutation are pooled, and genomic DNA extracted and digested with a restriction enzyme (EcoRI), and short barcoded adaptors are ligated to the fragments. The barcodes allow us to collect data for ~100 mutants per Illumina Hi Seq lane. Plotting the ratio of Hawaiian SNP reads to total reads for all sequenced SNPs defines roughly one megabase genome intervals that include the ts mutations.

1153B

him-5 functions in crossover control and DNA Damage Repair. **Olivia McGovern**[1], Kristie Jolliffe[3], Philip Meneely[2], Brent Derry[3], Judith Yanowitz[1]. 1) Magee-Womens Research Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA; 2) Dept of Biology, Haverford College, Haverford, PA; 3) Developmental and Stem Cell Biology Program The Hospital for Sick Children, Toronto, Ontario, Canada.

Meiosis promotes diversity within a population through crossover recombination. In fact, failure to execute recombination, through improper orchestration of double strand break (DSB) formation or repair, homologous chromosome pairing, or synapsis, can result in nondisjunction (NDJ), the erroneous segregation of chromosomes into gametes. With few exceptions, NDJ results in fatal outcomes accounting for ~50% of spontaneous abortions. While study of meiosis is extremely difficult in mammalian systems, it is specifically suited for study in *C. elegans* due to the fact that NDJ of the X chromosome leads to increased production of males, a completely viable outcome. The **H**igh **I**ncidence of **M**ales, or Him phenotype, has been used to identify and characterize nondisjunction mutants including the *him-5* locus described here.

The initial characterization of *him-5* by Jonathan Hodgkin revealed an X chromosome bias for *him-5*, with approximately 40% male self-progeny (compared to 0.2% in wild type) and high viability (indicating normal segregation of autosomes). Our characterization of *him-5* has revealed that it is required for DSB formation on the X, in some if not all nuclei. In addition, DSB formation on autosomes is also somewhat compromised in the mutant and appears to be exacerbated by age. Cytological analysis of *him-5* reveals a delay in meiotic progression, which can be rescued through induction of exogenous DSBs via irradiation, suggesting an active mechanism to monitor crossover exchange intermediates.

Through our studies we have also discovered redundant roles for *him-5* and *cep-1*, the *C. elegans* p53 homologue, in DNA damage repair. *him-5; cep-1* double mutants have significant embryonic lethality. These arise due to defects in meiosis as seen by analysis of diakinesis nuclei where *cep-1; him-5* mutants display Rad-51-like chromosomal fusions. Our more detailed characterization of these double mutants will be presented along with a framework to understand the role of these genes in DNA damage repair.

1154C

Novel Protein SPE-7 is Required for Meiotic Chromosome Segregation and Fibrous Body Assembly. **Kari Messina**, Marc Presler, Diane C. Shakes. Department of Biology, College of William and Mary, Williamsburg, VA.

The development of functional spermatozoon from uncommitted germ cells requires the progression of two distinct yet presumably interacting cellular programs: the meiotic and cell differentiation programs of spermatogenesis. One spermatogenesis-defective factor required for the normal progression of both of these programs is SPE-7. spe-7 mutant spermatocytes progress through the early phases of meiosis, detach from the rachis, and set up a metaphase I spindle. However meiotic chromosome segregation is aberrant and the spermatocytes never undergo cytokinesis. Ultimately, the spe-7 spermatocytes arrest with cortically localized actin and microtubules that reassemble into a network-like pattern. Surprisingly, we found that in developing wildtype spermatocytes, SPE-7 localizes to fibrous bodies, the paracrystaline storage form of the major sperm protein, MSP. During the meiotic divisions, the localization of SPE-7 is somewhat dynamic but it ultimately segregates with the fibrous bodies into the budding spermatids before disappearing altogether. This localization pattern is apparently functional as a reexamination of spe-7 spermatocytes revealed that they express MSP but are defective for fibrous body assembly. spe-7 encodes a novel protein with close homologs limited to other Caenorhabditis species. Both germline microarrays and western blots indicate that SPE-7 is expressed specifically during spermatogenesis, and it is one of the many genes regulated by the sperm-specific transcription factor, SPE-44 (see abstract by Kulkarni et al.). We are currently pursuing additional genetic and biochemical studies designed to understand the dynamic localization of SPE-7 as well as its interactions with other known spe genes.

1155A

Identification and characterization of new nuclear envelope proteins. **Adela Morales-Martinez**, Cristina Ayuso, Peter Askjaer. CABD-UPO-CSIC, SEVILLA, Spain.

The nuclear envelope (NE) constitutes a physical barrier between the nucleoplasm and the cytoplasm in eukaryotic cells. The NE is composed by the nuclear lamina and inner and

outer nuclear membranes, each enriched for numerous transmembrane and peripheral proteins. Within the NE nuclear pore complexes (NPC) composed of multiple copies of around 30 nucleoporins are responsible for transport in and out of the nucleus. In addition, the NE plays a critical role in organization of nuclear architecture and control of gene transcription by providing an anchoring surface for chromatin and transcription factors. Although the list of NE components has expanded considerably during recent years, it is conceivably that numerous, important, NE proteins remain to be identified. To identify novel NE proteins we have performed a genome-wide RNAi screen for genes that show synthetic lethality with mutations in genes encoding the nucleoporin Nup50/NPP-16 or the NE transmembrane protein LEM-2. NPP-16 plays an important role in resistance to anoxia (Hajeri et al, Mol Biol Cell 21, 712-24) but its function in NE assembly is unknown. LEM-2 shares redundant functions with emerin/EMR-1, another NE membrane protein, since simultaneous depletion of LEM-2 and EMR-1 causes severe cell division defects (Liu et al, PNAS 100, 4598-603). Our screen consisted in three steps: (1) genome-wide analysis in liquid cultures in 96-well plates; (2) rapid verification on single NGM plates; (3) quantitative analysis on NGM plates in triplicates. We are currently analyzing the strongest candidates by time-lapse and immunofluorescence microscopy to identify the precise cellular and developmental phenotypes. Moreover, we will present detailed descriptions of LEM-2 and NPP-16 in terms of expression pattern and dynamics during the cell cycle. Combined, our experiments provide novel insight into NE structure and function.

1156B

Identification and functional analysis of histone demethylase activity in response to UV-induced DNA damage in *Caenorhabditis elegans*. **Toshia Myers**, Alexandra Avram, Lisa Sacini. BRIC, Copenhagen University, Copenhagen, Denmark.

DNA damage can result from several external genotoxic agents including UV radiation. Cells typically respond to UV-induced damage with DNA damage response mechanisms such as cell cycle arrest, nucleotide excision repair (NER), or apoptosis. DNA damage sensing and signalling pathways are evolutionarily conserved across diverse species ranging from *C. elegans* to humans. The control of DNA repair, similar to other cellular processes, involves a high degree of regulation. One mechanism for imparting such regulation is the organization and modification of chromatin, for example through histone lysine methylation and demethylation. Several studies have suggested that histone methylation is important for DNA damage repair. However, the role that histone demethylation plays in DNA damage response mechanisms remains elusive. We have taken advantage of the *C. elegans* germline to conduct a small-scale screen using jumonji-domain containing putative histone demethylase homologues to identify genes regulating DNA damage mechanisms in response to UV radiation. Interesting candidates will be further characterized. We have identified a few potential candidates and are currently confirming the results. Once we have conclusively confirmed the candidates then a more comprehensive analysis will be undertaken to determine how these genes are acting in mechanisms regulating DNA damage response. Results of the screen and functional data will be reported.

1157C

ZHP-3 is a ubiquitin E3 ligase with multiple roles in meiosis. **Cate Randall Paschal**, Christian Nelson, Needhi Bhalla. Molecular, Cell, and Developmental Biology, University of California, Santa Cruz, CA.

During meiotic cell division, homologous chromosomes must pair and undergo crossover recombination, producing a physical linkage that promotes proper chromosome segregation. Recombination is accompanied by a structural reorganization of chromosomes around the crossover site to facilitate segregation on the Meiosis I spindle. In *C. elegans*, ZHP-3 is required for both crossover formation and for the proper restructuring of chromosomes in late prophase (Jantsch et al., 2004; Bhalla et al., 2008). The presence of a conserved RING finger motif in ZHP-3 suggested that it might act as a ubiquitin E3 ligase and modify substrates to alter their stability, localization, and/or activity. To characterize the ubiquitin ligase activity of ZHP-3, several experiments have been conducted. First, ZHP-3 purified from bacteria shows auto-ubiquitination activity. In addition, a ZHP-3 transgenic lines was generated by microparticle bombardment to facilitate purification of ZHP-3 from adult worms. The construct was designed with *zhp-3* genomic sequence under control of the *pie-1* promoter, with an N-terminal mCherry-TEV-S (*mCherry::zhp-3*) tag. The *mCherry::zhp-3* transgene can fully rescue the phenotype of the *zhp-3(jf61)* null allele, recapitulating the localization pattern of the endogenous protein, localizing along the synaptonemal complex in pachytene and as foci in diplotene. The transgenic line displays wildtype levels of viability at 15º and 20º C. At 25º C, there is a slight decrease in viability and an increase in the frequency of male self-progeny, which may be due to elevated expression of the transgene at higher temperatures. The *mCherry::zhp-3* strain undergoes crossover recombination, as indicated by the presence of 6 DAPI-staining bivalents at diakinesis. Bivalent chromosomes are properly structured, with HTP-1 localization restricted to the long arm of the bivalent. These data indicate that the *mCherry::zhp-3* transgene is competent to carry out both of ZHP-3's roles. In agreement with experiments performed with recombinant protein, mCherry-ZHP-3 immunopurified from adult worm lysate had ubiquitination activity. Mass spectrometry analysis will be performed to identify proteins that co-immunopurify with mCherry-ZHP-3, including targets of its ubiquitination activity. Additionally, candidate substrates will be in vitro transcribed and translated and included in the in vitro ubiquitination reaction. Together, these data help elucidate how ZHP-3's ubiquitin ligase activity facilitates the large-scale changes in chromosome structure in late prophase that are required for proper meiotic chromosome segregation.

1158A

Role of SPD-3 in Pairing and Synapsis of Meiotic Chromosomes. **Regina Rillo**[1,2], Abby Dernburg[1,2]. 1) UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

Faithful segregation of chromosomes at the first meiotic division is dependent on proper homologous pairing and synapsis. In *C. elegans*, specific regions on each chromosome, called Pairing Centers, recruit a family of zinc-finger proteins including ZIM-1, 2, 3 and HIM-8. Pairing Centers are essential for linking chromosomes to the microtubule cytoskeleton, thereby facilitating chromosome motion required for the proper coordination of pairing and synapsis.

Specific nuclear envelope proteins, as well as dynein, aggregate at sites of Pairing Centers in early prophase. Recent work from our lab has shown that inner nuclear membrane protein SUN-1 and the KASH-domain bearing protein ZYG-12 are required for homolog recognition; in their absence, chromosomes fail to pair and undergo inappropriate synapsis with nonhomologous partners. In contrast, loss of dynein results in extensive asynapsis, though chromosomes do pair with their homologs, suggesting that dynein function is essential for synapsis initiation. Taken together, these results support a model in which SUN-1 and ZYG-12 impose a barrier to synapsis that must be overcome by dynein to coordinate pairing and synapsis in early prophase. How nuclear envelope proteins, like SUN-1 and ZYG-12, inhibit synapsis and promote homolog pairing remain to be determined.

A candidate screen for new factors involved in meiotic chromosome dynamics revealed a potential role for SPD-3 in pairing and synapsis. A temperature-sensitive allele, *oj35*, results in variable meiotic defects when shifted to the restrictive temperature (25°C) at the L4 stage. To deplete SPD-3 more completely, I performed RNAi in *spd-3(oj35)* mutants at the restrictive temperature. *spd-3(oj35, RNAi)* mutants undergo extensive nonhomologous synapsis in pachytene, similar to that seen in *sun-1* loss of function mutants, indicating that SPD-3 may play a similar role in coordinating pairing and synapsis. Unlike *sun-1(lof)* mutants, however, synapsis does not occur immediately upon meiotic entry. Instead, chromosomes remain asynapsed for some time even if they have paired with their homologs. Previous studies showed that SPD-3 is required for proper spindle alignment in the early embryo and polar body extrusion in meiosis (Dinkelmann et al, 2007). SPD-3::GFP localizes to mitochondria in both embryos and the germline. How a mitochondrial protein affects chromosome dynamics within the nucleus may shed light on the mechanism by which pairing and synapsis are coordinated. I am continuing to analyze *spd-3* mutants and generating an antibody against the SPD-3 protein to look at its endogenous localization, and will present my latest findings.

1159B

Quantitative analysis of chromosome dynamics during *C. elegans* meiosis. **O. Rog**, D. J. Wynne, A. F. Dernburg. UC Berkeley / HHMI, Berkeley, CA.

Meiosis, the specialized cell division that produces gametes and enables sexual reproduction, involves the separation of homologous chromosomes, the two copies of each chromosome inherited from each parent. This segregation process requires that each chromosome first physically pair with its homolog and precisely exchange genetic information. These complex events are coordinated both spatially and temporally by synapsis - the regulated assembly of a protein polymer called the synaptonemal complex, between paired homologs. *C. elegans* presents an exceptional opportunity to visualize the process of pairing and synapsis in real-time in living animals. Our analysis of chromosome pairing reveals the existence of coordinate, dynein-driven motion, that dramatically translocates one end of each chromosome in order to assist in correct pairing. Surprisingly, we also identify a constraint on movement that is removed upon meiotic entry, enabling chromosomes to pair. We will also present our progress in developing tools to visualize synapsis and the concomitant changes in chromosome morphology. Quantitative analysis using these tools will provide rigorous, in depth characterization of chromosome dynamics.

1160C

A novel gene promoting meiotic double-strand break formation in C. elegans. **Simona Rosu**, Anne Villeneuve. Genetics, Stanford University, 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 (called chiasma) that hold the homologs together and ensure proper segregation at the meiosis I division. COs are generated by homologous recombination initiated by DNA double strand breaks (DSBs) formed by the meiotic SPO-11 protein. The regulation of meiotic DSB formation is not well understood, however it is important that organisms have mechanisms to guarantee at least one break per chromosome pair to provide the obligate chiasma.

We have identified a new gene (defined by the me96 mutation) involved in promoting DSB formation during C. elegans meiosis. The me96 allele was isolated from 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 exhibit a mixture of bivalents (homologous chromosomes joined by a chiasma) and univalents (achiasmate chromosomes) at diakinesis. Notably, the phenotype becomes progressively more severe as the worms get older. The me96 allele contains an early stop mutation in the F26H11.6 gene, defining a novel component of the meiotic machinery. Immunofluorescence experiments show that RAD-51 foci, which mark recombination intermediates, are reduced in the me96 mutant. Providing exogenous breaks by irradiation rescues both RAD-51 foci levels and chiasma formation in me96 worms. This suggests F26H11.6 is involved in promoting DSB formation, a step that is critical for meiotic recombination. An antibody against the F26H11.6 protein localizes to chromatin of nuclei in transition zone and early pachytene,

the stages of meiotic prophase corresponding to the presumed timing of DSB formation. This suggests that F26H11.6 may help create an environment competent for SPO-11 dependent DSB formation. Further characterization of this mutant will provide new insights into the regulation of endogenous meiotic DSB formation.

1161A

Multiple developmental processes collaborate to regulate *cdc-14* activity. **Sarah Roy**, Mako Saito. Genetics, Dartmouth Medical School, Hanover, NH.

Our understanding of the Cdc14 family of dual-specificity phosphatases is largely based on studies of yeasts. Fungal Cdc14 is an important regulator of M-phase events; however, the *C. elegans* homolog, *cdc-14*, is dispensable for mitosis. Rather, *cdc-14* regulates G1/S progression to establish developmental cell-cycle quiescence. Despite the importance of the Cdc14 family, the mechanisms by which activity is coordinated with development are largely unknown. We demonstrate that several processes conspire to focus the activity of *cdc-14*. First, while the *cdc-14* locus can produce at least six protein variants through alternative splicing, a single form, called CDC-14C, is the key variant acting during vulva development. Second, post-transcriptional regulation defines CDC-14C expression to a limited subset of cells, including the vulva precursors. Lastly, CDC-14C subcellular location, and thus its potential interactions with other regulatory proteins, is regulated by nucleocytoplasmic shuttling. The active export of CDC-14C from the nucleus during interphase is dependent on members of the Cyclin D and Crm1 families. Together these mechanisms collaborate to coordinate *cdc-14* activity with developmental progression.

In order to identify genes potentially acting together with CDC-14C, we used the yeast two-hybrid assay. Over 50 million potential two-hybrid interactions between CDC-14C and a mixed-stage *C. elegans* cDNA library were screened and the majority (117/123) of isolated clones represented a member of the A-kinase anchoring protein (AKAP) family. AKAPs were initially identified as binding partners of protein kinase A, but have subsequently been shown to interact with a wide variety of protein kinases and phosphatases. We determined that loss of AKAP activity through either RNAi or genetic mutation resulted in a weakly penetrant cell-cycle quiescence defect. Moreover, loss of the AKAP activity partially suppresses the Cye-1(lf) cell cycle phenotype. The overlapping mutant phenotype with Cdc-14 suggests that the CDC-14C-AKAP interaction identified by the yeast two-hybrid screen functions in the control of cell cycles during development. Specifically, we hypothesize that the AKAP interaction tethers CDC-14C to either a subcellular location or a functional partner that is necessary for CDC-14C function.

1162B

Characterizing the meiotic role of protein phosphatase 4. **Aya Sato**[1], Fumio Motegi[2], Asako Sugimoto[3], Peter Carlton[1]. 1) Institute for Integrated Cell-Material Sciences (iCeMS), Kyoto University, Kyoto, Japan; 2) Department of Molecular Biology and Genetics, Howard Hughes Medical Institute, Center for Cell Dynamics, Johns Hopkins School of Medicine, Baltimore, MD USA; 3) Graduate School of Life Sciences, Tohoku University, Sendai, Japan.

Meiosis is a specialized cell division that creates haploid gametes from diploid cells by executing two rounds of chromosome segregation after one round of replication. In meiotic prophase, chromosomes face the challenge of finding their homologous partners and establishing physical linkages (chiasmata) with them through crossover recombination. Chiasmata hold homologous chromosomes together and thus are essential for segregating homologous chromosomes in opposite directions at Meiosis I. Successful formation of chiasmata can be assayed by detecting six bivalent DAPI bodies in late meiotic prophase (diakinesis) in the worm gonad. Failures in chromosome pairing, synapsis or recombination often lead to more than six DAPI bodies, indicating the presence of univalent chromosomes. Currently we are analyzing a pph-4 (protein phosphatase 4) mutant which produces ~12 univalents in diakinesis. A previous study has shown that RNAi-mediated knockdown of pph-4 leads to univalents (Sumiyoshi et al. JSC, 2002), but the function of PPH-4 in meiosis is not understood. Protein phosphatase 4 is a conserved serine/threonine phosphatase, and is known to be involved in various cellular functions such as centrosome regulation, DNA damage repair and microtubule function (Sumiyoshi et al, JSC, 2002, Han et al., Genetics, 2009). We will report initial characterization of the pph-4 mutant and discuss its potential meiotic role in *C. elegans*.

1163C

Insulin signalling functions as somatic DNA damage checkpoint during development. Michael Müller[1], Maria Ermolaeva[1], Peter Frommolt[1,2], Sebastian Greiss[1], Jennifer Schneider[1], **Bjoern Schumacher**[1]. 1) CECAD, University of Cologne, Cologne, Germany1Cologne Excellence Cluster for Cellular Stress Responses in Aging Associated Diseases (CECAD), University of Cologne, Zülpicher Str. 47a, 50674 Cologne, Germany; 2) Cologne Center for Genomics, University of Cologne, Weyertal 115b, 50931 Cologne, Germany.

Congenital defects in genome maintenance systems cause complex disease phenotypes characterized by developmental failure, cancer susceptibility and premature aging. In contrast to well-characterized cellular DNA damage checkpoint mechanisms, it remains poorly understood how DNA damage responses affect organismal development and maintain functionality of tissues when DNA damage gradually accumulates with aging. Here we show that transcription-coupled repair defects that in human Cockayne syndrome patients lead to developmental growth defects and progeria, specifically impair somatic development upon UV damage in C. elegans. Repair proficient animals, in contrast, transiently arrest development. We demonstrate that persistent DNA damage activates the transcription factor DAF-16/FoxO and that the insulin/insulin-like growth factor signalling (IIS) is required for DNA damage induced developmental arrest. In response to persistent DNA damage in adult worms reduced IIS enhances somatic tissue functionality and lifespan. Our findings suggest that IIS functions as a somatic DNA damage checkpoint during development and that tolerance of persistent DNA damage contributes to extended longevity.

1164A

Regulation of Meiotic Double-Strand Break Formation in C. elegans. **Ericca Stamper**, Abby Dernburg. Department of Molecular and Cell Biology, UC Berkeley, Berkeley, CA.

Meiotic recombination is essential for the successful execution of meiosis, and therefore for sexual reproduction. Recombination is initiated by the formation of programmed DNA double-strand breaks (DSBs), catalyzed by the conserved endonuclease Spo11. If not properly repaired, DSBs pose a threat to genomic integrity. The activity of Spo11 is thought to be tightly regulated to control both the number and location of DSBs. In budding yeast, where the regulation of DSB formation is best understood, at least 9 proteins in addition to Spo11 are required for meiotic DSBs. However, little is understood about how recombination initiation and DSB formation is regulated in other organisms, including *C. elegans*, which has emerged as an important system for studying meiosis. To gain new insights into the regulation of SPO-11 and DSB formation in *C. elegans*, I have been characterizing a novel gene that we have named *dsb-1.* Animals lacking *dsb-1* function are viable and fertile, but do not make crossovers during meiosis and therefore produce many inviable progeny and a high incidence of males (Him). This defect in recombination can be rescued by irradiation, indicating that they are specifically defective in DSB formation and not repair. DSB-1 shares homology with another *C. elegans* gene implicated in DSB formation (Rosu et al., 2009), but lacks obvious homology with proteins outside of Caenorhabditis. I have found that epitope-tagged DSB-1, expressed from single-copy transgenes integrated by MosSCI, localizes as foci on meiotic chromosomes during early meiotic prophase, corresponding to the timing of DSB formation. One hypothesis is that DSB-1 may recruit SPO-11 to meiotic chromosomes. However, analysis by yeast two-hybrid (Y2H) assays failed to detect an interaction between DSB-1 and SPO-11. Y2H analysis did detect a weak interaction between DSB-1 and HTP-3, complementing previous studies that suggested a role for HTP-3 in DSB formation through the recruitment of recombination proteins to meiotic chromosomes (Goodyer et al., 2008). I plan to investigate DSB-1 function through co-immunoprecipitation and ChIP experiments. Future goals of my project aim to address some of the many remaining questions regarding the role of DSB-1 in promoting DSBs and the regulation of meiotic recombination initiation in *C. elegans*.

References: Goodyer W, Kaitna S, Couteau F, Ward JD, Boulton SJ, Zetka M. 2008. HTP-3 links DSB formation with homolog pairing and crossing over during C. elegans meiosis. Dev Cell 14:263-74. Rosu S, Tam A, Villeneuve A. 2009. Identification of novel components of the C. elegans meiotic machinery. International Worm Meeting.

1165B

The C. elegans adenomatous polyposis coli tumor suppressor homolog APR-1 prevents chromosomal instability by regulating centrosome duplication. **Attila Stetak**[1,2,3], Erika Froehli-Hoier[4], Alex Hajnal[4]. 1) University of Basel, Dept. of Psychology, Division of Molecular Neuroscience, Basel, Switzerland; 2) University of Basel, Biozentrum, Life Sciences Training Facility, Basel, Switzerland; 3) University of Basel, University Psychiatric Clinics, Basel, Switzerland; 4) University of Zürich, Institute of Molecular Life Sciences, Zürich, Switzerland.

