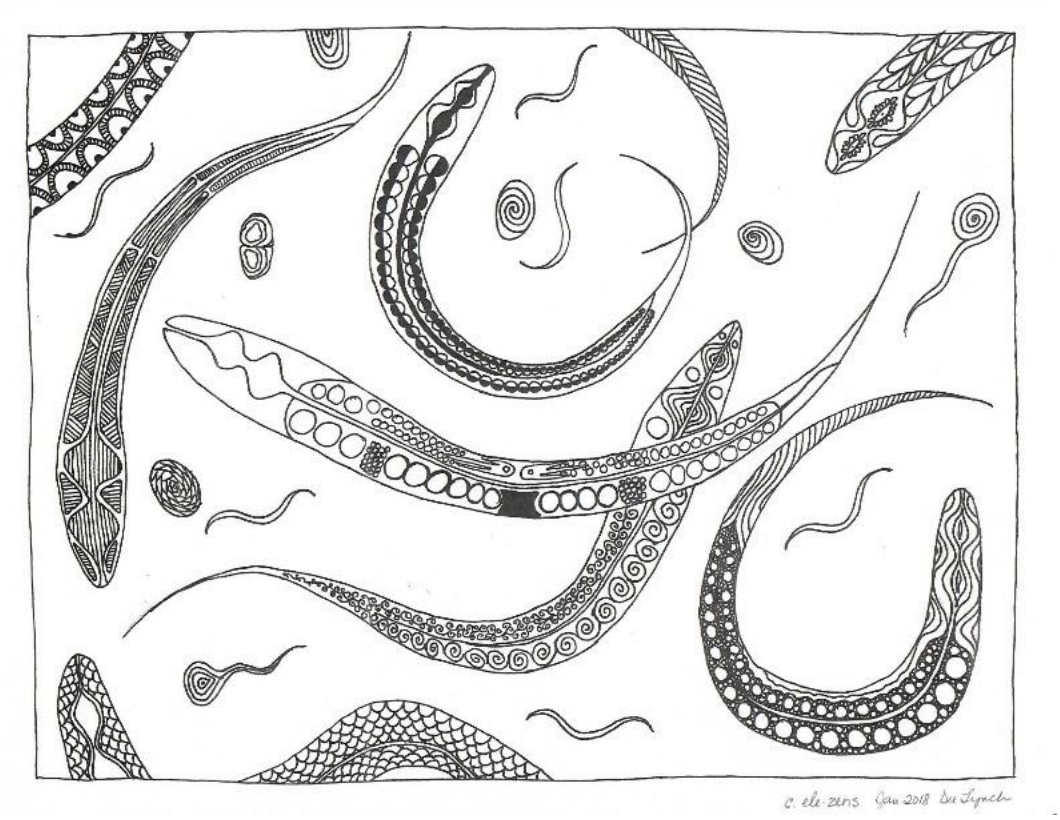
Chicago AREA WORM MEETING



2nd Chicago Area Worm Meeting

Thursday May 23rd, 2019

University of Illinois – Chicago

Web: www.chawm.org. Twitter: @ChicagoWorms, #ChAWM2019.

Keynote, Talks and Workshop

Moss Auditorium. Room 1020, College of Medicine Research Building (COMRB). 909 S. Wolcott Ave., Chicago, IL.

Keynote - 10:00-11:00AM.

Introduction to *microPublication Biology* and Control of Germline Stem Cell Differentiation in *C. elegans*.
Tim Schedl. Dept. of Genetics Washington University.

Session 1 - 11:00-12:00PM. Chair: Xantha Karp (12 min. talks, 3. min. questions).

1.- Germline RNA helicases drive the phase separation and perinuclear anchoring of germ granules to promote piRNA-mediated genome surveillance.
Wenjun Chen, Jordan Brown, Charles Lang, Donglei Zhang, Karen Bennett, Shikui Tu, Zhiping Weng, Ed Munro, Heng-Chi Lee.

2.- SYGL-1 and the molecular switch from GSC self-renewal to differentiation in *C. elegans*.
Tina Lynch, Charlotte Kanzler, Heaji Shin, and Judith Kimble.

3.- The *mir-44* family of microRNAs regulates the sperm/oocyte switch in *C. elegans* hermaphrodites.
Katherine Maniates, Benjamin S. Olson and Allison L. Abbott.

4.- Biochemical characterization of essential kinesin activity and regulation in *C. elegans* oocyte meiosis.
Ian Wolff and Sadie Wignall.

Lunch (12:00-1:00)

Session 2 (1:00-2:00PM). Chair: Claire de la Cova (12 min. talks, 3. min. questions).

5.- EXC-4/CLIC proteins are ancient regulators of heterotrimeric G-protein-Rho/Rac signaling.
Anthony Arena, De Yu Mao, Jan Kitajewski and Daniel Shaye.

6.- Wnt signaling antagonizes repression of germline genes in somatic cell nuclei.
Jerrin Cherian and Lisa Petrella.

7.- Muscular exertion is detrimental to viability in a nematode model of Duchenne muscular dystrophy.
Kiley Hughes, Anjelica Rodriguez, Kristen Flatt, Sneha Ray, Andrew Schuler, Brian Rodemoyer, Visalashki Veerappan, Kori Cuciarone, Alex Kullman, Calis Lim, Neha Gutta, Samantha Vemuri, Victoria Andriulis, Dana Wismonger, Lucas Barickman, Wolfgang Stein, Aakanksha Singhvi, Nathan Schroeder, Andres Vidal-Gadea.

8.- A Genetic Screen to Identify New FGFR Signaling Components.
Victoria Puccini de Castro, J. Palalay, J. E. Webb, C. Gaudenzi, X. Alava, O. Payan Parra, M. Stefinko, C. Voisine, T.-W Lo, and, M Stern.

Coffee Break (2:00-2:30PM)

Session 3 (2:30-3:30PM). Chair: Chiou-Fen Chuang (12 min. talks, 3. min. questions).

9.- Massive sampling of *Caenorhabditis elegans* across the Hawaiian Islands reveals remarkable genetic diversity on the islands and admixture with globally distributed populations.
Tim Crombie, Stefan Zdraljevic, Shannon Brady, Daniel Cook, Kathryn Evans, Steffen Hahnel, Daehan Lee, Briana Rodriguez, Robyn Tanny, Ye Wang, Gaotian Zhang, Joost van der Zwaag, and Erik Andersen.

10.- The role of DEX-1 in dauer-specific locomotion behaviors.
Kristen Flatt, and Nathan Schroeder.

11.- Investigating the Function of the ARID-type Transcription Factor CFI-1 in Cholinergic Motor Neurons of the Nematode *C. elegans*.
Yinan Li, and Paschalis Kratsios.

12.- Timing matters: Organismal responses to stress.
Srijit Das, Felicia Ooi, Johnny Cruz Corchado and Veena Prahlad.

Workshop (3:30-4:15PM): Genome Editing. Chair: Cindy Voisine (5 min. talks).

Short presentations by Daehan Lee (Northwestern), Alexandra Socovich (UIC), Olivia Gaylord (U. Chicago), Sarit Smolikove (U. Iowa), and Min Le (ThermoFisher Scientific), followed by a Q&A session and open discussion.

Posters (4:30-6:30PM): Faculty Alumni Lounge. Room 219, College of Medicine West (CMW) Building.

Page numbers below denote poster-board number in exhibit room. Poster-boards are 3' high x 6' long (36”x72”).
Presenters for odd-numbered posters should stand by their posters from 4:30 to 5:30PM.
Presenters for even-numbered posters should stand by their posters from 5:30 to 6:30PM.

13.- Duchenne Muscular Dystrophy in dystrophic *C. elegans* eggs.

14.- Dauer IL2 neurons use distinct and shared mechanisms with FLP and PVDs to regulate arborization.

15.- An excreted small-molecule signal promotes *C. elegans* reproductive development and aging.

16.- Investigating the role of mechanoreceptors in magnetic orientation in *C. elegans.*

17.- A nematode-specific gene underlies bleomycin-response variation in *Caenorhabditis elegans*.

18.- The role of the Integrator Complex in piRNA transcription termination.

19.- An autism-causing variant misregulates selective autophagy to alter axon targeting and behavior.

20.- ERAD for the SLOw and Sluggish.

21.- A role for the DREAM complex in germline apoptosis.

22.- Elucidating the role of the conserved transcription factor HLH-10/musculin (TCF21) in sex-specific motor neuron differentiation

23.- *In-silico* characterization of the *Caenorhabditis elegans* matrisome and proposal of a novel collagen classification.

24.- Identifying *ben-1*-independent resistance alleles of benzimidazoles.

25.- AIR-2/Aurora B kinase activity is required for critical events during *C. elegans* oocyte meiosis.

26.- The Role of NEKL-3 In the Regulation of Axon Termination.

27.- FBF partnerships and their role in regulation of germline fates.

28.- A toolkit for analyzing the role of heterotrimeric G-protein-Rho/Rac signaling, and its regulation by EXC-4/CLIC in the excretory canal cell.

29.- Natural variation underlies differential responses to zinc treatment in *Caenorhabditis elegans*.

30.- Securing motor neuron terminal identity throughout life by intercepting a Hox-mediated transcriptional switch.

31.- Developing a model to screen for small molecule treatments that reduce AGEs using *C. elegans*.

32.- Deciphering the functional network of FSGS-associated genes using *C. elegans*.

33.- A role for Cofilin (UNC60A) in early embryogenesis in *C. elegans*.

34.- SYGL-1 and the molecular switch from GSC self-renewal to differentiation in *C. elegans*.

35.- *daf-16* blocks expression of *let-7*-family microRNAs to promote multipotent cell fate during dauer.

36.- Cytoplasmic Aggregates of Human TDP-25 Protein in *C. elegans* Challenge Proteostasis.

37.- Functional analysis of microRNA regulatory roles in the *Caenorhabditis elegans* male gonad.

38.- High throughput analysis of magnetic orientation using the nematode *C. elegans*.

39.- A Potential New Component That Mediates the Regulation of Fluid Homeostasis in *C. elegans*.

40.- Determination of *mig-32* as a Class A or Class B synthetic multivulva (synMuv) gene.

41.- Effect of D-amino acids on *C. elegans* locomotion.

42.- Age-appropriate coordination of behavior and reproductive physiology via a shared neuronal circuit.

43.- Compromised Mating Ability and Reduced Sperm Transfer Explain Reduced Fertility in *C. elegans* Males at High Temperature.

44.- The excretory canal as a platform to discover kinase regulators of tubulogenesis and angiogenesis.

45.- Modified DSB repair program for exogenously induced DSBs in the *C. elegans* germline.

46.- The search for genes that prevent the progression of degeneration in Duchenne muscular dystrophy.

47.- Wnt Dependent Cell Fate Specification Requires Active SYS-1/β-Catenin Trafficking and Turnover at the Mitotic Centrosome.

48.- Cell to cell spreading of TDP-43 C-terminal fragments may lead to toxicity in *C. elegans*.

49.- Understanding the role of the conserved kinase PIG-1/MELK in tubulogenesis.

50.- Natural variation of *C. elegans* short tandem repeats.

51.- A genetically divergent *C. elegans* wild isolate exhibits extremely high male frequency.

Many thanks to our sponsors:

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Thanks also to Cold Rain Studios, for designing our logo, and to Tina Lynch and Dee Lynch for the "*C. ele-zens"* sketch used in our cover. Thanks to Kamil Czarnowski, Monica Horga and Laura Scott, from the Dept. of Physiology and Biophysics at UIC, for administrative assistance, and to Nicholas Kane, Chief Operating Officer of the UIC College of Medicine, for logistics support.

For WiFi access during ChAWM: connect to the “UIC-Guest” network, open a web browser, then authenticate with the login: chawm_3 and the password: roy3int3.
Follow us on Twitter (@ChicagoWorms) and tweet about the meeting with the hashtag #ChAWM2019.

Map and Directions:

- **By Public Transportation:** the closest train station is “Polk” on the Pink Line. It’s a 5-10-minute walk from this station to the College of Medicine Research Building (COMRB) at 909 S. Wolcott Ave., where Moss Auditorium is located (COMRB1020). The "Illinois Medical District" stop on the Blue Line (Damen Ave. exit) is also near, but a longer (10-15 minute) walk. CTA Bus Routes 12 and 157 have several stops nearby.

- **Driving:** COMRB is accessible from Interstate 290 (Kennedy Expressway) and several major roads.
    - From either direction on 290, take Exit 28A (Damen Ave./Illinois Medical District). Travel south on Damen Ave. then make a Left onto Taylor St. If dropping off at COMRB, make a Left onto Wolcott St. If driving to Parking Structures, make a Right on S. Wood St. or a Left on Paulina St.
    - If travelling on local roads, UIC College of Medicine is accessible from several major streets: Ashland Ave. on the East, Roosevelt Rd. on the South, Damen Ave. on the West, Polk St. on the North, and it is traversed by Taylor St.