Chromosome segregation is a critical process during mitosis that must be carried out precisely. Aneuploidy caused by missegregation of chromosomes results in the malignant transformation of cells leading to cancer in humans. The presence of only two centrosomes during mitosis is essential for the correct assembly of the mitotic spindle, otherwise alignment of chromosomes would be erroneous and lead to chromosome mis-segregation. The adenomatous polyposis coli (APC) tumor suppressor was previously found to be necessary to maintain genome stability in vertebrate cells. A C-terminal truncation of the *C. elegans* APC homolog *apr-1(ok3970)* results in complete sterility and the formation of multipolar spindles in the mitotic germline. In order to study the function of APC proteins in maintaining genome stability, we purified from HELA cell nuclear extracts proteins binding to the C-terminal 170 amino acids of human APC and analyzed them by mass-spectroscopy. We identified the heterogeneous nuclear ribonucleoprotein hnRNPA1 as a new APC binding protein. hnRNPA1 has previously been shown to bind to the microtubule-associated protein TOG-1/XMAP215, the mammalian ortholog of *C. elegans* ZYG-9. Using co-immunoprecipitation and GST pull-down experiments, we show that APC, hnRNPA1 and TOG-1 form a tripartite complex. Furthermore, all three proteins are localized in cultured mammalian cells to the centrosomes and along microtubules during metaphase. We then studied the function of the *C. elegans* orthologs APR-1 (APC), HRP-1 (hnRNPA1) and ZYG-9 (TOG-1) during mitotic cell divisions in the early embryo. The three *C. elegans* proteins show similar localization in dividing cells as their mammalian orthologs. APR-1 depletion by RNAi reduces localization of HRP-1 but not of ZYG-9 from the centrosomes, while depletion of HRP-1 or ZYG-9 has no effect on APR-1 localization. RNAi against each of the three proteins causes formation of extra centrosomes and multipolar spindles, suggesting that APR-1, HRP-1 and ZYG-9 act together to control centrosome stability. Taken together, we have identified a conserved tripartite protein complex composed of APR-1/APC, HRP-1/hnRNPA1 and ZYG-9/TOG-1 that regulates centrosome duplication and spindle integrity processes during mitotic cell divisions in *C. elegans*.

1166C

PKC-3 aPKC phosphorylates LIN-5 NuMA to position the mitotic spindle during

asymmetric division. Matilde Galli[1], Javier Muñoz[2], Vincent Portegijs[1], Mike Boxem[1], Albert Heck[2], **Sander van den Heuvel[1]**. 1) Developmental Biology, Utrecht Univiversity, Utrecht, The Netherlands; 2) Biomolecular Mass Spectrometry and Proteomics Group, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands.

Asymmetric cell divisions that create different daughter cells are critical for development and stem cell maintenance. The position of the mitotic spindle controls the plane of cell cleavage and determines if polarized cells divide symmetrically or asymmetrically. Studies in various animal systems have identified an evolutionarily conserved PAR-3/PAR-6/aPKC polarity complex that acts upstream of a conserved LIN-5 (NuMA)/GPR (Pins/LGN)/Gα pathway to position the mitotic spindle. However, the molecular interactions between polarity proteins and LIN-5/GPR/Gα remain to be identified. We found that the C. elegans LIN-5 protein is phosphorylated in vivo by the polarity kinase PKC-3 (aPKC), and that this phosphorylation directs the position of the mitotic spindle during asymmetric division. We developed a quantitative mass spectrometry approach for in vivo identification of protein kinase substrates. Applying this strategy to C. elegans embryos, we found that depletion of the polarity kinase PKC-3 results in severely reduced phosphorylation of four adjoining LIN-5 serine residues. These LIN-5 residues are also phosphorylated by PKC-3 in vitro. LIN-5 phosphorylation in vivo overlaps with PKC-3 localization at the anterior cell cortex and temporarily coincides with a switch from anterior- to posterior-directed spindle movements in the one-cell embryo. LIN-5 mutations that prevent phosphorylation increase anterior-directed spindle movement, while phosphomimetic mutations reduce spindle migration. Together, these results demonstrate that PKC-3 locally inhibits cortical microtubule pulling forces through direct phosphorylation of LIN-5, thereby promoting posterior migration of the spindle. These results reveal an important molecular mechanism by which polarity cues control the spindle-positioning machinery to instruct the cleavage plane during asymmetric cell division.

1167A

Purification of intact germline nuclei: Towards a high-resolution spatiotemporal map of meiotic chromatin in *C. elegans*. **Christina M. Whittle[1,2]**, Abby F. Dernburg[1,2]. 1) Dept of Molecular and Cell Biology, UC Berkeley, Berkeley, CA; 2) Howard Hughes Medical Institute.

C. elegans as a model has traditionally lent itself well to genetic and cytological studies with strengths being the well-characterized, invariant cell lineage leading to relatively few tissue types. However, biochemical studies thus far have been largely limited to whole animal extracts leaving tissue-specific studies consigned to the resolution afforded by cytology. *C. elegans* simplicity as a metazoan model gives it enormous untapped potential as a system in which to do tissue-specific biochemistry in primary cells. Methods for isolating specific tissues to analyze protein-protein or protein-DNA interactions would bridge the gap between cytological analysis, which provides low-resolution but tissue-specific information, and the molecular interaction data obtained from whole animal extracts, such as ChIP-Seq. Germline processes, such as meiosis and the specialized chromatin inherent to its execution, provide an ideal test case and intriguing subject for examination. We employed a nuclear envelope tagging strategy to tissue-specifically label nuclei with GFP and both employing and simplifying an approach recently reported in plants (1), in *C. elegans*. We obtained GFP::ZYG-12 driven specifically in the germline under the pie-1 promoter (2) and isolated intact nuclei. To circumvent the use of antibodies for purification, which could impair vital downstream applications such as ChIP, we utilized an unusual GFP binding protein (GBP) derived from camelid antibodies (3) to magnetically label only the GFP tagged nuclei, followed by bulk isolation. We are now optimizing the purification protocol, as well as generating meiosis-specific tagged nuclei. We will present preliminary results that demonstrate the feasibility and purity of germ nuclei isolated for biochemical applications such as ChIP-seq.

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1168B

CDC-48/p97 is required for meiotic chromosome segregation in *C. elegans*. Yohei Sasagawa[1,2], Teru Ogura[1], **Kunitoshi Yamanaka[1]**. 1) Dept Molecular Cell Biol, Kumamoto Univ, IMEG, Kumamoto, Japan; 2) Functional Genomics Unit, RIKEN CDB, Kobe, Japan.

Chromosome segregation requires regulated release of chromosome cohesion. In meiosis, cohesion of homologous chromosomes are released at the end of meiosis I, whereas association of sister chromatids has to be maintained until segregation at meiosis II. In *C. elegans*, Aurora B kinase (AIR-2) specifically localizes between homologous chromosomes, at which it promotes the release of meiotic cohesin REC-8 via phosphorylation, in meiosis I metaphase. Although this specific localization of AIR-2 is crucial for proper meiotic chromosome segregation, its precise mechanism has been unclear. CDC-48/p97 is a ubiquitin-selective AAA (ATPases associated with diverse cellular activities) chaperone and converts chemical energy generated from ATP hydrolysis into mechanical force used for protein conformational changes such as unfolding of proteins and disassembly of protein complexes. We previously reported that *C. elegans* CDC-48s (CDC-48.1 and CDC-48.2) are essential for progression of meiosis I metaphase. Here, we report that CDC-48s are required for meiotic chromosome segregation in *C. elegans*. In wild-type worms, at diakinesis phase, phosphorylation of histone H3, one of the known substrates for AIR-2, on meiosis I chromatids correlated with AIR-2 localization at the cohesion sites of homologous chromatids. On the other hand, the knock-down of CDC-48s resulted in significant expansion of signals for AIR-2 and phosphorylated histone H3 over the entire meiotic chromosomes, which leads to the defective chromosome segregation, while the amount of AIR-2 in total lysates was not changed by the knock-down of CDC-48s. The defective meiotic chromosome segregation of the knock-down of CDC-48s was suppressed by the simultaneous knock-down of AIR-2 and seems to be similar to that observed by the knock-down of PP1 phosphatase. However, the amount and localization of PP1 were not changed by the knock-down of CDC-48s. These results imply that CDC-48s determine the localization of Aurora B kinase to the cohesion sites of homologous chromatids at meiosis I prophase.

1169C

A mutation in *cya-1*, which encodes the *C. elegans* Cyclin A homolog, specifically affects the division of the NSM neuroblast. **Bo Yan[1,2]**, Barbara Conradt[1,2]. 1) Dept Gen, Dartmouth Medical School, Hanover, NH; 2) Department Biologie, Ludwig-Maximilians University, Munich, Germany.

We have identified a hypomorphic mutation of the *cya-1* gene, which encodes the *C. elegans* Cyclin A protein. *cya-1(bc416)* causes temperature-dependent phenotypes. In wild-type animals, the NSM neuroblast divides to give rise to the NSM, which differentiates into a serotonergic neuron, and the NSM sister cell, which undergoes programmed cell death. In contrast, in *cya-1(bc416)* animals grown at 15°C and 20°C, the division of the NSM neuroblast is blocked in the G2 phase of the cell cycle, and the non-dividing NSM neuroblast differentiates into a NSM-like cell that expresses a serotonergic marker, the gene *tph-1*. When grown at 25°C, most of *cya-1(bc416)* animals fail to undergo morphogenesis. We also demonstrate that at least at 25°C, bc416 affects splicing of the *cya-1* message, resulting in an aberrant mRNA that is predicted to produce a non-functional CYA-1 protein. A detailed analysis of *cya-1(bc416)* may provide insight into how the cell cycle can be controlled in a cell-type specific manner during development.

1170A

Illuminating the Formation and Regulation of Meiotic Crossovers with GFP:COSA-1. **Karl Zawadzki[1]**, Rayka Yokoo[2], Anne Villeneuve[1,2]. 1) Developmental Biology, Stanford University, Stanford, CA; 2) Dept of Genetics, Stanford University, Stanford, CA.

Faithful chromosome segregation during meiosis I requires crossover (CO) recombination events that form the basis of temporary links between homologous chromosomes. Surprisingly, rather than creating many COs between homologs, most organisms create only a small number of widely spaced COs while ensuring that each pair of homologs receives at least one. This regulation of CO number and placement is collectively termed “crossover control” but the underlying mechanisms are poorly understood. In C. elegans CO control is particularly robust, with each pair of homologs receiving one CO. We are investigating the formation and regulation of meiotic COs, building on our recent discovery of COSA-1 (Crossover Site Associated), a novel and conserved CO protein that is required for the formation of COs and localizes to CO sites during the late pachytene and diplotene stages of meiotic prophase. A functional GFP::COSA-1 fusion protein serves as a robust *in vivo* reporter for the CO control system, as GFP::COSA-1 reliably localizes to six foci per nucleus (*i.e.* one focus for each pair of homologous chromosomes), even in the context of a large excess of DSBs (which serve as initiating events for meiotic recombination). Further, in worms harboring fusions of two chromosomes GFP::COSA-1 localizes to five foci reflecting the reduced chromosome complement. We are taking two approaches that exploit GFP::COSA-1 to investigate the mechanisms of CO control. First, we are using immunoprecipitation of GFP::COSA-1 to identify proteins and DNA that associate with COSA-1. Second, we are conducting a genetic screen to identify mutations that alter the number of GFP::COSA-1 foci. To date, we have isolated four mutants with altered numbers of GFP::COSA-1 foci: three with reduced numbers of foci, perhaps due to early disruption of the meiotic program, and one with increased numbers of foci. Further characterization of these mutations and others generated by additional screening will help elucidate the factors that control CO number.

1171B

HAL-2 promotes homologous pairing during *C. elegans* meiosis by antagonizing inhibitory effects of SC proteins. **Weibin Zhang[1]**, A.J. MacQueen[2], N. Miley[1], M. Zastrow[1], A.M. Villeneuve[1]. 1) Stanford University, Stanford, CA; 2) Wesleyan University, Middletown, CT.

During meiosis, homologous chromosomes identify and pair with their correct partners, and the synaptonemal complex (SC) forms between them to stabilize homolog alignment. The SC assembles cooperatively but it is also indifferent to chromosome homology, so pairing and synapsis must be tightly regulated and coordinated to ensure successful homologous synapsis. We are investigating the roles of HAL-2, a novel component of the meiotic machinery, in coordinating these early meiotic events. We find that HAL-2 promotes homolog pairing largely by preventing inappropriate interaction of SYP proteins (SC central region proteins) with chromosomes. *hal-2* mutants fail to establish homolog pairing, lack the nuclear reorganization of chromosomes into a clustered configuration that normally accompanies pairing, and load SYP proteins incorrectly onto unpaired chromosomes. Analysis of *hal-2; syp-2* double mutants suggests that this inappropriate SYP localization impedes homolog pairing by inhibiting normal functioning of pairing centers (PCs), which are cis-acting regions near one end of each chromosome that connect chromosomes to cytoplasmic microtubules through a nuclear envelope-spanning complex that includes ZYG-12 and SUN-1. During the period of nuclear reorganization, SUN-1 is phosphorylated and ZYG-12/SUN-1 form mobile patches on the nuclear envelope that colocalize with PCs. SUN-1 phosphorylation, ZYG-12 patches, and pairing at PCs are all

HAL-2 dependent and are coordinately restored in *hal-2; syp-2* double mutants. These and other data indicate that HAL-2 enables function of the PCs, predominantly but not exclusively by counteracting the inhibitory effects of the SYP proteins. HAL-2 is broadly localized within the nuclei of wild type germ cells and colocalizes with SYP proteins in the aggregates that form when SC assembly is prevented in mutant germ cells. We are currently testing the working hypothesis that HAL-2 enables SYP proteins to accumulate but remain in a soluble state prior to licensing of SC assembly.

1172C

Mapping the PKD-2 localization defective mutant *cil-2(my2)*. **Deanna Michele De Vore**[1,2], Juan Wang[1], Karla Knobel[3]. 1) Genetics, Rutgers University, Piscataway, NJ; 2) GSBS, University of Medicine and Dentistry, Piscataway, NJ; 3) University of Wisconsin-Madison, Madison WI.

PKD2 encodes a transient receptor potential polycystin (TRPP) channel receptor protein located in non-motile, primary cilia of mammalian cells. In humans, PKD2 mutations result in Autosomal Dominant Polycystic Kidney Disease (ADPKD). In *C. elegans*, PKD-2 localizes to cilia of male-specific sensory neurons where it is required for male mating behaviors. Given the ancient and evolutionarily conserved role for the PKD-2 protein in cilia, we are using *C. elegans* as a tool to identify new genes required for ciliary receptor localization. To this end, we performed a forward genetics screen and identified several PKD-2::GFP ciliary localization (Cil) defective mutants (Bae et al 2008).

Here, we focus on the *cil-2(my2)* mutant that exhibits accumulation of PKD2::GFP in the ciliary base and proximal dendrites of *C. elegans* male specific CEM neurons and exhibits temperature sensitive sterility in hermaphrodites. Three factor, SNP, and deficiency mapping in conjunction with whole genome sequencing were used to determine the gene mutated in *cil-2(my2)* animals. *cil-2(my2)* maps between +22.9 and +24.4 on linkage group X with *mec-5, let-2, crb-1*, and *tps-1* as possible candidate genes. Complementation testing and single gene rescue experiments are in progress to determine the identity of *cil-2*.

Once we clone *cil-2*, our future plans include determining whether *cil-2* acts cell-autonomously in male-specific neurons and the mechanism by which CIL-2 affects PKD-2 ciliary receptor trafficking. *cil-2(my2)* hermaphrodite sterility suggests that CIL-2 plays a broader role in the animal, and we will also address this possibility. These studies will shed light on how sensory receptors like PKD-2 are targeted to cilia, and may advance the understanding and treatment of ADPKD.

Reference: Bae YK. (2008) Identification of Genes Involved in the Ciliary Trafficking of *C. elegans* PKD-2 *Developmental Dynamics* 237:2021-2029.

1173A

A new system for neuron-specific RNAi and its application to studying the function of essential genes in mature GABA neurons. **Christopher Firnhaber**, Marc Hammarlund. Department of Genetics and Program in Cellular Neuroscience, Neurodegeneration and Repair, Yale University School of Medicine, New Haven, CT.

Forward genetic screens in *C. elegans* are powerful tools that have advanced our understanding of many conserved biological processes, such as RNA interference, synaptic transmission, and autophagy. Conventional forward screens, however, often have difficulty identifying genes whose relevant functions are masked by pleiotropy. In particular, we are interested in discovering gene functions in mature neurons, but if loss of function results in sterility, lethality, or other severe pleiotropy, neuronal-specific functions cannot be readily analyzed.

To address this problem, we have developed a method in which sensitivity to RNAi is limited to a subset of neurons - the GABAergic motor neurons. Our method combines three previous approaches: a mosaic approach using *rde-1* [1]; a sensitizing approach using *eri-1* and *lin-15B* [2]; and another sensitizing approach using *sid-1* [3]. The GABA neurons of our animals are highly sensitive to RNAi, while other cells are resistant; for example, knockdown of ubiquitously expressed GFP eliminates fluorescence from GABA neurons but maintains it in other cells. As a result, these worms display GABA-specific behavioral phenotypes when fed RNAi against genes with important functions in GABA neurons, while remaining viable, fertile, and morphologically wild type, even if these genes have essential functions.

We are currently screening for the roles of essential genes in synaptic transmission, axon maintenance, and neuronal cell survival. We are also building strains in which RNAi sensitivity is limited to various other neuronal subpopulations. Since the function of most essential genes in mature neurons *in vivo* is all but unknown, we hope to identify novel functions for these important and well-conserved genes.

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1174B

MosSCI: Improved efficiency and new insertion sites. **C Frøkjær-Jensen**, MW Davis, M Ailion, EM Jorgensen. Biology, HHMI, U. of Utah, Salt Lake City, UT.

Mos1 mediated Single Copy Insertion (MosSCI) is a method to insert a single copy of exogenous DNA into a predetermined chromosomal location[A]. MosSCI works by excising a Mos1 transposon; repair inserts the transgene together with a positive selection marker (*unc-119*) in place of the Mos1 element. Insertions are generated by injection and are identified by the lack of fluorescent co-injection markers. The inserted DNA is stable, is expressed at approximately endogenous levels and can be expressed in the germline. To expand the utility of the technique we have generated additional insertion sites, significantly improved the insertion efficiency and developed a selection marker to kill animals with extrachromosomal arrays.

Insertion sites: We have generated 4x outcrossed insertion strains for a total of seven sites on Chr. I, II, IV and X. For all insertion sites, we have made regular cloning vectors and three-way Gateway compatible vectors. We have generated bright GFP insertions into all sites to validate expression from each site and for use as balancer strains.

Insertion efficiency: We exclusively use direct injection to generate insertions. We have tested modifications to the helper plasmid supplying Mos1 transposase (hyperactive mutations and different promoters) and to the injection conditions (decreased DNA concentrations). We have developed a protocol that results in few extrachromosomal arrays and insertions in 60% of the injected animals.

Selection against array: One of the main time constraints of generating MosSCI insertions is the screening process. Modifications to the injection conditions result in fewer arrays but do not eliminate them altogether. To further facilitate the screen for inserts we have generated a plasmid that encodes a heat-shock inducible toxin. By including the plasmid in the injection mix all animals with an extrachromosomal array can be killed by simple heat-shock. The method is fast (a couple of hours) and it greatly improves the ease of isolating insertions; in the absence of heat-shock the plasmid only very moderately decreases the efficiency of rescue.

Altogether, the improvements should significantly expand the usefulness and ease of generating transgene insertions by MosSCI.

(A) Frøkjær-Jensen et al. (2008) .

1175C

Development of three automated quantitative platforms for RNAi phenotyping. **Andrew G. Fraser**, Arun Ramani, Tungalag Chuluunbaatar, Hong Na. Molecular Genetics, Univ Toronto, Toronto, ON, Canada.

RNAi screens have revolutionized genetic screens in the worm. Like any genetic screen, an RNAi screen relies completely on phenotyping accuracy. However, to date there have been no published genome-scale screens using automated quantitative phenotyping — all phenotyping has been manual and at best semi-quantitative. Furthermore, although over 50 distinct phenotypes have been examined at genome-scale, the single most important phenotype for any evolutionary studies — fitness — has been completely ignored. We present here three complementary quantitative methods to direct assess the effect of RNAi on fitness and provide strong evidence that these methods are more sensitive than any manual screening as well as yielding highly reproducible quantitative measures of phenotypic strength. We illustrate how we have applied these methods to the study of natural variation in *C. elegans* and demonstrate the critical importance of quantitation to identify subtle defects and to correct for strain to strain growth differences.

1176A

*Mos*SCI-biotic: neomycin resistance gene as a co-insertion marker for *Mos1*-mediated single-copy insertion (*Mos*SCI). **R. Giordano-Santini**[1], D. Tu[2], R. Johnsen[2], D. Baillie[2], D. Dupuy[1]. 1) Genome Regulation and Evolution, INSERM U869, Institut Européen de Chimie et Biologie, Pessac, France; 2) Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, Canada.

Since the development of *Mos1*-mediated single-copy insertion (*Mos*SCI) a few years ago, *Mos*SCI has become the method of choice to introduce a single-copy of transgenes in the *C. elegans* genome (Frokjær-Jensen, C. et al., 2008; Nat. Genet. 40(11):1375-1383). This transposon-excision repair method has been developed using *unc-119* as a co-insertion marker, and two intergenic *Mos1* insertion sites have been used to date. In theory, every *Mos1* intergenic insertion from NemaGENETAG could be used for gene conversion and single-copy integration using *Mos*SCI. However, the use of *unc-119* as a co-insertion marker necessitates the introduction of the *unc-119(ed3)* allele in the genetic background beforehand, a requirement that may not be suitable for cases where the *unc-119(ed3)* mutant background may interfere with the study or if the desired *Mos1* insertion site is too close to the *unc-119* locus.

We have previously shown that the neomycin resistance gene (*neoR*) is a potent genetic marker for nematode transgenesis and that a single-copy of *neoR* is enough to confer resistance to G-418 in wild type animals (Giordano-Santini, R. et al., 2010; Nat. Methods 7(9):721-723). By using two different *Mos1* insertion lines, we demonstrated that this powerful selection system could be used in the context of *Mos*SCI and replace the *unc-119* co-insertion marker. Herein, we show that *neoR* is compatible with the *Mos*SCI heat-shock protocol as well as the direct protocol, in which the Transposase source is under the control of a heat-shock promoter or under the promoter of a germline specific gene respectively. We demonstrate that the majority of steps in these protocols are facilitated by the use of antibiotic resistance systems, hence proving that the benefits of antibiotic selection extend to single-copy transgene applications. We therefore believe that these benefits should contribute to the development of more flexible and efficient techniques for nematode transgenesis.

1177B

Construction of Arrayed Toxicology Reporter Panels using Transcript-activated Fluorescent Reporters in Transgenic Nematodes. **Miluka Gunaratna**[1], John Manfredi[2], Christopher Hopkins[1]. 1) Knudra Transgenics, Salt Lake City, UT; 2) Sfida BioLogic, Inc., Salt Lake City, UT.

Toxicology assessment of chemicals is used for government regulatory decisions, industrial and environmental safety, and design of new therapeutic compounds. We are developing a panel of nematodes for use in high-throughput toxicology assays. Promoters of toxin-responsive genes are fused to fluorescent-protein coding sequence and inserted C. elegans genome by the patented MosSCI transgenesis procedure. The resulting panel of 96 toxicology-reporter nematodes are exposed to toxin and scored for gene activation by fluorescent plate-reader assays. Detection of a gene-activation is observed as increased fluorescence in a reporter strain. The genes chosen for reporter development are involved in heat shock, oxidative stress, heavy metals, and xenobiotic response. Over 300 genes are

under consideration for development as toxin-responsive genes. Currently, 64 gene-activation reporters have been designed and 21 have progressed to transgenic strain candidates (hsp-1, hsp-3, hsp-4, hsp-6, hsp-16.2, hsp-16.41, hsp-17, hsp-60, dnj-13, daf-21, gst-4, gst-38, ugt-1, ugt-13, gcs-1, skn-1, cdr-1, mtl-2, cyp-13A7, cyp-14A3, cyp-35A2). To control for toxin-induced population differences, the reporter strains are made in the background of the constitutively-expressed unc-47::GFP reporter gene. To enable high-throughput liquid handling of strains, we have attempted to observe expression in liquid media using the hsp-16.41::hRFP reporter strain. Heat-shock activation of the reporter shows greatly reduced expression in liquid media relative to growth on NGM plates. We have developed an artificial-soil substitute to solve the low expression problem of liquid media. Expression of heat-shock reporters in the artificial soil gives expression comparable to NGM plates. Ease of application of artificial-soil to the 96-well format enables the design of a high-throughput platform for rapid gene-activation detection.

1178C

A systematic screen for secreted proteins using a synthetic lethal approach. **Samantha N. Hartin**[1], Vi Leitenberger[1], Martin L. Hudson[2], Brian D. Ackley[1]. 1) Molec Biosci, Univ Kansas, Lawrence, KS; 2) Dept of Biology & Physics, Kennesaw State University, Kennesaw GA.

Intercellular signaling molecules are essential for instructing distinct development events. Approximately 30% of the *C. elegans* genome encodes molecules with secretion signals. Using a bioinformatic approach we compiled a list (the Secretome) of ~6,700 genes from *C. elegans* that encode for signal peptides that would direct proteins to the cell surface or extracellular matrix. We prepared an RNAi targeting library of ~4200 genes present in the Secretome from the available Ahringer genomic clone RNAi library and are screening this library to identify genes that interact with molecules known to affect distinct aspects of development, *ptp-3* and *sdn-1*. The leukocyte-common antigen related receptor protein tyrosine phosphatase, (LAR/*ptp-3*), affects embryonic development, neuron outgrowth and synapse formation. The heparan sulfate proteoglycan, syndecan (*sdn-1*), also has been demonstrated to regulate embryonic and neuronal patterning. In *Drosophila*, LAR and SDN physically interact to facilitate synapse formation. Previously, we have found that animals lacking either *ptp-3* or *sdn-1* are viable, but double mutants are synthetic lethal (SynLet), indicating they function in parallel pathways that contribute to an important developmental process. To facilitate the identification of molecules that may function in LAR or Syndecan-dependent development, we are screening our Secretome library for clones that have a SynLet phenotype with either *ptp-3* or *sdn-1*. We will then begin determining how molecules identified in our screen may interact with either *ptp-3* or *sdn-1* as appropriate. We will use double mutants, where possible, coupled with cell-specific rescue to identify within which tissues these molecules are required.

1179A

Low-copy integration of transgenes by TMP/UV methods. **Eriko Kage-Nakadai**[1,2], Hiroyuki Kobuna[1], Osamu Funatsu[1], Muneyoshi Ootori[1], Keiko Gengyo-Ando[1,3], Sawako Yoshina[1,2], Sayaka Hori[1,2], Shohei Mitani[1,2]. 1) Dept Physiology, Tokyo Women's Med Univ Sch Med, Tokyo, Japan; 2) CREST, JST, Saitama, Japan; 3) Saitama Univ Brain Sci Inst, Saitama, Japan.

In *Caenorhabditis elegans*, transgenic strains are typically generated by injecting DNA into the germline to form high-copy extrachromosomal arrays. These transgenes are semi-stable and their expression are silenced in germline. In order to create single- or low-copy chromosomal integrated lines, methods using Mos1 transposon or microparticle bombardment have been developed. We have developed an alternative method by using TMP/UV, that produces low-copy integrations. We have successfully integrated low-copy of transgenes using positive selection with *vps-45* rescue fragment / temperature sensitivity and negative selection with *ben-1* rescue fragment / benomyl sensitivity. We confirmed that low-copy integrants express transgenes in germline. Currently, using this method, we are constructing a PhiC31-attBP / Cre-LoxP system.

1180B

Simple and rapid single nucleotide polymorphism (SNP) mapping method in C. elegans without the use of restriction fragment length polymorphisms (RFLP) analysis - made it possible by real-time PCR and high-resolution melting curve analysis (HRM). **Claudia Litterst**, Luis Ugozzoli. Bio-Rad, Hercules, CA., 94547.

Gene mapping in C. elegans is currently conducted by the use of SNPs as genetic markers. The current approach involves two closely related C. elegans strains that differ by a large number of polymorphic sites: a wild type C. elegans strain (N2 Bristol) and a closely related strain (CB4856 Hawaiian). The method is based on the detection of RFLPs located within polymerase chain reaction (PCR) -amplified DNA fragments that include nucleotide variations. One of the major disadvantages of this approach is that it requires the use of gel electrophoresis and is labor consuming. In addition, only SNPs that alter a restriction site (snip SNPs) are detected. Here, we present a novel method based on the use of real-time PCR and HRM analysis that enables SNPs detection directly from crude C. elegans lysates. We developed 12 assays which allow mapping of unknown genes to single chromosomes. Our approach represents a significant improvement for C. elegans gene mapping. In fact, the time to obtain results is shorter since the use of restriction enzymes and gel electrophoresis is not required.

1181C

RAD mapping and genomic interval pulldown sequencing for rapid mutation identification. **Sean O'Rourke**, Doug Turnbull, Nick Stiffler, John Yochem, Amy Connelly, Josh Lowry, Michael Miller, Eric Johnson, Bruce Bowerman. Inst Molec Biol, Univ Oregon, Eugene, OR.

Forward genetic screens in *C. elegans* and other model organisms represent a powerful approach to explore gene function by assigning phenotypes to mutated genes. However, determining the causal mutation by traditional mapping is often the rate-limiting step, especially when analyzing many mutants. We report two genomic approaches for more rapidly determining the identity of the affected genes in *C. elegans* strains. First, we report our use of restriction site associated DNA (RAD) markers for rapidly mapping mutations identified after chemical mutagenesis. We have used the N2 background to isolate mutants and crossed them to the polymorphic Hawaiian CB4856 strain for mapping. Approximately 200 F2 animals are used for the RAD mapping which simultaneously samples SNPs throughout the genome and can resolve genetic intervals smaller than one megabase. Second, we present our use of genomic interval pull-down sequencing to selectively capture and sequence portions of a genome up to several megabases in size. Many samples for both RAD mapping and genomic interval pull-down sequencing can be multiplexed into one Illumina sequencing run saving resources. Our methods should greatly decrease the time required to identify the causal mutation in *C. elegans* and other organisms.

1182A

An altered method of feeding RNAi that knocks down multiple genes simultaneously in the nematode *Caenorhabditis elegans*. **Yukari Omichi**[1], Kenji Gouda[1], Yohei Matsunaga[2], Takashi Iwasaki[1,2], Tsuyoshi Kawano[1,2]. 1) Department of Biochemistry and Biotechnology, Graduate School of Agriculture, Tottori University, Tottori, Japan; 2) Department of Bioresource Sciences, The United Graduated School of Agricultural Sciences, Tottori University, Tottori, Japan.

In reverse genetics, RNA interference (RNAi) which is substitutable for gene-disruption, is an outstanding method for knockdown of a gene's function. In *Caenorhabditis elegans*, feeding RNAi is most convenient, but this RNAi is not suitable for knockdown of multiple genes. When knockdown of two genes (double knockdown), animals are fed a mixture of two sorts of RNAi bacteria at the same time (double feeding). However, in some cases, only one gene's function is significantly inhibited, or both genes are only slightly knocked down. This is due to the lesser reliability of double feeding than single feeding. Given this background, we have established a method for more convenient, efficient, and reliable knockdown of more than two genes using feeding RNAi. We expected that bacteria producing two distinct dsRNAs bound to each other could induce simultaneous knockdown. For a model experiment, we chose *oma-1* and *oma-2* genes, which are functionally redundant to each other and responsible for embryonic development [1]. Only when both genes loss their function simultaneously, embryonic lethal occurs. We utilized this system for establishing double knockdown. In addition, for establishing triple knockdown, we attempted to suppress expression of GFP as well as OMA-1/OMA-2 in a transgenic animal expressing GFP. Quantitative RT-PCR and observation of phenotypes indicated that our method is much more efficient than the traditional one [2]. Our method is useful for investigating genes' functions in *C. elegans*. References: [1] Detwiler et al. (2001) Dev. Cell 1:187-199. [2] Gouda et al. (2010) Biosci. Biothech. Biochem. 74:2361-2365.

1183B

Incorporating Unnatural Amino Acids in *C. elegans*. **Angela Parrish**[1], Xingyu She[1], Zheng Xiang[1], Irene Coin[1], Andrew Dillin[2], Lei Wang[1]. 1) Chemical Biology and Proteomics Lab, Salk Insitute, La Jolla, CA; 2) Molecular and Cell Biology Laboratory, Salk Institute, La Jolla, CA.

Technologies to refine the study of protein interaction and localization in live organisms can be a key driving force for new research. One recent technology uses the cell's translation machinery to insert amino acids with novel functional groups at specific residues [1]. Amino acid residues of interest can be replaced by unnatural amino acids with various attributes, such as photo-crosslinking or environmentally sensitive fluorescence. By applying this technique, the role of certain sites of a protein in ligand binding or protein docking can be studied precisely, potentially by capturing an interaction that is transient using crosslinking. Proteins of interest could be fluorescently tagged by mutagenesis of a single amino acid, rather than appending an entire protein.

The basis of this technology is the introduction of a tRNA and an aminoacyl-tRNA synthetase from another kingdom of life, which functions with the *C. elegans* translation machinery to insert an unnatural amino acid in response to a unique codon. We use the amber stop codon (UAG) to eliminate competition between native and introduced tRNAs in the ribosome during translation. The introduced tRNA has a corresponding anticodon mutation to recognize the altered codon, while the cognate aminoacyl-tRNA synthetase has been evolved to charge a specific unnatural amino acid on the mutant tRNA. The unnatural amino acid is supplied in the media. Expression of all the system components leads to the incorporation of an unnatural amino acid at the specified site of the growing polypeptide chain. To develop this system in *C. elegans*, tRNA/aminoacyl-tRNA synthetase pairs were assessed to find candidates that express functional and aminoacylated tRNAs. Using body wall muscle as a pilot tissue, the components of the system were validated using a mutagenized fluorescent protein as a reporter for unnatural amino acid incorporation, and quantitative expression data was collected for several unnatural amino acids using a mutagenized firefly luciferase reporter. Further study into the potential functions of this tool includes suppressing amber alleles of *C. elegans* genes. In the future, this system could be used for a variety of experiments, including tissue specific CHIP or visualization of protein interactions in live animals. [1] Expanding the genetic code for biological studies. Wang, Q., Parrish, A.R., Wang, L. **Chem Biol.** 2009 16(3): 323-36.

1184C

A Three-pronged Method Employing *Caenorhabditis elegans* for Large Scale Screening of Potential Pharmacological Agents against Alzheimer's Disease. **Shreesh Raj Sammi**, Supinder Kaur, Pooja Jadiya, Aamir Nazir. Division of Toxicology, Central Drug Research Institute, CSIR, Lucknow, India.

Large scale screening of potential pharmacological compounds is usually a pilot task requiring larger efforts. Efficient and quick screenings rely on established models that can provide initial information on a high-throughput scale with a better sensitivity. Diseases like Alzheimer's disease (AD) have found no cure yet; though there has been an immense increase in understanding of the disease employing non-mammalian model systems like nematode C. elegans. The broad understanding that has been achieved, thus far, proves beyond doubt, the involvement of multiple factors that lead to devastating neurodegenerative AD. Amongst various factors involved, increased amyloid beta aggregation, increased reactive oxygen species and decreased acetylcholine levels are the hallmarks of AD. Thus any potential pharmacological agent or a possible combination therapy should be targeted at these multiple factors. Studies in this direction require robust model systems that could be used for high throughput screening of chemicals. In our research studies, we employ transgenic nematode strain CL2006 that expresses 'human' amyloid beta and serves as a model for Alzheimer's disease. The model has previously been shown to exhibit aggregation of amyloid beta and a proteomic expression pattern similar to human Alzheimer's patients. Herein we propose the use of a transgenic C. elegans based three pronged methodology that, we believe, could be immensely beneficial in carrying out large scale screening campaigns of potential pharmacological agents exhibiting anti-Alzheimer activity. Specifically we propose that studies on: a) amyloid beta expression (qualitative and quantitative), b) Acetylcholine activity and c) quantification of reactive oxygen species (ROS), in transgenic C. elegans model of Alzheimer's disease, could prove to be an effective regime of tests towards carrying out large scale screening campaigns. Since Alzheimer's disease is a multifactorial disease, a set of three tests targeting different factors could help in identifying potential pharmacologicals specifically suited to treat the multiple factors of the disease. Our studies demonstrate the feasibility of these methods aimed at establishing a sensitive test regime for screening drugs against Alzheimer's disease.

1185A

Update on the use of drug selection in nematodes. **Jennifer I. Semple**, Ben Lehner. Systems Biol Unit, Ctr Genomic Regulation, Barcelona, Spain.

We recently described the use of puromycin to select large populations of transgenic worms from lines carrying extrachromosomal arrays with the resistance gene (Nat Methods. 2010; 7(9):725-7.). Now, we have extended the technique to select for transgenic worms following bombardment. We have introduced some modifications in the standard bombardment protocol and created a three way Gateway compatible plasmid expressing both puromycin and neomycin resistance genes to make drug selection possible, despite the extremely high dilution ratio of transgenic to non-transgenic worms. The new protocol makes it possible to pick adult F1 generation worms only 8 days after bombardment, significantly faster than the most widely used selection protocol with *unc-119*. Furthermore, this drug selection regime does not require any particular genetic background and we have successfully generated lines with integrated transgenes in worms from various different strains and species, namely *C. elegans* (N2, CB4856), *C. briggsae* (AF16, HK104) and *C. remanei* (PB4641).

1186B

High throughput identification of mutations affecting aggregation using a novel Illumina sequencing analysis pipeline. **Katherine P. Weber[1]**, Felix Baier[1], Subhajyoti De[2], Mario de Bono[1]. 1) Cell Biology, MRC LMB, Cambridge, United Kingdom; 2) DFCI Biostatistics & Computational Biology, Harvard University, Boston, Massachusetts.

The standard *C. elegans* reference strain, N2, feeds in isolation, but wild-caught *C. elegans* aggregate on food or after starvation. Failure of N2 to aggregate is due to a gain-of-function mutation in the neuropeptide receptor *npr-1*: when this gene is knocked out N2 strongly aggregate. To identify molecules that promote or inihibt aggregation we mutagenized, in a series of independent screens, *npr-1(null)* or N2 animals and isolated mutants that suppressed or enhanced aggregation. This approach has been very fruitful: we have identified a number of genes involved in aggregation behavior. However, traditional genetic mapping and gene cloning is time-consuming, and recent work from other groups has shown that Illumina sequencing can be used to more rapidly identify mutations (Sarin *et al.* (2008); Doitsidou *et al.* (2010); Zuryn *et al.* (2010)).

To increase the throughput of aggregation gene cloning, we have established a pipeline to sequence more than 200 uncloned modifiers of aggregation using Illumina technology. Many of these mutants have been mapped roughly (usually to ~1 Mb) so examining their genome sequences allows us to rapidly identify the lesion responsible for their phenotype.

We have adapted a protocol from Dan Turner at the Sanger Institute to make amplificaton-free libraries and sequenced these on both the Illumina Genome Analyzer II (GAIIX) and on the Illumina HiSeq machine. The HiSeq has increased our output approximately six-fold. We have also assembled a bioinformatics pipeline to analyse the gigabases of data generated. Our downstream analysis can be completed on an entire flow cell (7 lanes of sequence) in approximately 2 days: it uses the BWA (Burrows Wheeler Algorithm) program to align filtered sequence reads to the reference genome, further filters the alignment output for high confidence polymorphisms, and then implements a custom pipeline to highlight which of these introduce exonic and regulatory changes, indicate genes affected and predict specific coding changes.

1187C

Computer automated forward-genetic screening using sub-cellular fluorescent reporters. **Matthew M Crane**[1], Peri T Kurshan[2], George J Wang[2], Jeffrey N Stirman[1,3], Kang Shen[2], Hang Lu[1,3]. 1) Bioengineering, Georgia Tech, Atlanta, GA; 2) Department of Biology, Howard Hughes Medical Institute, Stanford University, California, USA; 3) School of Chemical and Biomolecular Engineering, Georgia Tech, Atlanta, GA.

Here we present a comprehensive approach composed of a computer-driven pattern recognition system and a microfluidic system for phenotyping and screening fluorescent-based synaptic reporters in C. elegans. As fluorescent reporters have become increasingly subtle, phenotyping and screening for novel genes affecting these phenotypes has become an increasingly critical bottleneck handicapping C. elegans research. Imaging and phenotyping for subtle fluorescent expression is limited to manual microscopy, which can be slow, labor intensive and qualitative. To overcome these hurdles, we present a rapid microfluidic device that operates autonomously and employs high-level image recognition methods to automatically identify and phenotype animals in forward genetic screens.The unique and robust microfluidic platform developed for this task allows for automated handling of thousands of animals in a reliable and repeatable fashion to image and sort based on sub-cellular reporters using high-magnification. This microfluidic platform improves upon previous generations by allowing sustained operation for hours without failure and with no human intervention. For images of each animal in the screen library, the pattern recognition system uses a combination of local and regional information to automatically identify synapses with >99.99% accuracy, even if the reporter is dim and photobleaches quickly. Once synapses and landmarks are detected, a large number of specific features (such as distance between synapses, fluorescence intensity, distance to landmarks) are extracted and used to define the phenotypical space. The location of each animal on this phenotypical space is then used for classification as either wild-type or mutants.

We used the well-characterized DA-9 motorneuron synapses for our screens, primarily using a GFP-tagged synaptic vesicle marker, Rab-3. Using this system we have screened thousands of animals and identified >30 mutants with altered synaptic phenotypes including altered synapse locations, intensity of fluorescence reporter, synapse morphology, and number of synapses. We are characterizing several mutants from this screen, including one with a striking morphological phenotype: axons contain enlarged spine-like protrusions filled with synaptic markers. The results demonstrate the power of combining computer vision and automated microfluidics in large-scale genetic studies.

1188A

Automated sub-cellular imaging for drug screening in *C. elegans*. **Francesca Farina**, Betty Peignelin, Rémi Vernet, Clément Meiller, Frédéric Parmentier, Christian Néri. Inserm U894, Neuronal Cell Biology & Pathology, 75014 Paris, France.

Screening for drugs at the sub-cellular level may enhance drug discovery and development for degenerative disease. The nematode *C. elegans* has potential for drug screening at the sub-cellular level and in vivo as it is transparent at all stages of development and can be grown in multi-well plates. To develop drug screening at the sub-cellular level in nematodes, we took advantage of the Plate Runner HD® (Trophos, France), a 96/384-well device that collects fluorescence at resolutions ranging from 1024x1024 (1 px is 7.4 µm) to 8192x8192 (1 px is 1 µm). This device has high depth-of-field (about 40 µm at resolution of 7.4 µm; 8 µm at resolution of 1 µm), thus allowing fluorescent signals to be quantified from whole animals after paralysis. This device also has a wide-field objective that allows a single image of the whole well to be acquired at once.

We developed a drug screening assay at the sub-cellular level to search for compounds that may protect from the early-stage cytotoxicity of mutant PABPN1, the oculopharyngeal muscular dystrophy (OPMD) protein. Transgenic nematodes co-expressing nuclear GFP and mutant PABPN1 (PABPNA-A13) in body wall muscles show defective motility, a phenotype that is accompanied by a progressive loss of nuclear GFP signals. These phenotypes are aggravated by sirtuin (*sir-2.1*/SIRT1) activation and ameliorated by *sir-2.1* inhibition, and they can be also manipulated by pharmacological means [1,2]. The loss of GFP nuclei in mutant PABPN1-A13 animals provide an easily-assayable phenotype to screen for drugs that may rescue mutant PABPN1 toxicity. Results from screening greater than 2000 compounds indicated that our screen was robust and selective. As part of a drug discovery program on neuromuscular diseases, we are also exploring the development of a drug screening assay at the sub-cellular level to search for compounds that protect from defective axonal transport. To this end, we generated transgenic nematodes that express extrachromosomal arrays encoding a fluorescent pre-synaptic reporter in GABAergic motor neurons and that carry a temperature-sensitive loss-of-function allele in a gene encoding an axon motor gene. Preliminary results suggest that, at the restrictive temperature, this mutant allele induces a significant change in the expression pattern of pre-synaptic signals. This effect is unrelated to a change in transgene expression and can be quantified by the Plate Runner HD after paralysis of the animals in the 96-well plate. Results from the two approaches abovementioned will be presented and discussed.

[1] Catoire, H. *et al.* (2008) Hum Mol Genet 17, 2108-2117. [2] Pasco, M.Y. *et al.* (2010) J Med Chem 53, 1407-1411.

1189B

High-resolution *in vivo* Ca^{2+} imaging of neuromuscular system in *Caenorhabditis elegans*. **K. Gengyo-Ando**[1], A. Usami[2], Y. Nagamura[1], Y. Yoshida[1], N. Matsuki[2], Y. Ikegaya[2], J. Nakai[1]. 1) Brain Science Institute, Saitama University, Saitama, Japan; 2) Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.

Animal locomotion is based on the coordination of rhythmic muscle contractions, but fundamental mechanism underlying the rhythmic locomotion has not been fully understood. In *Caenorhabditis elegans*, which has a simple nervous system, the structural connectivity of motor neurons and muscles is well defined; however, their functional dynamics during locomotion still remain unexplored. In this study, we conducted large-scale and high-resolution Ca^{2+} imaging of the neuromuscular system in freely behaving *C. elegans* using G-CaMP4, a newly designed genetically encoded calcium indicator (GECI). We identified highly coordinated Ca^{2+} transients in muscle cells during sinusoidal movements, and the G-CaMP fluorescence correlated positively with the velocities of the Ca^{2+} waves and animal locomotion. Ca^{2+} imaging of neurons and muscles from wild-type and GABA-deficient mutant animals, respectively, revealed that GABA plays multiple and distinct roles in the forward and backward locomotion. Furthermore, Ca^{2+} transients in the body wall muscles during switching from forward to backward locomotion were different from those during switching from backward to forward locomotion. Our data indicate that forward and backward locomotion recruits different regulatory neuromuscular circuits in *C. elegans*.

1190C

Endrov - an open source framework for image processing and analysis. **Johan Henriksson**[1], Jürgen Hench[1,2], Thomas R Bürglin[1]. 1) Dept Biosciences & Nutrition, Karolinska Institutet, Stockholm, Sweden; 2) Department of Pathology, University Hospital Basel, Switzerland.