- **Parking:** There are several UIC lots open to the public.
    - The rate for the full day (anything more than 4.5 hours) is $15.
    - The nearest visitor parking lots are B2 and C4 (see map), but these are small and may be full. Instead we recommend the Wood Street Parking Structure, at 1100 South Wood St. (marked on the map above), or the Paulina Street Parking Structure at 915 South Paulina St. (corner of Taylor St. and Paulina St.). For directions to either of these parking structures, click on their addresses above.

TALK ABSTRACTS

Germline RNA helicases drive the phase separation and perinuclear anchoring of germ granules to promote piRNA-mediated genome surveillance.

Wenjun Chen, Jordan Brown, Charles Lang, Donglei Zhang, Karen Bennett, Shikui Tu, Zhiping Weng, Ed Munro, and Heng-Chi Lee.
Department of Molecular Genetics and Cell Biology, University of Chicago.

piRNAs function as a guardian of the genome through the silencing of foreign or selfish nucleic acids. However, it remains unknown how piRNAs effectively identify foreign/selfish RNAs among many endogenous mRNAs. Intriguingly, many piRNA pathway factors are enriched in germ granules, the major sites of RNA export in germ cells, implying their localization may facilitate the detection and silencing of foreign nucleic acids. Here we showed that two germline helicases, GLH-1 and GLH-4, play a redundant role in germ granule formation. In *glh-1; glh-4* double mutants, many germ factors, including PGL-1 and PRG-1, become completely dispersed into the cytoplasm. To investigate the roles of germline helicases in controlling germ granule formation, we created gene-edited worms that express various mutated GLH-1 helicases. We found that GLH-1 controls the formation and disassembly of germ granules through their binding and release of RNAs, respectively, which are coupled to distinct steps of its ATP hydrolysis cycle. Microscopic and proteomic analyses support a model that RNA binding by GLH-1 allows for the assembly of germ granule factors on RNAs, which promote the phase separation of germ granules. In addition, the FG repeats of GLH-1 is required for the robust anchoring of germ granules to perinuclear foci. Importantly, piRNA reporter analyses and small RNA cloning data suggest that normal germ granule dynamics and proper nuclear membrane association are both critical for the production of secondary small RNAs and gene silencing. Furthermore, we showed that these *glh-1* mutants with abnormal germ granules also exhibit defects in transgenerational gene silencing. Together, our research suggests that the localization and dynamics of germ granules are both controlled by germline helicases and their RNA-bound complexes drive the phase separation of germ granules. In addition, our data support a model that germ granules function as a checkpoint, where small RNAs survey mRNA transcripts as they exit nuclei to identify RNA targets for gene silencing.

SYGL-1 and the molecular switch from GSC self-renewal to differentiation in *C. elegans.*

Tina Lynch, Charlotte Kanzler, Heaji Shin, and Judith Kimble.
Department of Biochemistry and HHMI , University of Wisconsin - Madison.

A proper balance between self-renewal of germline stem cells (GSCs) and generation of gametes is crucial for survival of the species. The *C. elegans* germline provides a well-established model for studying this fundamental biological issue. Notch signaling from the niche directly activates transcription of *sygl-1* to maintain GSC self-renewal (Kershner 2014). Downstream of Notch and *sygl-1*, two FBF RNA-binding proteins repress differentiation-promoting mRNAs (e.g. Crittenden 2002). Self-renewal pushes GSC daughters proximally and out of the niche, a process coincident with their "switch" from a naïve stem cell-like state to a state primed for differentiation. The molecular basis of this switch is not understood.

We previously showed that SYGL-1 protein interacts with FBF, that SYGL-1 represses FBF target RNAs required for differentiation (e.g. *gld-1*), and that the extent of SYGL-1 expression determines size of the GSC pool (Shin, Haupt 2017). The challenge now is to understand the role of SYGL-1 in triggering the fate switch to begin differentiation. We hypothesize that lowering SYGL-1 abundance below a critical threshold disrupts the SYGL-1::FBF repressive complex, and triggers the molecular switch.

We are taking two approaches to test this hypothesis. First, we are defining the SYGL-1 threshold below which GSCs switch to differentiation. Second, we are analyzing the molecular basis of the SYGL-1::FBF interaction in an attempt to compromise complex formation. To define the SYGL-1 threshold, we have systematically engineered a series of *sygl-1* promoter mutants to precisely tune the quantity of SYGL-1 in the germline. We quantitate amounts of *sygl-1* RNA and SYGL-1 protein as a function of position and estimate the position of the switch by standard methods. To interrogate the FBF::SYGL-1 binding interface and learn how it functions in the switch, we identified FBF binding motifs in SYGL-1, generated mutants of those motifs and found them to abrogate GSC self-renewal. Our results reveal how a key stem cell maintenance regulator functions within a macromolecular complex to balance self-renewal and differentiation in an in vivo context. Our plan for the future is to seek regulators of SYGL-1 abundance.

References:

Crittenden, S.L., et al., Nature, 2002. 417(6889): p. 660-663.
Kershner, A.M., et al., Proc Natl Acad Sci U S A, 2014. 111(10): p. 3739-44.
Shin, H., et al., PLoS Genet, 2017. 13(12): p. e1007121.

The *mir-44* family of microRNAs regulates the sperm/oocyte switch in *C. elegans* hermaphrodites.

Katherine Maniates, Benjamin S. Olson, and Allison L. Abbott.
Department of Biological Sciences, Marquette University.

Germline sex determination is a complex process that is necessary for the formation of sexually dimorphic gametes. *C. elegans* hermaphrodites switch from generating sperm to oocytes in the fourth larval stage. Mutations in genes in this pathway results in an increase or decrease the number of sperm that are generated. We have identified a family of microRNAs that regulate the switch from producing sperm to producing oocytes in *C. elegans*. The *miR-44* family comprises four miRNAs: *miR-44, miR-45, miR-61*, and *miR-247* that share a common seed sequence and thus are predicted to regulate shared target mRNAs. Interestingly, *miR-44* and *miR-45* share an identical mature sequence and are located only ~9kb apart on chromosome II. This close proximity precluded the generation of double mutants through conventional genetic crosses. Using CRISPR/Cas9, we generated worms that lacked both *mir-44* and *mir-45*: *mir-45(xw11) mir-42/43/44(nDf49)*, which are referred to as "*mir-44/45*" mutants. *mir-44/45* mutants have a significantly decreased brood size and generate an increased number of unfertilized oocytes. The low number of progeny and high number of unfertilized oocytes can be accounted for by a decreased number of sperm. To determine if the decreased number of sperm was due to a premature switch from producing sperm to producing oocytes, we analyzed worms at L4 molt+5 hours and saw that few wild-type worms had any embryos compared to 66% of *mir-44/45* mutants at the same time point that have at least one embryo, indicating that *mir-44/45* mutants switch earlier. To understand the genetic relationship between *mir-44/45* and the genes that regulate the switch from producing sperm to oocytes, we examined a component of the pathway which modulates sperm number, FBF-1. *fbf-1(ok91)* mutants generate an increased number of sperm and show a delayed switch to producing oocytes (Crittenden et al. 2002). In a *mir-44/45; fbf-1* multiply mutant strain, we saw that loss of *fbf-1* activity suppressed the decreased sperm and early embryo phenotypes observed in *mir-44/45* mutants, indicating that *mir-44/45* likely functions upstream of *fbf-1* to regulate the germline sex determination pathway.

Biochemical characterization of essential kinesin activity and regulation in *C. elegans* oocyte meiosis.

Ian Wolff, and Sadie Wignall.
Dept. of Molecular Biosciences, Northwestern University.

Meiosis is a reductional cell division that segregates chromosomes into haploid gametes. In female germ cells, or oocytes, this division is mediated by a microtubule-based spindle that is built and stabilized in the absence of centrosomes. In contrast, male meiotic and mitotic divisions require centrosomes. While much is known about the proteins and forces underlying centrosome-based spindle assembly, due to the difficulty in isolating mammalian oocytes little is known about the mechanisms underlying acentrosomal spindle assembly and maintenance in an in vivo system. Our previous work established *C. elegans* as a model system to investigate the fundamental mechanisms of acentrosomal spindle dynamics, and identified KLP-18, a kinesin-12 family microtubule motor, and MESP-1, a rapidly evolving adaptor protein, as essential for spindle assembly. However, the biochemical mechanism of how these proteins bind to spindle microtubules and generate force was unknown. Here, we employ a combination of in vitro and in vivo approaches to gain insight into this important problem. First, to investigate the nature of KLP-18 and MESP-1's interaction with microtubules, we purified recombinant truncations of the KLP-18 coiled-coil stalk domain along with full length MESP-1 to use in microtubule binding experiments in vitro. We identified a novel microtubule binding site at the C-terminus of the KLP-18 stalk and found that KLP-18 exists in an auto-inhibited conformation until a direct interaction with MESP-1 activates microtubule binding. We then tested the importance of this domain in vivo using a temperature sensitive mutant strain containing two adjacent amino acid substitutions. Prolonged incubation at the restrictive temperature caused spindle assembly defects that are identical to those observed following depletion of KLP-18 by RNAi. In addition, we found that short incubation of this mutant at the restrictive temperature caused the collapse of already formed spindles. In both cases, staining by immunofluorescence showed KLP-18 still localized to aberrant spindles, indicating the protein is present but non-functional. These results demonstrate that the microtubule binding site we identified in vitro is required for both spindle assembly and the maintenance of spindle bipolarity in vivo. This work sets the basis for further investigation into how microtubule associated proteins govern spindle assembly and maintenance specifically in a system lacking centrosomes.

EXC-4/CLIC proteins are ancient regulators of heterotrimeric G-protein-Rho/Rac signaling.

Anthony Arena, De Yu Mao, Jan Kitajewski, and Daniel Shaye.
Dept. of Physiology and Biophysics, University of Illinois – Chicago.

G-protein coupled receptors (GPCRs) regulate many aspects of physiology and are implicated in various pathological conditions. Several GPCR signaling pathways are vital for angiogenesis, the formation of new blood vessels from existing ones. We are interested in understanding this, and other forms, of biological tube formation (tubulogenesis). To this end, we use the C. elegans excretory canal (*ExCa*) as a model to find novel and conserved regulators of angiogenesis. Our collaborative studies of *ExCa* tubulogenesis and mammalian angiogenesis led us to discover a new class of GPCR regulators: The Chloride Intracellular Channel (CLIC) family of proteins. Although CLICs have been implicated in development and disease, their function has remained largely unknown. Here we provide evidence for a novel and conserved role for CLICs as regulators of GPCR-heterotrimeric G-protein (Gα/ß/γ)-Rho/Rac signaling.

A role for CLICs in tubulogenesis was first defined by studies of a *C. elegans* CLIC ortholog called *exc-4*. Subsequently, we and others showed that two mammalian CLICs, CLIC1 and CLIC4, function in endothelial (blood vessel) cells to promote migration, growth, and tube formation during angiogenesis. While EXC-4 is constitutively localized to the plasma membrane in the *ExCa*, and this localization is critical for its function, in mammalian cells CLIC1 and CLIC4 are cytoplasmic. However, these CLICs are recruited to the plasma membrane upon activation of various GPCRs, including the S1P family of receptors (S1PRs). These receptors are potent regulators of angiogenesis that act through $G_{\alpha}i$, $G_{\alpha}12/13$, RhoA and Rac1. Therefore, this regulation of CLIC localization by S1PRs led us to hypothesize that EXC-4/CLICs function in G_{α}-Rho/Rac signaling. Our data demonstrate that CLIC1 and CLIC4 regulate RhoA and Rac1 in endothelial cells. Additionally, we find that *exc-4* genetically interacts with *goa-1*/G_{α}o, *gpa-12*/$G_{\alpha}12/13$ and two of the worm Rac orthologs, *ced-10* and *mig-2*, in *ExCa* tubulogenesis. This is the first evidence that G_{α} and Rac proteins function in *ExCa* tubulogenesis. We are currently generating more tools to further test genetic and physical interactions between *exc-4* and other components of $G_{\alpha/ß/\gamma}$-Rho/Rac signaling in the *ExCa* (see Julianna Escudero poster). Taken together, our data suggests that regulation of $G_{\alpha/ß/\gamma}$-Rho/Rac is a primordial function of EXC-4/CLIC proteins.

Wnt signaling antagonizes repression of germline genes in somatic cell nuclei.

Jerrin Cherian, and Lisa Petrella.
Dept. of Biological Sciences, Marquette University.