During our work on Virtual-Worm Base (a database of spatio-temporal gene expression patterns during C. elegans development), we found that the current tool chain for microscopy is insufficient. Commercial software did not provide the necessary flexibility and could not adapted for our purposes. We also evaluated free solutions. But we found that, for example, ImageJ, the de facto-standard image software, is not designed for demanding 4D microscopy needs. Adding the necessary code would be difficult, and still not result in the desired end product.To solve these problems we have developed a new platform - Endrov (www.endrov.net) - that covers the entire microscopy chain. It is a plug-in framework consisting of 160 000 lines of Java, running on all operating systems. The following are Endrov's features

- It has advanced control of the microscope hardware (via Micro-manager)
- It can view, annotate, process and analyze recordings. New functional modules can be implemented either with a graphical programming language (flows) if they are simple, with interpreted Java, or with new plugins. Endrov can also be used as a library, allowing therefore batch processing of large data sets.
- Presently, there are about 130 image processing filters and 80 plugins available in Endrov. An important aspect is image data handling; a new scheme for data storage was developed which handles arbitrary metadata, 6D recordings, mixed resolutions and compression (both lossy and lossless). This allows handling of large data sets, and - given todays hardware limitations such as hard-drive data transfer speeds - the speed of most operations is unaffected by the size of datasets. Endrov supports most file formats (via Bio-formats).

Our framework is open and free of charge. It is highly flexible and can be adopted to any needs in current research, in fact it is not limited to *C. elegans*. As an application example, we have used our framework to quantify the expression levels of a large number of genes (see abstract: J. Henriksson et al,) and to study one of them in particular detail (see abstract: Lois Tang).

1191A

In vivo calcium imaging of the pharyngeal muscles in freely moving animals. **I. Kotera**[1], L. Su[1], S. Wong[2], K. Truong[2], H. Suzuki[1,3]. 1) Tanz CRND, University of Toronot, Toronto, ON, Canada; 2) Inst of BBE, University of Toronto, Toronto, ON, Canada; 3) Dep Physiology, University of Toronto, Toronto, ON, Canada.

Imaging Ca^{2+} concentrations in live cells provides temporospatial dynamics of cellular activities, which is of vital importance in understanding how multi-cellular systems function. The first *in vivo* Ca^{2+} imaging in *C. elegans* has been established in the pharynx using a genetically encoded Ca^{2+} probe, cameleon; the pharynx is an attractive model for its neuronal regulation because the pharyngeal nervous system has few neurons, and functions semi-autonomously. However, previously reported imaging methods require immobilization of the worms and exogenously added serotonin just to observe pharyngeal activity. We have developed a new imaging system, which combines two independent imaging units to enable two simultaneous imaging, along with “worm tracker” functionality to perform imaging on a freely moving worm. We have also developed a pattern matching algorithm, which recognizes the expression pattern of the probe, and automatically extracts Ca^{2+} dynamics of the pharynx. Pharyngeal Ca^{2+} dynamics of freely-moving worms was measured in the presence of serotonin. Both the rise time (0.14s) and firing rate (3.14 Hz) of the transients in the pharynx are in good agreement with the previous report, confirming the validity of our new system. The free worms showed pharyngeal activity even in the absence of exogenous serotonin, albeit a reduced firing rate (0.24 Hz). The rise slope of the transients stayed relatively unchanged with or without serotonin (106%/s and 65%/s), but the rise duration

has increased from 0.14s with serotonin to 0.39s without it. With an incorporation of new GCaMP, our result has demonstrated over 10-fold improvement in both the temporal resolution and dynamic range when used on our system. By employing all the techniques developed, an intriguing relation was revealed between the pharyngeal firing rate and locomotion direction. When worms are moving forward, the firing rate was similar to what we have measured with the serotonin-stimulated worms. However, when the worms autonomously reversed its direction, the firings quickly ceased; this pharyngeal firing was restored when the worm resumed forward movement. Thus we have developed a novel imaging system which is capable of concurrently performing Ca^{2+} imaging and transmission image acquisition on a freely moving worm. The ability to record multiple behaviors of the worm provides a unique opportunity to investigate how multiple systems are interacted and coordinated; further investigation of the seemingly isolated pharyngeal and somatic nervous systems should yield a compelling model for the inter-systems relation.

1192B

The Metal Biology of *C. elegans*. **Gawain McColl**[1,2], Simon James[3], Blaine Roberts[1,2], Martin de Jonge[4], Robert Cherny[1,5], Ashley Bush[1,5]. 1) Mental Health Research Institute, University of Melbourne, Australia; 2) Center for Neuroscience, University of Melbourne, Australia; 3) Commonwealth Science and Industrial Research Organisation, Australia; 4) Australian Synchrotron, Australia; 5) Department of Pathology, University of Melbourne, Australia.

Metal ions are essential for life, with ~30% of all proteins being metalloproteins. The metallome (the metal complement of cells and tissues with associated proteins and transport systems) plays a critical role in many fundamental biological processes. Metal ion cofactors enable electron transfer reactions, providing catalytic potential as well as having significant effects on protein stability. Redox active transition metals (like Fe and Cu) are intrinsically involved in the generation of free radicals and highly reactive oxygen species (ROS). ROS have required roles as secondary messengers in cellular signal transduction and innate immunity. However, free radicals can also damage cellular macromolecules and ultimately impair function. There has been much interest in oxidative stress as a contributor to pathogenesis of many diseases. However, despite the fact that metal-mediated catalysis is the origin of most oxidative stress, remarkably little work has focused on metals. Until now the means to visualize and characterize metals in biology have been lacking. Furthermore, assessing the elemental composition of complex structures, such as cells and organelles, without destroying the intricate distribution has been a significant challenge. Developing analytical technologies partnered with an appropriate model system, like *C. elegans*, offers an ideal means to study the eukaryotic metallome. X-ray fluorescence microscopy (XFM) is well suited for investigating elemental distributions within biological specimens. Using XFM imaging we have mapped elements (Sulfur to Zinc, 16<Z<30) at ~1 μm resolution in adult *C. elegans*. Subsequent tomographic analysis has allowed identification of features not readily discernible in 2-D. To compliment these spatial data we have begun to apply a metal-based approach to systematically characterize and identify the native metal-protein complexes of *C. elegans*. We have combined native size exclusion chromatography (SEC) with Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) to characterize and identify protein-metal complexes.

1193C

The GLOWorm repository & GLOWormJ: a system for distribution and analysis of multidimensional embryological images. **William A. Mohler**, Ion Moraru, Ariel B. Isaacson, Frank Morgan, Dan Vasilescu, Jeffrey Dutton, Glenn Duzy. Dept Genetics & Dev Biol and Center for Cell Analysis & Modeling, Univ Connecticut Health Ctr, Farmington, CT.

All wild-type *C. elegans* embryos re-enact the same series of cell divisions, deaths, movements and fates within a nearly identical space and time-scale. 4-dimensional recordings of embryos expressing single fluorescent transgenes are, therefore, conceptually superimposable for synchronized comparison and correlation. Technical hurdles that prevent such comparisons from being more commonly useful include diverse image data types, the sheer size of the data, and the likelihood that any single embryo might vary slightly in its spatial dimensions, its rate of development, or stochastic quirks in its cells' positioning and movements. Our system allows anyone in the community to access the contents of multi-gigabyte data sets via a web-facilitated file-sharing interface, downloading only those fragments of 4D movies that are of particular interest to each viewer. Visualized scenes can combine an arbitrary collection of different genotypes presented in synchrony, with interactive controls for fitting each to a common spatiotemporal frame. A variety of public-domain algorithms packaged within the GLOWormJ adaptation of ImageJ permit enhancements, measurements, and annotation of features within each embryo. The saved adjustments and markings for any embryo in the collection can be recalled along with those of other embryos to foster progressively better comparisons and richer insights.

1194A

Investigation of Low-cost Fluorescence Microscopy. **Andy Papp**, Chris Aldrich, David Perry. Tritech Research, Los Angeles, CA. www.TritechResearch.com.

Small genes encoding fluorescent proteins (e.g. EGFP, mCherry and dsRed), are used extensively as visible markers to study the location and timing of gene expression. Fluorescent fusion proteins are especially well-suited to studying *in vivo* gene expression patterns in the transparent *C. elegans* (Tursun et al., 2009). Perhaps one of the most significant limitations to their use is the high cost of the equipment needed to observe fluorescent proteins microscopically — the fluorescence dissecting stereomicroscope for screening and picking mutant worms and epi-fluorescent compound microscopes for more detailed studies.

Commercially available fluorescence dissecting stereomicroscopes typically cost between US$12,000 and US$50,000. They are offered by only the "high-end" microscope companies, based upon their most expensive dissecting scopes, and incorporate premium-priced mercury arc-lamp illuminators, power supplies and epi-fluorescence modules. In a previous study, we demonstrated that significant cost savings could be achieved in EGFP detection by substituting high-flux blue spectrum LEDs for the mercury arc-lamps, and incident illumination for epi-illumination, if appropriate high-quality optical filters were employed (Papp et al., 2009). The resultant US$7,000 prototype system was compared with a US$50,000 top-of-the-line Leica fluorescence dissecting stereomicroscope system using the same plate of EGFP positive worms and the systems gave similar results.

In the current study, we investigate the possibility of extending our findings to fluorophores with different spectral properties, such as dsRed and mCherry. We examined various LEDs combined with different excitation and emission filters. While the commercially available high-flux green spectrum LEDs that we studied do not have emission peaks that line up well with the excitation peaks of the red fluorophores, we were still able to demonstrate a highly usable level of detection by using appropriate optical filters. Since the human eye and fluorescence cameras can detect very low light levels, the absolute fluorescence level is not as important as the fluorescence signal-to-noise ratio. We will investigate sources of noise and present data (possibly with a demonstration) regarding techniques to improve the signal:noise ratio by reducing background fluorescence.

1195B

Characterizing development by lineage tracing. **Julia L Richards**, Joshua Burdick, Travis Walton, Elicia Preston, John Murray. Genetics, University of Pennsylvania, Philadelphia, PA.

We previously developed automated lineage tracing methods and used these methods to characterize the expression pattern of over 120 gene reporters in C. elegans embryos through the 350-cell stage. Current work in our lab aims to leverage and improve this technology to understand how the lineage controls fate specification. Using a new resonance-scanning confocal microscope, we can collect images with improved axial resolution (0.5 micron) and higher throughput (4-5 embryos at once) while maintaining normal development. By combining the improved images with improved image analysis methods, we can trace the lineage of C. elegans embryos to ~600 cells with about 8 hours of manual curation. We are using a collection of wild type cell lineages to quantitatively analyze normal variability in cell division timing and orientation and cell migration at later stages of embryogenesis, allowing us to more comprehensively answer the question "how invariant is the cell lineage of C. elegans?" We are using these improved methods to understand how gene expression is regulated across the lineage during development. The existing collection of gene expression data shows different spatial and temporal patterns for transcription factors (TFs). We are testing regulatory models within individual cell lineages by using RNAi to perturbing TFs expressed early and measuring changes in potential targets expressed later in the same lineage. We have tested this approach with pal-1, a known regulator of C lineage fate; for some genes, RNAi depletion of pal-1 results in loss of C lineage expression as expected, while for others it results in altered patterns of C lineage expression rather than loss. We are currently expanding this approach to candidate novel lineage regulators and also to understand how developmental decisions are limited to the appropriate time. Regulatory network inference would be more powerful if we knew how all genes' expression varied across the lineage. Genome-wide analysis of FACS sorted cells allows analysis of particular cells or cell types, but fluorescent reporters uniquely and specifically expressed in each of the 558 embryonic cells do not exist, and the cost of sorting and analyzing each of the 558 cells individually would be substantial. We have generated and tested a theoretical framework for deconvoluting the full lineage expression pattern from a smaller set of FACS experiments on 30 reporters chosen to maximize information content. Simulations suggest that this approach could generate high-resolution and accurate measurements of all genes' expression across the full lineage.

1196C

Subcellular *in vivo* time-lapse imaging and optical manipulation of *C. elegans* in standard multiwell plates. **Christopher Rohde**, Mehmet Fatih Yanik. Massachusetts Institute of Technology, Cambridge, MA.

High-throughput high-resolution *in vivo* time-lapse assays require repeated immobilization and imaging of large numbers of organisms. We report a rapid, simple, and minimally invasive technology for screening *Caenorhabditis elegans* at cellular resolution over its entire lifespan entirely inside standard multiwell plates alone using repeated immobilization, imaging and optical manipulation (in press, *Nature communications*). Recently, we and others developed microfluidic approaches for immobilization of *C. elegans* for high-throughput and high-content screening. Despite their capabilities, microfluidic systems are still too complex to manufacture, operate, maintain and scale for mainstream use. Integration of these techniques into existing high-throughput screening (HTS) platforms in order to enable delivery of library compounds to *C. elegans* incubated on-chip has been too challenging because of well-known 'world-to-chip' fluidic interface issues. Although *C. elegans* can be anaesthetized in HTS-compatible multiwell plates for immobilization and imaging, the effects of anesthesia are slow and variable and removal of the anesthetic media without losing animals is highly unreliable. Here, we demonstrate a simple method to rapidly image and optically manipulate *C. elegans* at cellular resolution in standard multiwell plates at frequent time points without the use of anesthesia. Our

technology is fully compatible with existing instruments, robotics and protocols used in industrial HTS platforms. Furthermore, our system does not use any fluidic or mechanical components, and can operate for tens of thousands of cycles without failure. It allows forward and reverse genetic and chemical screens to be performed at cellular resolution and can screen an entire 96-well plate in approximately 30 minutes. Animals are immobilized only during the brief period they are imaged, thus minimizing stress and frequent immobilization and imaging of *C. elegans* over several days does not affect animal health. This technique also enables the use of subcellular-precision optical manipulation methods such as *in vivo* femtosecond laser microsurgery, and we use this to study the time-dependent regeneration dynamics of single neurons after microsurgery in standard multiwell plates. Our single-neuron in vivo time-lapse analysis shows that the neurite regrowth over short time windows is greater than that predicted by ensemble averaging over many animals. Our technology can significantly accelerate most *C. elegans* investigations, especially developmental and lifespan studies which require repeated imaging and tracking of thousands of individual animals over an extended duration.

1197A

Cell lineaging for single cell, high throughput analysis. **Anthony Santella**, Zhirong Bao. Developmental Biology, Sloan Kettering Inst, NY, NY.

The study of embryonic phenotypes at high detail and large scale requires accurate and fast assembly of detected nuclei into a cell lineage. Accuracy is essential. Every error limits analysis, requiring either manual correction or a retreat from the goal of single cell tracking. In addition, high throughput work limits the effort that can be spent on correction and quality control of results. The time it would take to confirm, unguided, the correctness of even a perfectly accurate cell lineage may be unsupportable. As such, a measure of the local reliability of results is just as important as low error. Existing methods do not to provide this combination of features. Common, simple methods such as nearest neighbor association across time are error prone and lack a model that can provide confidence in results. More sophisticated general tracking methods such as particle filters lack an explicit model of cell division, a key source of ambiguity and error. These requirements lead to an approach that scores lineage configurations against a learned model. The key design decision is what aspects of the lineage, as revealed via imaging and cell detection, to include in the model used to judge alternative tree configurations. Our desire is for a general method applicable to any embryo capable of normal cell division. As such, we model key local behaviors of nuclei, firstly their individual change over time (in appearance and position), and secondly the correlation in these measures expected between daughter cells at division. We also model two key aspects of the detection method that provides the raw nuclear positions for lineage assembly, firstly the relationship between nuclear appearance and the probability of a detection being a false positive and secondly the probability of a hypothesized series of detection failures. This model, which can be learned from a set of corrected lineages, is relatively simple but sufficient to produce accurate results and to highlight ambiguous areas for human inspection.

1198B

Development of a High Throughput Infrared Detection Method for Toxicity and Pharmacological Testing in *C. elegans*. Dario R. Bichara, **Sergio H. Simonetta**. Fundacion Instituto Leloir, Buenos Aires, Argentina.

Although *Caenorhabditis elegans* has raised the understanding of new drugs mechanism, its potential application for pharmaceutical drug discovery requires novel techniques with high throughput screening capability. In this concern, the analysis of animal movement has been successfully employed to study *C. elegans* viability, stress resistance and lifespan. This particular behavior is the result of complex interactions between neural networks, cell physiology and molecular pathways, some of them target of pharmacological compounds. We had previously developed an automated method to quantify *C. elegans* movement in which swimming worms are detected as they cross through infrared microbeams. In this work we present the adaptation of this methodology to implement it for high throughput analyses. In this regard, we had developed a 384 channel apparatus and successfully recorded the behavioral changes produced by toxic compounds, environmental modulation and aging. Worm populations were cultured in 96 or 24 well microplates using liquid buffer or NGM medium, and their swimming behavior was automatically estimated from infrared sensor information. The IR-microbeam interruption rate showed a linear correlation with animal population size and length of recorded period. This readout allowed us to determine the lethal concentration (LC50) for standard chemicals (Na, K, Cd, Cr and SDS), evaluate metabolic modulators (mutations and temperature effects), and perform automatic lifespan measurements in non-invasive long-term experiments. This system, that we have named ”Worm Microtracker”, might be useful for the worm community to develop toxicity and paralysis assays in an easier and faster way, opening the possibility of performing high throughput studies in *C. elegans*.

1199C

Addition of the genetically encoded, red-shifted Ca2+ sensor RCaMP to the *C. elegans* optogenetic toolbox. **Sebastian Wabnig**[1], Jasper Akerboom[2], Jeffrey Stirman[3], Hang Lu[3], Loren Looger[2], Alexander Gottschalk[1,4]. 1) Institute of Biochemistry, Goethe-University Frankfurt, Germany; 2) Janelia Farm Research Campus, Ashburn, USA; 3) Georgia Institute of Technology, Atlanta, USA; 4) Frankfurt Molecular Life Sciences Institute, Goethe-University Frankfurt, Germany.

Calcium is a universal 2nd messenger affecting a broad variety of cellular events. To assess Ca2+ signalling in vivo, several groups developed genetically encoded calcium indicators (GECIs). These include GCaMPs (~470nm ex.) or Cameleons (~415nm ex.) to detect Ca2+ responses1,2,3. To stimulate neurons in live animals, we have introduced Channelrhodopsin-2 (ChR2), photoactivated adenylyl cyclase (PACα), and bacteriorhodopsin as optogenetic tools which are excited by blue light (~470nm)4,5,6. These are useful tools to investigate effects of stimulation of specific neurons, but until now, investigating C. elegans neural network responses depends more or less on behavior as a readout for network function4,5,6. Since all of the current GECI are based on GFP, CFP or YFP1,2,3, simultaneous PACα or ChR2 (~470 nm) based optogenetic stimulation combined with calcium imaging requires complicated setups to handle the overlapping excitation spectra of these tools7. To overcome these problems, we introduced a newly red-shifted GECI to the C. elegans optogenetic toolbox. RCaMP is a red fluorescent protein, coupled to calmodulin and M13 domain, which increases its fluorescence emission intensity in response to Ca2+ binding. We expressed RCaMP under the pmyo3 promoter in body wall muscles (BWMs) of animals expressing ChR2 from the punc-17 promoter in cholinergic motor neurons (transgene zxIs6). Measurements were done on an inverted fluorescence microscope equipped with two high power light-emitting diodes (LEDs; 590 and 470nm) coupled with a beam splitter that allowed simultaneous illumination at two wavelengths. Separating the excitation wavelengths 470nm (for ChR2) and 590nm (for RCaMP) allowed Ca2+ measurements in the BWMs without excitation of ChR2. Here we show RCaMP based Ca2+ measurements of BWM activation caused by ChR2-mediated photostimulation of cholinergic motor neurons. We currently test the possibility to analyze neuronal networks using both tools in neurons. The RCaMP/ChR2 combination promises a wide range of applications to investigate cell-cell interactions and network function in live animals, even if no behavior is evoked, on a simple imaging setup. 1 Tian et al. (2009), Nat Meth 6, 875-81 2 Nakai et al. (2001) Nat Biotech 19, 137-41 3 Miyawaki (1997) Nature 388, 882-7 4 Nagel et al. (2005) Curr Biol 15, 2279-84 5 Weissenberger et al (2011) J Neurochem 116, 616-25 6 Stirman et al. (2011) Nat Meth 8, 153-8 7 Guo et al. (2009) Nat Meth 6, 891-896.

1200A

Automated alignment of TEM image stacks to simplify anatomical reconstruction. Greg Hood[1], **Arthur Wetzel**[1], Meng Xu[2], David Hall[2], Scott Emmons[2]. 1) National Resource for Biomedical Supercomputing, Pittsburgh Supercomputing Ctr, Carnegie Mellon Univ, Pittsburgh, PA; 2) Albert Einstein College of Medicine, Bronx, NY.

The National Resource for Biomedical Supercomputing (NRBSC) conducts core research at the interface of supercomputing and the life sciences and NBRSC scientists develop collaborations with biomedical researchers around the country. As part of this effort we have developed methods for assembling, viewing and analyzing extremely large volumetric datasets, such as serial section transmission electron microscopy (SSTEM) image stacks, that presently reach more than 10 Tvoxels from a single specimen. SSTEM currently provides the most cost effective means to capture the full anatomical detail of neural circuits in C. elegans and other organisms. However, the usefulness of large SSTEM stacks has often been limited by the difficulties of studying them in an accurate 3-dimensional context. The NRBSC has developed a suite of programs to assemble coherent volumetric datasets by registering TEM images in both 2D and 3D and providing uniform contrast and brightness transforms so that researchers can easily trace structures, such as neurons, through complex 3-dimensional paths that often span hundreds of microns. A version of this software has been deployed on a SUN computing cluster at the Albert Einstein College of Medicine (AECOM) to enable large scale C. elegans nervous system studies by David Hall and Scott Emmons' research groups. In this case the output of the registration process feeds into additional analysis processes that have been developed at AECOM. The biological results of those studies are described in separate presentations at this conference. This poster outlines the processes used to produce aligned volumes using our methods. Although the software is primarily designed for application to the largest possible image sets by applying high-performance parallel computing it can also be used to align smaller datasets on laptop or desktop Linux/UNIX computers.

1201B

Rapid and accurate phenotyping of embryonic lethality via image analysis of *C. elegans* developmental stages from high-throughput image data. **Amelia G White**[1,2], Huey-Ling Kao[1], Patricia G Cipriani[1], Eliana Munarriz[1], Annalise Paaby[1], Davi Geiger[3], Eduardo Sontag[4], Kristin C Gunsalus[1], Fabio Piano[1]. 1) Center for Genomics and Systems Biology and Department of Biology, New York University, New York, NY, USA; 2) BioMaPS Institute, Rutgers University, Piscataway, NJ, USA; 3) Department of Computer Science, New York University, New York, NY, USA; 4) Department of Mathematics, Rutgers University, Piscataway, NJ, USA.

We present an automated image analysis system (DevStaR) for quantitative phenotyping of *C. elegans* embryonic lethality and sterility phenotypes. Our image analysis system counts each developmental stage in an image of a *C. elegans* population, allowing efficient high throughput calculation of *C. elegans* viability phenotypes. DevStaR is an object recognition machine comprising several hierarchical layers that build successively more sophisticated representations of the objects (developmental stages) to be classified. The algorithm segments the objects, decomposes the objects into parts, extracts features from these parts, and classifies them using an SVM (support Vector Machine) and global shape information. This enables correct classifications in the presence of complicated occlusions and deformations of the animals. Features of the classified objects are then used to obtain a count of each developmental stage. We are currently using this system to analyze phenotypic data from *C. elegans* high-throughput genetic screens, and have processed over one million images for lab users so far. Validation of DevStaR measurements will be shown by comparing DevStaR output to both manual counting of developmental stages and manual

scores of quantitative phenotypes. DevStaR can provide an accurate measurement of quantitative phenotype and is comparable to manual scoring. DevStaR has been used to score a *C. elegans* genome wide RNAi screen with up to 30 repeats per clone tested at up to 5 temperatures per clone. The screen consists of over 600,000 images each scored by DevStaR, Analysis of these data illustrate the convenience of DevStaR scoring and the use of a quantitative phenotype. Our system overcomes a previous bottleneck in image analysis by achieving near real-time scoring of image data in a fully automated manner. Our system reduces the need for human evaluation of images and provides rapid quantitative output that is not feasible at high throughput by manual scoring.

1202C

Cell type-specific profiling of the transcriptome in *C. elegans*. **Meenakshi K. Doma**[1], Igor Antoshechkin[2], Paul Sternberg[1]. 1) HHMI and Dept Biol, California Inst Technology, Pasadena, CA; 2) Millard and Muriel Jacobs Genetics and Genomics Laboratory, CALTECH, CA.

Cell type-specific gene expression patterns underlie much of biology including development, physiology and behavior. Currently, analysis of cell-specific gene expression in *C. elegans* include strategies such as microarray analysis of RNA from surgically dissected tissue or sorting of GFP-tagged embryonic culture cells and an mRNA tagging technique used to profile specific cells and tissues. Each of these methods is limited in their applications. We are developing a new strategy to isolate RNA from specific cells that will enable cell type-specific transcriptome analysis in *C. elegans*. Recent studies have shown that uracil phosphoribosyltransferase (UPRT) from *Toxoplasma gondii* can be used for biosynthetic labeling of newly synthesized RNA from specific cells in multicellular organisms like *Drosophila*. We have adapted this method to *C. elegans* and designed transgenes that express TgUPRT in a spatially-restricted manner using cell type-specific promoters. Preliminary results show that on providing the uracil analog, 4-thiouracil (TU), it is modified and incorporated in newly synthesized RNA only from cells that express the TgUPRT. Our on-going and future efforts will be directed at purification of TU-tagged RNA followed by deep sequencing using RNA-seq in the context of specific biological questions. This method will provide *C. elegans* researchers a powerful tool that may provide novel insights into the regulation of gene expression at the single cell or tissue level.

1203A

A method for purifying nuclei from different tissues. **Florian A Steiner**[1], James R Priess[1,2], Steven Henikoff[1,2]. 1) Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA; 2) Howard Hughes Medical Institute, Fred Hutchinson Cancer Research Center, Seattle, WA.

Obtaining tissue- or cell-type specific expression profile and chromatin profiles is one of the challenges in creating complete pictures of the genome landscapes. Our group recently published a simple method for the cell-type specific purification of nuclei from Arabidopsis roots[1]. The method, termed INTACT (Isolation of Nuclei TAgged in specific Cell Types), relies on a nuclear envelope tag that is biotinylated in vivo, which allows for affinity purification of the biotin-tagged nuclei. We have adapted the INTACT method to C. elegans tissues. In a first proof of principle strain, an outer nuclear pore complex protein is fused to mCherry for visualization and a BLRP tag (Biotin Ligase Recognition Peptide), and its expression is driven by the muscle-specific myo-3 promoter. The E. coli biotin ligase BirA is expressed ubiquitously, mediating in vivo biotinylation. Using this approach, muscle nuclei can be efficiently purified. Specificity of the pull-down is shown by an enrichment of mRNAs from muscle-specific genes. The system will allow purification of nuclei from different tissues at different developmental stages from embryos to adults. 1) A Simple Method for Gene Expression and Chromatin Profiling of Individual Cell Types within a Tissue. Deal RB, Henikoff S. Dev Cell. 2010 18(6) 1030-40.

1204B

An assessment of the quality of histone-modification antibodies. Thea Egelhofer[1], Aki Minoda[2], Sarit Klugman[3], Kyungjoon Lee[4], Paulina Kolasinska-Zwierz[5], Julie Ahringer Lab[5], Mitzi Kuroda Lab[4], Sarah Elgin Lab[6], Mark Perry Lab[7], Vincenzo Pirrotta Lab[8], Bing Ren Lab[3], **Susan Strome**[1], Peter Park Lab[4], Gary Karpen Lab[2], R. David Hawkins[3], Jason Lieb[9]. 1) University of California Santa Cruz; 2) Lawrence Berkeley National Lab; 3) University of California San Diego School of Medicine; 4) Harvard Medical School; 5) University of Cambridge; 6) Washington University; 7) Ontario Institute for Cancer Research; 8) Rutgers University; 9) University of North Carolina at Chapel Hill.

Investigations of transcriptional regulation and chromatin organization often rely heavily on antibodies to various post-translational modifications on histones. The reproducibility and biological relevance of histone-modification studies depends on the specificity and performance of the antibodies, most of which are now provided commercially. As part of our activities in the NIH modENCODE and Roadmap Reference Epigenome initiative, we are using antibodies in conjunction with chromatin immunoprecipitation (ChIP) to determine the genomic distributions of histone modifications in *C. elegans*, *Drosophila*, and human cells. We have tested the specificity and utility of 246 antibodies raised against 3 unmodified histones and 57 different histone modifications (Egelhofer et al. Nature Struc & Molec Biol 18: 91-93). Although most antibodies performed well, more than 25% failed specificity tests by dot blot or western blot analysis. Among specific antibodies, more than 20% failed in ChIP experiments. We discuss our tests of antibody specificity and our criteria for successful performance. We advise rigorous testing of histone-modification antibodies in the organism of interest before use, and we provide a website for posting new results (http://compbio.med.harvard.edu/antibodies/).

1205C

Revising the DAF-12 ligand biosynthetic pathway in *C. elegans*: A comprehensive NMR-based approach. **Neelanjan Bose**[1], Parag Mahanti[1], Axel Bethke[1], Joshua Judkins[1], Joshua Wollam[2], Adam Antebi[2], Patrick Hu[3], Frank Schroeder[1]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 2) Max Planck Institute for Biology of Ageing, Cologne, Germany; 3) Departments of Medicinal Chemistry, University of Michigan, Ann Arbor, MI.

In *C. elegans*, the nuclear hormone receptor DAF-12 plays a central role in regulating developmental progression from larva to adult. Two steroid hormones Δ4 and Δ7 -dafachronic acids have been predicted to function as ligands of DAF-12, promoting reproductive development and suppressing entry into larval diapause (dauer)[1].

Previous investigations[1,2,3] predominantly based on indirect feeding experiments and phenotypic based proposed a putative biosynthetic pathway for Δ4 and Δ7 -dafachronic acids involving a cytochrome P450, DAF-9; a Rieske oxygenase, DAF-36 and a hydroxysterol dehydrogenase, HSD-1. Using NMR based comparative metabolomics, we show that the biosynthetic pathway for DAF-12 ligands requires substantial revision. For example, comparison of the metabolome of *hsd-1* mutants with that of hormone deficient *daf-9:daf-12* mutant worms revealed the presence of a novel endogenous DAF-12 ligand that is absent in wild-type worms. In contrast, *hsd-1* mutants lacked a major wild-type ligand proposed to be upregulated in *hsd-1*. In addition we report changes in dafachronic acid biosynthesis in several other mutant strains, including *daf-36, dhs-16* and *strm-1*.

Separate ligand biosynthetic pathways for DAF-12 leading to structurally different ligands may represent a mechanism for controlling DAF-12 function[4]. The identification of new components and the association of the known compounds to specific mutant backgrounds will provide a better understanding of the convergence of signals from the upstream dauer network: Guanylyl Cyclase, TGF-β and insulin/IGF-1 pathways on to the central regulator DAF-12.

1. Motola DL, Cummins CL, Rottiers V, Sharma KK, Li T, Li Y, Suino-Powell K, Xu HE, Auchus RJ, Antebi A, Mangelsdorf DJ (2006). *Cell* 124, 1209.

2. Rottiers, V, Motola, DL, Gerisch, B, Cummins, CL, Nishiwaki, K, Mangelsdorf, DJ, and Antebi, A (2006). *Dev. Cell*.10, 473.

3. Patel DS, Fang LL, Svy DK, Ruvkun G, Li W. (2008). *Development*. 135, 2239.

4. Dumas KJ, Guo C, Wang X, Burkhart KB, Adams EJ, Alam H, Hu PJ. (2010). *Dev Biol*. 340, 605.

1206A

Optimising metabolic profiling approaches towards an improved understanding of metabolism during development and aging in *C.elegans*. **Florian M. Geier**[1], Gabriel Valbuena[1], Sarah K. Davies[2], Jake G. Bundy[1], Armand M. Leroi[2]. 1) Surgery & Cancer, Imperial College, London, United Kingdom; 2) Department of Biology, Imperial College, London, United Kingdom.

Development and ageing are strongly interlinked with metabolism. Especially in *C.elegans* this relationships becomes apparent with the insulin like signalling pathway (IIS), mitochondrial activity and dietary restriction having a direct effect on lifespan. We recently showed (Fuchs *et. al.* 2010) that untargeted metabolic profiling (metabolomics | metabonomics) can be used to find a signature of long-life by comparing different longevity mutants and old worms.

By optimising tissue extraction protocols and incorporating chromatography mass spectrometry coupled approaches (GC-MS, LC-MS) we have refined our methodology to achieve a higher coverage of *C.elegans* metabolites (pathways) and increased sensitivity, aiming at single worm concentrations. We also use stable isotopes (^{13}C, ^{15}N) to label the *C.elegans* metabolome to help identify (novel) metabolites to increase coverage and trace the fate of dietary uptake.

By providing this array of new tools to the *C.elegans* (metabolomics) community, we hope to help improve the understanding of metabolism in this important model organism.

1207B

Connecting genotype with metabotype in *C. elegans*: 2D-NMR based metabolomics uncovers small molecules dependent on peroxisomal beta-oxidation via DAF-22. **Yevgeniy Izrayelit**[1], Steven Robinette[2], Arthur Edison[3], Frank Schroeder[1]. 1) Boyce Thompson Institute and Dept. of Chemistry and Chemical Biology, Cornell Univ., Ithaca, NY; 2) Dept. of Surgery and Cancer, Imperial College London, London, UK; 3) Dept. of Biochemistry & Molecular Biology, Univ. of Florida, Gainesville, FL.

We present a novel approach to 2D-NMR based metabolomics and demonstrate its utility for the identification of diverse sets of small molecules that differentiate genetically distinct *C. elegans* strains. We show the value of this method by comparing two *daf-22* alleles to wild type (N2) worms. *daf-22* catalyzes the final step in peroxisomal fatty acid beta-oxidation to produce ascarosides, a class of small molecules that regulate dauer formation as well as several different social behaviors. Many of these ascarosides were identified by comparing 2D-NMR spectra of wild-type and *daf-22* worms using a simple overlay technique (DANS)[1]. However, DANS was limited to detecting highly conspicuous metabolic changes. Here we report a semi-automated method for comprehensive comparison of metabolomes that combines 2D-NMR with statistical analysis.

To investigate changes in the metabolomes associated with the *daf-22* mutation, we grew liquid cultures of wild type and two different *daf-22* mutant alleles. dqfCOSY spectra, a particularly information-rich form of 2D-NMR spectra, were acquired for both the wild-type and mutant metabolomes. A peak recognition algorithm and principle component analysis (PCA) were utilized to compare the spectra from the three *C. elegans* strains. Spectral back projections of principle components (PC) were visualized as COSY-like 2D spectra to connect statistical analysis with molecular structure. PCA effectively separated the spectral data sets of the three genotypes; PC1 differentiated both *daf-22* mutant alleles from wild-type *C. elegans*, whereas PC2 separated the two *daf-22* alleles from each other. Significantly, back projection of PC loadings onto the COSY spectra enabled identification of a large number of metabolites that were up or down regulated in the *daf-22* mutant sets.

The analysis provides insight into the role of peroxisomal beta-oxidation and ascaroside signaling in *C. elegans* metabolism. We highlight the wide applicability of this metabolomics approach.

(1) Pungaliya C, Srinivasan J, Fox BW, Malik RU, Ludewig AH, Sternberg PW, Schroeder FC. A shortcut to identifying small molecule signals that regulate behavior and development in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 2009 May 12;106(19):7708-13.

1208C

Second generation synthesis and bioactivity of novel endogenous ligands of the nuclear hormone receptor DAF-12. **Joshua C. Judkins**[1], Neelanjan Bose[1], Parag Mahanti[1], Jacob Hoffman[2], Frank Schroeder[1]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 2) Heidelberg University, Tiffin, OH.

Nuclear hormone receptors (NHR's) are critical components in metazoan signaling cascades, and it is estimated that 284 NHRs exist in *C. elegans*.[1] Although the function of most NHR's may be regulated by small molecule ligands, endogenous ligands for only one *C. elegans* NHR have been proposed. The two bile-acid-like steroids, Δ^4- and Δ^7-dafachronic acids were predicted as endogenous transactivators of DAF-12[2], an NHR with high homology to vertebrate Vitamin D and LXR receptors.[3] Recent studies by the Schroeder group have revealed the presence of additional ligands of DAF-12.

The structural diversity of the newly revealed dafachronic acids highlights the importance of a high yielding and flexible approach to chemically synthesize DAF-12 ligands for biological investigation.

Here we present a novel second generation synthesis that is shorter, more efficient, and more versatile than those previously published.[4-8] In addition, our synthesis converges on an aldehyde intermediate that allows divergent access to both previously described and novel dafachronic acids. Our synthetic approach provides Δ^4- and Δ^7-dafachronic acids in 6 and 9 steps, respectively. Key features of this synthesis include multiple transformations in a one-pot reaction and a late-stage chiral hydrogenation.

The variety of structures attainable through this synthetic method has allowed us to investigate the role of these and related molecules in *C. elegans* signaling pathways and to examine structure activity relationships (SAR's) of several dafachronic acid variants. We present bioactivity data for several natural and non-natural dafachronic acids. In addition, we compared SAR's obtained from i*n-vitro* experiments with the results from *in-vivo* studies.

[1] Robinson-Techavi et al.; *J. Mol. Evol.* **2005**, *60*, 577. [2] Motola et al.; *Cell*. **2006**, *124*, 1209. [3] Antebi et al.; *Genes & Dev.*, **2000**, *14*, 1512. [4] Giroux et al.; *Org. Lett.*, **2008**, *16*, 3643. [5] Giroux et al.; *Org. Lett.*, **2008**, *5*, 801. [6] Giroux et al.; *JACS*, **2007**, *129*, 9866. [7] Martin et al.; *Org. Biomol. Chem.*, **2010**, *8*, 739. [8] Gioiello et al.; *Tetrahedron*, **2011**, *67*, 1924.

1209A

SILAN - an Effective Method for Quantitative Proteomics in *C. elegans* . **M. Larance**, A. Bailly, A. Gartner, A.I. Lamond. Wellcome Trust Centre for Gene Regulation and Expression, University of Dundee, Dundee, United Kingdom.

We report here the development of an effective strategy for conducting quantitative, mass spectrometry-based proteomic studies on the nematode *Caenorhabditis elegans*, i.e., Stable Isotope Labelling of Amino acids in Nematodes (SILAN). We demonstrate efficient whole worm metabolic labelling with heavy isotope-substituted amino acids and show that this can be readily combined with RNAi knock-down of targeted genes. The nematode *Caenorhabditis elegans* is one of the simplest and most genetically tractable model metazoan organisms. However, while it has been used extensively for genetic and cell biological studies, there have been few biochemical and proteomic analyses in the nematode system, due in part to the limitations of previously available proteomics tools. To label whole worms using stable isotope-labelled amino acids requires their efficient metabolic incorporation into all proteins. Typically this uses arginine and lysine, which are the sites for trypsin cleavage. One issue affecting efficient implementation of the SILAC technique in multi-cellular organisms has been the metabolic conversion of labelled arginine into proline. To allow for simultaneous RNAi feeding and metabolic labelling with isotope substituted forms of lysine and arginine, we generated a derivative of HT115 that is auxotrophic for arginine and lysine. Upon label incorporation we extract and fractionate the *C. elegans* proteome by denaturing size exclusion chromatography prior to trypsin digestion and mass spectroscopy on a LTQ-Velos-Orbitrap instrument. Using this approach we have identified over 1,700 nematode proteins and quantified changes in their levels before and after heat shock treatment. This detected upregulation of small heat shock proteins while the abundance of a number of fatty acid desaturase enzymes decreased following heat shock, consistent with the need to decrease membrane fluidity at high temperature. Adapting SILAN to simultaneously compare three differentially labelled samples requires blocking the conversion of labelled arginine to proline. We have achieved this by using RNAi to inactivate ornithine aminotransferase, an enzyme required for proline biogenesis. We are currently testing changes in the nematode proteome in response to other stress treatments, such as ionizing irradiation, in various genetic backgrounds. We are also characterizing global changes in the nematode proteome in response to the induction of apoptosis. To

increase the number of proteins identified by SILAN we are optimizing protein separation of nematode extracts by combining size and charge based chromatography. We anticipate that the SILAN approach will have wide applications in future studies on nematode biology and responses.

1210B

Measuring Protein Abundance across Developmental Stages of *C. elegans* via Molecular Weight Fractionation and High-Resolution Mass Spectrometry. **Gennifer E. Merrihew**[1], Giana Angelo[2], Michael J. MacCoss[1]. 1) Dept Genome Sciences, Univ Washington, Seattle, WA; 2) Fred Hutchinson Cancer Research Center, Seattle, WA.

A vast majority of the genome annotation of "protein coding genes" is based on experimental data collected on the transcript level. While powerful, the analysis of some proteins will only be possible at the protein level. Mass spectrometry is a powerful experimental tool that can contribute significantly to the annotation of genomes at the protein level. Combining mass spectrometry with molecular weight fractionation techniques strengthens our ability to identify more proteins at varying abundances and can resolve different protein isoforms. In addition, analyzing different developmental stages of an organism will improve our knowledge of changes of protein abundance during development.

We are currently analyzing 12 developmental stages of *C. elegans* - N2 mixed embryo, N2 L1, N2 L2, N2 L3, N2 L4, N2 YA, N2 dauer, *spe-9* L4, *spe-9* YA, *spe-9* adult, *spe-9* adult reproductive diapause (ARD) and *him- 8*. The developmental stages are separated into 16 molecular weight fractions ranging from 3.5 - 500 kDa using the GelFree 8100™ fractionation system (Protein Discoveries). An unfractionated set is also analyzed for all developmental stages. The peptides from each fraction are analyzed using a 120-minute LC-MS/MS run on a Thermo LTQ-Orbi-Velos mass spectrometer. A biological and analytical replicate is performed for each sample. The MS/MS spectra are assigned to peptides using the SEQUEST algorithm, q-values are assigned at the peptide level using Percolator and the peptides are assembled into proteins using an in-house implementation of IDPicker.

Presently, we have analyzed the unfractionated samples and the half of the first molecular weight fraction (3.5-18 kDa). We identified 2736 non-redundant proteins and 14558 peptides in the unfractionated sample set and 1828 non-redundant proteins and 7122 peptides in the first molecular weight fraction at a very stringent peptide q-value cut-off of 0.01. Extrapolation of the current results out to all biochemical fractions suggests we will have sufficient depth of coverage to provide quantitative measures of protein abundance and confirmation of protein coding genes for approximately half the protein coding genes in the genome. Additionally, because we have data about the molecular weight fraction that the tryptic peptides were identified in, we will show examples of proteolytic processing of the protein coding gene to a smaller functional protein.

1211C

Searching for DAF-16 partners. **A Nazari Daftari**[1], Y Wang[1], R Baumeister[1,2,3,4]. 1) University of Freiburg,Bioinformatics and Molecular Genetics (Faculty of Biology),BIO III, Schänzlestr.1, D- 79104 Freiburg, Germany; 2) FRIAS Freiburg Institute of Advanced Studies, School of Life Sciences (LIFENET); 3) Center for Biochemistry and Molecular Cell Research (Faculty of Medicine); 4) Center for Systems Biology (ZBSA).

In *Caenorhabditis elegans*, the insulin/IGF-1 signaling pathway is comprised of many genes including the insulin/IGF-1 signalling receptor (DAF-2) that signals through a conserved PI-kinase/AKT pathway and ultimately down-regulates DAF-16, a forkhead transcription factor (FOXO). Besides, DAF-16 also receives input from several other pathways such as germline and JNK pathway. In fact it may sit at the centre of a complex network where it accepts multiple upstream signals and then translates them into diverse transcriptomes. All together it decides many biological aspects such as life span, fat storage, dauer diapauses, metabolism, development, reproduction and stress response. However there are still many things unknown about DAF-16, for example which other kinases beside the known ones might phophorylate DAF-16, or which proteins may bind to DAF-16 to interfere its subcellular localization and transcriptional activity. To answer these questions, we performed split-ubiquitin screen in Yeast. We used *C.elegans* library containing proteins expressed in different developmental stages. We also combined full length DAF-16 together with C-terminal and N-terminal truncated version of the protein as Bait. We got 8 possible candidates and we choose some of those for further analyzing their detailed relevance with DAF-16, using proteomics and genetic methods. We believe the results can help us better understanding the regulatory mechanism of DAF-16.

1212A

Comprehensive analysis of *C. elegans'* ascarosides, a modular library of small molecule signals. **Stephan von Reuss**[1], Jagan Srinivasan[2], Neelanjan Bose[1], Paul Sternberg[2], Frank Schroeder[1]. 1) Boyce Thompson Institute and Department of Chemistry and Chemical Biology, Cornell University, Ithaca, NY; 2) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA.

Recent work has shown that a group of small molecule signals, the ascarosides control population density sensing, male mating, and aggregation. These glycosides of the didesoxysugar ascarylose feature various fatty acid derived side chains originating from peroxisomal beta-oxidation. New ascaroside structures are continuously being discovered which often act synergistically to modulate a variety of behaviors [1,2].

Given the general importance of ascarosides in *C. elegans* biology, we aimed to develop a fast and reliable method for detection and quantitation of known and previously unidentified components. Here we describe the use of HPLC-MS/MS for ascaroside-screening of metabolite extracts from wild-type (N2) worms as well as three mutants defective in specific components of peroxisomal beta-oxidation, *acox-1*, *dhs-28*, and *daf-22*. These analyses showed that the known ascarosides, ascr#1-ascr#8 are members of a much larger group of over 80 components. We identified several new series of ascarosides including compounds that feature omega-linked side chains or amino acid-derived indole-3-carbonyl or 4-hydroxybenzoyl moieties, many of which serve as potent signaling molecules. In addition, we elucidated the precise roles of three genes implicated in ascaroside biosynthesis, *acox-1*, *dhs-28*, and *daf-22*. Given their structural diversity, the ascarosides appear as a modular library of small molecule signals that integrate inputs from several basic metabolic pathways, including carbohydrate (ascarylose core unit), peroxisomal beta-oxidation (fatty acid side chain), and amino acid metabolism (indole-3-carbonyl or 4-hydroxybenzoyl units). Mass spectrometry-based ascaroside profiling provides a powerful tool to study the effect of environmental conditions and genetic modifications on ascaroside expression profiles and associated phenotypes.

[1] Pungaliya, C., et al. (2009). A shortcut to identifying small molecule signals that regulate behavior and development in *Caenorhabditis elegans*. *PNAS 106*, 7708.

[2] Srinivasan, J., et al. (2011). A modular library of small molecule signals regulates social behaviors in *Caenorhabditis elegans*. Submitted.

1213B

Proteomic profiling dietary restriction pathways with 13C-labeled Caenorhabditis elegans. **Y. Yuan**[1], C. Kadiyala[2], T. Ching[3], S. Saha[2], H. Xu[2], K. Kramp[1], R. Ewing[2,4], A. Hsu[3,5], M. Miyagi[1,2], Z. Feng[1]. 1) Dept Pharmacology, Case Western Reserve Univ, School of Medicine, Cleveland, OH; 2) Center for Proteomics and Bioinformatics, Case Western Reserve University, School of Medicine, Cleveland, OH; 3) Department of Internal Medicine, Division of Geriatric Medicine, University of Michigan, Ann Arbor, MI; 4) Department of Genetics, Case Western Reserve University, School of Medicine, Cleveland, OH; 5) Department of Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI.