Organisms need to maintain proper gene expression at all times. The DREAM complex helps proper gene expression maintenance in somatic cells by repressing germline gene expression. DREAM complex mutants have close to normal gene expression at 20ºC; however, at 26ºC, DREAM complex mutants have increased misexpression of germline genes ectopically in the soma, and they also show a High Temperature larval Arrest (HTA) phenotype. The mechanism by which germline gene activation occurs at 26ºC is still not fully known. To understand how DREAM target loci are activated, I am working on identifying transcription factors (TFs) involved in misexpression of the DREAM target germline genes at 26ºC. I conducted an RNAi screen to determine the TFs that when knocked-down result in suppression of the HTA phenotype in DREAM mutants. I filtered out a set of TFs that are predicted to bind known DREAM targets and are expressed in wild type intestinal cells. I found that knockdown of 20 out of 107 TFs tested were able to significantly suppress the HTA phenotype. I performed a secondary RNAi knockdown screen against the 20 identified TFs using immunostaining to see if germline gene (PGL-1) misexpression is reduced in DREAM mutants showing the HTA suppression phenotype. Knockdown of 13 out of 20 TFs show significant reduction in PGL-1 misexpression in DREAM mutants. This reveals that the TFs are possibly able to activate germline genes directly by binding to DREAM target promoters or indirectly by activating other TFs downstream in the signaling pathway. I performed a Gene Ontology (GO) Enrichment Analysis using PANTHER and gProfiler module and found that the TFs are enriched for the GO terms; cell fate specification, neuron fate specification, pattern specification process and Wnt signaling. Based on this, I knocked down 42 Wnt pathway associated genes in DREAM mutant background to see if there is suppression of HTA phenotype at 26ºC. I found that knock down of 17 Wnt factors were able to significantly suppress HTA phenotype in DREAM mutants. This indicates that Wnt signaling might play a role in germline gene misexpression in the intestine. Overall, this study helped identify the signaling pathways that can act opportunistically in disrupting cell fate maintenance.

Muscular exertion is detrimental to viability in a nematode model of Duchenne muscular dystrophy.

Kiley Hughes, Anjelica Rodriguez, Kristen Flatt, Sneha Ray, Andrew Schuler, Brian Rodemoyer, Visalashki Veerappan, Kori Cuciarone, Alex Kullman, Calis Lim, Neha Gutta, Samantha Vemuri, Victoria Andriulis, Dana Wismonger, Lucas Barickman, Wolfgang Stein, Aakanksha Singhvi, Nathan Schroeder, and Andres Vidal-Gadea.
School of Biological Sciences, Illinois State University.
Neuroscience Program, University of Illinois - Urbana-Champaign.

Duchenne muscular dystrophy (DMD) is a genetic disorder caused by loss of dystrophin, a protein required in muscles for proper force transfer out of the cell. DMD presents at a young age, and causes developmental delays, loss of ambulation, and muscle necrosis resulting in early death. Increased sarcoplasmic calcium levels and damage to the muscle ultrastructure are evident in dystrophic muscles, but how loss of dystrophin leads to muscle death remains unresolved. Understanding the molecular mechanisms responsible for the dysregulation of calcium, and for the ultrastructural decline observed in dystrophic muscles is of paramount importance. It will allow the development of treatments that specifically target the most susceptible steps in the progression of the disease.

C. elegans worms lacking dystrophin (*dys-1(eg33)* mutants) recreate most of the pathologies associated with DMD. We leveraged the experimental amenability of *C. elegans* to investigate the proximal causes of calcium dysregulation, and to evaluate if physical exertion treatments might help prevent muscle decline. We found that sarcoplasmic calcium dysregulation was associated with impaired calcium clearance following contraction and could be mitigated by reducing calmodulin expression. High exertion activity produced signs of muscle hypertrophy but did not improve longevity in these animals. The high degree of phenotypic conservation between dystrophic worms and humans provides a unique opportunity to gain insights into DMD's underlying pathology and to assess potential treatment strategies.

A Genetic Screen to Identify New FGFR Signaling Components.

Victoria Puccini de Castro, J. Palalay, J. E. Webb, C. Gaudenzi, X. Alava, O. Payan Parra, M. Stefinko, C. Voisine, T-W. Lo, and M. Stern.
Department of Biology, Ithaca College, and Department of Biology, Northeastern Illinois University.

Fibroblast growth factor receptors (FGFRs) are cell-surface receptor tyrosine-kinases (RTKs) that phosphorylate specific tyrosine residues to trigger downstream responses. In *C. elegans*, the sole FGFR, EGL-15, is involved in multiple functions, including serving as a receptor for a chemoattractive guidance cue for the migrating sex myoblasts, ensuring that the egg-laying muscles are in functional positions. EGL-15 also regulates internal fluid levels: hyperactivation of EGL-15 causes excessive accumulation of clear fluid inside the worm's body (the Clr phenotype). The isolation of Suppressor Of Clr (*soc*) mutants has led to the identification of many of the core components of EGL-15 signaling: the original set of *soc* mutations identified the Grb2/SEM-5 adaptor protein that links RTK signaling to the RAS/MAPK pathway.

egl-15(n1457) is a nonsense mutation that is predicted to truncate EGL-15's carboxy-terminal domain (ΔCTD) and eliminates the two-known direct SEM-5 binding sites on EGL-15. Interestingly, *n1457* confers an Egl phenotype, but does not confer a Soc phenotype. Mutation of the two YXNX SEM-5 binding sites in the CTD mimics the phenotype of *n1457*. Since SEM-5 is required for EGL-15 signaling, these data suggest the existence of an alternate pathway that links EGL-15 to SEM-5/Grb2 in its role in mediating fluid homeostasis.

To identify components of this alternate pathway, we repeated the screen for Soc mutants in an *n1457*(ΔCTD) background. Preliminary characterization of these enhancer alleles indicates that the screen was successful in two ways. First, the behavior of the enhancer alleles supports two cooperative pathways that transduce EGL-15 signaling: many of the enhancer mutations show only partial Soc phenotypes on their own, and are enhanced by the *n1457*(ΔCTD) mutation. Second, the characterization of these *soc* enhancer mutations suggests that they define up to three new *soc* genes that potentially function in the alternate pathway. Further genetic analysis and whole-genome sequencing will be used to identify the molecular identities of these new FGFR signaling genes.

Massive sampling of *Caenorhabditis elegans* across the Hawaiian Islands reveals remarkable genetic diversity on the islands and admixture with globally distributed populations.

Tim Crombie, Stefan Zdraljevic, Shannon Brady, Daniel Cook, Kathryn Evans, Steffen Hahnel, Daehan Lee, Briana Rodriguez, Robyn Tanny, Ye Wang, Gaotian Zhang, Joost van der Zwaag, and Erik Andersen.
Department of Molecular Biosciences, Northwestern University.

Global surveys of *Caenorhabditis elegans* genetic diversity suggest that large-scale selective sweeps on chromosomes I, IV, V, and X are associated with reduced diversity in some regions of the world. These sweeps are thought to be caused by adaptation to human-associated environments, so swept strains might not represent the natural diversity present before human influence. Interestingly, strains isolated from the Hawaiian Islands are generally highly diverged from the rest of the global population and contain little evidence of these sweeps. Therefore, we sought to better characterize the natural diversity of the species by collecting *C. elegans* from the Hawaiian Islands. At two different times, we used a collection protocol to sample nematodes from a total of 3,264 sites across five Hawaiian Islands. Among the 4,558 nematodes we isolated, we identified five *Caenorhabditis* species including *C. elegans, C. briggsae, C. tropicalis, C. kamaaina*, and a new species, *C. oiwi*. On the first collection trip, we discovered that *C. elegans* was generally found in cooler environments at higher elevations than other members of the genus. These data enabled improvements to our collection protocols that increased our success rate for isolating *C. elegans* from 1.8% to 4.1% on the second collection trip. We identified 26 distinct *C. elegans* strains from our first collection trip, increasing the total number of Hawaiian strains to 43. The mean genome-wide nucleotide diversity of these 43 Hawaiian strains is three-fold higher than the 233 non-Hawaiian strains from around the globe. We used ADMIXTURE to investigate the ancestry of all 276 strains and identified six distinct ancestral populations. Surprisingly, we saw evidence of admixture between some of the Hawaiian strains and one predominantly swept globally distributed population. Taken together, these findings confirm that divergent strains can be collected reliably from the Hawaiian Islands, but raise new questions about the direction, magnitude, and timing of gene flow among swept and non-swept populations that has contributed to the global pattern of diversity in the species.

The role of DEX-1 in dauer-specific locomotion behaviors.

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The *C. elegans* dauer larvae undergo extensive remodeling of several tissues which facilitate survival in unfavorable environments. Along with changes in morphology, dauer larvae also exhibit alterations in behavior likely due to extensive neuronal remodeling. To better understand these morphological and behavioral adaptations, we are currently investigating the role of the extracellular matrix (ECM) protein, DEX-1, in dauer remodeling. DEX-1 was previously found to be required for proper dendrite morphology during embryogenesis. We have uncovered an additional role for *dex-1* in dauer-specific radial shrinkage and alae formation. Furthermore, we observed that *dex-1* mutants have defects in dauer-specific behaviors. Wild-type dauers are often behaviorally quiescent, but are capable of rapid movement when mechanically stimulated. We found that although *dex-1* mutant dauers initially respond to mechanical stimulation, they are sluggish and uncoordinated compared to wild-type. This effect is dauer-specific, as non-dauer *dex-1* mutant locomotion is wild-type. Expression analysis revealed that *dex-1* is expressed in the lateral seam cells, and tissue-specific rescue showed that DEX-1 functions as a tightly localized secreted protein to facilitate seam cell remodeling during dauer. Interestingly, seam cell-specific expression of *dex-1* rescues radial shrinkage and alae formation in a mosaic pattern, while locomotion is rescued to wild-type levels. This suggests a possible role for *dex-1* in dauer-specific locomotion. Indeed, previous work shows that a knockdown of *dex-1* results in defects in D-type motor neuron commissures in the locomotor circuit, indicating a role for DEX-1 in maintaining the integrity of the neuromuscular system during dauer. Muscles make neuromuscular junctions with motor neurons via extensions of muscle tissue called muscle arms, and dauer larvae extend additional, dauer-specific muscle arms that are predicted to facilitate rapid locomotion. Our preliminary data suggests that *dex-1* mutant dauers have defects in muscle arm formation, and we hypothesize that this may affect their ability to initiate rapid movements. Ongoing investigations seek to uncover the molecular mechanisms through which DEX-1 functions to mediate dauer behavior. Additionally, we are utilizing electron microscopy technologies to investigate these structural alterations in the relatively unexplored dauer nervous system.

Investigating the Function of the ARID-type Transcription Factor CFI-1 in Cholinergic Motor Neurons of the Nematode *C. elegans*.

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Neuronal diversity critically relies on precise spatiotemporal regulation of gene expression in the nervous system. The cholinergic motor neurons (MNs) of the *C. elegans* nerve cord provide a prime model to investigate the gene regulatory mechanisms underlying neuronal diversity. These MNs are grouped into six classes (SAB, DA, DB, VA, VB, AS) based on anatomical and molecular criteria. A remarkable wealth of MN class-specific markers provides a unique entry point into the problem of neuronal diversity. Such markers report expression of terminal identity genes, whose continuous expression is essential for MN function. It has been previously reported that the phylogenetically conserved Collier/Olf/Ebf (COE)-type transcription factor (TF) UNC-3 is essential for cholinergic MN diversity by directly activating a large battery of MN class-specific terminal identity genes. However, an important mechanistic question remains: since UNC-3 is expressed in all cholinergic MN classes, what are the mechanisms that prevent UNC-3 from activating class-specific genes more broadly, i.e., in all MN classes? A recent study identified seven MN class-specific TFs that act as transcriptional repressors to counteract UNC-3's ability to activate MN class-specific genes. However, the underlying mechanism is unknown. Here, we focus on one repressor, the AT-rich Interaction Domain (ARID) protein CFI-1. CFI-1 is expressed specifically in head muscles, interneurons and DA, DB, VA, VB cholinergic MNs. We found that *cfi-1(-)* mutants exhibit severe locomotion defects, suggesting a critical role for CFI-1 in MNs. To uncover the downstream targets of CFI-1 in MNs, we are currently employing RNA-seq and ChIP-seq, as only one target of CFI-1 (glutamate receptor subunit *glr-4*) has been described to date. In wildtype animals, *glr-4* is activated by UNC-3 in SAB neurons but not in DA or DB MNs, due to repression by CFI-1. Overexpression of CFI-1 in SAB neurons leads to downregulation of *glr-4*, suggesting that CFI-1 is sufficient to silence *glr-4*. Lastly, we investigated the mechanisms that induce and maintain *cfi-1* expression in MNs. We found that the midbody HOX genes *lin-39* and *mab-5* are required for *cfi-1* induction during early development, while UNC-3 appears to be indispensable for *cfi-1* maintenance in adulthood. Together, our experiments aim to shed light into the mechanisms upstream and downstream of CFI-1, a critical regulator of cholinergic MN diversity.

Timing matters: Organismal responses to stress.

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Cells possess natural defense mechanisms to counteract protein misfolding. One such mechanism is the activation of a conserved gene expression program, the so-called heat-shock response that increases the cellular protein quality control (QC) capacity to help refold and/or degrade misfolded proteins. Experimentally activating this response ameliorates disease pathology, making it a prime target for medical intervention. Yet, in neurodegenerative diseases, cells accumulate misfolded and aggregated proteins but fail to naturally activate this response. As in human disease, we have shown that cells of the metazoan *C. elegans* also do not naturally activate their protein QC machinery upon protein misfolding: neuronal activity inhibits the cells' natural defense against misfolding. However, upon a *sensed threat* in the environment, the nervous system activates the cellular defense response against protein damage. Specifically, we showed that *C. elegans* can be trained to initiate HSF-1-dependent chaperone gene expression prior to, and in anticipation of, a proteotoxic encounter, through olfactory exposure to specific smells that signify threat. This occurs through the release of the neuromodulator serotonin. We have since discovered that serotonergic activation of HSF1 is conserved in mammalian cortical neurons and occurs through the cAMP-PKA axis. Interestingly, we find that serotonin release in *C. elegans* acts as a timer to control the initiation and duration of the stress response, through modulation of the histone chaperone FACT at *hsp* genes. We propose that the neural networks control stress-dependent gene transcription though epigenetic changes allowing organisms to implement acute, cytoprotective responses to stress, in opposition to chronic, detrimental responses.

POSTER ABSTRACTS

Duchenne Muscular Dystrophy in dystrophic *C. elegans* eggs.

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Duchenne Muscular Dystrophy (DMD) is caused by mutations impairing dystrophin. Patients display severe muscle weakness, eventually leading to loss of ambulation, and death. The tight regulation of cytosolic calcium release and clearance is essential for normal muscle contraction. Elevated cytosolic calcium is well established in DMD patients. The proximal causes leading to high calcium levels remains unresolved. Previously we reported high levels of cytosolic calcium in dystrophic L1 larvae. We also found that excess calcium persisted in relaxing muscles due to impaired calcium clearance. Here, we report cytosolic calcium using the calcium indicator GCaMP2 in embryonic wild type and dystrophic muscles. Preliminary data suggests that once dystrophic animals begin moving *in ovo*, cytosolic calcium persists in relaxed muscles. Understanding the chain of events leading to calcium dysregulation in dystrophic muscles might provide us with therapeutic opportunities to improve the quality of life of individuals affected by this incurable disease.

Dauer IL2 neurons use distinct and shared mechanisms with FLP and PVDs to regulate arborization.

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Environmental stress can significantly influence the shape of dendrites, and dendrite morphology plays a key role in proper neural signaling. We wanted to determine the underlying molecules that regulate stress-induced dendritic arborization. When *C. elegans* are grown under well-fed conditions, the two FLP neurons located in the head begin to arborize rapidly during L4, these arbors persist through adulthood. During the stress-induced dauer stage, four quadrant IL2 neurons arborize similarly to the FLPs, extending arbors out to the body wall and covering the head of the worm. We observed that the FLPs maintain an unbranched state during dauer. Using a forward genetic screen, we identified the membrane bound receptor DMA-1, which was previously shown to regulate FLP branching, as essential for dauer-specific IL2 arborization. We found that the IL2s use identical DMA-1 binding partners during IL2 dauer arborization. In adult animals a DMA-1::GFP translational reporter clearly localizes to the FLP. Using this same reporter, we observed DMA-1 localization in the IL2 dendrite during dauer, though not during other developmental stages. However, overexpression in the IL2s did not induce branching, suggesting that additional dauer-specific components regulate IL2 arborization during dauer and that the IL2s are inhibited from branching in the adult animal. Intracellularly, DMA-1 enables actin polymerization through interactions with HPO-30 and TIAM-1. Our mutant analysis shows that HPO-30 and TIAM-1 are necessary for IL2 arborization. While the DMA-1 complex is needed, we have found that several of the regulators of the complex in FLP/PVD are dispensable in the IL2s. For example, the unfolded protein response protein IRE-1 is required for proper DMA-1 localization in the PVD/FLPs, but is dispensable in dauer IL2s. However, the FOXO transcription factor, DAF-16 can compensate for a blocked UPR. We found that *daf-16; daf-7* partial dauers are defective for IL2 arborization while the FLPs remain unaffected. Altogether our results show that DMA-1 functions during dauer to mediate stress-induced dendrite plasticity in the IL2 neurons. The role of DMA-1 during dauer mirrors its function in the adult FLPs. Both extracellular and intracellular DMA-1 binding partners are necessary for IL2 arborization. However, DMA-1, HPO-30 and SAX-7 are regulated uniquely during dauer.

An excreted small-molecule signal promotes *C. elegans* reproductive development and aging.

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Excreted small-molecule signals can bias developmental trajectories and physiology in diverse animal species. However, the chemical identity of these signals remains largely obscure. Here we report identification of an unusual N-acylated glutamine derivative, nacq#1, that accelerates reproductive development and shortens lifespan in *C. elegans*. Produced predominantly by *C. elegans* males, nacq#1 hastens onset of sexual maturity in hermaphrodites by promoting exit from the larval dauer diapause and by accelerating late larval development. Even at picomolar concentrations, nacq#1 shortens hermaphrodite lifespan, suggesting a trade-off between reproductive investment and longevity. Acceleration of development by nacq#1 requires chemosensation and depends on three homologs of vertebrate steroid hormone receptors. Unlike ascaroside pheromones, which are restricted to nematodes, fatty acylated amino acid derivatives similar to nacq#1 have been reported from humans and invertebrates, suggesting that related compounds may serve signaling functions throughout metazoa.

Investigating the role of mechanoreceptors in magnetic orientation in *C. elegans.*

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Many organisms, from bacteria to mammals, rely on magnetic fields to orient within their environment and perform impressive feats of navigation. Despite the prevalence of magnetic orientation across taxa, mechanisms for magnetoreception remain poorly understood. A favored model for magnetoreception proposes coupling of magnetic particles or proteins to mechanical-force sensitive channels (mechanoreceptors). Mechanoreceptors are thought to transduce the mechanical forces of particle alignment with magnetic fields into electrical signals for sensory cells. Mechanoreceptors are functionally versatile and recruited to transduce diverse sensory modalities, making them promising candidates for magnetoreceptors. We previously found that *C. elegans* detects and orients to magnetic fields with a pair of magnetosensory neurons (AFDs). *C. elegans* has about 50 described mechanoreceptors.

We are using the nematode *C. elegans* to test the potential role of mechanoreceptors in magnetic field transduction. To approach this question, I will use a combination of RNA interference to silence mechanoreceptor expression and automated machine vision to assess mechanoreceptor necessity for magnetic orientation. To support their role as potential magnetoreceptors, mechanoreceptors will be fluorescently labelled to determine their proximity to AFD neurons. Our results have the potential to identify the first working magnetoreceptor system in any animal.

A nematode-specific gene underlies bleomycin-response variation in *Caenorhabditis elegans*.

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Bleomycin is a powerful chemotherapeutic drug used to treat a variety of cancers. However, individual patients vary in their responses to bleomycin. The identification of genetic differences that underlie this response variation could improve treatment outcomes by tailoring bleomycin dosages to each patient. We used the model organism Caenorhabditis elegans to identify genetic determinants of bleomycin-response differences by performing linkage mapping on recombinants derived from a cross between the laboratory strain (N2) and a wild strain (CB4856). This approach identified a small genomic region on chromosome V that underlies bleomycin-response variation. Using near-isogenic lines and strains with CRISPR-Cas9 mediated deletions and allele replacements, we discovered that a novel nematode-specific gene (scb-1) is required for bleomycin resistance. Although the mechanism by which this gene causes variation in bleomycin responses is unknown, we suggest that a rare variant present in the CB4856 strain might cause differences in the potential stress-response function of scb-1 between the N2 and CB4856 strains, thereby leading to differences in bleomycin resistance.

The role of the Integrator Complex in piRNA transcription termination

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Transposons are mobile genetic elements that can cause infertility and disease by altering an organism's genome.

Recent studies have shown that metazoans guard their germlines against transposons and other foreign transcripts with small RNAs, in particular with PIWI-interacting RNAs (piRNAs). In *Caenorhabditis elegans*, thousands of piRNAs are produced from independent transcriptional units by RNA pol II. Intriguingly, the transcribed precursor sequences are only approximately 30 nucleotides in length despite piRNA loci not possessing a detectable termination sequence. One key question that remains to be answered is how cellular machinery can terminate transcription from these thousands of independent nascent transcripts and generate the extreme diversity that makes the piRNA defense system successful in genome defense. An interesting candidate for piRNA transcription termination is the multimeric Integrator Complex, which is recruited to the CTD of RNA pol II and terminates the transcription of small nuclear RNA (snRNA). The Integrator cleaves snRNA precursors approximately 9-19 nucleotides downstream of the mature transcripts' 3'end. Using a piRNA reporter that is uniquely sensitive to loss of mature piRNAs, we have shown that knocking down a subset of Integrator components results in a reduction in piRNA mediated silencing. We have confirmed that the knockdown of these components results in depletion of both mature piRNAs and piRNA precursors in adult worms. Our finding suggests that *C. elegans* have adapted extant cellular machinery to help develop the more recently evolved piRNA-meditated genome surveillance system.

An autism-causing variant misregulates selective autophagy to alter axon targeting and behavior.

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Common and rare variants of the CACNA1C gene have been associated with autism and other neurodevelopmental disorders including schizophrenia, bipolar disorder and ADHD. However, little is known about how CACNA1C variants affect cellular processes to alter neurodevelopment. The Timothy syndrome mutation is a rare, gain-of-function variant in CACNA1C that causes autism with high penetrance, providing a powerful avenue into investigating the role of CACNA1C variants in neurodevelopmental disorders. Here, we show that an *egl-19(gof)* mutation that is equivalent to the Timothy syndrome mutation can alter axon targeting and affect behavior in C. elegans. We find that wildtype *egl-19* functions independently of RPM-1 to negatively regulate axon termination. The *egl-19(gof)* mutation represses axon termination to cause axon targeting defects that lead to the misplacement of electrical synapses and alterations in habituation to light touch. Moreover, genetic analysis indicates that selective autophagy acts downstream of the *egl-19(gof)* mutation to mediate its effects on both axon termination and behavior. These results reveal a novel mechanism whereby an autism-causing variant of CACNA1C misregulates selective autophagy to alter circuit formation and affect behavior.

ERAD for the SLOw and Sluggish,

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The SLO-1 BK Channel is a calcium-activated potassium channel that controls synaptic transmission and muscle excitation. The overall density of SLO-1 channels is primarily regulated at the level of channel trafficking. However, the mechanism underlying BK channel trafficking has yet to be clearly defined. In a previous *C. elegans* genetic study, the ER membrane protein ERG-28 was identified as a regulator of SLO-1 trafficking from the ER to the Golgi complex; SLO-1 level is drastically reduced in the absence of ERG-28. We hypothesize that without ERG-28, SLO-1 is recognized by the ERAD (ER associated degradation) system and inactivation of ERAD increases SLO-1 channel level. Using a candidate gene approach, we identified *sel-11*, encoding an ER-resident E3 ubiquitin ligase, as an important regulator of SLO-1 degradation in *erg-28* mutants. Introduction of *sel-11* mutation to *erg-28* mutants resulted in significant recovery of SLO-1 levels at the plasma membrane. Moreover, the recovered SLO-1 at the plasma membrane is functional as *sel-11* mutation suppressed *erg-28* mutant phenotype. To further ascertain this finding, we determined whether members of the canonical SEL-11-mediated degradation pathway influence SLO-1 channel levels. We indeed found that *sel-1* and *cdc-48.2* mediate SLO-1 degradation in *erg-28* mutants. We previously uncovered that *ddi-1*, a gene encoding an aspartic protease, participates in the degradation of SLO-1. Based on our new findings, we examined the relationship between *ddi-1* and *sel-11*. The SLO-1 channel levels of *ddi-1; sel-11 erg-28* triple mutant and *sel-11 erg-28* or *ddi-1; erg-28* double mutants were not significantly different, suggesting a shared pathway of SLO-1 degradation between SEL-11 and DDI-1. Together, our data show that the overall level of SLO-1 channels is regulated in the ER by the concerted action between ERG-28 and the ERAD machinery, in which SEL-11 and DDI-1 are major components.