Dietary restriction (DR) modifies the metabolism of animals and humans, extends lifespan and improves age-related health of various animals. The underlying mechanism of these physiological outputs of DR and their interplay remains to be clarified. Here, we developed a quantitative proteomic method called stable isotope labeling by amino acids in C. elegans (SILACE) by labeling worms with isotope labeled amino acids. Using SILACE, we reliably quantified the abundance of 1805 proteins. The average and median levels of these 1805 proteins of dietary restricted worms were lower than that of control worms when comparing both the same number and the same weight of worms, indicating that DR systematically decreases protein synthesis. In this background, we were able to identify 237 proteins with quantities significantly up- or down- regulated by DR. We found that DR specifically modified the expression of key genes involved in the catabolism/synthesis of carbohydrates, fatty acids, amino acids and other small molecules, as well as mitochondrial/ribosome functions and stress responses. Many transcriptional factors, translational factors and signaling molecules that are associated with or play a key role in the pathology of human metabolic, cancer and other diseases were also identified as significantly altered in abundance by DR. Most of these proteins have a previously unknown relationship with both DR and lifespan. Lifespan and qPCR assays confirmed some of these findings. In summary, DR has a profound effect on metabolisms of this organism and elicits consequent signaling and physiological responses. We also concluded that SILACE provides sensitive, reliable and scalable proteomic analysis.

1214C

A Website to Annotate SNP Data. John Rummage, Kenneth G. Miller, **Robert J. Barstead**. Genetic Models of Disease, Oklahoma Medical Res Fndn, Oklahoma City, OK.

The first step in analyzing whole genome sequence alignments is to detect variant sequences and associate them with genome annotations. Most genome sequence facilities have access to developers who run command line driven scripts to make this association. Our mission is to simplify this and make the annotation of SNP and Indel calls accessible to the average investigator. To this end we have launched Whole Genomes, a website where investigators can upload SNP-called files for custom annotations (http://seqreport.omrf.org/genome: open to all on May 1, 2011). Administratively, the site design is flexible, allowing one to easily create annotation tables based on tab delimitated flat files like the GFF files distributed by Wormbase. Further, the site uses simple forms to establish persistent templates that describe the format for uploaded SNP files. All files are imported into a MySQL database and all comparisons are done via MySQL queries that offer a much more dynamic interaction with the data compared to a simple script driven approach. Users do not need to know anything about annotation files, templates, or MySQL to use the site.

Whole Genomes allows users to map SNPs to any combination of features in the annotation database, such as coding sequence, potential splice sites, untranslated regions, or microRNAs. For examples, a coding sequence query would report the specific amino acid changes for SNPs that fall within translatable sequence along with a metric of evolutionary conservation for that particular change. The site also allows the user to compare SNPs in two strains to identify both unique and common variations. In addition, if a user has minimal or no mapping information Whole Genomes can compare two allelic strains to find the gene that is affected in both. Each annotated SNP is associated with a unique "Download" link that lets users download the DNA sequence associated with the annotated feature. Finally, for any given SNP, the site will design PCR primers to isolate DNA for SNP verification.

1215A

The Virtual Worm: A Three-Dimensional Model of the Anatomy of *Caenorhabditis elegans* at Cellular Resolution. **Christian A. Grove**, Paul W. Sternberg. Division of Biology, California Institute of Technology, Pasadena, CA.

Painstaking analysis over three and a half decades has elucidated the detailed cellular anatomy of *Caenorhabditis elegans* to sub-cellular resolution. Presented here is a three-dimensional model of the adult hermaphrodite of *C. elegans* at cellular resolution, consisting of 693 objects, each one representing an individual somatic cell. The morphology and position of each cell in the model has been deduced from a variety of data sources including WormAtlas.org, the "Mind of a Worm", and the *C. elegans* Atlas book (see References). Cells have been colored according to the Worm Atlas color code for consistency. The goal of developing this model is to: (1) Provide students, teachers, and researchers an interactive tool to learn about and explore the cellular anatomy of *C. elegans*, (2) provide a three-dimensional framework for the integration and archiving of cellular and molecular biological data, (3) promote hypothesis generation and discovery by providing novel visualizations and perspectives, and (4) encourage constructive debate over the details of *C. elegans* anatomy for the purpose of refining current working models. A user interface has been developed within the 3D graphics program, Blender, to allow users to explore the model in three dimensions while customizing their views and perspectives by toggling visibility, transparency, Worm Atlas color, and cell names. Cells have been grouped according to similar tissue, location, and function and can be selected for visualization on the basis of these groupings or by individual cell name. Future plans for the model include: (1) Construction of the larval and embryonic stages of *C. elegans* hermaphrodite development, (2) construction of the male and its developmental stages, (3) incorporation of sub-cellular organelles including, but not limited to, nuclei and mitochondria, (4) incorporation of meta data including gene expression, lineage, and anatomy function data, (5) incorporation (where possible) of molecular information including molecular composition of cytoskeleton, organelles, and cytoplasm, (6) placement of neuronal synapses (chemical and gap junctions), and (7) development of an interactive web display to be incorporated into WormBase.org for "on the fly" interaction with the model.

1216B

Hey, you, get ON our cloud: easy private installations of WormBase through cloud computing. **Todd W. Harris**. Ontario Institute For Cancer Research, Toronto, Ontario, Canada.

WormBase is a large and complicated suite of software, databases, and computational services. Although local or private installations would be useful for many researchers, the complexity of the system makes installation a time consuming task. The frequency with which WormBase is updated means local installations must be actively maintained from month-to-month. Skill and dedication aside, it is the size of the database and requisite download times that all but preclude the feasibility of individual local installations.

Cloud computing makes running your own private instance of WormBase not just feasible, but trivial, requiring a minimum amount of time and only nominal expense.

We've placed all WormBase data and software -- preinstalled, configured, and ready to run -- "in the cloud". Launching a new instance requires a matter of minutes with no knowledge of the software infrastructure of WormBase required. When a new version of WormBase is released, one simply shuts down the old private instance and launches the new one.

Stop by this interactive poster and we'll show you how to get your own instance of WormBase up and running.

1217C

New and Improved: WormImage 2.0 and WormAtlas Mobile. **L.A. Herndon**, Z.F. Altun, C.A. Wolkow, K. Fisher, C. Crocker, T. Stephney, M. Xu, D.H. Hall. Center for *C. elegans* Anatomy, AECOM, Bronx, NY.

We announce the release of WormImage 2.0. The WormImage database houses over 55,000 unpublished electron micrographs and their related metadata. This new version of WormImage still includes access to this enormous collection of *C. elegans* micrographs, but now features a new look and a new search interface making the data more easily accessible to users. To streamline the website, users can now search directly from the front page. As before, search criteria allow users to narrow results by sex, genotype, age, body portion and tissue type of the animal, but now this is found all on one page. Additionally, with new expandable menu options, one can now select a single tissue or multiple tissue types with just a few clicks. Search results are presented in a new format that is simpler, making it easier to screen through and navigate among all the images. The dataset offered by WormImage continues to expand and welcomes all laboratories to share their best archival TEM and SEM images so that this resource can continue to grow and serve the *C. elegans* community.

The WormAtlas website is also growing and changing. To adapt to the increased demand for mobile-ready content, WormAtlas is now available in a format optimized for access from mobile devices. This allows for users to quickly navigate through the handbook chapters without worrying about zooming in on small icons and menu bars. We are also pleased to announce that WormAtlas will soon feature a new handbook section on the Dauer Larva. Sections for this new handbook will be posted as they become available. In addition to adding new content, we are also continually updating material on WormAtlas to keep the information available to users current. As such, the Individual Neuron pages are being revised and feature new images and data. Slidable Worm is expanding as well, with the ongoing addition of new slices.

This work is supported by NIHRR 12596 to DHH.

1218A

Synthesizing Biological Theories. Cory Plock, Andy Roberts, **Hillel Kugler**. Microsoft Research Cambridge.

We present a new software tool we call Synthesizing Biological Theories which enables experimental biologists and modelers to construct high-level theories and models of biological systems, capturing biological hypotheses, inferred mechanisms, and experimental results within the same framework. Among the key features of the tool are convenient ways to represent several competing theories and the interactive nature of building and running the models using an intuitive, rigorous scenario-based visual language. The tool is being used to model several aspects of *C. elegans* biology including the embryonic lineage, germline population dynamics and VPC fate specification, more information is available at http://research.microsoft.com/SBT/ .

1219B

The Biogemix knowledge base project: cross-species and network-based data integration for Huntington's disease research. François-Xavier Lejeune[1], Lilia Mesrob[1], Frederic Parmentier[1], Cédric Bicep[1], Jean-Philippe Vert[2], **Christian Neri[1]**. 1) Laboratory of Neuronal Cell Biology and Pathology, INSERM Unit 894, 75014, Paris, France; 2) Institut Curie, INSERM Unit 900, 75005 Paris, France.

The identification and validation of neuroprotective targets is of primary importance in research on neurodegenerative diseases such as Huntington's disease (HD). The development of genetically tractable models of disease and their use in genome-wide screens has generated a large amount of data in several species. A current challenge is the unbiased integration of these data sets in order to prioritize candidate target genes. The Biogemix knowledge base project has been developed with the European HD network (Euro-HD) to integrate 'omics' data from models of HD pathogenesis as available in several species (invertebrates, mammalian cells, mice, human samples). This project relies on the combination of network-based and cross-species procedures to unlock the biological information buried into disease data sets. The Biogemix procedure is a method that relies on the use of molecular networks for the unbiased integration of 'omics' data across different species. This method is particularly suited to the analysis of data sets for which the number of genes analyzed clearly exceeds the number of conditions tested. Single data sets are firstly processed with respect to a reference molecular network (for instance, use of MouseNet to analyze mouse data) to extract clusters (modules) that are made of highly interconnected genes, enriched in HD-relevant information and automatically annotated for their biological role and biomedical potential. In a second step, cross-species clusters (meta-modules) are calculated by balancing gene/protein connectivity with protein sequence similarities. In a third step, all of the Biogemix products are ranked according to topological and biological features of interest, which is part of a larger prioritisation system that uses several criteria to classify modules and genes of high interest. A user-friendly graphical interface and query system is being developed to allow the users to browse and select Biogemix information of interest. Further developments will aim at fine-tuning data analysis and information display in view of making the Biogemix knowledge base v 1.0 publically available on-line. Preliminary results will be shown to illustrate how the Biogemix system might be useful for basic research and disease research.

1220C

OrthoList, a compendium of *C. elegans* genes with human orthologs. **Daniel Shaye**[1], Iva Greenwald[1,2,3,4]. 1) Howard Hughes Medical Institute; 2) Dept. of Biochemistry and Molecular Biology; 3) Dept. of Genetics and Development; 4) Columbia University College of Physicians and Surgeons. New York, NY 10032.

BACKGROUND: *C. elegans* is an important model for genetic studies relevant to human biology and disease. We sought to assess the orthology between *C. elegans* and human genes to understand better the relationship between their genomes and to generate a compelling list of candidates to streamline RNAi-based screens in this model.

RESULTS: We performed a meta-analysis of results from four orthology prediction programs and generated a compendium, "OrthoList", containing 7,663 *C. elegans* protein-coding genes. Various assessments indicate that OrthoList has extensive coverage with low false-positive and false-negative rates. Part of this evaluation examined the conservation of components of the receptor tyrosine kinase, Notch, Wnt, TGF-β and insulin signaling pathways, and led us to update compendia of conserved *C. elegans* kinases, nuclear hormone receptors, F-box proteins, and transcription factors. Comparison with two published genome-wide RNAi screens indicated that virtually all of the conserved hits would have been obtained had just the OrthoList set (~38% of the genome) been targeted. We compiled Ortholist by InterPro domains and Gene Ontology annotation, making it easy to identify *C. elegans* orthologs of human disease genes for potential functional analysis.

CONCLUSIONS: We anticipate that OrthoList will be of considerable utility for streamlining RNAi screens by focusing on genes with apparent human orthologs and thus reducing screening effort by ~60%. Moreover, we find that OrthoList provides a useful basis for annotating orthology and reveals more *C. elegans* orthologs of human genes in various functional groups, such as transcription factors, than previously described.

1221A

Exploiting Simple Geometries in Microfluidic Chips to Orient *C. elegans* for Rapid Visual Screens. **Ivan Cáceres**[1], Nick Valmas[2], Massimo Hilliard[2], Hang Lu[1]. 1) Interdisciplinary Bioengineering Graduate Program, Georgia Institute of Technology, Atlanta, GA; 2) Queensland Brain Institute, University of Queensland, Brisbane, QLD.

Forward genetic screens are an invaluable resource for discovery in *C. elegans*, with key genes identified in this fashion for a large variety of biological processes. Visual screens to identify mutant animals with defects in neuronal development (using GFP-expressing neurons) require significant amounts of manual operations and can be very time consuming. Microfluidics is a powerful tool that enables higher throughput experiments by eliminating the need of manual picking and use of anesthetics. However, orientation of nematodes during screening is usually random and can hinder visual inspection. Additionally, existing microfluidic devices do not allow for dorso-ventral orientation, important to examine morphological features along the antero-posterior axis.

We present a simple microfluidic design that allows passive and reliable orientation of *C. elegans* within microchannels by exploiting device channel geometry. With this strategy, we can position more than 80% of animals into lateral orientations, compared to less than 25% using regular methods. We validated our technology by performing on-chip analysis and high-throughput visual screens to isolate mutant animals involved in neural development and degeneration. Additionally, we show that this design is harmless to both animal survival and reproduction capabilities. The advantages of this system are threefold: i) curved channel geometry passively orients animals allowing analysis of strains in lateral orientation; ii) the curved channel design increases nematode body area within the microscope field of view, allowing larger body regions to be examined; iii) the system is simple, requiring no additional mechanical, cooling, or fluid systems.

Using the transgenic strain *juIs76* (*Punc-25*::GFP), expressing GFP in GABAergic neurons (Huang et al., 2002), we performed an EMS pilot screen on-chip and isolated six alleles of interest. Alleles a070, a071, and a073 exhibit axonal guidance and connectivity abnormalities, such as extra or misguided commissural connections. Alleles a074, a076, and a077 present a novel axonal degeneration phenotype with numerous breaks along both nerve cords and commissures. Our results demonstrate that our simple microfluidic device allows for visual screens of *C. elegans* to be performed at a consistent rate of 500 animals per hour, without the use anesthetics, and with controlled animal orientation.

1222B

Effects of electrical stimulation on the ASH chemosensory neuron in *Caenorhabditis elegans*. **Trushal V. Chokshi**[1], Nikos Chronis[2]. 1) Department of Electrical and Computer Engineering, Univ Michigan, Ann Arbor, MI; 2) Department of Mechanical Engineering, Univ Michigan, Ann Arbor, MI.

Electrical stimulation has been widely used to modulate and study the functionality of the nervous system in-vivo and in-vitro. Here, we characterized the effect of electrical stimulation on ASH neuron in *C. elegans* and employed it to probe the neuron's age dependent properties. We utilized an automated microfluidic-based platform to acquire calcium imaging data from the ASH neuron in response to an electric current passing through the worm's body. We characterized the ASH neuronal activity in response to electric currents of varying strength and electrical polarity. For positive polarities (the worm's head faces the positive electrode), ASH depolarization was proportional to the magnitude of electric current while for negative polarities (the worm's head faces the negative electrode), it was inversely related. Interestingly, the ASH neuron hyperpolarized for higher negative polarity electric currents. Further, the neuronal activity in response to electrical stimulation showed significant differences across worms of different ages, indicating that the effect of electrical stimulation is age-dependent.

1223C

Red-shifted optical excitation with a *Chlamydomonas* / *Volvox* hybrid Channelrhodopsin. **Karen Erbguth**[1], Matthias Prigge[2], Franziska Schneider[2], Jeffrey N. Stirman[3], Hang Lu[3], Peter Hegemann[2], Alexander Gottschalk[1,4]. 1) Institute of Biochemistry, Goethe-University Frankfurt, Germany; 2) Experimental Biophysics, Humboldt-University, Berlin, Germany; 3) Georgia Institute of Technology, Atlanta, USA; 4) Frankfurt Molecular Life Sciences Institute, Goethe-University, Germany.

How neural circuits evoke behavior is one of the most fascinating questions in the neurosciences. To aid the functional elucidation of neural networks, several optogenetic tools of microbial origin have been adopted for use in *C. elegans*. *Chlamydomonas* Channelrhodopsin-2 (ChR2) (1, 2) is a well established optical tool to trigger the depolarization of neurons with blue light, while yellow-green light-triggered hyperpolarization can be achieved using Cl^- pumps like *Natronomonas pharaonis* Halorhodopsin (NpHR) (3) or proton pumps like *Leptosphaeria maculans* bacteriorhodopsin (MAC) (4). Along with the investigation of tissue specific expression systems, optogenetic tools pave the way for a straightforward and systematic approach to dissect neural networks in vivo. To expand possibilities for multimodal investigation of neural networks, activation of different cell types of one circuit in a temporally and spectrally independent manner is desired. Channelrhodopsin-1 from *Volvox carteri* (vChR1), another depolarizing cation channel, exhibits red-shifted absorption properties compared to ChR2 (5). However, vChR1 expresses only weakly in metazoan cells including, as we found, *C. elegans*. Thus, an improved version would help to expand the optogenetic toolbox. To assess alternatives to vChR1, we generated different chimeras of vChR1 and *Chlamydomonas* Channelrhodopsins (from now on termed cChRs), and screened for robust expression and beneficial properties, i.e. red-shifted action spectrum and light-sensitivity. Candidate proteins were compared to cChR2, and tested for optical compatibility and distinguishable functionality in different cell types. Finally, we identified C1V1duo, a hybrid with specific point mutations that showed peak activation at ~540nm (cChR2 peaks at ~460nm). We could activate both proteins with 400 or 570nm light, respectively, in mechanosensory neurons, evoking withdrawal behavior. cChR2 expressed in GABAergic neurons (activated at 400nm) could counteract activity of C1V1duo in muscles (570nm). C1V1duo appears to be more light-sensitive and efficient than cChR2. We currently assess the compatibility of C1V1duo with yellow Cameleons for Ca^{2+}-imaging as a readout of network activity. (1) Nagel *et al*. (2005): Curr Biol 15, 2279-84; (2) Liewald *et al*. (2008): Nat Methods 5, 895-902; (3) Zhang *et al*. (2007): Nature 466, 633-9; (4) Stirman *et al*. (2011): Nat Methods 8, 153-8; (5) Zhang *et al*. (2008): Nat Neurosci 11, 631-3.

1224A

Large-scale *in vivo* laser neurosurgery for discovery of small-molecule enhancers of regeneration. **Cody L. Gilleland**[1], Christopher B. Rohde[1], Chrystanthi Samara[1], Stephanie Norton[2], Stephen J. Haggarty[2], Mehmet Fatih Yanik[1]. 1) MIT; 2) Harvard.

The ability to rapidly identify cell-permeable compounds that affect regeneration of neurites *in vivo* can accelerate the discovery and development of treatments against neurodegenerative diseases, brain trauma, stroke and spinal cord damage. By integrating microfluidics, laser microsurgery, and a multiwell interface with chemical libraries, we demonstrate large-scale chemical screens for identification of modulators of *in vivo* neurite regeneration using *C. elegans* (Samara *et al*. PNAS 2010). Chemical screening to identify modulators of neurite regeneration have been performed mainly in *in vitro* cell culture systems. Such studies have yielded numerous chemical effectors of neuronal regeneration. However, most remain untested *in vivo*, and potential off-target, toxic or lethal *in vivo* effects are not known. *In vivo* investigations have been limited until recently to higher organisms, such as mice and rats where large-scale screens are not feasible. The stochasticity of the neurite regeneration process requires precise neuronal injury to be repeatedly conducted on large populations of animals with low genetic variability. *C. elegans* is a powerful model for nerve regeneration studies due to its small size, short life cycle and inexpensive culture, along with its ability to hermaphroditically produce large isogenic populations and its fully mapped neural network. We enhanced the reproducibility of *C. elegans* assays with software assistance by repeatedly axotomizing the same neurons at a precise distance from the soma. We previously developed microfluidic methods to rapidly orient and mechanically immobilize physiologically-active *C. elegans* without anesthesia (Rohde *et al*. PNAS '07). We also previously used femtosecond laser microsurgery to study neurite regeneration in *C. elegans* (Yanik *et al*. Nature '04). Combining microfluidic immobilization with microsurgery allows precise and high-throughput injury of a single axon. To enable large-scale screens, we developed a system to rapidly load/unload nematodes from/to multiwell plates to microfluidic devices with excellent reliability. We performed thousands of microsurgeries at single-axon precision. Using software we designed, we can load, image, perform femtosecond laser microsurgery, and unload *C. elegans* within 20 s per animal. We screened a hand-curated chemical library for its effects on neurite regeneration. We demonstrate that structurally distinct PKC inhibitors modulate regeneration in a concentration- and neuronal type-specific manner. We show that prostratin, a PKC activator, significantly increases regeneration. We have also recently identified other classes of targets and chemicals that modulate regeneration in *C. elegans*.

1225B

Microfabricated device for acute drug treatment in the early C. elegans embryos. **Alex Groisman**[1], Ana Carvalho[2], Sara Olson[2], Edgar Gutierrez[1], Kelly Zhang[2], Lisa Noble[2], Arshad Desai[2], Karen Oegema[2]. 1) Department of Physics, University of California, San Diego, La Jolla, CA; 2) Ludwig Institute for Cancer Research, Dept. of Cellular and Molecular Medicine, UCSD, 9500 Gilman Drive, La Jolla, CA 92093.

Small molecule inhibitors are a valuable tool for the analysis of fundamental cellular functions and an entry point for the development of therapeutic agents. Their primary advantage is temporal control, which is especially powerful when combined with live imaging. In C. elegans embryos the use of small molecule inhibitors has been limited by eggshell impermeability. C. elegans eggshells have been permeabilized with laser puncturing, pushing against a coverslip, and by passage through a narrow mouth pipette after weakening by sequential treatment with bleach and chitinase. However, these techniques suffer from problems with consistency, non-uniform permeability, and disruption of embryonic processes. To address these problems, we identified a gene whose inhibition renders the eggshell permeable without disrupting events during the early embryonic division and built a specialized microdevice for in situ worm dissection and high resolution imaging of embryos. The microdevice has a rectangular well (8x6x3mm) with an array of microwells (0.3x0.3x0.15mm) at the bottom and an integrated dissection board near the microwell array. Worms are soaked in dsRNA to inhibit the gene and make the embryos permeable. Treated worms are placed on the dissection board, cut with a scalpel and the fragile permeable embryos are gently swept towards the microwell array using an eyelash tool. Embryos settle onto the bottom of the microwells, where they rest on a coverslip, being accessible to high-resolution imaging and protected from flow by the microwell walls. Permeabilized embryos are acutely exposed to drugs after the medium in the microwells is exchanged by aspiration of the existing medium from the macroscopic well and dispensing of a medium with the drug into the well with a pipette. Embryos remain motionless and within ~7 sec are exposed to a drug concentration that is ~60% of that in the added medium. 90 sec is sufficient for ~99% medium exchange, including removal of a drug from the embryo environment. The technique was tested with 4 common inhibitors: the microtubule

inhibitor nocodazole, actin inhibitor latrunculin A, myosin inhibitor blebbistatin, and proteasome inhibitor c-lactocystin-β-lactone. All 4 inhibitors had the expected effects on permeable embryos and no effect on control impermeable embryos. These results establish that our method can be used to combine precisely controlled inhibitor treatments with live imaging in the early C. elegans embryo.