A role for the DREAM complex in germline apoptosis.

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Organisms exist in environments that are subject to changes and fluctuations that may incur stress upon them; thus, they have means to cope with or adapt to stress and ensure their survival and propagation. One means of dealing with stress that organisms have is to induce programmed cell death through apoptosis in cells that have been damaged by the stressful condition. We are studying apoptosis in response to stresses in the *C. elegans* germline. The germline is the only tissue in the adult animal that undergoes apoptosis. *lin-35*, the single worm homolog of the Retinoblastoma (pRB) tumor suppressor has been shown to be important for the promotion of several of the apoptotic pathways in the germline. However, LIN-35 does not appear to work at the same point in the apoptotic pathways as other members of the E2F complex, EFL-1 and DPL-1. Another complex that LIN-35 interacts with is the conserved MuvB core of the DREAM complex which is made up of synMuv B proteins. We have found that DREAM complex mutants, like *lin-35* mutants, have reduced germline apoptosis in response to DNA damage. It is of note that in embryonic tissues, the DREAM complex and LIN-35 bind at the promoter of *ced-9/Bcl2* which is the anti-apoptotic regulator of the core apoptotic machinery. This could indicate that LIN-35 may be working with the DREAM complex to regulate germline apoptosis. We predict that DREAM complex mutants, like *lin-35* mutants, will have increased levels of *ced-9* expression but not that of *ced-4* or *ced-3* in the germline. We also predict that DREAM complex mutants will have an even more pronounced defect in germline apoptosis in an engulfment mutant background, like *lin-35* mutants. Together these would indicate that the DREAM complex is a good candidate to be the complex with which LIN-35 is modulating germline apoptosis.

Elucidating the role of the conserved transcription factor HLH-10 / musculin (TCF21) in sex-specific motor neuron differentiation.

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In every nervous system, there are neurons common to both sexes, known as sex-shared neurons. However, there are also sex-specific neurons, with sex-specific specialized functions. Sex-shared neuron differentiation has been intensively studied, but a significant gap remains to be filled in understanding the molecular mechanisms underlying sex-specific neuron differentiation. To bridge this knowledge gap, the diverse motor neuron (MN) subtypes populating the ventral nerve cord (VNC) of the nematode *Caenorhabditis elegans* provide an ideal model. The anatomy, connectivity, and function of these MNs has been well described in both *C. elegans* sexes (males and hermaphrodites). In addition, there is a remarkable variety of markers for both sex-shared MN subtypes (SAB, DA, DB, VA, VB, AS) and sex-specific MN subtypes (VC MNs in hermaphrodites, CA/CP MNs in males) that populate the *C. elegans* VNC. We have previously shown that the evolutionarily conserved Collier/Olf/Ebf (COE)-type transcription factor (TF) UNC-3 co-regulates the expression of cholinergic terminal features in the majority of VNC cholinergic MNs in *C. elegans*. While UNC-3 controls terminal differentiation in sex-shared MNs, UNC-3 is not expressed in sex-specific MNs. Thereby, the mechanism through which sex-specific MNs acquire and maintain their terminal features remains elusive. We hypothesize that the TF HLH-10, which is expressed in VC and CA sex-specific MNs and suppressed by UNC-3 in sex-shared MNs, plays a key role in the development and maintenance of sex-specific cholinergic MNs. To test this hypothesis, we will perform genetic crosses between animals carrying a deletion allele for HLH-10 and available transgenic reporter animals for VC MNs, CA MNs, and the TF UNC-3. We predict that a percentage of these markers will exhibit loss of expression in VC and CA MNs. Moreover, since UNC-3 represses HLH-10 in sex-shared MNs, we will test whether HLH-10 in turn represses UNC-3 in sex-specific MNs. Lastly, we will determine if HLH-10 is essential for sex-specific VC MN function (e.g. egg laying). We predict that animals carrying a deletion allele for HLH-10 will display a decrease in egg laying efficiency. Uncovering the molecular features that distinguish sex-specific from sex-shared MNs may advance our understanding of neurological diseases, such as amyotrophic lateral sclerosis (ALS); as in humans with ALS, sex-shared MNs degenerate while sex-specific remain fully functional.

In-silico characterization of the _Caenorhabditis elegans_ matrisome and proposal of a novel collagen classification.

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By pairing the big data capabilities of bioinformatics with proteomic screening and validation we have defined the matrisome - a detailed inventory of extracellular matrix (ECM) and ECM-associated proteins - in humans and mice [1]. To do so we took advantage of the conserved domain-based organization of ECM proteins. We constructed a list of InterPro domains (such as Fibronectin type I-III, Laminin G, Collagen triple helix repeats) to interrogate the proteomes of both species and identify their ECM components. Combined with proteomic analysis of ECM-enriched tissues and knowledge-based curation, we identified 1027 human and 1110 mouse matrisome genes. The matrisome of the zebrafish was also recently described [2]. We have now utilized the well-annotated genome and proteome of *C. elegans* to develop a new bioinformatic pipeline to define the matrisome of this widely-used model organism. Similar to the human genome, we found that ~4% of the C. elegans genome (719 out of ~20,000 genes) encodes matrisome proteins, including 181 collagens, 35 glycoproteins, 10 proteoglycans, and 493 matrisome-associated proteins [3]. We also found that 173 out of the 181 collagen genes are unique to nematodes and are predicted to encode cuticular collagens, which we propose to group into five clusters.

To facilitate the use of our lists and classification, we have developed an annotation tool to identify ECM components in large datasets (http://ce-matrisome-annotator.permalink.cc/). We have also established a novel database of all *C. elegans* collagens called CeColDB (http://cecoldb.permalink.cc). We hope these tools will be valuable for the researchers using C. elegans to study the role of the ECM in development and disease.

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Identifying *ben-1*-independent resistance alleles of benzimidazoles.

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Parasitic nematodes are a major burden on livestock and human health around the world, infecting billions every year. Anthelmintic drugs are the front line to fight these infections, however, their widespread use has led to resistance appearing around the world. To fight this resistance, a thorough understanding of the genetics and mechanisms of resistance to all major drug classes are essential, especially for the most commonly used benzimidazole (BZ) class. Most studies on BZ resistance have focused on the beta-tubulin isotype-1 locus *ben-1* leading to the identification of a number of alleles correlated with resistance to BZs. However, in field isolated parasite populations a huge amount of variation exists in populations with the same allele at the beta-tubulin isotype-1 locus. Thus, making identification of additional resistance alleles essential for proper control of resistance. We have investigated this phenomenon through the use of the natural genomic diversity present in *Caenorhabditis elegans* by utilizing quantitative genetics approaches. Utilizing a combination of linkage mapping and genome wide association studies we have identified a region correlated with differences in response to the benzimidazole thiabendazole. We generated near isogenic lines that provide some evidence that important causal variants lie within this region. We have also identified a potential resistance allele that could prove an important marker for parasite resistance research.

AIR-2/Aurora B kinase activity is required for critical events during *C. elegans* oocyte meiosis.

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Oocyte spindles assemble in the absence of centrosomes through unique, poorly understood mechanisms. In *C. elegans* oocyte meiosis, microtubules form lateral associations with chromosomes and a multi-protein ring complex (RC), which is essential for both congression and segregation, forms at the center of each bivalent. The RC consists of more than fifteen proteins including the conserved chromosomal passenger complex (CPC), which contains AIR-2 (Aurora B kinase). Previous studies have shown that AIR-2 is required for targeting all known ring components, but how AIR-2 promotes ring assembly is not understood. One idea is that AIR-2 serves as a scaffold upon which other proteins can assemble. This idea stems from a study that proposed that SUMOylation of various ring components enables the recruitment of other components; this study also demonstrated that AIR-2 can be SUMOylated *in vitro* and proposed that this SUMOylation event was crucial for ring assembly. However, whether AIR-2 simply serves as a scaffold or whether its kinase activity is also important for ring assembly is not known.

To address this question, we used a novel auxin-inducible degron-based strategy to inhibit the kinase activity of AIR-2 *in vivo* and then assess effects on oocyte meiosis. These studies revealed that AIR-2 kinase activity is essential for the accurate patterning of the CPC on chromosomes and for the overall assembly of the RC. We also observed SUMO aggregates around bivalents, suggesting a role for AIR-2 dependent phosphorylation in the SUMOylation pathway. In addition to these functions, we found that AIR-2's kinase activity is required for acentrosomal spindle bipolarity and for chromosome alignment and segregation, independent of AIR-2's role in RC formation. Thus, our studies have revealed that AIR-2 kinase activity is essential for multiple essential events in *C. elegans* oocytes, providing new insights into mechanisms driving this important specialized cell division.

The Role of NEKL-3 In the Regulation of Axon Termination.

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Formation of a healthy and stable nervous system requires precise regulation of axon development. Failures in this process can affect axon development and stability, leading to diseases such as Amyotrophic Lateral Sclerosis (ALS). Recent work has uncovered an association between ALS and NEK kinases, suggesting that they can affect axons. However, the molecular mechanisms of NEK kinase function in axons are unknown. Our research utilizes the model *C. elegans* to explore the role of NEKL-3 NEK kinase in the PLM (Posterior Lateral Microtubule) neuron. Here, we show that loss of *nekl-3* function causes PLM axon termination defects. Additionally, fluorescently labelled NEKL-3 was observed in clusters along the PLM axon. These observations suggest that NEKL-3 regulates axon termination in neurons. To investigate the function of NEKL-3 in axon development, we are currently conducting double mutant analysis with mutations in other genes that regulate axon development. We aspire for our research to aide in the understanding of the role of NEK kinases in axon development and provide insight into how NEK kinases contribute to ALS.

FBF partnerships and their role in regulation of germline fates.

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PUF RNA-binding proteins regulate stem cells throughout animal phylogeny. In the *C. elegans* germline, two PUF proteins, collectively called FBF, maintain germline stem cells (GSCs) by repressing differentiation RNAs (e.g. Crittenden et al., 2002). We are analyzing how FBF-1 and FBF-2 work with key protein partners to regulate stem cells. Our approach is two-fold. The first is to focus on a key FBF partner and dissect its role in regulating germline fates. We will describe our progress with one such partner, LST-1. Briefly, we found the minimal fragment in LST-1 that drives stem cell self-renewal normally and can drive tumor formation when overexpressed. The second is to identify amino acid residues within FBF and its partners that are essential for their interaction. We will describe our progress with both FBF-2 and LST-1. Briefly, we found one site in FBF-2 and two sites in LST-1 that are essential for their interaction in yeast. The biological roles of these sites will be described in our poster.

A toolkit for analyzing the role of heterotrimeric G-protein-Rho/Rac signaling, and its regulation by EXC-4 in the excretory canal cell.

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We study the genetic regulation and cell biological processes that underlie biological tube formation (tubulogenesis) in *C. elegans* and during angiogenesis; a process of blood vessel formation. To this end, we study the excretory canal (*ExCa*) a unicellular tube that elongates throughout the worms' entire body. Some genes that regulate *ExCa* tubulogenesis are also involved in angiogenesis (see Socovich abstract). One example is the gene *exc-4*, which encodes a chloride intracellular channel (CLIC). The first mutants in this gene were retrieved in a pioneering screen conducted by Buechner et al., looking for animals with cystic excretory canals (the *exc* screen), and *exc-4* was later cloned by the Hobert Lab. Subsequently, work from our collaborator, Dr. Jan Kitajewski, and his lab implicated two vertebrate orthologs, CLIC1 and CLIC4, in angiogenesis *in vitro* (using cultured human umbilical vascular endothelial cells, or HUVEC) and *in vivo* (in mice). In collaboration with the Kitajewski Lab we have now shown that EXC-4 in the worm, and CLIC1 and CLIC4 in endothelial cells, modulate signaling pathways that act via heterotrimeric G proteins ($G_{\alpha/ß/\gamma}$) and the small GTPases Rho and Rac. This suggests that EXC-4/CLIC proteins are conserved regulators of Rho/Rac signaling (see Arena abstract).

We want to use genetic and expression analyses to define how EXC-4 and Gα/ß/γ-Rho/Rac signaling components interact to achieve this regulation. Genetic analysis of these players is confounded by lethality caused by null mutations in some of them (e.g. *gsa-1/*G_{α}*s, gpb-1/*$G_{ß}$*, gpc-2/*G_{γ}*, rho-1/Rho* and *ced-10/Rac*) or potential redundancy among others (e.g. between different $G_{\alpha/ß/\gamma}$-encoding genes, or between the two Rac homologs *ced-10* and *mig-2*). To circumvent these issues, we are generating a toolkit to activate or deplete G_{α}, Rho and Rac homologs specifically in the *ExCa*, as well as to visualize protein localization of these components in this cell. Our goal is to combine these tools with exc-4/CLIC mutants to define the conserved mechanisms by which EXC-4/CLIC regulates $G_{\alpha/ß/\gamma}$-Rho/Rac signaling.

Natural variation underlies differential responses to zinc treatment in *Caenorhabditis elegans*.

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Zinc is an essential element for growth and development, acting as a co-factor for more than 300 enzymes and transcription factors in the cell. For this reason, altering intracellular zinc levels can produce dramatic defects, ranging from cell proliferation to cell death. Furthermore, genetic variation in these zinc-associated proteins might affect the ability of a protein to interact with zinc and similarly cause zinc-dependent defects. Leveraging the power of *Caenorhabditis elegans* as a tractable metazoan model for quantitative genetics, we found that the N2 strain is resistant to micromolar amounts of zinc supplementation (N2), and a Hawaiian natural isolate (CB4856) is more sensitive to such perturbations. Using a panel of recombinant inbred lines constructed from a cross between these two genetically and phenotypically divergent strains, we mapped two large-effect quantitative trait loci (QTL) in response to zinc on the left arm of chromosome III and the center of chromosome V. Here, we validate and narrow both QTL using near-isogenic lines (NILs) and generate a list of prioritized candidate genes to further evaluate via CRISPR-Cas9-mediated deletions. Our work provides a context of natural variation in nematode zinc biology and aims to identify novel genetic factors underlying zinc-induced toxicity.

Securing motor neuron terminal identity throughout life by intercepting a Hox-mediated transcriptional switch.

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Nervous system function critically relies on continuous expression of neuron type-specific terminal identity features, such as neurotransmitter receptors, ion channels and neuropeptides. How post-mitotic neurons select and maintain their terminal identity features is poorly understood. We elucidate the mechanistic basis of this fundamental process in the context of *C. elegans* ventral cord cholinergic motor neurons (MNs) by uncovering a dual role for the conserved terminal selector UNC-3 (Collier/Ebf). In developing and adult MNs, UNC-3 is continuously required to activate cholinergic MN identity genes, and simultaneously prevent expression of terminal identity features normally found in other ventral cord neurons. This dual outcome is achieved through an unconventional *unc-3*-dependent mechanism that blocks a switch in the transcriptional targets of the mid-body Hox protein LIN-39 (Scr/Dfd/Hox4-5). The strategy of a terminal selector preventing a Hox-mediated transcriptional switch may constitute a general principle for consolidating neuronal terminal identity features throughout life.

Developing a model to screen for small molecule treatments that reduce AGEs using *C. elegans*.

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Prostate cancer is the second leading cause of cancer death among men in the United States. Race and ethnicity significantly influence prostate cancer incidence and its level of aggressiveness. Recent studies have found that advanced glycation end products (AGEs) are elevated in the serum of African-American compared to European-American men. AGEs are harmful compounds that form when glucose covalently attaches to proteins, lipids or nucleic acids in a non-enzymatic reaction. Failure by protective cellular pathways leads to AGE accumulation and this accumulation may serve as a potential biomarker for aggressive forms of Prostate Cancer. Since the model organism *Caenorhabditis elegans* shares conserved components of AGE detoxification pathways that reduce AGE levels, we are developing a high throughput screen to identify small molecules that reduce AGE accumulation in the nematode. Our first objective was to induce rapid accumulation of AGEs by feeding the animals a high glucose diet and adding exogenous methylglyoxal, a precursor to AGE formation. We then monitored AGE accumulation using a green fluorescent protein (GFP) reporter, where an increase in fluorescence reflects an increase in the level of AGEs. Preliminary results suggest that a 2% glucose diet and an exogenous treatment with a high concentration of methylglyoxal (7mM) for 6 hours increase fluorescence. This breakthrough in our research gives way for high throughput assays to test for drug candidates that reduce AGEs. Our long-term goal is to use *C. elegans* to assist in our understanding of underlying cellular mechanisms that contribute to prostate cancer disparities.

Deciphering the functional network of FSGS-associated genes using *C. elegans*.

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Focal segmental glomerulosclerosis (FSGS) is a kidney disease defined by morphological changes to, and eventual loss of, podocytes: specialized cells that form the kidney filtration barrier. Podocyte loss due to FSGS leads to end-stage kidney disease. Genetic studies have identified mutations in >20 genes associated with FSGS. Current therapies are ineffective when the underlying FSGS is due to a single genetic cause (i.e., monogenic). Thus, for monogenic FSGS it is critical to understand 1) how do the proteins encoded by FSGS-causing genes function? 2) how do disease-causing mutations alter them? and 3) do they function in a network that could be manipulated to provide new therapeutics?

One of the most commonly mutated genes in monogenic FSGS is INF2, a member of the formin family of actin-polymerizing factors. Previous models of INF2 in FSGS centered on its canonical role as an F-actin regulator. However, our work showed that INF2 function is more complex. We previously characterized the two *C. elegans* INF2 orthologs, *exc-6* and *inft-2*, by studying their function in the excretory canal (*ExCa*): a unicellular tube required for osmoregulation. We showed that *exc-6* not only regulates F-actin accumulation, but also microtubule (MT) dynamics, raising the possibility that MTs may be affected in FSGS. We also showed that disease-causing forms of human INF2 rescue *exc-6*, demonstrating conserved function. As for *inft-2*, we showed that it promotes F-actin accumulation and that this activity is inhibited by *cyk-1*, the sole ortholog of the mammalian formin Diaphanous.

How gain-of-function mutations in INF2 lead to the changes in podocyte morphology and FSGS remains unknown. To study the effects of FSGS-causing mutations, we are mutating the conserved, disease-associated, residues in *inft-2*, to ask how they affect INFT-2 localization and the cellular functions regulated by this formin. FSGS-causing mutations have also been identified in seven other genes whose products are cytoskeletal regulators or cytoskeleton-associated proteins (*TRPC6, CD2AP, ANLN, ACTN4, ARHGAP24, ARHGDIA* and *MYOE1*). Whether these genes, and INF2, function in one or several pathways to regulate the podocyte cytoskeleton has not been explored. *C. elegans* encodes orthologs for all these genes. We are assessing their expression and function in the *ExCa*, as we did for INF2 homologs, and are testing genetic interactions between these genes and *exc-6* and *inft-2*, to define a functional network of FSGS-associated genes.

A role for Cofilin (UNC60A) in early embryogenesis in *C. elegans*.

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During the first cell division of the fertilized embryo in the *C. elegans*, the actin cytoskeleton undergoes a series of cortical contractions, disassembly, reorganization, and ultimately builds the cytokinetic ring in a short period of time. These processes require precisely timed rapid disassembly and re-assembly of actin filaments. Mutants or small molecules that decrease actin filament disassembly have a dramatic effect on the cortical actin network, causing massive disruptions in the integrity of the network, and can prevent cytokinesis from occurring. As such, understanding the requirement for actin disassembly factors in the embryo will provide insights into how actin network turnover occurs in a dynamic environment and why it is crucial for cytokinesis. I have purified the predominant cofilin isoform in the early embryo, UNC60A. By visualizing the effect of UNC60A on polymerizing actin filaments using TIRF microscopy, I've found that it can promote both severing of filaments and depolymerization from the actin filament pointed end. I am generating a labeled UNC60A to visualize its binding to actin filaments in vitro, and I am also making a *C. elegans* line with a fluorescently tagged UNC60A to visualize its effect on the cortical actin network in vivo during early embryogenesis.

SYGL-1 and the molecular switch from GSC self-renewal to differentiation in *C. elegans*.

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A proper balance between self-renewal of germline stem cells (GSCs) and generation of gametes is crucial for survival of the species. The *C. elegans* germline provides a well-established model for studying this fundamental biological issue. Notch signaling from the niche directly activates transcription of *sygl-1* to maintain GSC self-renewal (Kershner 2014). Downstream of Notch and *sygl-1*, two FBF RNA-binding proteins repress differentiation-promoting mRNAs (e.g. Crittenden 2002). Self-renewal pushes GSC daughters proximally and out of the niche, a process coincident with their "switch" from a naïve stem cell-like state to a state primed for differentiation. The molecular basis of this switch is not understood.

We previously showed that SYGL-1 protein interacts with FBF, that SYGL-1 represses FBF target RNAs required for differentiation (e.g. gld-1), and that the extent of SYGL-1 expression determines size of the GSC pool (Shin, Haupt 2017). The challenge now is to understand the role of SYGL-1 in triggering the fate switch to begin differentiation. We hypothesize that lowering SYGL-1 abundance below a critical threshold disrupts the SYGL-1::FBF repressive complex, and triggers the molecular switch.

We are taking two approaches to test this hypothesis. First, we are defining the SYGL-1 threshold below which GSCs switch to differentiation. Second, we are analyzing the molecular basis of the SYGL-1::FBF interaction in an attempt to compromise complex formation. To define the SYGL-1 threshold, we have systematically engineered a series of sygl-1 promoter mutants to precisely tune the quantity of SYGL-1 in the germline. We quantitate amounts of sygl-1 RNA and SYGL-1 protein as a function of position and estimate the position of the switch by standard methods. To interrogate the FBF::SYGL-1 binding interface and learn how it functions in the switch, we identified FBF binding motifs in SYGL-1, generated mutants of those motifs and found them to abrogate GSC self-renewal. Our results reveal how a key stem cell maintenance regulator functions within a macromolecular complex to balance self-renewal and differentiation in an in vivo context. Our plan for the future is to seek regulators of SYGL-1 abundance.

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daf-16 blocks expression of _let-7_-family microRNAs to promote multipotent cell fate during dauer.

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Maintenance of multipotency during lengthy periods of cellular quiescence is critical to stem cell function, yet the mechanisms linking multipotency to quiescence remain poorly understood. One candidate for coordinating these processes is the conserved DAF-16/FOXO transcription factor. In response to adverse conditions, *daf-16* promotes entry into the quiescent dauer larva stage midway through larval development. We previously found that during dauer, *daf-16* blocks EGFR/Ras and LIN-12/Notch signaling to promote multipotency in vulval precursor cells. We now show that *daf-16* acts via a distinct mechanism to promote multipotency in hypodermal seam cells during dauer. Larval seam cells are self-renewing and multipotent, whereas adult seam cells are differentiated. MicroRNAs within the heterochronic gene network promote adult seam cell fate. Using RNA-seq and transgenic reporter strains, we find that *daf-16(0)* dauer larvae express adult cell fate markers, including *col-19::gfp*. Consistent with the aberrant expression of adult cell fate markers, *let-7*-family microRNAs are highly and specifically overexpressed in *daf-16(0)* dauer larvae. Genetic experiments show that this overexpression contributes to the aberrant *col-19::gfp* expression in *daf-16(0)* dauers. The LIN-29 transcription factor is the most downstream heterochronic gene and directly activates expression of *col-19* in adults. Surprisingly, *lin-29* is dispensable for the *col-19::gfp* phenotype in *daf-16(0)* dauer larvae, suggesting that *let-7*-family microRNAs act via a novel mechanism to control *col-19::gfp* during dauer. As expected, given overexpression of *let-7*-family microRNAs, RNA-seq data shows that most predicted *let-7*-family targets are downregulated in *daf-16(0)* dauer larvae. All together, our data suggest a model whereby *daf-16* opposes the transcription of *let-7*-family microRNAs, allowing the expression of novel *let-7* target genes that inhibit adult cell fate and maintain multipotency during dauer.

Cytoplasmic Aggregates of Human TDP-25 Protein in *C. elegans* Challenge Proteostasis.

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Proteostasis, the process by which a cell maintains protein production, folding, and degradation is critical for survival; however, the fidelity of this process declines with age. Disturbance of proteostasis contributes to many age-related neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis (ALS), leading to the accumulation of misfolded proteins. Patients with this disease accumulate an aggregated form of the ALS associated protein TDP-43 in neurons, suggesting a disruption in proteostasis. Here, we are using the nematode *C. elegans* to examine how a toxic fragment of TDP-43, called TDP-25, challenges proteostasis. *C. elegans* is our model of choice because of its short life cycle, its transparent nature, and conservation of genes with human homologues. We have generated multiple transgenic lines expressing fluorescently tagged TDP-25 in the body wall muscles of the animal. Using gel electrophoresis followed by Western Blot Analysis, I will evaluate the steady state level of TDP-25 in each of the three transgenic lines during development and aging. Furthermore, the aggregation state of TDP-25 will be monitored with an expectation that the aggregation level increases with age. The strain with the highest steady state level will be identified and then aggregation will be examined. We anticipate a high level of TDP-25 will lead to an increase in aggregation in aging animals by challenging proteostasis.