1226C

Microfluidic vacuum actuated trap for gentle immobilization and high-resolution microscopy of *C. elegans* across developmental stages. **Edgar Gutierrez**[1], Rebecca Green[2], Karen Oegema[2], Alex Groisman[1]. 1) Biophysics, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA., 92093, USA; 2) Ludwig Institute for Cancer Research, Dept. of Cellular and Molecular Medicine (UCSD), 9500 Gilman Drive, La Jolla, CA, 92093, USA.

A major challenge for studying dynamic developmental processes in *C. elegans* has been the logistical difficulties associated with long-term high-resolution imaging of worms. Worms cannot be anesthetized over long time periods, ingest the immobilization beads when anesthesia is avoided, and their immobilization with the existing trapping techniques is incomplete, making it very difficult to track single animal throughout the course of development. Here we present a technique that uses a micro-machined elastomer chip that is pushed against a thin layer of a silicone gel (~20 μm) on a glass coverslip by the application of vacuum to trap worms between the elastomer and gel. Loading the device is simple and minimizes the loss of specially treated animals. Worms are transferred, one-by-one, to a ~5 μl droplet on the coverslip which is directly applied to the device. The device is compatible with short working distance microscope objectives (<200 μm) and has an array of continuously perfused micro-compartments with individual worms sequestered in them. The pushing force is widely adjustable by varying the level of vacuum, making the device suitable for a variety of worm sizes and enabling a user-defined degree of immobilization. A high level of vacuum is applied to completely immobilize worms during short intervals of high-resolution imaging; the vacuum is reduced between imaging to allow worms to feed and develop. Upon reduction of vacuum pressure, the worms recover their normal behavior and pharynx contractions, while staying confined in their micro-compartments. Thus multiple worms can be tracked in parallel during a long (hours to days) time course. Worms can also be easily recovered from the device into a minimal volume of buffer (<200 μl) for further propagation, by separating the elastomer chip from the gel substrate. In pilot experiments, we have successfully used this device and technique to immobilize and image worms at different developmental stages over several days, without loss of viability. Specifically, we have imaged the developmental time course of the gonad region, using DIC and two-color fluorescence confocal microscopy. This innovation makes it feasible to perform long-term high-resolution imaging on living worms, allowing for direct observation and characterization of dynamic developmental processes.

1227A

Design of a C. elegans sorting microchip based on structure dependent swimming behavior. **Bicheng Han**, Daeyeon Kim, Jennifer H Shin. Korea Advanced of Science and Technology (KAIST), Daejeon, Korea.

Caenorhabditis elegans (C. elegans) is a popular model organism widely used in the fields of molecular and developmental biology thanks to its very short life span of 5 distinctive developmental stages. Sorting worms by their stage is required to investigate the stage-specific characteristics of C. elegans. We developed a novel PDMS microchannel for sorting C. elegans by their developmental stages which can replace a current costly and massive sorter system. The microchannel device features micropillars arrayed in 2 layers of hexagonal lattice. Worms weave their way through the micropillars along the diagonal directions and exhibit an enhanced locomotion by combining the fast oscillations of swimming gait and the reduced slip in crawling modes. We optimized the structure of the channels by varying the diameter and spacing of micropillars so that targeted sized worms have the fastest swimming speed. Additionally, to induce a biased directional movement, electric field is applied between two ends of the microchannel based on cathodal electrotaxis behavior of C. elegans. This device can further be utilized in the characterization of locomotion defects of mutants as well as the sorting worms by different stages for researches in C. elegans biology. This work was supported by the National Research Foundation (NRF) grant 2010-0016886.

1228B

A multi-worm optogenetic system. **Yuya Kawazoe**[1], Hiromu Yawo[2], Kotaro Kimura[1]. 1) Department of Biological Sciences, Osaka University, Toyonaka, Osaka, Japan; 2) Tohoku University Graduate School of Life Sciences, Sendai, Japan.

Optogenetic techniques are extremely useful for proving that the particular activity pattern of neuron(s) is the causal reason for a specific neural function. One of the major problems associated with optogenetics, however, is the requirement of a strong light for the activation. To analyze *C. elegans* behavior with optogenetics, most methods use a microscope with a strong light source to obtain enough light intensity and a computer-controlled motorized stage to keep the worm in the field of view. However, such methods limit the observation to one animal per assay, require multiple expensive equipments, and may not be suitable for experiments with delicate signal gradients such as odor or temperature. To overcome the limitations associated with such methods, we have established a simple and easy-to-use optogenetic system in combination with a strong LED ring and an improved channelrhodopsin (ChR). First, we developed a strong LED ring that allows exposure of an entire 9-cm plate at 0.5 mW/mm2 with 470 nm light; the intensity is comparable to the strongest light intensity obtained from a mercury lamp for GFP excitation. The LED can also be used to expose a small area of the plate at 1.2 mW/mm2. We have also developed a custom-made program with LabVIEW to control LED intensity in synchronization with capturing the images via a USB camera. Because of the high resolution of the camera (approximately 2.5 k × 2 k), the system does not need a microscope or a motorized stage, and we can record the behavior of multiple worms on a 9-cm plate simultaneously. Thus, using this system we are able to efficiently conduct optogenetic behavioral analysis for delicate signal gradients. In addition, this system is relatively inexpensive: the total cost, including the LED ring, camera, and PC is approximately $14,000. To prove that the LED can actually activate the optogenetic ion channel, we expressed an improved ChR, ChRGR (Wen et al., PLoS ONE, 2010), in the body wall muscles with a *myo-3* promoter. Upon light stimulation, the movement of transgenic animals was significantly inhibited in the presence of all-trans-retinal, indicating that the LED intensity was strong enough to activate ChRGR. We are currently trying to express ChRGR in the AWB sensory neurons to understand how the activity of AWB regulates avoidance behavior to repulsive odor 2-nonanone, which is enhanced by preexposure to the odor in a dopamine-dependent manner (Kimura et al., J. Neurosci., 2010).

1229C

A simple microfluidic device for long-term *in vivo* imaging of mitochondrial dynamics in *C. elegans*. Sudip Mondal, **Sandhya Koushika**. Dept Neurobiology, NCBS-TIFR, Bangalore, India.

To track development and stability of mitochondrial distributions in identified animals, we developed a growth cum imaging microfluidic device for *C. elegans*. In wild type animals, mitochondria numbers co-relate linearly with neuronal process length of the touch receptor neurons. In short time-scales we observe that all large mitochondria are stationary and only about 15-20% of mitochondria, all small in size, move. Distribution of mitochondria along the neuronal process is regulated such that few mitochondria are placed less then 3 microns from each other. We wished to investigate how these large stationary mitochondria arise by tracking individual organisms throughout their development. The growth and imaging device utilizes a deformable PDMS membrane to immobilize *C. elegans* for high magnification mitochondrial matrix::GFP fluorescence tracking, with wide enough channel geometry to track developmental parameters such as body length and body diameter inside the device at low magnification. The worm is fed using a simple hydrostatically driven *E. coli* supply in liquid culture media using 200 μL pipette tips. Preliminary observation suggests that *C. elegans* grown in the microfluidic device shows similar developmental and behavioural parameters as compared to animals grown on NGM plates. We tracked mitochondrial positions over 10 hours in animals grown in a microfluidic device. This tracking shows greater mitochondrial dynamics in the first 100 μm of the neuronal process as compared to the middle of the neuronal process. Currently we are optimizing imaging conditions to avoid fluorescence bleaching to be able to track individual animals for all developmental stages.

1230A

A 96-well plated based liquid phase C. elegans behavioral quantification system. **Maohua Zheng**, Olga Gorelenkova, Zhaoyang Feng. Case Western Reserve University, Cleveland, OH.

Motor activity of C. elegans is commonly used to study the mechanisms ranging from basic neuronal functions to a number of human diseases. It may also serves as a paradigm to screen for potential therapeutic reagents treating these human diseases. Here, we developed an automated, 96-well plate/liquid-phase based system that quantifies worm motor activity in real time. Using this system, we confirmed an adult-onset, ageing-associated motor activity loss that accompanied dopaminergic specific neuron degeneration in a worm line expressing human pathogenic G2019S mutant LRRK2 (Leucine-rich repeat kinase 2). LRRK2 is a leading genetic cause of Parkinson's Disease (PD) characterized by dopaminergic neuron degeneration and motor deficient mainly in elder citizens. Thus, our system may be used as a platform for high-throughput screening for potential PD therapeutic drugs. It could be also used to monitor motor activity of large population worms in other similar scenario.

1231B

Nicotine exposure response in Caenorhabditis elegans: a model for evaluating smoking cessation agents. Danny Tudor[1], Aaron Behm[2], David Montgomerie[2], Chad Ahia[1], Patrick Yasukawa[2], Supakit Wongwiwatthananukit[3], Anthony Otsuka[1], **Leng Chee Chang[1]**. 1) Department of Pharmaceutical Sciences, College of Pharmacy, University of Hawaii Hilo, Hilo, HI, 96720; 2) Department of Biology, College of Arts and Sciences, University of Hawaii Hilo, Hilo, HI, 96720; 3) Department of Pharmacy Practice, College of Pharmacy, University of Hawaii Hilo, Hilo, HI, 96720.

Nicotine dependence is a significant health concern as it represents the chief preventable cause of worldwide morbidity and mortality. Nicotine exposure has distinct effects on the physiology of C. elegans that can be easily observed, including increase in egg-laying behavior, pharyngeal pumping, locomotion, and hypercontraction of body-wall muscles. These physiological changes may serve as a useful model for identifying compounds as pharmacotherapies to aid in smoking cessation. As a proof-of-principle experiment, we hypothesized that currently approved smoking cessation medications used in humans would exhibit similar effects in a C. elegans model. These medications include bupropion (Zyban), varenicline (Chantix), and mecamylamine (Inversine). First, an egg-laying assay was used to investigate each medication's ability to block the egg-laying response in C. elegans acutely exposed to nicotine. Results showed all three medications produced a significant decrease in egg-laying behavior when C. elegans were exposed to subsequent nicotine treatment, compared with a control that was not treated with the test medication. The second nicotine-motivated behavioral assay was developed to investigate the ability of each medication to behaviorally block treated C. elegans from seeking nicotine. A 50 µl aliquot of 50mM nicotine was placed in a circle on one side of an unseeded agar plate and allowed to absorb into the agar. Subsequently, C. elegans were placed in the center of the plate and allowed to move freely. Then the plates were placed into a freezer to stop locomotion. The number of C. elegans on the nicotine side of the plate and the non- nicotine side of the plate were determined. Cohorts naïve to nicotine were attracted to nicotine on plates with no food. Our hypothesis was that C. elegans treated with medications/compounds that aid in smoking cessation would demonstrate a more even distribution on each side of the plate. As expected, the nicotine side was favored by naïve C. elegans compared with the non-nicotine side of the plate. Compared to naïve C. elegans, those C. elegans, those treated with smoking cessation medications were less attracted toward the nicotine side of the plate. Our ultimate goal is to use this method for identifying and evaluating any products or remedies with smoking cessation claims.

1232C

Identification of a New Ascaroside, Female Attracting Pheromone in *Panagrellus redivivus*. **Tatsuji Chuman[1]**, Ramadan Ajredini[1], Hans Alborn[2], Andrea Choe[3], Stephan von Reuss[4], Fatma Kaplan[2], Frank C. Schroeder[4], Paul W. Sternberg[3], Arthur S. Edison[1]. 1) Dept of Biochemistry & Molecular Biology, University of Florida; 2) USDA Laboratory, Gainesville, FL; 3) HHMI and Dept of Biology, Caltech; 4) Boyce Thompson Institute, Cornell University.

Panagrellus redivivus, a free-living nematode related to the well-known model organism, *Caenorhabiditis elegans*, has been studied in the laboratory for decades and is therefore useful for comparative biological studies with *C. elegans*. *P. redivivus* can be easily cultured in the laboratory using conditions similar to those used for *C. elegans*, and the two species share many desirable traits such as short generation time. Whereas *C. elegans* has self-fertilizing hermaphrodites and males, *P. redivivus* has females and males and requires mating for reproduction. *P. redivivus* females can specifically attract males and males can specifically attract females but the chemical nature of this attraction has until now not been known. We used a protocol, previously developed for *C. elegans*, to collect large volume liquid co-cultures with bacterial food as well as biologically active worm water samples of *P. redivivus*. In addition we developed a robust bioassay to test for female attraction using the worm water samples. By activity-guided fractionation, in combination with NMR and LC-MS analyses, we found a pheromone component, component-1, as a female attractant from its worm water sample. Component-1 is a new ascaroside compound and its structure is elucidated by MS and NMR analyses after purification. The synthesis of component-1 for confirmation of the proposed structure is now undergoing. These results suggest a highly conserved and complex system of nematode pheromones and may one day lead to new approaches to the control of parasitic species[1,2]. **References** 1.Srinivasan, J., Kaplan, F., Ajredini, R., Zachariah, C., Alborn, H. T., Teal, P. E., Malik, R. U., Edison, A. S., Sternberg, P. W., and Schroeder, F. C. 2008. A blend of small molecules regulates both mating and development in *Caenorhabditits elegans*. Nature. 454:1115-1118. 2.Edison, A. S. 2009. Caenorhabditis elegans pheromones regulate multiple complex behaviors, *Curr Opin Neurobiol* 19, 378-388.

1233A

The Characterization of Enhanced Environmental RNAi Mutants in Pristionchus pacificus. **Jessica Cinkornpumin**, Jeffrey Shibata, Maryn Cook, Neomal Muthumala, Ray Hong. Biology, California State University Northridge, Northridge, CA.

The lack of environmental RNA interference outside of the Caenorhabditis crown group presents an unexplained evolutionary phenomenon as well as a major obstacle to carrying out functional analysis by forward genetics in non-Caenorhabditis species. In the necromenic nematode Pristionchus pacificus, effective RNAi has been limited to the enhancement of weak alleles via injection of dsRNA into the gonads. Thus, systematic RNAi may exist in P. pacificus but there has not been any evidence of environmental RNAi. To understand the genes involved in the environmental uptake of dsRNA as well as to enable future functional gene analyses in Pristionchus species, our lab carried out a forward genetic screen for Pristionchus enhanced RNAi mutants, or peri's. Specifically, we looked for mutant F1 and F2 individuals in the dominant prl-1 roller gain-of-function mutant background that moved in a wildtype manner when fed on prl-1 dsRNA bacteria but reverts to rolling when fed on OP50. This nonsaturating screen has yielded only one putative peri mutant but demonstrates the potential of this approach to better understand the evolution of systematic RNA interference as well as augment the methodology for Pristionchus species.

1234B

Mass Spectrometric Imaging and Behavioral Assays of *Pristionchus pacificus*. **Chaevien S Clendinen[1]**, Ramadan Ajredini[1], Robert Menger[2], Ghulam Khan[1], Richard Yost[2], Arthur S Edison[1]. 1) Biochemisttry and Molecular Biology, University of Florida, Gainesville, FL; 2) Chemistry, University of Florida, Gainesville, FL.

Nematodes are one of the most numerous and diverse multicellular organisms on earth. They have a profound impact on human health, agriculture, and economy. Like many other organisms, they synthesize and use small molecules to communicate within and between their species. In *Caenorhabditis elegans*, ascarosides have been found to control dauer formation (Butcher, et al., 2007), mating (Srinivasan, et al., 2008), aggregation (Macosko, et al., 2009), and olfaction (Yamada, et al., 2010). *Pristionchus pacificus* is a useful satellite organism for comparative studies with *C. elegans* (Hong & Sommer, 2006). We are developing a bioassay for gender specific chemotaxis (e.g. mating behavior) in *P. pacificus* for activity-guided fractionation of the mating cue. The mating assays are complicated by aggregation behavior in males under some conditions. We are trying to understand the chemical and environmental factors that control these different behaviors. Several behaviors, including aggregation may be mediated or enhanced by chemical cues on the cuticle surface of the nematodes. Therefore, we are trying to develop an alternative approach to chemical ecology studies in nematodes using matrix-assisted laser desorption/ionization (MALDI) mass spectrometric imaging (MSI) (Garrett & Yost, 2006). This technique could potentially not only allow us to detect metabolites on the surface of nematodes, but also visualize the spatial distribution of these small molecules. Using a combination of MALDI MSI and principal component analysis (PCA), we can compare nematodes of different species and find differences in mass spectral profiles resulting from gender, developmental stage, strain or species. We are currently examining *C. elegans* strain N2 (a standard wild-type) and *C. elegans* daf-22 (dauer-defective mutants). Daf-22 mutants are unable to produce short chain ascarosides such as the dauer pheromones common in the N2 strain. Once this technique is developed by the use of *C. elegans*, we will begin to analyze the surface molecules of *P. pacificus* nematodes. Bibliography: Yamada, K., et al. (2010). *Science*, 329 (5999), 1647-1650. Butcher, R. et al. (2007). *Nature Chemical Biology*, 3 (7), 420-422. Garrett, T., & Yost, R. (2006). *Analytical Chemistry*, 78, 2465-2469. Hong, R., & Sommer, R. (2006). *BioEssays*, 28, 651-659. Macosko, E., et al. (2009). *Nature* (458), 1171-1175. Srinivasan, J., et al. (2008). *Nature*, 1115-18.

1235C

Classification and population analyses of soil nematodes by using an improved barcode-sequencing method. **Toshihiko Eki**, Erika Miyazaki, Shoko Yoshimitsu, Hisashi Morise. Dept Environ. Life Sci., Toyohashi Univ. Tech., Toyohashi, Aichi, Japan.

Nematodes are the most abundant animal species on the Earth and soil nematodes including *C. elegans* play an important role in the soil food web. Recent studies indicate that soil nematodes can be used as a suitable indicator for assessing soil environment, because it has been shown that the population balance among soil nematodes are affected by their surrounding environment. Since the current morphology-based methods for soil nematode identification require high level of skills and experience, we aimed to establish an improved method for soil nematode identification by the sequence barcode-based approach.

Here, we have developed the improved procedures for the DNA barcode analysis with 18S ribosomal RNA (18S rRNA) gene in nuclear DNA and the cytochrome *c* oxidase subunit I (*cox-1*) gene in mitochondrial DNA. Using the procedure, we have isolated nematodes from the soils of the flower bed in the campus and the field cultivated with soy bean and successfully clarified taxonomic structures of the soil nematode community based on the sequence analysis of 18S rRNA gene. This analysis showed abundant distributions of nematode species in the genera *Xiphniema* and *Mylonchulus* in the flower bed soil, and the genera *Cephalobus*, *Acrobeloides* and *Distolabrellus* in the field soil, respectively. Furthermore, we have comparatively analyzed the nematode population in the field cultivated with soy bean by the barcode analyses with the 18S rRNA gene and *cox-1* gene, suggesting that the barcode analysis with the *cox-1* gene could clarify more detailed taxonomic structure than that with the 18S rRNA gene. In conclusion, the barcode-based analysis of soil nematodes may be useful for further assessment of soil environment.

1236A

Update: Progress made toward clinical use of the *Bacillus thuringiensis* (*Bt*) Cry5B protein as an anthelmintic. **Brian L. Ellis**, Yan Hu, Ying Liu, Raffi Aroian. Division of Cell Biology, University of California, San Diego, San Diego, CA.

There are approximately 7 billion people in the world and about one quarter to one third are infected with intestinal parasitic nematodes including hookworms, whipworms, and Ascaris. Most of these people are the poorest in the world. These parasitic infections are a leading cause of morbidity worldwide, causing detrimental effects on human growth, nutrition, cognition, school attendance and performance, earnings, productivity, and pregnancy, and, furthermore, can cause increased susceptibility and severity to other infections including HIV, malaria, and tuberculosis. Currently, albendazole is the only drug that shows enough efficacy against these soil-transmitted helminths (STHs) for global mass drug administration and recent data indicate that these parasites are becoming resistant. We

are exploring a new alternative to conventional chemotherapy for these parasites, namely Crystal (Cry) proteins from the Gram-positive soil bacterium *Bacillus thuringiensis* (*Bt*). We have demonstrated that Cry5B, when used in hamsters infected with *Ancylosarom ceylanicum* (hookworm, a blood-feeding parasite), causes a significant reduction in worm burden and egg release, and a significant increase in hamster weight and hemoglobin level. Secondly, we have shown that Cry5B induces a significant decrease in egg release and worm burden in mice infected with *Heligmosomoides bakeri* (a.k.a *H. bakeri*, a luminal parasite). Thirdly, we have shown that Cry5B can act synergistically with other known anthelmintics in *C. elegans* and has interesting combinatorial properties with these anthelmintics. Here, we present an update for the continued work on developing Cry5B and *in vitro* and *in vivo* studies against intestinal parasites as we continue on the path to human clinical trials.

1237B

Crystal structure and function of novel nematicide Bacillus thurigiensis Cry5B. Hui Fan[1], **Yan Hu[2]**, Alan Kelleher[2], Raffi Aroian[2], Partho Ghosh[1]. 1) Department of Chemistry & Biochemistry, University of California, San Diego,La Jolla, CA 92093-0375 U.S.A;; 2) Davison of Cell Biology, University of California, San Diego, La Jolla, CA 92093-0322, U.S.A.

Crystal (Cry) proteins from the soil bacterium Bacillus thuringiensis (Bt) are well known as natural insecticides used in transgenic and organic farming. Our group has shown they may have tremendous value also in the control of soil-transmitted nematodes (aka soil-transmitted helminths or STHs). STHs are amongst the most prevalent human parasites, infecting more than 1 billion of the poorest peoples around the world. New powerful anthelmintics (anti-worm drugs) with novel mechanism of action are in urgent need, and Cry proteins, such as Cry5B, are leading candidates. As part of understanding better how Cry5B works, we set out to solve the three-dimensional structure of this protein. Here we describe our findings from X-ray crystallographic studies, which provide the first structural knowledge of a nematicidal Cry protein, highlighting the similarities and differences between nematicidal and insectidal proteins in this family. In addition, the three-dimensional structure is being used to direct mutagenesis studies in the flexible loop regions of Cry5B to ascertain which residues are critical for specific Cry5B functions.

1238C

Exploring the Biological Targets of a Novel Class of Stilbenoid Compounds with Anthelmintic Activity. **J.A. Miskowski[1]**, O. Awoyinka[1], M. Gross[1], A. Monte[2]. 1) Dept Biol, Univ Wisconsin-La Crosse, La Crosse, WI; 2) Dept Chem, Univ Wisconsin-La Crosse, La Crosse, WI.

Parasitic worms, called helminths, infect plants, animals, and humans worldwide leading to a decreased food supply, economic hardship, and significant levels of morbidity and mortality. Anthelmintics are drugs used to treat helminth infections, and the misuse of these pharmaceuticals has contributed to widespread anthelmintic resistance in worms that infect livestock and emerging drug resistance in human-infecting helminths. The identification of new means to target helminths is imperative. The non-parasitic nematode Caenorhabditis elegans has long been a model system for helminths. We developed two microassays to screen a unique collection of synthetically-derived compounds for anthelmintic activity using C. elegans. These derivatives were based on a parent stilbene compound purified from sweet fern, Comptonia peregrina, that exhibits pharmacological activity. The motility assay screens the animals for paralysis, which is the final outcome of treatment with most existing anthelmintics. To detect activities that might negatively affect other apects of nematode function, the developmental assay screens animals for developmental arrest prior to sexual maturity, a decrease in fecundity, or lethality. Both assays have been successfully adapted to 96-well microtiter plates. 67 stilbenoid compounds were tested in the motility assay and six caused reduced motility, paralysis, or death in worms at the lowest concentrations tested. 15 of the compounds were also tested in the developmental assay and two elicited strong effects. These data led to five stilbenoid test compounds prioritized for further study. The test compounds possess a unique structure compared to existing anthelmintics, yet the potency of the compounds is not sufficient to justify their development as bona fide anthelmintics. Hence, the novel test compounds will be used as probes to explore mechanisms by which worms might be targeted. To this end, the stilbene derivatives are being tested against mutant C. elegans strains that are resistant to existing anthelmintics using our assays for anthelmintic activity. This will reveal if any of the new compounds acts via a novel molecular mechanism. The data from these assays, combined with the potency of each compound, will be used to prioritize one compound for further study. A genetic screen will be performed to identify mutants that are resistant to the prioritized compounds, and the gene(s) harboring the molecular lesion in the mutants will be identified. This knowledge will be extended to helminths and potentially contribute to new treatments.