Functional analysis of microRNA regulatory roles in the *Caenorhabditis elegans* male gonad.

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microRNAs are short non-coding RNAs with a length of ~ 22 nucleotides that regulate gene expression at the post-transcriptional level. Disruption of miRNA function is associated with a wide array of human diseases, including male factor infertility. While evidence suggests that miRNA function is needed for production of functional sperm, the specific regulatory roles of miRNAs in this process are largely unknown. To address this question in *Caenorhabditis elegans*. I used small RNA sequencing on isolated gonad tissues from adult hermaphrodites and males to identify gonad-enriched microRNAs. This analysis revealed a differential expression pattern of microRNAs between hermaphrodite and male gonads. Specifically, 27 miRNAs were found to be lower and 25 miRNAs were found to be higher in the hermaphrodite gonad compared with the male gonad. Then two major approaches will be used to analyze these candidate microRNAs. First, functional analysis is being carried out on existing microRNA deletion mutants or microRNA mutants built with CRISPR genome editing tool. Specifically, mating assays are being used to screen potential miRNAs for regulation of male fertility and fecundity. Defects in sperm formation or quality would result in higher percentage of self-progeny compared to control. Preliminary studies with some microRNA mutants have identified two miRNA mutants with increased percent of self-progeny compared to control. Future studies will include phenotypic characterization on those mutants with increased percent of self-progeny. Secondly, computational analysis will be conducted using gonad-expressed miRNAs, miRNA target predictions, and transcriptome data sets to identify potential microRNA regulatory networks, which can allow systematic understanding of miRNA regulatory roles in the male germline. Taken together, this integrative study will provide important clues for our understanding of microRNA regulatory roles in sperm formation and function.

High throughput analysis of magnetic orientation using the nematode *C. elegans*.

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Many organisms, from bacteria to whales can detect and orient to the magnetic field of the Earth. Many of these magnetosensitive species use magnetic fields for large migrations. While much work has been done on which animals use the magnetic field, the mechanism for magnetic field detection remains unknown. This is in large part due to the difficulty of studying such large-scale migratory behaviors, and the lack of genetic tools in magnetosensitive species to uncover genes involved in magnetoreception. Thus, studying mechanisms for magnetic field detection would be aided by being able to study this behavior on a smaller scale in an animal amenable to genetic approaches. We recently showed that the nematode *C. elegans* can detect and orient to the magnetic field of the Earth. Since *C. elegans* are small organisms, they are able to be monitored in the lab. Many genetic tools are available to study *C. elegans*. This provides us a manageable experimental framework from which to study this behavior, and positions us to uncover the mechanisms for magnetic orientation.

A Potential New Component That Mediates the Regulation of Fluid Homeostasis in *C. elegans*.

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Fibroblast growth factor receptors (FGFRs) belong to a larger family of receptor tyrosine-kinase (RTK) cell-surface receptors which phosphorylate specific tyrosine residues to trigger downstream responses such as cell proliferation, migration, and differentiation. The study of the EGL-15 FGFR in *C. elegans* has long been used as a paradigm to understand principles of RTK signaling, since defects in the processes mediated by EGL-15 result in striking phenotypes that provide powerful tools for genetic analysis. One such process is the regulation of fluid homeostasis. EGL-15 hyperactivation causes excessive accumulation of clear fluid inside the worm's body (the Clear (Clr) phenotype). The isolation of Clr suppressors, termed suppressor of clr (*soc*) mutants, has led to the identification of many of the core components of EGL-15 signaling. For example, the original set of *soc* mutations identified the Grb2/SEM-5 adaptor protein that links RTK activation to the activation of the RAS/MAPK pathway. Although SEM-5 is required for EGL-15 signaling, a key component that links activated EGL-15 to SEM-5 has yet to be identified, since the *egl-15(n1457)* mutation that eliminates the known, direct SEM-5 binding sites on EGL-15 does not confer a Soc phenotype. To identify these missing components, we conducted a modified "enhancer" Soc screen in an *egl-15(n1457)* background. This screen identified new mutations that confer a strong Soc phenotype in an *egl-15(n1457)* background. Of sixteen characterized enhancers, six have been shown to be autosomal. Two are alleles of the known, major *soc* gene *soc-1*. Using complementation tests, we have shown that the remaining four autosomal mutations (*ay157, ay169, ay174, and ay195*) are allelic to one another, and are unlinked to any known *soc* genes. When isolated from *egl-15(n1457)*, *soc(ay195)* is not significantly Soc, consistent with it functioning as a redundant link between EGL-15 and SEM-5. Further characterization of this autosomal complementation group may lead to the discovery of a new component of EGL-15 signaling.

Determination of *mig-32* as a Class A or Class B synthetic multivulva (synMuv) gene.

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We are interested in how gene expression is regulated during development in *C. elegans*, and specifically the regulation of gene expression through changes in chromatin modifications. Our research has shown that many of these chromatin regulatory pathways and subsequent gene expression pathways are temperature sensitive. One of the group of chromatin regulators we study are synMuv B proteins. The synthetic Multivulva (synMuv) class of A and B genes function redundantly to induce proper vulva growth in *C. elegans*. Class A synMuv genes encode novel proteins, and Class B synMuv genes encode proteins that are involved in chromatin regulation. Single mutations in either class do not produce a multivulva phenotype, however; when a double mutation of class A and B is created, a characteristic phenotype of multivulva is apparent. Previously published research indicated that the gene *mig-32* may be a newly defined synMuv gene. When a double mutant between *mig-32* and a temperature sensitive synMuv AB mutant *lin15(n765ts)* were created they showed a 100% synMuv phenotype at 15°C (Karakuzu et al. 2009). Therefore, we wanted to determine whether *mig-32* is a synMuv A or synMuv B gene. To replicate and observe these findings, we first created a *mig-32(n4275); lin-15B(n744)* double mutant strain and scored for the synMuv phenotype at both 20°C and 24°C. We saw no synMuv phenotype at either temperature and saw a weak but statistical difference in the P-vulva phenotype in the double mutant when compared to the controls. Next, we created a *mig-32(n4275); lin-15A(n767)* double mutant strain and scored for the synMuv phenotype at 20°C and 24°C. Again, there was no appearance of the synMuv phenotype at either temperature. Lastly, we are currently creating a *mig-32(n4275); lin-15AB(n765)* strain to score for the presence of the synMuv phenotype as to replicate previously published experiments. Once the final strain has been successfully made, we will be able to conclude whether *mig-32* is a synMuv A or synMuv B gene or not a part of this synMuv gene class.

Karakuzu O., S. Cameron, and D. P. Wang, 2009 MIG-32 and SPAT-3A are PRC1 homologs that control neuronal migration in *Caenorhabditis elegans*. Development 136: 943 – 953.

Effect of D-amino acids on *C. elegans* locomotion.

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D-amino acids (D-AAs) are the D-enantiomers of the prevalent L-amino acids and were previously regarded as non-functional. However, recent evidence has shown that D-AAs have functional roles in biological systems ranging from microbes to mammals. Mammalian brains can synthesize D-aspartate (D-Asp) and D-serine (D-Ser) to activate N-methyl-D-aspartate receptors. However, many D-AAs that are found in mammals are likely to be from exogenous sources, either from diet or gut microbiota. For instance, D-alanine (D-Ala) is an essential building block in peptidoglycan bacterial cell walls and is found in the neuroendocrine glands of mammals; however, its physiological role in higher animals is unclear. Here we use *Caenorhabditis elegans* as a model to understand the effect of exogenous D-AAs, including D-Ser, D-Asp and D-Ala, on C. elegans locomotion by using a thrashing assay. As metabolism of D-AAs in animals includes racemases for D-AA synthesis and oxidases to degrade D-AAs, we used both wild type strain N2 and a serine racemase homolog-knockout mutant (*T01H8.2*) for testing.

Age-appropriate coordination of behavior and reproductive physiology via a shared neuronal circuit.

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Successful reproduction in animals involves orchestration of numerous behaviors and physiological processes. A promising path to understanding how suites of reproduction-related traits are coordinated is to investigate how sex pheromones can induce both “releaser” (behavioral) and “priming” (physiological) effects. Here we describe a neuronal circuit that couples the reproductive system and behavior in adult *Caenorhabditis eleg*ans hermaphrodites. We found that the response of the oogenic germline to the male pheromone requires serotonin signal from NSM and HSN neurons acting via the *mod-1* receptor in AIY and RIF interneurons and antagonized by pigment-dispersing factor (PDF). Surprisingly, the same neurons and pathways have been previously implicated in regulation of exploratory behavior in the absence of male-produced signals. We demonstrate that in the presence of male pheromone, this circuit acts in hermaphrodites to reduce exploration and decrease mating latency. By affecting the germline and reproduction-related behavior the circuit tunes fitness-proximal processes that unfold on different time scales. Critically, only sexually mature hermaphrodites with a functional egg-laying apparatus modify their behavior in response to male pheromone. We show that this age-appropriate response is due to feedback from the vulva muscles that reports ongoing reproduction to the nervous system. Our results demonstrate a mechanism by which a single circuit could coordinate distinct responses to the environment and reveal an activity-dependent conduit by which the reproductive system continuously licenses adult responses to the pheromones of the opposite sex.

Compromised Mating Ability and Reduced Sperm Transfer Explain Reduced Fertility in *C. elegans* Males at High Temperature.

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Despite the numerous advantages sexual reproduction confers, it has one ancient and deeply conserved flaw: it is temperature sensitive. As temperature rises, fertility decreases. Previous work in our lab has shown that *C. elegans* wild isolate males raised at 27°C are almost all sterile, while males raised at 20°C, but up-shifted to 27°C for mating, are almost all fertile. Male fertility depends upon the production of fully functional sperm, and the ability to find and successfully mate with a hermaphrodite. Therefore, the greatly diminished male fertility we observe at high temperature could be explained by changes in: (1) sperm count, (2) sperm activation, (3) male mating behavior, (4) sperm motility, or some combination of these factors. We investigated these possibilities in wild isolate strains LKC34, JU1171, and the lab wild type N2. We did not find any significant differences in sperm count between males raised at 20°C and 27°C. Thus, as has been observed in hermaphrodites, male sperm count is not greatly impacted by high temperature stress. At 27°C, males showed decreased rates of *in vitro* sperm activation in some instances (10-25%), but these differences are not great enough to fully explain the near sterility we observe. Using a four-minute male mating interest assay, we found that males from all strains showed declines in multiple stages of courtship behavior at 27°C; and the decrease in total successful mating events at 27°C differed significantly from that at 20°C. We also tested the ability of males to transfer fluorescent sperm to strain-specific hermaphrodites given a 45 min. mating window. Significantly fewer males exposed to 27°C transferred sperm to hermaphrodites than their counterparts at 20°C. Finally, we tested if fluorescent sperm could migrate properly in the hermaphrodite reproductive tract over an additional 45 min period. The lab adapted wild type N2 sperm were significantly less likely to migrate to the spermatheca when exposed to 27°C. Our data indicate that reduced sperm count, and activation are not the main cause of low fertility at 27°C, but rather that decreased mating interest, sperm transfer, and sperm motility within the hermaphrodite reproductive tract may play more principle roles.

The excretory canal as a platform to discover kinase regulators of tubulogenesis and angiogenesis.

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Tubulogenesis, the process by which organisms form biological tubes, plays an integral role in development, including in blood vessel formation (angiogenesis) and function. Moreover, defects in tubulogenesis can lead to congenital disease (e.g., capillary malformations) and dysregulated tubulogenesis due to tumor-induced angiogenesis is associated with cancer progression and metastasis. The excretory canal (*ExCa*) provides a tractable model to study tubulogenesis. Human orthologs of several genes that regulate *ExCa* tubulogenesis, for example orthologs of the CLIC channel *exc-4* regulate Rho/Rac signaling in endothelial cells (see Arena, and Escudero, abstracts). Moreover, the kinases *gck-1, gck-3,* and *wnk-1*, all previously implicated in *ExCa* tubulogenesis, regulate angiogenic behaviors in human endothelial cells, or have been implicated in vascular disease. Our hypothesis is that we can find novel regulators of angiogenesis by screening for new players in *ExCa* tubulogenesis. Kinases are critical regulators of key cellular functions, with approximately 30% of human proteins being phosphorylated by kinases. Using OrthoList, a compendium of *C. elegans* genes with human orthologs (ortholist.shaye-lab.org), we identified 248 *C. elegans* kinases that have human orthologs. An RNAi screen against these kinases led to discovery of nine whose loss leads to *ExCa* defects. Among these are four (*gck-1, gck-3, mrck-1* and *wnk-1*) previously implicated in *ExCa* tubulogenesis, indicating a low false-negative rate. Alleles for the five new kinases have confirmed the RNAi results, indicating a low false-positive rate. We are characterizing the phenotypes caused by loss of these kinases, using available nulls and conditional alleles we are generating by CRISPR, to understand their roles in tubulogenesis. We have found that orthologs of all the kinases identified in the ExCa screen are expressed in human umbilical vein endothelial cells (HUVEC); a canonical model for studying endothelial cell behavior and angiogenesis in cell culture. We are using lentiviral shRNA constructs to knockdown the expression of these kinases and performing angiogenesis assays to define conserved roles that kinases discovered in *C. elegans* may have in angiogenesis.

Modified DSB repair program for exogenously induced DSBs in the *C. elegans* germline.

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In the germline, numerous DNA double-strand breaks (DSBs) are formed in a programmed manner by the germline-specific SPO-11 nuclease, forming DSBs that are committed to repair via the homologous recombination (HR) pathway. In addition to SPO-11 DSBs, germline nuclei also acquire DNA damage from exogenous sources, as found in other tissues. It is unknown if these exogenously induced DSBs are committed to error free HR, or can be repaired by the error prone non-homologous end joining (NHEJ) pathway. To study the germline's response to DSBs caused by exogenous sources we have adopted the laser microirradiation (MIR) system for the induction of DSBs in a way that allows following the recruitment of DSB repair proteins in a live, whole, worms. Utilizing the spatiotemporal organization of nuclei in the C. elegans germline we can follow DSBs in different cell cycle stages, acquiring information about recruitment time of DSB repair proteins to DSBs and their spatial distribution over time. We have previously shown that HR proteins (GFP::RPA-1 and GFP::RAD-51) form foci specific to nuclei exposed to MIR and are recruited to MIR breaks at ~10 and ~20 min respectively, as expected from their biochemical functions. We are currently investigating early events of DSB repair through recruitment of nucleases to MIR breaks. EXO-1 and MRE-11 are two major nucleases in the HR pathway. Both nucleases form foci within ~1 and 3 min following MIR respectively, consistent with their role as germline nucleases. However, while both nucleases are recruited to DSBs in mitotic and early meiotic nuclei, only MRE-11 is found on MIR foci of mid-prophase nuclei. This indicates that EXO-1 is not required for resection in early meiotic nuclei, which is surprising since EXO-1 is the only nuclease proposed to be involved in long-range resection in the C. elegans germline. MRE-11 MIR foci recruit RAD-51, as do SPO-11-induced DSBs. However, MIR foci also contain cKU-80, an obligatory member of the canonical NHEJ pathway, which does not localize to SPO-11-induced DSBs. In the absence of cKU-80, MRE-11 is recruited faster to breaks, but only in mitotic and mid-prophase I nuclei. Our studies suggest that unlike SPO-11-induced DSBs, MIR-induced DSBs can be substrates for error prone repair pathways even when HR is functional. If so, repair of exogenous DSBs may be more mutagenic compared to repair of endogenous, SPO-11-induced, DSBs.

The search for genes that prevent the progression of degeneration in Duchenne muscular dystrophy.

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Duchenne muscular dystrophy (DMD) is a degenerative neuromuscular disorder that affects 1 in 3,500 males and is characterized by severe muscle atrophy, mobility loss, intracellular calcium accumulation, degradation of mitochondria, and decreased muscle fibers. DMD is caused by an absence of the dystrophin protein. Within a muscle cell, dystrophin acts to bridge the cytoskeleton to the surrounding extracellular matrix through association with other proteins to form the dystrophin glycoprotein complex (DGC). This connection provides support for the muscle membrane during contraction, and the absence of dystrophin increases the muscle's susceptibility to damage. It is well known that muscle fiber integrity is lost in DMD patients, but it is not yet understood how the loss of dystrophin results in different molecular mechanisms that lead to degeneration. To examine these molecular mechanisms, we are investigating gene expression in dystrophic and healthy *Caenorhabditis elegans*. After undergoing locomotion requiring high levels of muscular exertion, animals with loss of function mutations in the *C. elegans* homolog of dystrophin, display symptomatic similarity to DMD patients. We have found that genes associated with mitochondrial stress and contractile machinery are upregulated in dystrophic worms. Understanding the molecular mechanisms underlying degeneration in DMD will help us to fine tune our search for potential molecular therapeutics.

Wnt Dependent Cell Fate Specification Requires Active SYS-1/β-Catenin Trafficking and Turnover at the Mitotic Centrosome.

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Asymmetric cell division, the unequal distribution of cell fate determinants between daughter cells, is a critical system underlying the development and maintenance of varied tissue types in a multicellular organism. Despite widespread utilization of such divisions, the mechanisms responsible for induction of asymmetry are less understood due to the pleiotropic, and often functionally redundant signaling systems involved. Throughout *C. elegans* development, the Wnt/β-catenin asymmetry pathway induces asymmetric distribution of Wnt signaling components to polarize a mother cell and differentially regulate Wnt target genes in its daughters. Despite this asymmetry in its regulation, cytoplasmic SYS-1 localizes symmetrically to mitotic centrosomes in a manner consistent with conserved β-Catenin localization. The sub-functionalization of *C. elegans'* 4 β-catenin homologs allow us to investigate the role of this localization on the transcriptional regulation of the Wnt/β-catenin asymmetry pathway. Recent data indicate that centrosomal localization of SYS-1 serves as a clearance mechanism to increase the robustness of Wnt-mediated polarity. However, the method of SYS-1 accumulation at the centrosome is unknown. In the early embryo, centrosomal localization of SYS-1 is dependent on the centrosomal scaffolding protein RSA-2. Depletion of RSA-2 by RNAi prevents SYS-1 centrosomal loading, forcing it to accumulate in the approximate region of kinetochore microtubules. This localization, with minus-end directed movement implied by rapid accumulation at a microtubule organizing center, suggests a role for dynein and dynactin components as the motor necessary. Data from microtubule-destabilizing and ATP-depleting chemical treatment indicate that both ATP and microtubules are important for SYS-1 centrosomal localization, and a temperature-sensitive allele of the dynein heavy chain shows a disproportionate effect on SYS-1 localization. Additionally, an RNAi screen depleting these microtubule transport proteins identified several dynein subunits that promote proper centrosomal localization of embryonic SYS-1/ β-catenin. Some of these are enough to induce SYS-1 mediated cell fate changes These results suggest an active role for the multimeric dynein/dynactin cargo binding complex in SYS-1/β-catenin regulation and signaling status.

Cell to cell spreading of TDP-43 C-terminal fragments may lead to toxicity in *C. elegans*.

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Many neurodegenerative disorders are characterized by abnormal accumulation of disease proteins in nerve cells leading to selective neurotoxicity. Moreover, a prion-like spreading mechanism might play a role in disease progression, where misfolded proteins spread from affected to unaffected neurons. Interestingly, amyotrophic lateral sclerosis (ALS) exhibits a focal clinical onset followed by a regional spreading of protein misfolding and cell death. Evidence points towards TAR DNA-binding protein 43 (TDP-43) as the major pathological protein in sporadic and certain familial forms of ALS where aggregates in affected neurons contain full length and fragmented forms of TDP-43. Despite recent advances in research on ALS disease associated proteins like TDP-43, a mechanistic explanation that links toxicity with cell to cell transmission remains unclear.

To explore whether TDP-43 spreads from cell to cell, we established a *C. elegans* model that expresses a human TDP-43 C-terminal fragment (TDP-25) fused to red fluorescent protein in the body wall muscle (BWM) cells. We employed high-resolution time-lapse imaging and observed the intercellular movement of TDP-25 from BWM cells to the hypodermis, intestinal cells and gonad. These results confirm that at least certain fragments of TDP-43 are released from donor cells into neighboring receiving cells. To determine if the accumulation of TDP-43 C-terminal fragments in receiving tissues leads to toxicity, we monitored the function of the gonad. We found that accumulation of TDP-25 had no significant effect on fecundity or embryogenesis compared to wild type animals. Furthermore, expression of TDP-25 in BWM did not reduce thrashing activity suggesting that the expression of TDP-43 C-terminal fragments in the BWM alone does not produce a toxic phenotype. Currently, we are mapping the movement of TDP-43 C-terminal fragments from donor cells to receiving cells using strains expressing tagged lysosomal and endosomal components. Evidence of phosphorylation and co-localization would support the model that the cell to cell spreading of toxic TDP-43 fragments contributes to the progression of disease pathology.

Understanding the role of the conserved kinase PIG-1/MELK in tubulogenesis.

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Biological tube formation, or tubulogenesis, is a conserved process critical for the formation and function of most organs. We use the *C. elegans* excretory canal (*ExCa*), a single-cell tube, to discover and study new conserved regulators of tubulogenesis. Kinases are central regulators of diverse signaling pathways and cell biological processes. The *C. elegans* genome encodes ~500 protein kinases, of which 248 are conserved with humans. In a previous genetic screen from our lab, focused on these conserved kinases, five new kinase regulators of *ExCa* tubulogenesis were found. The main goal of this project is to define the role of PIG-1, one of these new kinases. The human homolog of PIG-1 is called MELK, and its role is not well understood, although high MELK expression is correlated with aggressiveness and poor prognosis for several types of cancer. One possible target of PIG-1 is the protein EXC-6; a worm homolog of the human protein INF2, which is mutated in congenital kidney disease and Charcot-Marie-Tooth neuropathy. The *ExCa* phenotypes seen in *exc-6* mutants are almost identical to those seen in *pig-1* mutants, leading us to hypothesize that *exc-6* and *pig-1* function in the same pathway. To test this hypothesis we have mapped a transgene expressing fluorescently-tagged EXC-6 (mCherry::EXC-6) and are crossing this construct into an *pig-1* null (0) background to analyze the effects that loss of *pig-1* has on mCherry::EXC-6 localization using confocal microscopy. We have also mapped the genomic location of several other transgenes expressing fluorescent proteins that mark different sub-cellular compartments (e.g. Golgi, ER and Mitochondria) in the *ExCa*, and we will cross these into *pig-1(0)* and *exc-6(0)* to further analyze the common, or different, effects that loss of these proteins have on cellular architecture.

Natural variation of *C. elegans* short tandem repeats.

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Short tandem repeats (STRs) can rapidly evolve and have been implicated in the regulation of gene expression, genetic diseases, and complex traits. Despite the increased recognition of their functional importance, little is known about the variation of STRs across the *Caenorhabditis elegans* species. To understand how STRs evolve across the *C. elegans* population, we analyzed whole-genome sequencing data of 330 wild isolates and leveraged HipSTRs to infer the genetic variation of STRs. First, we identified 11,193 high-confidence polymorphic STRs, which are unevenly distributed across the genome. Second, we determined the allele frequencies of STRs across the population and found STRs that fall into coding regions show features of functional constraint, depleted heterozygosity, and increased interrupted allele frequencies. Third, we analyzed the distribution of STR motifs across the genome. We characterized motif-specific enrichment and depletion among the intergenic region, promoter, coding sequences (CDS), intron, and UTRs. Taken together, we discovered a large number of population-wide STR polymorphisms, of which variation could be shaped by natural selection in a motif-specific manner.

A genetically divergent *C. elegans* wild isolate exhibits extremely high male frequency.

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Caenorhabditis elegans is an androdioecious nematode with hermaphrodites and males. A self-fertilizing hermaphrodite rarely produces males, with frequencies of approximately 0.1% in the laboratory strain N2. By contrast, hermaphrodites fertilized by males have 50% male progeny. Many studies have suggested that self-fertilization is the primary reproduction mode of *C. elegans* in nature, and the transition from obligately outcrossing might be recent (Cutter et al., 2008). Natural variation of male frequencies were also observed in *C. elegans*, ranging from 0.1% to 35% (Teotónio et al. 2006; Anderson et al., 2010). In a recent sampling of *Caenorhabditis* in Hawaii (see abstract by Crombie *et al*.), we isolated a *C. elegans* strain, named ECA701, which exhibited extremely high male frequencies of 34% - 57% and low brood sizes of 80 - 170 by selfing of hermaphrodites. Mating of ECA701 between hermaphrodites and males showed male frequencies of 47% - 70% and brood sizes of 150 - 290. We further found that ECA701 produced embryos with high levels of embryonic lethality (30% in young adults), which is exacerbated in older adults (60% in two-days old adults). These phenotypes indicate that ECA701 is potentially defective in the meiotic processes. Notably, using whole genome sequencing and phylogenetic analysis, we also found that ECA701 is one of the most divergent strains that have yet been described. Taken together, ECA701 provides a great opportunity to improve our understanding on the possible ancestral state and the evolution of reproduction mode in *C. elegans*.