1239A

A can of worms - challenges and strategies for next generation nematode genomics. **Michael Paulini**. Wellcome Trust Sanger Institute, Cambridge, United Kingdom.

Next generation sequencing has provided many laboratories with the means to sequence whole nematode genomes. At WormBase we have created standards to streamline the integration and presentation of this data. This enables small nematode communities to use the comprehensive information available for Caenorhabditids in context of their research species. Using comparative genomics with/against highly curated nematodes, sparse and rough genomes can have interesting features annotated. I will outline the integration process using the examples of Trichinella spiralis and Strongyloides ratti and describe some possible use cases, such as creating list of potential anti-helminthic drug targets.

1240B

Unveiling the unique biology of nematodes living in a cold methane seep on the ocean floor and their symbiosis with chemosynthetic bacteria. **Amir Sapir[1,5]**, Adler R. Dillman[1,5], Manuel Mundo-Ocampo[2,4], James G. Baldwin[2], Victoria J. Orphan[3], Paul W. Sternberg[1]. 1) Howard Hughes Medical Institute and Division of Biology, California Institute of Technology, Pasadena, CA 91125, USA; 2) Department of Nematology, University of California, Riverside, CA 92521, USA; 3) Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, CA 91125, USA; 4) CIIDIR-IPN, Unidad Sinaloa, Guasave, Sin. Mex C.P. 81000; 5) equal contribution.

The complex life of microbes in deep sea chemosynthetic environments is being actively deciphered with a focus on microbes' ecology and metabolism. In contrast, the biology of several nematode species that were reported to live in these habitats remains largely elusive. To start addressing questions of nematodes ecology, metabolism, and symbiosis with microbes in chemosynthetic environments we sampled and sorted worms from Hydrate Ridge, a cold methane seep off the Oregon coast 774 meters below the sea surface. This niche is characterized by low oxygen levels (anoxic/ microoxic), low temperature, and high concentration of hydrogen sulfide. Chemotrophic microorganisms drive primary production in this deep-sea ecosystem, deriving energy through sulfate-dependent methane oxidation or sulfide-oxidation. Stable isotopic analyses suggest that worms and other metazoans in this niche rely on these microbes as a food source. A molecular survey of nematodes from this seep site revealed a surprising level of diversity, representing a number of understudied phylogenetic clades. In contrast to many terrestrial free-living nematodes that are found in the wild primarily as dauers, the majority of nematodes identified in the samples were reproducing adults. This may suggest that at the time of sampling food was not the limiting factor. Combining DAPI staining with light and scanning electron microscopy we discovered a complex nematode-bacteria relationship including the distribution of external and internal symbionts: The worm's hypodermis is covered with microbes and in two different worm species we identified two morphologically different microbes in the body cavity suggesting species-specific interactions between nematodes and microbes. Preliminary profiling of the external and internal microbes in selected species identified the associated microbes as bacteria. We report the characterization of symbiont diversity with respect to nematode hosts as a first step toward understanding worm-microbe symbiosis and worms' adaptation to extreme chemosynthetic environments.

1241C

Characterizing wild nematode isolates in an undergraduate research lab. **Robin L. Hill**. Natural Science, The University Of Virginia's College at Wise, Wise, VA.

Small Public Liberal Arts Colleges pose multiple challenges, particularly in teaching laboratory courses. Resources are limited and the majority of students have no lab experience. In addition, faculty struggle to maintain a full-time active research program due to heavy teaching loads. A significant percentage of students expressing an interest in the sciences also express a desire for graduate or professional studies. As many of these programs desire "hands-on" scientific experience, providing research opportunities could be vital to their future success. To address these issues, a full semester research-based project was developed around isolating and identifying wild nematode isolates. An overview of nematode phylogeny and population genetics studies is coupled with students working in small groups to develop a research project, isolate nematodes and characterize the isolates using morphological and molecular analysis. Results are presented in a format similar to graduate lab meetings. The molecular techniques utilized during the pilot project included species-specific PCR (*glp-1*) (1) as well as sequencing of the 18S ribosomal RNA gene (2). I adapted universal rice primers (URP) as a fingerprint assay (3). Morphological analysis included buccal (lips, buccal tube, pharynx) and tail (shape, papillae and spicule structure) morphology as well as mating system (gonochoristic vs. hermaphroditic). Strains from the CGC have been used for comparison (both morphological and molecular) to wild isolates. Mating crosses with *C.elegans* and *C.briggsae* males have been performed with hermaphroditic species. Males from gonochoristic strains can be employed for gonochoristic isolates. Hermaphroditic isolates were obtained from leaf litter in wooded settings, open grasslands and under a rotting pumpkin. Species-specific PCR as well as mating crosses reject identification as *C.elegans* or *C.briggsae*. Sequencing of the 18S RNA gene is ongoing. Gonorchorisitc isolates were obtained from both household and grass cutting compost. Morphological analysis based on tail morphology suggests the genus *Rhabditis*, subgenus *Rhabditella* and *Cephalopoides*. Student feedback was uniformly positive. Isolates were easily obtained and the self-directed nature of the projects deepened their understanding of lab methods and research design. The range of analysis methods allows the course to be adapted to resource availability, therefore providing a method of research experience while enhancing knowledge of the model species. (1) Barriere, A., and Felix M.A. (2005). *Curr. Biol*. 15,1176-184. (2) Floyd, R. et al. (2002). *Mol Ecol*. 11, 839-850. (3) Kang, H. W. et al. (2002). *Mol. Cells*. 2, 281-287.

1242A

Application of medium-throughput mutagenesis techniques in the molecular laboratory environment. **Jonathan E. Karpel**, J. Cole Rasch. Dept Biol, Southern Utah Univ, Cedar City, UT.

The inclusion of original research has been an integral component of the molecular biology laboratory class at Southern Utah University. As part of an NSF grant proposal, we are investigating potential group projects that can be incorporated into the molecular laboratory. One such project is the isolation and characterization of mutants in putative spliceosomal-related genes. Here we used the TMP/UV worm mutagenesis technique to create large, random deletions in C. elegans, and, using the 35 students enrolled we were able to collect and analyze upwards of 750 mutant F_1 worms. Students were required to maintain liquid C. elegans cultures in 20-well plates and then, using standard PCR techniques, identify potential mutants of interest. Although no mutants were discovered in the genes of interest with this particular attempt, we feel that this process can easily be scaled up to cover more of the nematode genome. The expected outcomes of this project are to expand the knowledge of the spliceosome in this model organism, to subject undergraduates to original research in a laboratory setting, and to allow these students to share their experience and train others in the fundamental molecular techniques undertaken.

1243B

A writing intensive *Caenorhabditis elegans* laboratory exercise for undergraduates examining oxidative stress and antioxidants. Taylor Bell, Justine Betzu, Loreal Brown, Marissa Fontana, Christopher Frymoyer, Christina Hamilton, Amy Hartl, Lauren King, Amanda March, Caitlyn McLarnon, Lauren Meeley, Kathleen Murley, Katherine Murphy, Matthew Pall, Rabiul Ryan, Erin Slaunwhite, **Rebecca E. Kohn**. Biol Dept, Ursinus Col, Collegeville, PA.

An open-ended laboratory exercise was developed for an undergraduate Molecular Neurobiology course. The goal of the exercise was for students to design and carry out an experiment to examine how oxidative stress affects *Caenorhabditis elegans* strains with mutations affecting nervous system function and whether an antioxidant could protect the worms from damage. A writing intensive component of the laboratory was included for students to submit their findings to a peer-reviewed journal. This exercise would be accessible for a variety of upper level courses, including neuroscience and cell biology. Students successfully designed their experiments based on information in the Materials and Methods sections in related scientific journal articles. Students chose conditions for inducing oxidative stress and for incorporating an antioxidant in the experiment. The professor teaching the course and a student teaching assistant experienced with *C. elegans* research guided students in experimental design, trouble shooting difficulties, and analyzing findings. Students' results showed that strains with defects in neurotransmitter release had a higher percentage of lethality than a strain with a wild type nervous system. The antioxidant they chose to work with, L-ascorbic acid, decreased the percentage of lethality for some strains. Students worked in groups of four during scheduled laboratory times as well as during additional times to maintain their strains and perform experimental trials. During laboratory meeting times, students spent part of their time discussing effective approaches for writing scientific papers. Drafts of student manuscripts went through student peer-review and review by their instructor to prepare for submission to a journal. Three student groups chose to submit their manuscripts to the journal, *IMPULSE, An Undergraduate Journal for Neuroscience*, and one group chose to submit to *The Journal of Young Investigators*. Both journals are designed for undergraduate authors.

1244C

Problem-solving and project-assisted learning of genetics, molecular biology and biological pathways in the study of Protein Kinase C in *C.elegans*. **Marianne Land**. New York Institute of Technology, Old Westbury, NY.

Undergraduate students at New York Inst. of Tech. (NYIT) participate in my research, to elucidate the function of the *C. elegans*, Ca^{2+} and diacylglycerol activated Protein Kinase C-2 (PKC-2), in a Biomedical Research course (BIOL-425). This research reinforces many biological and physiological processes that are learned in Pre-Med. and B.S. programs. Potential PKC-2 substrates are cloned and expressed in *E.coli*. and genetic crosses are performed to elucidate the biological pathways of PKC-2. During the cloning of *C.elegans* cDNAs into *E.coli* expression vectors, the importance of maintaining the correct reading frame, that the genetic code is read in triplets, the universality of the genetic code, the necessity of stop codons and details of the *lac* operon are learned. Protein purification is carried out using affinity chromatography and the elution of histidine-fusion proteins from a metal affinity column demonstrates that imidazole mimics histidine. Testing wild type and mutant PKC-2 substrates for activity shows the importance of the charge and ionization constant of amino acids in the function of a protein. Problem solving skills are practiced when an experiment does not produce the expected outcome, for example if a *C.elegans* protein is poorly expressed or insoluble. Initially students perform a literature search on the research topic and present their findings to the rest of the group. Because students have a limited amount of time to perform research, a team-based approach is utilized, in which a collaboration is set up between students, to complete an experiment. During the expression and purification of bacterially expressed proteins there are many suitable stopping points at which an experiment may be suspended and proteins may be stored without sacrificing the integrity of the purified protein, until the purification procedure can be completed. Students are required to keep a detailed record of all experimental procedures as they are carried out. During group meetings each student explained the part of the experimental process they had performed. Upon completion of an individual experiment a laboratory report was written in scientific format, along with suggested future experiments that included suitable controls. Other methods of assessment include Journal Club presentations at group meetings and research presentations at NYIT's annual undergraduate research symposium, SOURCE. Response to this inquiry-based course has been very positive. Students frequently remark that our work clarifies concepts, for example, affinity chromatography and Mendel's Laws, which they are required to have knowledge of in other courses and for standardized tests, such as the MCAT exam and biology GRE.

1245A

Using Nematodes for Discovery Based Learning. **Candace C. LaRue**, Pamela Padilla. Biological Sci, Univ North Texas, Denton, TX.

Despite *C. elegans* popularity in genetics and developmental biology research, its use in the classroom is not widespread. One obstacle in using *C. elegans* for educational purposes is that available resources are primarily targeted to professionals in the researcher community, creating difficulty with implementation beyond the realm of graduate research education. We are working to engage students by incorporating nematodes into the science curriculum at the K-12 and undergraduate level. We *hypothesize* that key concepts in Genetics can be better understood if students take part in a "discovery based" lesson plan rather than a "cookbook" lesson plan or traditional lecture. To test this hypothesis, a change was implemented in the format of the Genetics lab at UNT. Students conducted a forward genetic screen to understand the biology and genetics of *C. elegans* and this powerful experimental approach often used to identify genes involved with biological processes. The original format of the lab included "cookbook" style labs and a *Drosophila melanogaster* project based on crosses resulting in a known/expected set of results. The new discovery based lab format includes a project in which the identification and isolation of *C. elegans* mutants is based on phenotypes students wish to investigate. Assessments evaluating student understanding were conducted at the beginning and end of the lab course. Data analysis identifies trends in student learning following the change in lab format that indicate: 1. shifts in student understanding, 2. areas that students continue to find conceptually difficult and 3. alternative concepts that students continue to hold.

In addition to the undergraduate science education projects involving nematodes, we are developing projects for grade school students. We *hypothesize* that these students will better understand scientific reasoning and the Nature of Science by engaging in discovery based, outdoor lessons. To test this hypothesis students isolate nematodes from soil or compost and systematically compare these species to the well studied model system, *C. elegans*. Participating students are from three groups: elementary (3rd-5th grade), middle school (6th-8th grade) and high school (9th-10th grade). Integration of the discovery based instruction was carried out via collaboration with the teachers, student and parents at these schools. Assessments of this approach to teach aspects of scientific discovery were conducted. The benefit of this work on the scientific community includes access to new curriculum and the potential identification of new species for evolution studies.

1246B

Student Projects using C. elegans to Investigate Animal Physiology. **Tim H. Lindblom**. Div Sci, Lyon College, Batesville, AR.

As a supplement to traditional physiology laboratory exercises, I require my comparative physiology students to design and implement physiology research projects involving C. elegans. Although I tend to guide the student's projects as little as possible, the level of instructor involvement is be quite variable. By requiring students to write a research proposal prior to initiating their experiments, they can be directed towards realistic projects likely to succeed. The only stipulations are that 1. the research investigate some area of physiology and 2. they not reproduce published data or experiments. At the first laboratory meeting, I introduce C. elegans as a research organism and ask them to consider their favorite area of physiology. From this interest, I guide them towards the relevant C. elegans literature and ultimately to a research plan. Prior to spring break, the students have formulated a research plan that includes a hypothesis, a detailed experimental design, and identification of the relevant controls. This way, during the break, I can gather the required reagents, strains, and supplies. As the groups begin their experimentation, it is important to regularly check their progress in the event that experimental design troubleshooting is required. Ultimately, the students are responsible for initiating the experiments, experiment troubleshooting, data collection, and assembly of the data into a research paper. I required that the research papers adhere to a specific journal format, typically one that is readily available for examples, such as Current Biology. This way, they experience the many steps in the process of converting great research ideas into meaningful publications. At the end of the semester, student groups present their hypothesis, experimental design, data, and conclusions to their colleagues in order to gain experience in oral research presentations and expose all the students to the various research projects. This presentation, along with the paper, is reviewed by other research teams as a mechanism to introduce them to peer review. Here, I present some recent examples of these projects.

1247C

Using *C. elegans* as a model to assay bacterial pathogenesis in an undergraduate research survey course. **Edith M Myers**, June H Middleton. Department of Biology, Fairleigh Dickinson University, Madison, NJ.

The department of biology has recently introduced a research survey course for advanced sophomore students who have expressed interest in doing undergraduate research with faculty within our department. The purpose of this course is to introduce students to basic concepts such as reading primary literature, designing an experiment, analyzing data, and presenting data, which are essential to biological research. Each semester-long course consists of two eight-week research modules performed with two different faculty members. One module, introduced this year, consisted of using *C. elegans* as a model organism to assay pathogenesis of several different strains of bacteria.

Students read several review and research articles about bacterial pathogenesis in *C. elegans* before designing and performing survival assays using different strains of mildly pathogenic bacteria. Students then assayed *C. elegans* survival on strains of *Enterococcus* isolated from the feces of resident and migratory Canada geese. In a previous module of this course, the same students used a biochemical key to determine putative *E. faecalis* isolates from the fecal samples. *E. faecalis* isolates were more common in resident than in migratory geese. Students then screened the *E. faecalis* isolates for antibiotic resistance profiles and the presence of several expressed virulence factors including production of gelatinase, hemolysins and cytolysins. Strains exhibiting different patterns of antibiotic resistance and virulence factor expression were evaluated using *C. elegans*. Students designed, performed, and modified assays to test survival on each strain of bacteria. Each bacterial strain was examined to determine the extent to which the microbe colonized the worm intestine. Using a video capture system, students documented differences in intestinal colonization. Students presented their findings in a poster at a local undergraduate research conference.

1248A

Combining old fashioned gene mapping and new fangled genetic analysis in an upperlevel Genetics course. **Maureen A. Peters**, Katherine Cullen. Biology Department, Oberlin College, Oberlin, OH.

Integrating a Genetics laboratory into a complementary lecture course strengthens the understanding of genetic concepts and introduces the process of experimental investigation. Five iterations of a combined lecture and lab course have been offered at a small liberal arts college, with each enrolling twenty or fewer students. Weekly three-hour lab meetings are scheduled. Students must also work outside of class time to complete the long-term experiments. The lecture portion of the class covers classical and molecular genetics with a focus on problem solving and genetic analysis.

The lab portion is divided into two experimental sequences: the first uses classical genetics, the second, molecular biology and/or reverse genetics. The classical sequence focuses on the concepts of dominance, chromosomal segregation, linkage, and recombination. Each pair of students are given a *C. elegans* uncoordinated (Unc) mutant whose identity is unknown. Students perform a dominance test, map their mutant to an approximate genetic map position, identify a candidate Unc using Wormbase and test their predication using a complementation test. This series of experiments builds microscopic manipulation and experimental planning skills, data analysis expertise, and provides familiarity with *C. elegans* biology and bioinformatic resources. The molecular biology and/or reverse genetics sequence advances student understanding of one of the concepts covered in the classical sequence, but does so through the active research program of a department member. In this latter portion, the lab emulates an active research lab, with each student engaging in open ended experiments. Thus this portion of lab varies each year. Several experiments have utilized single nucleotide polymorphism (SNP) mapping of single or double mutants. RNA interference screens using a feeding bacterial library have also been performed.

Students generally enjoy the independence and engagement provided by these investigative lab experiments as well as the integration of lecture concepts in lab exercises. Pre- and post-test surveys suggest that students gain confidence in their ability to analyze data and to propose new experiments.

1249B

Using C. elegans to teach transmission genetics in a large introductory biology lab. Penny L. Sadler, **Diane C. Shakes**. Dept Biol, Col William & Mary, Williamsburg, VA.

Transmission genetics is a core topic in most introductory biology courses and yet many students rarely think about genetics beyond Mendel's classical experiments with peas. In our introductory biology laboratory course at the College of William and Mary, we expand students' notions of transmission genetics by using C. elegans as the model system to determine whether an "unknown" Unc mutation is linked or unlinked to a known Dpy marker. In a course that serves not only biology majors but also neuroscience majors, pre-meds, and non-majors, the students see direct relevance in studying mutations with direct links to human neurological conditions. Our genetics module is a four-week session that culminates in a writing workshop designed to aid students in writing their first formal lab report. The first week serves as an introduction to best practices in using a dissecting scope, sexing the worms, distinguishing single mutant morphological phenotypes, and setting up a Po cross with 1mm long males and hermaphrodites. During the second week, the students score their P0 cross, set up their F1 crosses, and practice distinguishing wild type, single mutants and double mutant hermaphrodites. During the third week, the students score their F2 progeny, analyze their data, and test their data set against a null hypothesis that the two genes are unlinked using Chi-square. The format of the lab report is introduced and discussed as a class. During the fourth week, student teams meet with their lab instructor to go over a rough draft of their lab report. This one-on-two time dedicated to improving the content of the report allows for correction of major misconceptions and errors in formatting the report. The fourth lab week is also dedicated to a self-guided online Wormbase bioinformatics exercise which is designed to help students formulate their ideas for the future directions sections of their lab report. The poster will also present student learning objectives and assessment, and discuss the logistics of preparing the materials that enable over 400 freshmen carry out a series of genetic crosses during a four week lab module under the guidance of masters level graduate teaching assistants and a single, full-time lab coordinator.

1250C

Preparing Undergraduates to Work at the Intersection of Biology and Mathematics. **Timothy D. Walston**[1], Barbara Kramer[2], Jason Miller[3]. 1) Dept Biol, Truman State Univ, Kirksville, MO; 2) Dept Chem, Truman State Univ, Kirksville, MO; 3) Dept Math and CS, Truman State Univ, Kirksville, MO.

The workforce need for Science, Technology, Engineering and Mathematics (STEM) majors who are trained in interdisciplinary methods is rapidly increasing. In response to this need, Truman State University leveraged its expertise in undergraduate research and its commitment to interdisciplinary learning to develop an undergraduate program in mathematical biology. Here describe our undergraduate research program in mathematical biology (including projects involving *Caenorhabditis elegans*) , a portfolio-based minor in mathematical biology, interdisciplinary elective courses that count towards majors in Biology, Mathematics, and Computer Science, and efforts to increase exposure of students to interdisciplinary topics earlier in their college career. We also will discuss benefits and challenges to implementing an interdisciplinary program.

1251A

The novel non-coding RNA *lep-5* is a heterochronic regulator of male tail tip morphogenesis. **Edward Vuong**[1], Karin Kiontke[2], Antonio Herrera[2], Douglas Portman[1], David Fitch[2]. 1) Univ Rochester, Rochester, NY; 2) New York University, New York, NY.

The *C. elegans* male tail is an ideal system to understand how developmental cues converge to regulate morphogenesis. During L4, the four hypodermal cells of the male tail tip fuse and retract to form the rounded tail tip syncytium of the adult. At the core of the regulatory network controlling this process lie three transcription factors, NHR-25, DMD-3 and MAB-3, that regulate downstream effectors of morphogenesis (see poster by Herrera et al.). The onset of morphogenesis is controlled by the heterochronic pathway, which regulates the timing of *dmd-3* expression in the tail tip. Precocious heterochronic mutants (e.g., *lin-41(lf)*) display early *dmd-3* expression and premature retraction, while retarded mutants (e.g., *let-7* and *lin-41(gf)*) exhibit delayed or absent *dmd-3* expression and unretracted and unfused ("Lep") tail tips even in adults. In mutagenesis screens for male tail mutants, we identified two alleles of a novel heterochronic gene, *lep-5*. These mutants exhibit severe Lep tails and show delayed activation of *dmd-3* in the posteriormost tail tip cell, hyp10. *lep-5* also has other heterochronic characteristics: *lep-5* mutants of both sexes undergo supernumerary molts as adults, and *lep-5* phenotypes are suppressed by transition through the dauer stage. Epistasis experiments indicate that *lep-5* acts upstream of or in parallel with *let-7* and *lin-41*. *lep-5(ny10)* is a large deletion removing several genes, whereas *lep-5(fs8)* is a point mutation in the 5' regulatory region of a predicted protein of unknown function. ESTs and RT-PCR analysis have shown that *lep-5* produces a transcript that is SL1 trans-spliced and polyadenylated. However, several lines of evidence suggest that *lep-5* is not a protein, but rather a non-coding RNA. First, the single point mutation (G>A) found in *lep-5(fs8)* disrupts the predicted secondary structures of two stem loops at the 5' end of the *lep-5* transcript. Restoration of the secondary structure in this area with a compensatory mutation rescues the mutant phenotype. Second, there is no conservation of the *lep-5* sequence in *Caenorhabditis* at the protein level, although the nucleotide sequence is well conserved. Third, the *lep-5(ny10)* mutant can be fully rescued by the homologous sequence from *C. briggsae*. Interestingly, the sequence of the 5' region of the transcript (including SL1) has the potential to fold into a stem loop bearing a sequence similar to a known miRNA with which *lep-5* has some functional redundancy. We are currently testing the possibility that *lep-5* functions as a miRNA.

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